

The Lid Wiper Contains Goblet Cells and Goblet Cell Crypts for Ocular Surface Lubrication During the Blink

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Purpose: The conjunctival side of the upper and lower inner eyelid borders, termed the lid wiper, has a thickened epithelial lip for apposition to the globe, assumed to distribute the preocular tear film. The human lid wiper structure and its goblet cells are investigated.

Methods: Conjunctival whole mounts, including lid margins from 13 human body donors, were investigated by routine histology and semithin plastic sections, using histology, histochemistry, and immunohistochemistry.

Results: In routine histology, the conjunctival lid wiper epithelium regularly showed goblet cells, single and in clusters, at the luminal surface and also deep within the epithelium without apparent surface contact. Semithin sections revealed that the deep goblet cells were often connected to cryptal epithelial infoldings that opened to the surface, hence making their mucins available at the surface. The goblet cells produced mucins of neutral (periodic acid-Schiff) and acidic (Alcian blue) type and stained positive for the gel-forming mucin MUC5AC. Surprisingly, MUC5AC-negative goblet cells were also observed in the lid wiper.

Conclusions: Contrary to conventional assumptions, the lid wiper is part of the conjunctiva. It contains previously undescribed goblet cell crypts deep in the epithelium, suitable as an internal lubrication system for reduction of friction between the lid margin and the globe. This provides the first evidence of the morphological basis for the hydrodynamic type of lubrication and a more conclusive understanding of lid-margin lubrication and tear film distribution. It is another strong indication that the lid wiper is that area in apposition with the globe for distributing the thin preocular tear film during the blink.

Key Words: eyelid margin, lid wiper, goblet cells, goblet cell crypts, mucins, tear film, lubrication, blinking, mucocutaneous junction, conjunctiva, human

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The lid wiper is a specialized zone located at the conjunctival side of the inner (ie, posterior) lid border of the upper and lower eyelids. It is an epithelial thickening that represents the region most closely apposed to the globe, and it contributes to the formation of a relatively sharp angle of the inner lid border compared with the more rounded outer lid border. Because of its location, it can be assumed that the lid wiper represents the zone of the lid margin that actually wipes over the bulbar ocular surface during a blink.^{1–5}

Contemporary knowledge that the inner lid border is “sharp” and “lies in contact with the globe” was documented at least as far back as 1904 by Parsons.⁶ In addition, a thickened epithelium in this region was described as early as 1877 by Sattler⁷; Virchow,⁸ in 1910, termed it as the “admarginal Zone” (admarginal zone) without considering a potential specific function. The thickening of the marginal epithelium was again described by Ehlers¹ who noticed the immediate functional implications of an elevated epithelium at the inner lid border for the distribution of the preocular tear film over the bulbar surface. Ehlers noticed that the “lid margin in normal subjects is closely pressed against the bulbus” and that “during blinking the posterior palpebral limbus moves very closely against the bulbus.” He postulated that “it is highly probable that it is only the squamous epithelium-lined part of the lid that rubs against the bulbus during blinking” and that “this soft (unkeratinized) bead gliding over the cornea must be assumed to be a perfect ‘wind screen wiper’.”

Although this epithelial formation at the inner lid border is conceivably of significant importance for tear film distribution and hence for ocular surface integrity, it has received increased interest only in recent years^{2–5,9–11} (Knop et al 2007, Abstract at the TFOS 2007 Conference). Korb et al² termed it the “lid wiper” because it represents a structural feature of the eyelid. It became of increased interest again with the discovery of epithelial alterations in this region that were visible upon vital staining with fluorescein and rose bengal in a clinical setting. This alteration was termed “lid wiper epitheliopathy” (LWE).² In patients with a dry eye, LWE was seen distinctly more frequently than in normals,⁴ and it occurred as the first sign of tear film deficiency even in the absence of other conventional signs (such as Schirmer test and FBUT).³ LWE was hence suggested as a sensitive early indicator of tear film instability and dry eye disease.³

The exact structure of the epithelium at the inner lid border was, however, not exactly clear. Virchow⁸ described an epithelial zone that showed an increased stratification

and thickness with cuboidal to columnar morphology that contained “Schleimzellen” (mucus cells), which refers to goblet cells. In contrast, Ehlers¹ reported, at the “posterior palpebral limbus” (inner, ie, posterior lid border), a squamous unkeratinized epithelium that was “twice as thick as the epidermis of the lid” but without goblet cells. It was described to extend for 1 to 2 mm until “rather abruptly it continued in a single or multi-layered, almost cuboidal epithelium with goblet cells” at the tarsal conjunctiva. A specific zone in this location with similar width is supported by earlier observations.^{6,8,12} Ehlers suggested that the lid wiper glided over the cornea and that the natural, vital stainable line of Marx¹³ that occurs at the inner lid border was possibly a result of increased friction between this epithelium and the bulbar surface. Recently, we have described the zonal differentiation at the inner lid border¹⁴ and identified goblet cells in an unusual deep position inside the lid wiper epithelium.

However, the exact structure of the lid wiper and the significance of deep goblet cells remain unclear as well as its relation to the natural stainable line of Marx. In the present study, the epithelium at the inner lid margin is investigated by conventional and thin-section histology, histochemistry, and immunohistochemistry (IHC) with a focus on the occurrence of goblet cells and mucins. Potential functional implications of the observed lid wiper structure and its relevance for the formation and distribution of a thin precocular tear film are discussed.

MATERIALS AND METHODS

Tissues

Whole mounts of conjunctival sacs including the lid margin from 17 eyes were obtained from deceased donors ($n = 13$; average age, 78.5 ± 14.4 years) of a white population in Germany with a macroscopically normal ocular surface. Time after death to harvesting of tissues was 12 to 36 hours during which the cadavers were cold stored. Before death, body donors had given informed consent; this study complies with the Declaration of Helsinki and was approved by the institutional review board.

Preparation and Histological Staining

The complete conjunctival sac was excised 1 to 2 mm distal/external to the outer lid border along the tarsal and orbital lid region toward the corneal limbus as previously described.¹⁵ The conjunctival tissue remained connected at the nasal canthus while the lateral canthus was divided. Whole-mount specimens were then placed on a plastic board and gently flattened without touching the conjunctival surface or lid margin. The tissues were immediately fixed by immersion in a 4% paraformaldehyde solution in 0.01 M phosphate buffer at 4°C and then embedded either in paraffin or in plastic blocks (Technovit 7100; Heraeus-Kulzer, Wehrheim, Germany). Serial sections of a thickness of 10 to 5 μm were cut from paraffin blocks with a rotary microtome (HM 355S; Microm, Walldorf, Germany), and semi-thin sections (1–2 μm) were cut from plastic blocks. Sections

were stained by hematoxylin and eosin (H&E) and methylene blue–fuchsin. Other sections were stained by histochemistry using either Alcian blue or periodic acid-Schiff reaction or both in a sequential manner, with and without hematoxylin counterstain or by IHC.

Histochemistry

Histochemistry was performed using Alcian blue and PAS stains. Both stains were performed either separately or combined (Alcian blue—PAS) according to Romeis.¹⁶ Briefly, after rehydration, the sections were treated as follows. Alcian blue: after 3 minutes incubation in 3% acetic acid, the sections were stained for 30 minutes with 1% Alcian blue solution in 3% acetic acid, rinsed with 3% acetic acid, and washed for 5 minutes in distilled water. Alternatively or in addition, the sections were stained by the PAS reaction: after 10 minutes incubation with 1% periodic acid and 10 minutes washing in distilled water, sections were immersed in Schiff reagent (Merck, Darmstadt, Germany), incubated for $\times 3$ 2 minutes in 0.5% sodium metabisulfite and washed in distilled water. Counterstaining was performed with hematoxylin if necessary.

Immunohistochemistry

IHC was performed by the highly sensitive ABC technique (37 Hsu’81), after mild enzymatic pretreatment (0.1% trypsin for 5 minutes) of the tissue sections for antigen retrieval (36 Shi’03), using a primary antibody against MUC5AC (generous gift from Dr Ilene Gipson, The Schepens Eye Research Institute, Boston, MA) diluted $\times 100$ and incubated on the sections at 4°C overnight. The primary antibody was detected with biotinylated secondary antibodies from the goat (Jackson/Dianova, Hamburg, Germany) incubated over night in a refrigerator and visualized by streptavidin-coupled peroxidase incubated for 30 minutes at room temperature. Diaminobenzidine (Hoechst, Ingelheim, Germany) was used as chromogen. Single staining steps of the IHC procedure were interrupted by repeated washings in phosphate buffer.

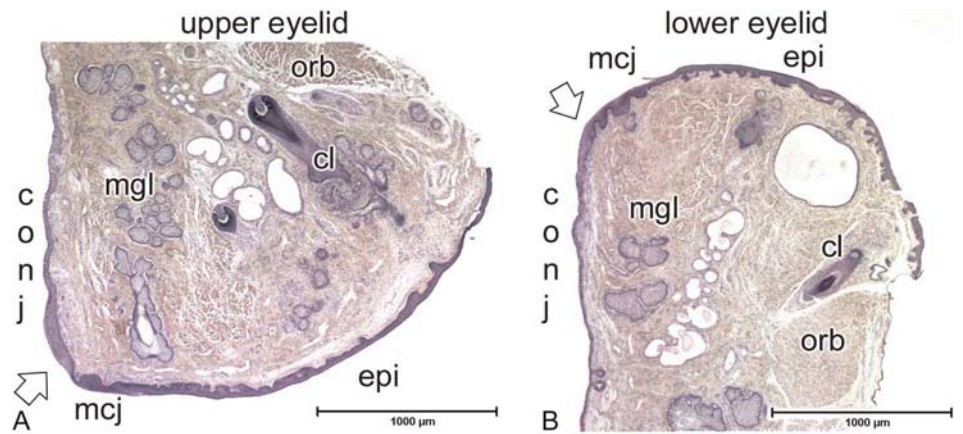
Microscopy and Photography

All sections were examined with a light microscope (Leica DMRB; Leica, Bensheim, Germany) and photographs were obtained using a digital camera (Spot Insight; Diagnostic Instruments, Sterling Heights, MI) with the Spot software V4.5.

RESULTS

In overview, the epithelium at the inner lid border of both the upper (Fig. 1A) and lower (Fig. 1B) eyelids was seen to form an elevation in the region directly apposed to the globe. This was inconspicuous in overview but showed clear structural characteristics in higher magnification. This epithelium at the inner lid border was thicker compared with the epidermis of the free lid margin and the tarsal conjunctiva.

FIGURE 1. The distinct epithelial elevation of the lid wiper (open arrow) is seen in overview at the inner (ie, posterior) lid border of an upper (A) and a lower (B) eyelid. The epithelial lining of the lid margin, consisting of the free lid margin epidermis (epi), mucocutaneous junction (mcj), and conjunctiva (conj), is seen together with the internal lid tissues (meibomian gland, mgl inside the tarsal plate; a ciliary hair follicle, cl; and the orbicularis muscle, orb). Paraffin histology, methylene blue–fuchsin; size marker in A and B = 1000 μm (1 mm)



Interposed between the lid wiper elevation and the termination of the free lid margin epidermis, as indicated by the absence of the granular and the cornified epithelial layers, was the mucocutaneous junction (MCJ). The MCJ had a multilayered stratified squamous epithelium (Fig. 2A) with a width of about 0.2 to 0.3 mm. The squamous cells of the MCJ were parakeratinized (ie, had a densely stained cytoplasm in H&E, a flat cell shape, and a highly condensed elongated nucleus; Fig. 2B). In the tarsal direction, ordinary squamous cells were interspersed, but goblet cells did not usually occur in the zone of the MCJ.

On the crest of the inner lid border at the start of the lid wiper elevation, the epithelial morphology changed, with cuboidal and occasionally columnar cells occurring at the surface. Goblet cells were usually seen from the start of this epithelium (Fig. 3A). Single squamous cells and some

parakeratinized cells were interspersed in places (Fig. 3B), but the majority of cells were cuboidal or columnar. Also, the internal composition of the epithelium starting on the crest of the inner lid border was different (Figs. 3A, B and 4A, B) from that of the MCJ because the lid wiper epithelium was composed of less intensely stained larger cells of cuboidal shape with larger and less compact nuclei. These cells were more loosely arranged and clearly resembled the structure of the conjunctival epithelium. The lid wiper epithelium was distinctly thicker than that of the tarsal conjunctiva, typically initially composed of approximately 8 to 12 cell layers but reached up to 15 cell layers in places. It

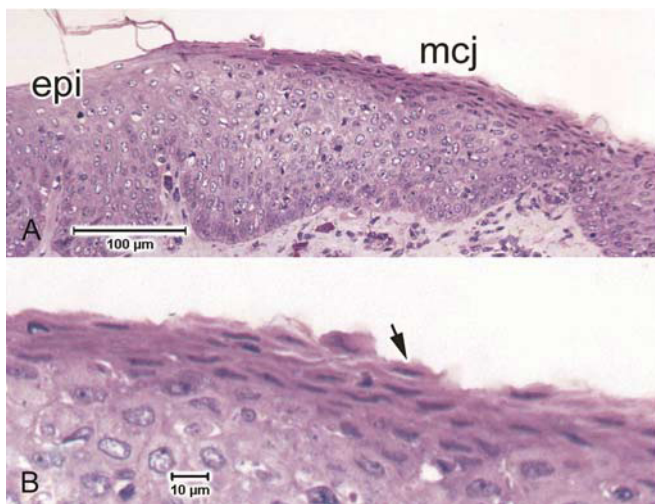


FIGURE 2. Anterior to the crest of the inner lid border (ie, to the skin side) is the stratified squamous epithelium of the mucocutaneous junction (A, mcj), which is followed by the free lid margin epidermis (A, epi). The mucocutaneous junction has several layers of parakeratinized cells at the surface that have an intensely stained flat cytoplasm and a highly compact nucleus (B, arrow). Semithin (2 μm) plastic section, methylene blue–fuchsin; size marker: A = 100 μm , B = 10 μm .

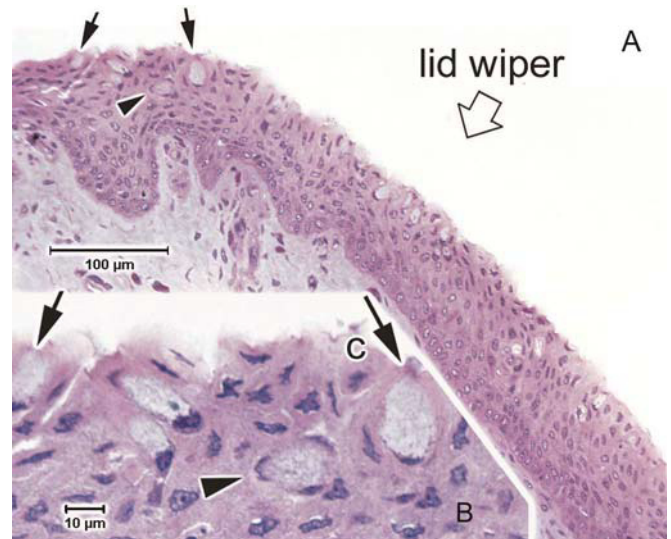


FIGURE 3. A, The start of the lid wiper on the crest of the inner lid border, or slightly anterior to it as seen here, is indicated by a change of the epithelial morphology. Cuboidal to columnar cells occur in the superficial epithelial layer and goblet cells are observed at the surface (arrows) and deep in the epithelium (arrowhead); the same cells are seen in higher enlargement (B). The epithelial structure is of the conjunctival type with larger, cuboidal (c) and less dense cells, some parakeratinized cells are interspersed. This lid wiper is about 100 μm thick and composed of about 10 cell layers. Semithin (2 μm) plastic section, methylene blue–fuchsin; size marker: A = 100 μm , B = 10 μm .

had a typical thickness of approximately 100 μm but could reach up to 150 μm . The exact height and number of cell layers varied among different individuals, but an epithelial thickening was always present. The lid wiper epithelium gradually thinned down in the tarsal direction toward the epithelium of the subtarsal fold. The lid wiper epithelium was the most elevated zone of the inner lid margin and, hence, directly apposed to the tear film overlying the bulbar conjunctiva and cornea of the globe.

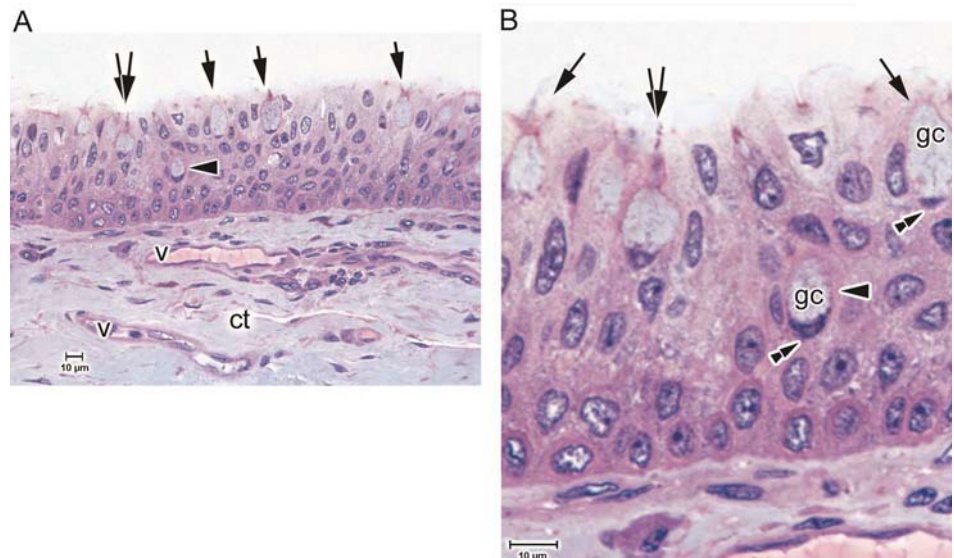
The lid wiper contained frequent goblet cells arranged as single cells among the ordinary epithelial cells, but they also clustered into smaller or larger groups. Some of the goblet cells were located in the topmost epithelial layer of the lid wiper and opened to the surface of the epithelium (Figs. 3, 4). Goblet cells also occurred somewhat deeper in the lid wiper epithelium with unclear but plausible connection to the surface as indicated by the presence of granular material connecting their apical pole to the epithelial surface (Figs. 4A, B). Other cells with typical goblet cell morphology occurred deep in the epithelium without apparent connection to the luminal surface (Figs. 4A, B). All such cells had the typical histological characteristics of goblet cells, such as, for example, a usually larger size of about 20 to 25 μm in length and about 10 to 15 μm width, a decreased staining intensity in H&E stain, a foamy appearance of their cytoplasm with occasional presence of faint internal reticular networks originating from mucin granules and with a flat or indented nucleus in a basal location (Fig. 4B).

In H&E-stained routine paraffin sections of about 10- μm thickness, groups of goblet cells inside the epithelium were seen as spots of decreased staining intensity (Figs. 5A, B). In higher magnification, goblet cells could be detected by their roundish shape and the presence of dense basal indented

nuclei. Goblet cells were further identified by histochemical reactions. In the PAS reaction, a strong pink staining (Figs. 5C–E) was observed in the goblet cells (Figs. 5C, E, F) and to a lesser extent but clearly detectable also among the epithelial cells of the MCJ (Fig. 5D). Goblet cell clusters were spherical in shape (Figs. 5C, E, F) or formed elongated arrangements with the long axis oriented toward the surface of the epithelium (Fig. 5B). Occasionally, intraepithelial goblet cell clusters were associated with narrow, less densely stained spaces inside the epithelium. In some sections, it could be seen that such spaces pointed into the direction of indentions or openings of the surface of the epithelium (Fig. 5B). In routine paraffin histology, it was frequently unclear as to whether the goblet cell-associated narrow hollow spaces represented infoldings of the surface into the depth of the lid wiper, resembling conjunctival crypts (Figs. 5B, C) or artifacts due to the tissue processing.

The exact relation of such goblet cell-related hollow luminal spaces to the epithelial surface was more clearly displayed in semithin sections of plastic embedded tissues (Figs. 6A, B). The luminal surface of the lining epithelial cells in such spaces formed a thin dense line and they hence resembled crypts. The cryptal lumina could be identified as natural structures as opposed to the potential presence of artificial gaps and clefts within the tissue that could possibly arise because of the preparation. Goblet cells were located along the lumen of such crypts with their apical pole directed to, and being in continuity with, the cryptal lumen (Fig. 6B). Occasionally, mucin material was seen to be delivered into the lumen. These epithelial infoldings resembled the tarsal crypts of Henle or Stieda, but in the lid wiper, they were restricted to the thickness of the epithelium and did not extend into the subepithelial connective tissue (Figs. 5A, 6A). Even

FIGURE 4. Several goblet cells are observed in this lid wiper epithelium (here about 8 cell layers, 80 μm thick) and occur in different location (A). Some are located in the topmost epithelial layer and open to the epithelial surface (indicated by arrows), whereas others are in a somewhat deeper subsurface location (double arrows) but granular material is still seen to connect their apical pole with the epithelial surface as in detectable in magnification (B). Frequently goblet cells also occur deep in the epithelium (arrowhead in A, B) without apparent connection to the surface. The goblet cells (gc) have the typical morphological characteristics such as large size, roundish to elongated shape, faint occasionally reticular staining and a dense, basally located indented nucleus (double arrowheads). Semithin (2 μm) plastic section, methylene blue–fuchsin; size marker in A, B = 10 μm .



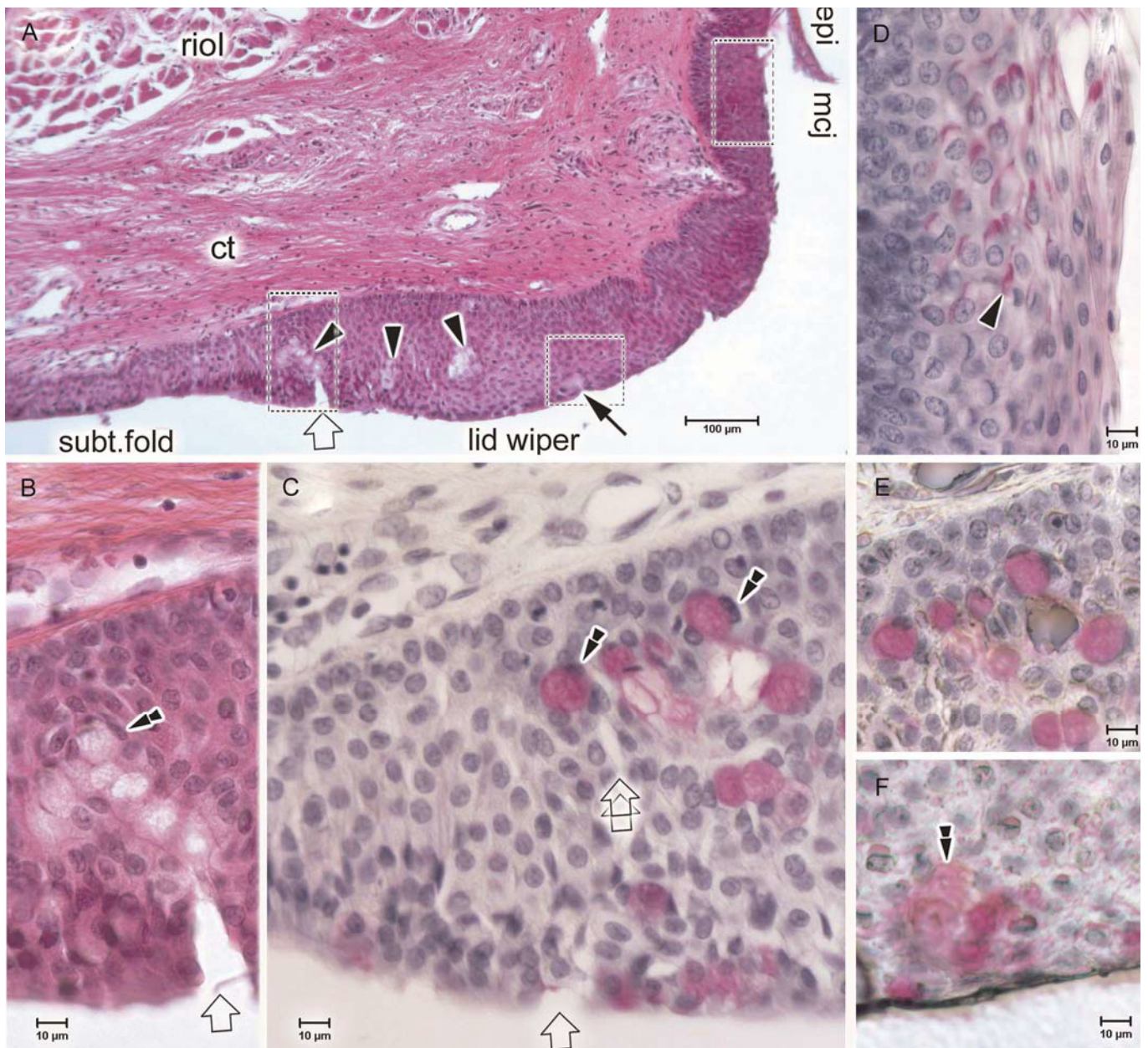
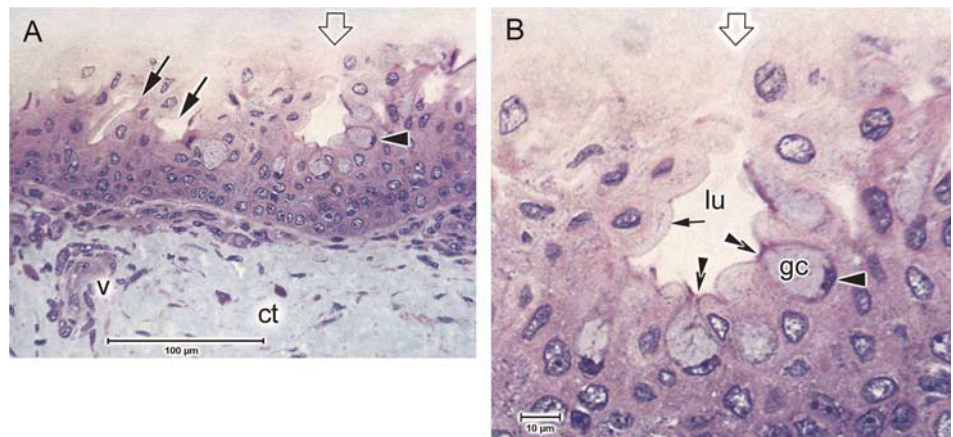


FIGURE 5. In routine paraffin histology, goblet cell clusters occur as roundish structures of decreased staining intensity at the surface (arrow) and deep within the epithelium (arrowheads, A). Their possible connection to the surface of the lid wiper is not apparent. This lid wiper in a midtemporal position along the lid margin is about 150 μm thick, consists of about 15 cell layers, and is about 0.8 mm long until it transforms into the subtarsal fold epithelium. Underneath is the tarsal connective tissue (ct) and fibers of Riolan's muscle (riol). In higher enlargement (B), individual goblet cells are identified by their roundish shape with faint staining and basally located, dark, and indented nucleus (double arrowheads). They are arranged in a cluster of elongated shape that is related to a luminal opening of about 15 μm diameter. The opening is continuous with the surface of the lid wiper (open arrows in A, B). In parallel sections, goblet cells are clearly identified by the pink staining in the PAS reaction (C–F). Here, the luminal opening is almost closed (C, open arrow) but a very narrow luminal space (C, double open arrows) is vaguely detectable deep in the epithelium and points to the goblet cells. In another parallel section (E), this space is lost. Goblet cell clusters also occur near the surface (F, double arrowheads) of the lid wiper and PAS reaction also stains diffuse material, without the presence of goblet cells, in the epithelium of the mucocutaneous junction (D, arrowhead). Paraffin histology (10 μm), (A and B) stained by H&E, (C–F) stained by PAS–hematoxylin; square frames indicated in (A) are enlarged in (B–F); size markers in A = 100 μm , in B–F = 10 μm .

FIGURE 6. In a semithin plastic section, it can be observed that a cryptal infolding (open arrow, in A and B) of the epithelial surface extends into the depth of the lid wiper epithelium and forms a spherical lumen. Further narrow lumina (arrows) occur in the vicinity. The lid wiper is about 10 cell layers thick, with underlying connective tissue (ct), and a blood vessel (v). Goblet cells (one marked by an arrowhead) are located along the crypt. In higher enlargement (B), the cryptal lumen is seen to be limited by a thin dense line (small arrow) constituted by the apical cell surface of the lining cells. Goblet cells (gc) point with their apical pole to the crypt lumen (lu) and are in contact with it (double arrowheads). Semithin (2 μm) plastic section, methylene blue–fuchsin; size marker in A = 100 μm , B = 10 μm



in serial sections, not all intraepithelial goblet cells could be identified to be related to epithelial crypts; some seemed to lie isolated deep in the epithelium.

Histochemical investigation of the content of goblet cells in serial sections of the lid wiper showed that these stained with PAS (Fig. 7A), indicating neural mucins, and with Alcian blue (Figs. 7B, C), indicating acidic mucins. In double-staining experiments (Alcian blue followed by PAS), most goblet cells were double positive for both the stains, although some had a preference for either PAS or Alcian blue and had a respective color (Figs. 7D, E). Stained material from goblet cells deep in the epithelium was occasionally seen to form very thin extensions located among adjacent ordinary epithelial cells and directed toward the epithelial surface (Fig. 7C), possibly indicating the access of the mucins from deeper goblet cells to the surface of the lid wiper. Stained material of deep goblet cells was also observed to be continuous from the body of goblet cells into the lumen of cryptal spaces, suggesting delivery of mucus into crypts (Fig. 7E).

IHC for MUC5AC showed that the cells with morphological characteristics of goblet cells indeed stained positive for this goblet cell–secreted gel-forming mucin, further verifying their identity as goblet cells (Fig. 7F). An MUC5AC-positive coat was also seen deposited at the epithelial surface in places. Not all cells with goblet cell morphology, which were stained with PAS–Alcian blue, also stained positive for MUC5AC as seen in serial sections. Such MUC5AC-negative goblet cells were identified by their typical morphology as seen in differential interference contrast microscopy (Fig. 7G).

DISCUSSION

The present study demonstrates that the elevated lid wiper epithelium of conjunctival structure at the inner lid

border is directly apposed to the tear film overlying the bulbar surface. The lid wiper contains goblet cells with characteristics different from those of the typical palpebral conjunctiva. The goblet cells of the lid wiper occur in different arrangements (single and in clusters) and in different locations (at the surface and deep in the epithelium) compared with the typical conjunctiva with only surface goblet cells. Their cytoplasmic content stains positive in histochemistry with PAS reaction and Alcian blue and in IHC using antibodies for the gel-forming mucin type, MUC5AC. However, MUC5AC-negative goblet cells also occur in the lid wiper. Goblet cells deep in the lid wiper epithelium were frequently identified to be located along epithelial crypts, and hence connected to the epithelial surface.

These findings differ from 2 previous histological studies,^{1,6} both of which reported that the epithelial elevation in this position had a stratified squamous epithelium without goblet cells. Another theoretical publication¹⁷ on lubrication and contact lens wear also depicted an elevation at the inner lid margin, but without original histological data and consideration of the preceding lid margin literature and without making mention of goblet cells. Our findings are, however, supported by the historical descriptions of Virchow⁸ who observed a cuboidal epithelium with “Schleimzellen” (mucus cells) in an “admarginal zone” at the inner lid border. From the figures shown in the respective publications, only Virchow depicts this epithelium in high magnification, which may imply that he has studied this epithelium in greater detail than the other authors.

Convention suggests that surfaces exposed to a certain degree of mechanical friction are typically composed of squamous epithelium, such as the cornea, oral epithelium, or esophagus.¹⁸ Thus, it appeared to make sense that the epithelium of the lid wiper had been reported to be squamous, without goblet cells, and described as a “soft bead gliding

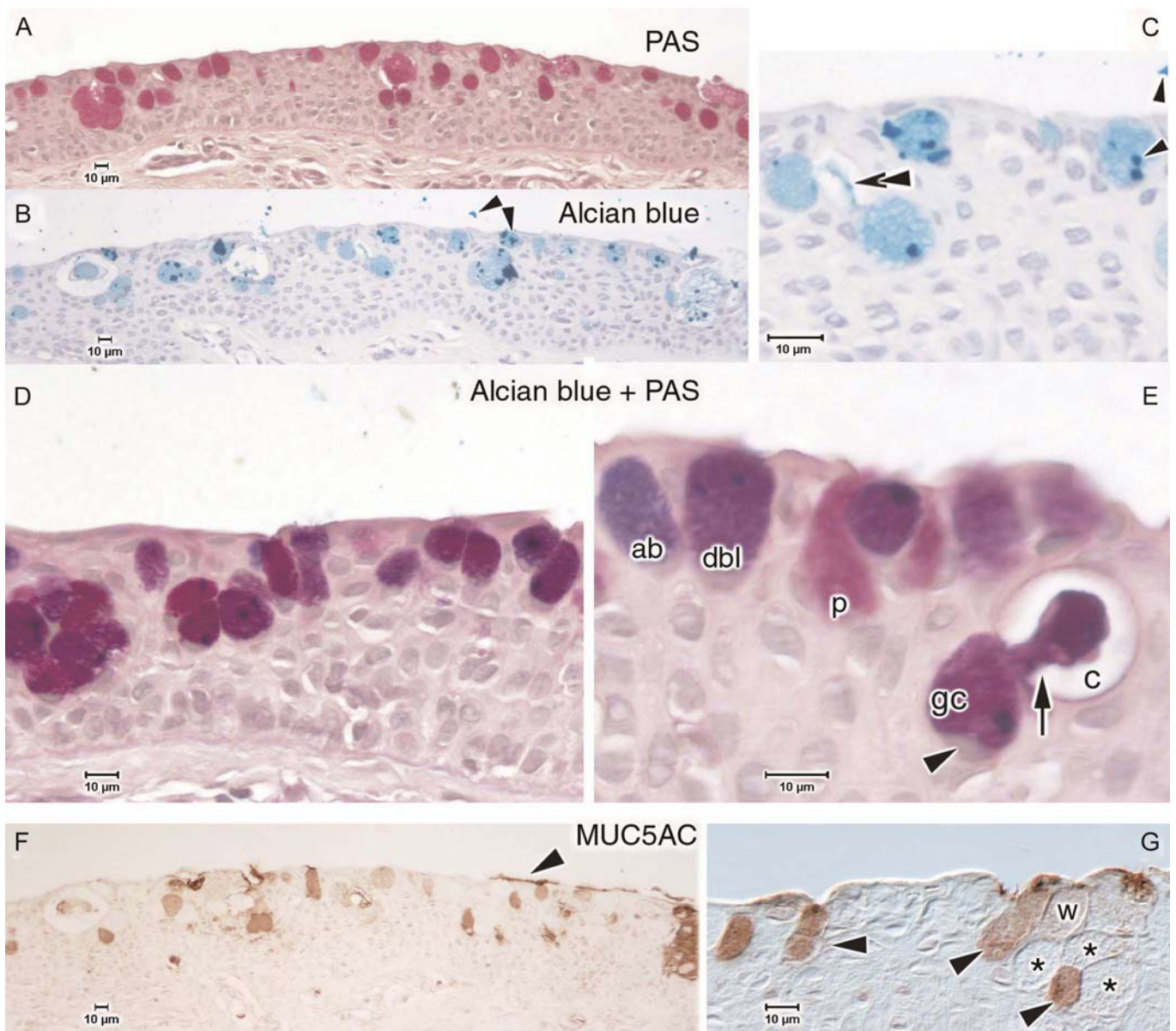


FIGURE 7. Overview of a lid wiper shows several goblet cells, some of them arranged in groups at the surface and others deep in the epithelium. Goblet cells are stained in serial sections either by PAS (A) and Alcian blue (B, C) or by combined staining (D, E). One isolated goblet cell deep inside the epithelium (C, Alcian blue) shows a narrow stained extension (double arrowhead) of the cytoplasmic content that points to the surface and conceivably indicates the delivery of mucus into a cryptal lumen or directly toward the surface. Staining with Alcian blue plus PAS (D, E) shows that most goblet cells are double stained (dbl in E) and have an intermediate color. G, Some goblet cells stain preferably for either Alcian blue (ab) or PAS (p) and have a respective color. Stained mucus material from a goblet cell (gc in E) with basal flat nucleus (arrowhead) deep in the epithelium of the lid wiper is seen to be continuous (arrow) with mucus in a cryptal lumen (c) suggesting delivery of mucus into a luminal space. IHC for MUC5AC (F, G) revealed that some goblet cells are strongly positive for MUC5AC (arrow heads in G), whereas others are weakly stained (w) and yet others are negative (asterisks). In places, MUC5AC-positive material is seen deposited on the epithelial surface (F, arrowhead). Note that Alcian blue shows artificial stain concretions (B, C small arrowheads) on the section and on the glass slide. Paraffin histology (A) PAS; (B, C) Alcian blue; (D, E) Alcian blue plus PAS; (F, G) MUC5AC; size marker in A–G = 10 μm.

over the cornea” that “must be assumed to be a perfect ‘wind screen wiper’.”¹ Therefore, at first sight, it may appear surprising that this zone apposed to the globe does not, in fact, have a squamous epithelium. However, a stratified cuboidal

surface that contains goblet cells and is hence covered by secreted mucins, forming a hydrodynamic fluid layer between the lid wiper and the bulbar surface, may appear much more suitable for the task to distribute the precorneal tears into

a thin film during blinking to form an optically perfect tissue–air interface without resulting in trauma to the ocular surface epithelia.

The observation of goblet cells in the more loosely arranged conjunctival epithelial elevation of the lid wiper indicates that it has a different surface and a different internal structure compared with the more anterior zone of the MCJ with tightly packed, small parakeratinized cells with dense cytoplasm. Because the zones of the MCJ, the surface of which is the line of Marx,¹⁴ and of the lid wiper have a distinctly different structure, it may be assumed that the MCJ is not simply a product of external factors such as an increased friction of the anterior part of the lid wiper elevation as previously suggested.¹ The MCJ, and hence the line of Marx, may more likely represent a separate specific entity, which is also supported by the structure of the deeper epithelial layers and of the underlying connective tissue and related structures.¹⁴

Evidence for the lid wiper being the only zone of the inner lid border that is in close apposition with the bulbar ocular surfaces and for wiping of the tear film during blinking includes the following: (1) it represents a surface elevation, ie, the highest point, at the inner lid border, and (2) this zone is, because of the lid geometry, in direct apposition to the globe. This assumption is (3) further supported by observations of Kessing¹⁹ who found that at least in the upper lid only the inner lid border was in touch with the globe, whereas the tarsal conjunctiva was separated from the globe by a deeper retropalpebral tear lake, which was termed Kessing space.^{4,17} (4) A functional clinical study suggested that a zone in the width of about 600 μm , which would be within the range of the width of the lid wiper^{5,10,14} (Knop et al 2007, Abstract at the TFOS 2007 Conference), is the contact zone of the eyelid border with the globe.²⁰

However, a previous study by the same group had reported some evidence that the line of Marx of about 100- μm width was the primary site of contact with the globe.²¹ This assumption of the line of Marx being the wiping zone was also shared by other authors.^{22,23} Based on the considerations (1–4) explained above and the following considerations, it must be assumed that the lid wiper is the primary contact zone of the eyelids with the globe. (5) The position of the lid wiper at the inner aspect of the inner lid border and apposed to the globe, is clearly established by the histological findings, whereas the line of Marx, the surface of the MCJ,¹⁴ is located on the outer aspect of the inner lid border. (6) In line with this, another clinical study had verified that “Marx’s line of the upper lid is visible in upgaze without lid eversion, suggesting that is not the contact area for the upper lid.”²⁴ (7) In lid wiper epitheliopathy, a condition that conceivably occurs because of increased friction, the lid wiper is the first zone that shows epithelial alterations in dry eye conditions.^{2–4}

The surface of the lid wiper is subjected to a higher risk of increased friction compared with the more proximal tarsal conjunctiva as it passes over the bulbar surface and the cornea during every blink. This must be diminished to prevent wounding of the apposed epithelia of the conjunctival lid

margin and globe (ie, corneal epithelium and bulbar conjunctiva). The presence of mucins secreted onto the lid wiper surface by the immediate goblet cells is uniquely suited to reduce any potential for friction and/or trauma to the lid wiper and the ocular surfaces.

Goblet Cells

As indicated by histochemistry and IHC, the goblet cells produce neutral mucins (stained by PAS) and acidic mucins (stained by Alcian blue). Most goblet cells stained for both neutral and acidic mucins, but interestingly not all goblet cells stained positive for MUC5AC because MUC5AC-negative goblet cells were also observed in the lid wiper epithelium. It has been reported that all human conjunctival goblet cells stain positive for this gel-forming mucin, but this study was restricted to bulbar conjunctival specimens.²⁵ MUC5AC-negative goblet cells in principle are a common finding in other mucosal tissues, such as in the intestine,²⁶ and they also occur in the human nasal mucosa where the majority of goblet cells are MUC5AC negative.²⁷ In contrast, in the airways, MUC5AC is the predominant gel-forming goblet cell mucin.²⁶ Our identification of MUC5AC-negative goblet cells in the lid wiper may indicate that other secreted mucins, such as, for example, MUC2, MUC5AB, or MUC6,²⁸ are produced by the goblet cells of the lid wiper; and probably membrane-bound mucins²⁹ assist the function of gel-forming mucins.

Goblet Cell Crypts

Goblet cells deep inside the lid wiper epithelium without apparent contact to the surface are an unusual finding because they normally deliver their mucins onto a luminal surface. Goblet cells deep in the lid wiper epithelium had been observed in a preceding study by the authors,¹⁴ but their significance had remained enigmatic in conventional histology. A delivery of mucins from such subsurface goblet cells onto the surface of the lid wiper is suggested in the present study, using thin-section morphology and advanced staining, by the findings that the goblet cells (1) were frequently located along cryptal epithelial infoldings of the luminal surface into the depth of the epithelium; (2) their apical surfaces were in contact with the cryptal lumen; and (3) Alcian blue and PAS-stained mucin material from the goblet cells was continuous with the respective material inside the associated cryptal lumina. Because these subsurface goblet cells and their secreted mucins are hence, indirectly, connected to the surface of the lid wiper, their secretions would be available at the surface.

These lid wiper crypts resemble those of Stieda³⁰ and Henle,³¹ also called “mucus crypts” or “mucus glands” by Kessing,³² because they contain many goblet cells along their wall. They are thought to serve as primordial gland-like structures that provide increased amounts of mucus for the conjunctival surface. In addition, the crypts of Henle and Stieda along the tarsal conjunctiva were also shown to serve an immunological function for immune defense by increased amounts of secretory immunoglobulin A³³ that is transcytosed

here through the epithelium.³⁴ But, in contrast to the crypts of Henle and Stieda, the lid wiper crypts observed in the present study do not extend into the subepithelial connective tissue and hence seem to represent previously undescribed structures. Cryptal epithelial infoldings in general provide an enlarged epithelial surface that can accommodate a higher functional capacity. This is also true for the intraepithelial goblet cell crypts of the lid wiper because they provide space for more goblet cells than could be accommodated at the surface without crypts. These intraepithelial lid wiper crypts hence amplify the availability of mucus at the surface of the lid wiper.

In subsurface goblet cells where access to a distinct cryptal luminal surface could not be found, the connection to a crypt may have been missed even though serial sections were performed. Even when they are in fact not all directly connected to a crypt, their mucous secretory product may still have access to the surface via narrow intercellular clefts as could be assumed from the presence of narrow extensions of PAS and Alcian blue–stained material from goblet cells into the direction of the surface (Fig. 7C). Isolated goblet cells below the surface may also represent developing goblet cells within the epithelium that only later contact the lumen. Developing goblet cells could indicate an increased requirement of lubricative mucins in the lid wiper zone. In the conjunctiva of the rat, at least during development, newly arising goblet cells are first smaller than in the adult,³⁵ which also applies to some of the observed subsurface goblet cells in the present study. In the human conjunctiva, it has been shown that the goblet cells and the ordinary epithelial cells share the same (bi-potential) stem cell that gives rise to both cell populations.³⁶ Thus, new goblet cells may arise among and from ordinary epithelial cells.

The presence of goblet cells in the lid wiper that can produce soluble mucins secreted locally onto the lid wiper surface meets a need for an increased lubrication in this region that constantly wipes over the globe (Fig. 8). Such lubrication would not be available to the same extent on a squamous surface without goblet cells that would require passive lubrication by the mucins dissolved in the aqueous tear film. The lid wiper can thus be considered to have an internal lubrication system providing a distinct advantage for the function of tear distribution. The secretion of mucins from the goblet cells in the lid wiper may be controlled by a self-regulating process. In the bronchial airways of the lung, it has been shown that increased chronic mechanical stress is able to induce increased presence of goblet cells (Park 2009). Therefore, the finding of goblet cells in the lid wiper epithelium may suggest that it is subjected to a chronic mechanical stress resulting from its constant movement over the bulbar surface during the blink. Expression of frequent goblet cells in the lid wiper could therefore reflect the successful adaptation of the amount of lubricative mucins to the increased mechanical requirements in this region. Too much friction however, as occurs in dry eye conditions, may override the adaptive capacity of the epithelium because dry eye is shown to result in squamous metaplasia with a lack of goblet cells,⁴⁰ at least on the bulbar conjunctival surface. This conceivably also explains the pathological staining observed in lid wiper

epitheliopathy^{2,3} at the lid margin that was recently shown to consist of parakeratinized cells.^{4,14}

Lubrication

One important requirement for the lid wiper to travel over the bulbar surface without wounding it is to keep a minimal (to spread a thin tear film layer) but secure (in order to avoid wounding) physical distance to the bulbar cell surface during the movement. Goblet cell mucins are very large and highly glycosylated proteins with a high water-binding capacity for the formation of a mucin–water gel that is assumed to represent the major component of the precorneal tear film for lubrication of the ocular surface.^{28,39,41–44} Adequate local mucins on the lid wiper surface would hence provide a thick local overlying mucus–water gel to maintain a sufficient physical distance between the epithelia of the lid wiper and bulbar surface to prevent cellular damage by mechanical friction. Such a thick mucin–water gel is also suggested to serve as a mechanical bumper for attenuation of mechanical forces between the eyelid and bulbar surface.⁴³ The goblet cells in the lid wiper would hence represent the structural prerequisite for increased lubrication that cannot be available at a squamous goblet cell–free epithelium. Mechanical friction occurs in particular because of the natural blinking movement. Because blinking occurs at a frequency of typically about 10 to 12 times per minute, the lid wiper travels, if 10 mm of vertical interpalpebral aperture and 16 hours of day activity are assumed, over a distance of at least 100 m everyday over the bulbar surface, which represents an enormous exposure to friction and subsequent risk of epithelial damage.

A second requirement for the lid wiper is not only to be able to push the tear film in front of it, as shown for the lower lid border,¹ but also to be able to pull the tear fluid, as applies to the upper lid. This is necessary because the main lid movement during a blink is performed by the upper lid. A lid wiper surface with a copious supply of mucins would result in high water-binding properties and be able to lift adequate aqueous tears against the capillary “suction” force¹ of the lower meniscus tear reservoir. This results in the desired thin precorneal tear film layer that is reformed during the up-phase of every blink.^{38,39,43,45–48}

In addition, it may be a conceivable but yet an unproven requirement for the inner lid border structure and hence for the lid wiper epithelium, that a thick mucin–water gel may also be necessary to contribute to a proposed function of the lid wiper in providing a seal that inhibits the outflow of the retropalpebral tear lake behind the upper eyelid. A proposed additional sealing function of the lid wiper against the flux of tears along the inner lid border from the retropalpebral tears into the tear meniscus and into the actual precorneal tear film may also be supported by the relatively similar structure of the lid wiper in both the upper and lower eyelids. This is in contrast to the fact that the main wiping movement during a blink is performed by the upper lid, whereas the lower lid border moves little during the blink but may still require a proposed sealing function by the thickened lid wiper epithelium and by a thick mucin–water gel on its surface.

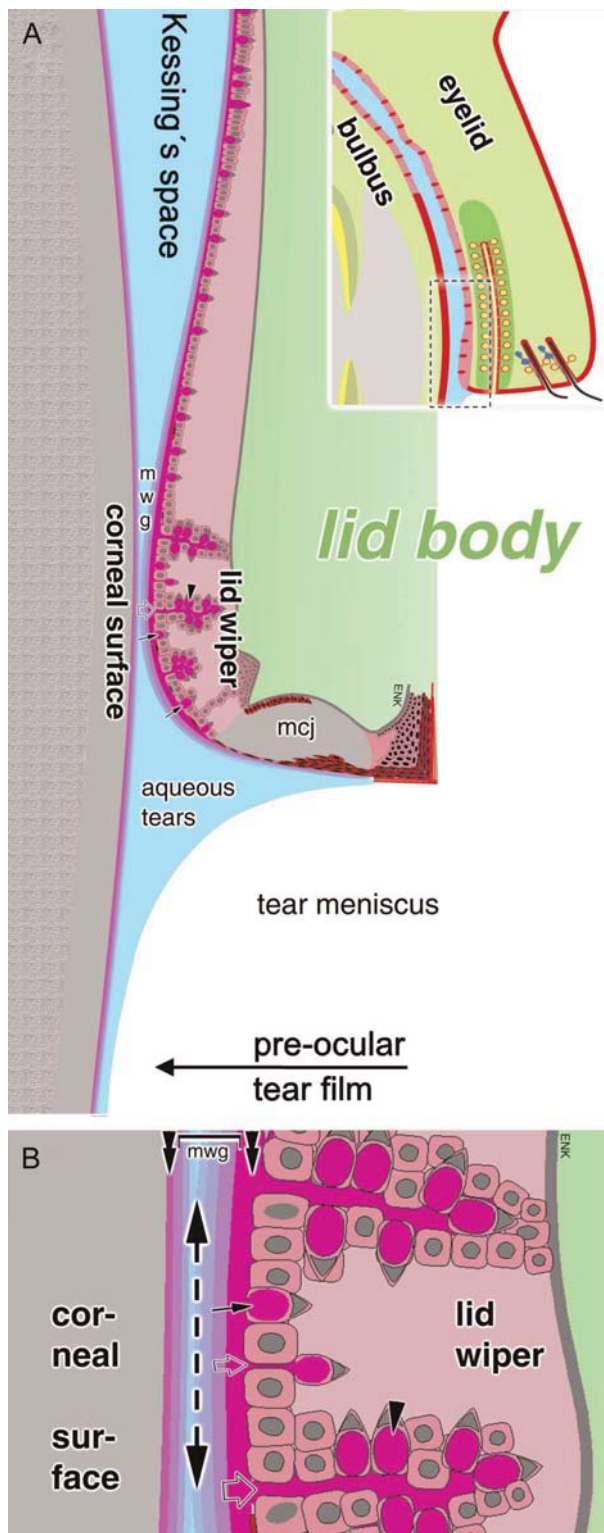


FIGURE 8. Schematic drawing of the structural organization of the goblet cells in the lid wiper at the human inner lid border and the proposed relation of the lid wiper to the ocular surface and tear film. The area indicated by a dotted rectangle in the overview (inset) is enlarged in (A). A, The thickened epithelial elevation of the lid wiper forms a lip-like structure

The goblet cells and goblet cell crypts in the lid wiper, as observed here, with a consequently thick mucin–water gel on its surface, suggest that the lubrication required for gliding of the lid wiper over the cornea, is maintained by a hydrodynamic type of lubrication. This employs a relatively thick liquid lubricant layer and avoids direct contact of the epithelial surfaces. Korb et al⁴ have recently discussed possible models for lubrication at the lid border to understand pathological epithelial alterations of the lid wiper, termed LWE. LWE occurs in dry eye conditions when the lubrication is reduced by tear film deficiencies and hence the friction is increased.

Lubrication in general is the process that reduces mechanical shear stress, friction, wear, and loss of energy on surfaces that move in close proximity relative to each other, and it is maintained by an interposed lubricant substance between them. In biological systems, the lubricant is usually a liquid. Depending on the thickness of the lubricant layer, two types of lubrication are mainly differentiated. One employs a very thin lubricant layer of molecular dimensions (boundary lubrication), whereas another is characterized by a relatively thick, usually hydrated, lubricant layer (hydrodynamic lubrication). There is a gradual transformation between both types of lubrication, and intermediate types exist. Apart from the thickness of the lubricant layer, lubrication, and hence the resulting shear stress, is also influenced by other factors such as the viscosity of the

directly apposed to the surface of the globe, here the corneal surface, with a slope toward the subtarsal fold. This is suitable to distribute the very thin preocular tear film layer. Further in tarsal direction, the lid is separated from the globe by a retro-palpebral tear fluid space (Kessing space).¹⁹ Goblet cells (shown in a pink color that refers to the PAS staining) are located at the surface (arrows in A, B) and in the depth (arrowhead in A, B) of the lid wiper. Those in the depth are often found along cryptal epithelial infoldings that open to the surface (open arrow in A, B) and can hence deliver their mucins onto the surface of the lid wiper. The local goblet cells of the lid wiper can provide a rich mucin layer (pink color; double arrowheads in B) and a respective thick mucin–water gel (mwg, shading indicates increasing dilution of mucin). (B, Higher magnification shows a subsurface goblet cell that can still deliver mucin through a narrow intercellular cleft (narrow open arrow) to the surface. The thick local mucin–water gel (mwg) is suitable to provide improved lubrication. This can diminish potential mechanical friction from the movement of the apposed surfaces of the lid wiper and cornea/conjunctiva along each other (double-headed arrow). The observed morphology suggests a hydrodynamic type of lubrication between the lid and bulbus. Known physiological parameters are represented in the drawing. The thickness of the lid wiper is about 100 μm .^{10,14} The MCJ, the surface of which represents the natural stainable line of Marx,¹⁰ is covered by the aqueous tears of the meniscus and represents the bottom of the meniscus.³⁷ The meniscus has a curvature of $r = 0.24$ mm.³⁸ Individual epithelial cells are in most parts only indicated at the tissue surface and along the goblet cell crypts. The thickness of the preocular tear film³⁹ and in particular the distance between lid wiper and corneal surface is not exactly known.

lubricant and the velocity of movement of the opposing surfaces.^{1,49,50}

Historically, Ehlers¹ had considered different types of lubrication that could apply to the ocular surface. In view of his description of a stratified squamous epithelium without goblet cells at the inner lid border he had suggested, that lubrication in this region must be of the boundary type because lid margin and bulbar surfaces were in contact and only, if at all, separated by a very thin film of molecular dimensions. Holly⁵¹ in contrast, had theoretically assumed that the opposed biological surfaces would still have a relatively high degree of roughness that would only allow a hydrodynamic lubrication by a relatively thick film to overcome the roughness and hence prevent wounding because of friction by direct contact. More recent theoretical models also seem to point into the direction of a hydrodynamic type of lubrication with a sufficiently thick mucin–water gel. This would be able to keep a secure distance between the opposing surfaces and to reduce sheer stress because of a proposed slip interface within the mucin–water gel.⁴³ Another theoretical, mathematical model predicted a hydrodynamic lubrication together with an elastic mattress model that was based on a proposed soft and elastic nature of the respective tissues.²³ This model, as the previous models, still took for granted the assumption that the inner lid border had a stratified squamous epithelium. This type of epithelium can be assumed to have a relatively smooth surface but only a limited degree of softness and is not provided with lubricant-secreting goblet cells. A conjunctival structure with goblet cells, as observed in the present study, would better serve not only for a higher elasticity but also for a built-in lubrication compared with a squamous epithelium.

CONCLUSIONS

The finding in the present study of goblet cells and goblet cell crypts in the lid wiper provides the first demonstration of the structural prerequisite for a relatively thick overlying mucin–water gel at the surface of the lid wiper that would allow for a hydrodynamic type of lubrication between the lid wiper and the bulbar surface during the blink motion that distributes the thin precorneal tear film. This offers first evidence of the structural basis for a more conclusive understanding of lubrication and tear film distribution at the inner lid border than previously available.

REFERENCES

- Ehlers N. The precorneal film. Biomicroscopical, histological and chemical investigations. *Acta Ophthalmol Suppl.* 1965;(suppl 81):1–134.
- Korb DR, Greiner JV, Herman JP, et al. Lid-wiper epitheliopathy and dry-eye symptoms in contact lens wearers. *CLAO J.* 2002;28:211–216.
- Korb DR, Herman JP, Greiner JV, et al. Lid wiper epitheliopathy and dry eye symptoms. *Eye Contact Lens.* 2005;31:2–8.
- Korb DR, Herman JP, Blackie CA, et al. Prevalence of lid wiper epitheliopathy in subjects with dry eye signs and symptoms. *Cornea.* 2010; 29:377–383.
- Knop E, Korb DR, Blackie CA, et al. The lid margin is an underestimated structure for preservation of ocular surface health and development of dry eye disease. In: Brewitt H, ed. *Research Projects in Dry Eye.* Basel, Switzerland: S Karger; 2010:108–122.
- Parsons JH. *Pathology of the Eye. Vol. 1. Histology.* London, United Kingdom: Holder and Stoughton; 1904.
- Sattler H. Beitrag zur Kenntnis der normalen Bindehaut des Menschen [in German]. *Graefes Arch Ophthalmol.* 1877;23:1–28.
- Virchow H. Mikroskopische Anatomie der äusseren Augenhaut und des Lidapparates. In: Saemisch T, ed. *Graefe-Saemisch Handbuch der gesamten Augenheilkunde, Band 1, 1. Abteilung, Kapitel II.* 2nd ed. Leipzig, Germany: Verlag Wilhelm Engelmann; 1910:431.
- McMonnies CW. Incomplete blinking: exposure keratopathy, lid wiper epitheliopathy, dry eye, refractive surgery, and dry contact lenses. *Cont Lens Anterior Eye.* 2007;30:37–51.
- Knop E, Knop N, Zhivov A, et al. Definition of different subzones, including the lid wiper, in the marginal human conjunctiva by histology and in vivo confocal microscopy. *Invest Ophthalmol Vis Sci.* 2008; 49:5299.
- Knop E, Knop N, Zhivov A, et al. Zonal differentiation of the epithelium including the lid wiper at the human lid margin. *Acta Ophthalmologica.* 2008;86:0.
- Wolff E. The mucocutaneous junction of the lid margin and the distribution of the tear fluid. *Trans Ophthalmol Soc UK.* 1946;66:291–308.
- Marx E. Über vitale Färbungen am Auge und an den Lidern I. über Anatomie, Physiologie und Pathologie des Augenlidrandes und der Tränenpunkte [in German]. *Graefes Arch Clin Exp Ophthalmol.* 1924;114: 465–482.
- Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat.* 2011;218:449–461.
- Knop N, Knop E. Conjunctiva-associated lymphoid tissue in the human eye. *Invest Ophthalmol Vis Sci.* 2000;41:1270–1279.
- Böck P. *Romeis Mikroskopische Technik.* 17th ed. München, Germany: Urban & Schwarzenberg; 1989.
- Fatt I. Architecture of the lid–cornea junction. *CLAO J.* 1992;18:187–192.
- Gray H, Standring S, Ellis H, et al. *Gray's Anatomy: The Anatomical Basis of Clinical Practice.* Edinburgh, United Kingdom: Elsevier Churchill Livingstone; 2005.
- Kessing SV. A new division of the conjunctiva on the basis of x-ray examination. *Acta Ophthalmol (Copenh).* 1967;45:680–683.
- Shaw AJ, Collins MJ, Davis BA, et al. Eyelid pressure and contact with the ocular surface. *Invest Ophthalmol Vis Sci.* 2010;51:1911–1917.
- Shaw AJ, Collins MJ, Davis BA, et al. Eyelid pressure: inferences from corneal topographic changes. *Cornea.* 2009;28:181–188.
- Donald C, Hamilton L, Doughty MJ. A quantitative assessment of the location and width of Marx's line along the marginal zone of the human eyelid. *Optom Vis Sci.* 2003;80:564–572.
- Jones M, Fulford GR, Please CP, et al. Elastohydrodynamics of the eyelid wiper. *Bull Math Biol.* 2008;70:323–343.
- Korb DR, Blackie CA. Marx's line of the upper lid is visible in upgaze without lid eversion. *Eye Contact Lens.* 2010;36:149–151.
- Gipson IK. Distribution of mucins at the ocular surface. *Exp Eye Res.* 2004;78:379–388.
- Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep.* 2010;12:319–330.
- Kim CH, Song KS, Kim SS, et al. Expression of MUC5AC mRNA in the goblet cells of human nasal mucosa. *Laryngoscope.* 2000;110:2110–2113.
- Gipson IK, Hori Y, Argüeso P. Character of ocular surface mucins and their alteration in dry eye disease. *Ocul Surf.* 2004;2:131–148.
- Govindarajan B, Gipson IK. Membrane-tethered mucins have multiple functions on the ocular surface. *Exp Eye Res.* 2010;90:655–663.
- Stieda L. Ueber den Bau der Augenlid bindehaut des Menschen [in German]. *Archive Mikroskopischer Anatomie.* 1867;3:357–365.
- Henle J. *Handbuch der Systematischen Anatomie des Menschen.* Braunschweig, Germany: F. Vieweg und Sohn; 1866.
- Kessing SV. Mucous gland system of the conjunctiva. A quantitative normal anatomical study. *Acta Ophthalmol (Copenh).* 1968;(suppl 95):1–133.
- Knop E, Knop N, Claus P. Local production of secretory IgA in the eye-associated lymphoid tissue (EALT) of the normal human ocular surface. *Invest Ophthalmol Vis Sci.* 2008;49:2322–2329.
- Knop N, Knop E. The crypt system of the human conjunctiva. *Adv Exp Med Biol.* 2002;506(pt B):867–872.
- Rios JD, Forde K, Diebold Y, et al. Development of conjunctival goblet cells and their neuroreceptor subtype expression. *Invest Ophthalmol Vis Sci.* 2000;41:2127–2137.

36. Pellegrini G, Golisano O, Paterna P, et al. Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J Cell Biol.* 1999;145:769–782.
37. Norn MS. Vital staining of the canaliculus lacrimalis and the palpebral border (Marx' line). *Acta Ophthalmol (Copenh).* 1966;44:948–959.
38. Yokoi N, Bron AJ, Tiffany JM, et al. Relationship between tear volume and tear meniscus curvature. *Arch Ophthalmol.* 2004;122:1265–1269.
39. King-Smith PE, Fink BA, Hill RM, et al. The thickness of the tear film. *Curr Eye Res.* 2004;29:357–368.
40. Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology.* 1985;92:728–733.
41. Jumblatt JE, Jumblatt MM. Detection and quantification of conjunctival mucins. *Adv Exp Med Biol.* 1998;438:239–246.
42. Braun RJ, King-Smith PE. Model problems for the tear film in a blink cycle: single-equation models. *J Fluid Mech.* 2007;586:465–490.
43. Cher I. A new look at lubrication of the ocular surface: fluid mechanics behind the blinking eyelids. *Ocul Surf.* 2008;6:79–86.
44. Jones MB, Please CP, McElwain DL, et al. Dynamics of tear film deposition and draining. *Math Med Biol.* 2005;22:265–288.
45. Tiffany JM. The normal tear film. *Dev Ophthalmol.* 2008;41:1–20.
46. Yokoi N, Yamada H, Mizukusa Y, et al. Rheology of tear film lipid layer spread in normal and aqueous tear-deficient dry eyes. *Invest Ophthalmol Vis Sci.* 2008;49:5319–5324.
47. Tiffany JM. Surface tension in tears. *Arch Soc Esp Ophthalmol.* 2006;81:363–366.
48. Cher I. Another way to think of tears: blood, sweat, and... "dacruon". *Ocul Surf.* 2007;5:251–254.
49. Coles JM, Chang DP, Zauscher S. Molecular mechanisms of aqueous boundary lubrication by mucinous glycoproteins. *Curr Opin Colloid Interface Sci.* 2010;15:406–416.
50. McFadden C, Soto C, Spencer ND. Adsorption and surface chemistry in tribology. *Tribol Int.* 1997;30:881–888.
51. Holly FJ, Holly TF. Advances in ocular tribology. *Adv Exp Med Biol.* 1994;350:275–283.