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The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an *in vivo* confocal and histological study

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Abstract

The inner border of the eyelid margin is critically important for ocular surface integrity because it guarantees the thin spread of the tear film. Its exact morphology in the human is still insufficiently known. The histology in serial sections of upper and lower lid margins in whole-mount specimens from 10 human body donors was compared to *in vivo* confocal microscopy of eight eyes with a Heidelberg retina-tomograph (HRT II) and attached Rostock cornea module. Behind the posterior margin of the Meibomian orifices, the cornified epidermis stopped abruptly and was replaced by a continuous layer of para-keratinized (pk) cells followed by discontinuous pk cells. The pk cells covered the muco-cutaneous junction (MCJ), the surface of which corresponded to the line of Marx (0.2–0.3 mm wide). Then a stratified epithelium with a conjunctival structure of cuboidal cells, some pk cells, and goblet cells formed an epithelial elevation of typically about 100 μm initial thickness (lid wiper). This continued for 0.3–1.5 mm and formed a slope. The MCJ and lid wiper extended all along the lid margin from nasal to temporal positions in the upper and lower lids. Details of the epithelium and connective tissue were also detectable using the Rostock cornea module. The human inner lid border has distinct zones. Due to its location and morphology, the epithelial lip of the lid wiper appears a suitable structure to spread the tear film and is distinct from the MCJ/line of Marx. Better knowledge of the lid margin appears important for understanding dry eye disease and its morphology can be analysed clinically by *in vivo* confocal microscopy.
Key words: conjunctiva; dry eye disease; eyelid margin; human; *in vivo* confocal microscopy; lid wiper; Marx's line; muco-cutaneous junction; tear film.

Introduction

The human eyelid margins are an important but incompletely understood structure for the maintenance of the pre-ocular tear film, which helps preserve ocular surface integrity (Holly, 1980; Zierhut et al. 2002; Bron et al. 2004; King-Smith et al. 2004; Knop & Knop, 2008). When considering the role of tears, the primary foci are usually the glandular secretions, which represent the tears, and the lubricative mucins of the ocular surface epithelia, which serve to adhere the tears to the ocular surface (Holly & Lemp, 1971; Jumblatt & Jumblatt, 1998; Argueso & Gipson, 2001; Cher, 2008). The lid margins, however, are the prerequisite for the thin spread of a stable tear film and its re-formation with every blink, to achieve a thin, optically perfect tissue–air interface (Lemp, 1981; Korb et al. 1994; Tsubota & Nakamori, 1995; Begley et al. 2002; Nichols et al. 2002; King-Smith et al. 2004; Millar et al. 2006; Paugh et al. 2008).

It was noticed by Parsons as early as 1904 that the inner lid border is 'sharp', 'lies in contact with the globe' and could contribute to the distribution of tears (Parsons, 1904). A thickened epithelium in this area had already been described by Sattler (1877) and Virchow (1910). Even though an epithelial elevation in this position has immediate functional implications, the assumption that it could act as the actual device for the distribution of the tear film like

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a 'wind-screen wiper' was not mentioned before Ehlers (1965). Since then it has received limited attention, its exact structure remaining insufficiently known, and only recently has it become of interest (Knop et al. 2010) with the discovery of lid wiper epitheliopathy (LWE) (Korb et al. 2002, 2005, 2010).

LWE is an alteration of the epithelium of that portion of the marginal conjunctiva of the upper eyelid that wipes the ocular surface; it is diagnosed by vital staining and is correlated to dry eye symptoms and disease (Korb et al. 2002, 2005). LWE occurs more frequently in patients with dry eye symptoms than in normal patients, and as it may also occur in the absence of conventional signs (Schirmers test and FBUT), it was suggested as a sensitive early indicator of tear film instability and dry eye disease. However, knowledge of the anatomy, histology and histopathology of this area of the lid margin is scant (Sattler, 1877; Parsons, 1904; Virchow, 1910; Wolff, 1946; Ehlers, 1965).

Clinically, it is known that anatomical changes of the lid margin often are associated with ocular surface disease (Hykin & Bron, 1992; Holbach, 1995; Foulks & Bron, 2003; Hirotani et al. 2003; McCulley & Shine, 2003; Di Pascuale et al. 2005; Den et al. 2006). The lid margin is also important for ocular surface integrity because it is the presumed location of conjunctival stem cells that appear to move towards the fornical conjunctiva (Pe'er et al. 1996; Wirtschafter et al. 1999; Liu et al. 2007).

In the present investigation of human lid margin morphology (Knop et al. 2008; Knop & Knop, 2009), it became apparent that the anatomy and histology of the different zones of the lid margin have not been precisely defined. This imprecision has resulted in difficulties in the description of the normal morphology and its alterations in disease states. Clinically, the whole free end of the lid is usually addressed as 'margin' and sub-zones are not specifically differentiated (e.g. free *margin* vs. anterior and posterior *border*). In particular, the MCJ is sometimes thought to extend onto the tarsal side (Wirtschafter et al. 1999; Wolfram-Gabel & Sick, 2002), which would include the region identified in the present study as the lid wiper. Furthermore, the nature and localization of the line of Marx is unclear. Marx (1924) originally described a zone at the inner lid border that could be stained by several vital dyes (Korb, 2010; Pult et al. 2010). Although it has received recent attention (Doughty et al. 2004; Yamaguchi et al. 2006), it is not clear what is actually stained.

The zonal differentiation at the inner lid border remains unclear with respect to the MCJ, the line of Marx and the lid wiper. As this region is of the utmost importance for the continuous distribution and re-formation of the pre-ocular tear film with every blink, it has eminent implications for ocular surface health and integrity. In this study, the histological morphology is analysed and compared with the widely used clinical investigation method of *in vivo* confocal microscopy. Morphological changes in this region could serve as a sensitive clinical early indicator of dry eye disease and as a control parameter for therapeutic outcome in a clinical setting.

Materials and methods

In vivo confocal microscopy

Subjects

Both eyes of healthy human volunteers ($n = 4$; average age 44.25 years) were studied using *in vivo* confocal microscopy. One to two drops of anesthetic (Proparakain-POS; Ursapharm, Saarbrücken, Germany) were applied topically into the lower conjunctival sac of each eye. A drop of carbomer gel (Visidic; Dr Mann Pharma, Berlin, Germany) served as coupling medium. With their chin positioned on a shelf, the subject fixated with the contralateral eye on a target to minimize eye movements. Examination time per eye was < 10 min, after which the subject was removed from the confocal microscope. After 60 min, the contralateral eye was examined.

Confocal microscopy

In vivo confocal microscopy was performed by a combination of the Heidelberg Retina Tomograph II (HRT, using a diode laser beam of 670 nm wavelength) connected to the Rostock Cornea Module (RLSM, Heidelberg, Germany) (Stave et al. 2002) and equipped with a computer-controlled hydraulic linear scanning device (Nikon hydraulic micromanipulator, Tokyo, Japan). A water-contact objective (63×/NA 0.95W; Zeiss, Jena, Germany) provides sufficient resolution to see cellular details over an image area with a side length of 400 μm. The focus level can be defined and changed in the axial direction by both external manual and internal *z*-scan. Thus, the Rostock Cornea Module allows the generation of images of the tissue with precise information on the depth in the form of a single image or as consecutive images along the *z*-axis (Zhivov et al. 2006; Stave et al. 2002) depending on the translucence of the tissue (Guthoff & Stave, 2006).

Histology

Tissues

Whole-mounts of conjunctival sacs including the lid margin from 14 eyes were obtained from cold stored cadavers ($n = 10$; average age 77.1 years) with a macroscopically normal ocular surface. Time after death to harvesting of tissues was 12–36 h. Prior to death, body donors had given informed consent. This study complies with the Declaration of Helsinki and was approved by institutional review.

Preparation

The complete conjunctival sac was excised from 1 to 2 mm distal/external to the outer lid border along the whole extension of the lid margin in the upper and lower eyelids. The posterior lamella of the lid including the tarsus, and in addition an adherent narrow outer zone of epidermis, was then separated by extending the preparation along the tarsal and orbital lid region towards the corneal limbus as previously described (Knop & Knop, 2000). Using this procedure, the whole posterior lamella and conjunctiva of both eyelids were obtained in one piece. This tissue of the upper and lower lids remained connected at the

nasal canthus, whereas the lateral canthus was divided. This resulted in one whole-mount strip of tissue. Whole-mount specimens were then placed on a plastic board and gently flattened without touching the conjunctival surface or lid margin. Tissues were immediately fixed by immersion in a 4% paraformaldehyde solution in 0.01 M phosphate buffer at 4 °C, then washed in the same buffer and embedded in paraffin. The flat paraffin-embedded whole-mount was then divided, from the lid margin to the bulbar side, into 5-mm-wide strips that could be sectioned separately, allowing the morphology in different regions of the lid margin to be investigated (Knop & Knop, 2000).

Sectioning, staining and photo documentation

Serial sections (5–10 μm) were cut from paraffin blocks with a rotary microtome (HM 355S; Microm, Walldorf, Germany). Sections were stained with haematoxylin & eosin (H&E) and Masson–Goldner's trichrome stain (MG stain). Sections were examined with a Leica DMRB light microscope (Leica, Bensheim, Germany) and photographs were taken with a digital camera (Spot Insight; Diagnostic Instruments, Sterling Heights, MI, USA). Morphometric analysis was performed with SPOT software v4.5.

Results

Histological examination of the lid margin in cross-section showed a clear difference in shape of the sharp inner lid border compared to the more rounded outer one (Fig. 1). The inner lid border was elevated and hence directly apposed to the globe (Fig. 1B,C).

Free lid margin epidermis

The stratified squamous cornified epidermis of the lid usually had well developed interdigitations (rete pegs) with connective tissue papillae (Fig. 2A). The epidermis was about 6–8 cell layers thick (ca. 50 μm) over dermal papillae and thicker over rete pegs. The basal cells were more deeply stained and had numerous basal processes at which dense bundles of filaments terminated (Fig. 2B). The processes were increasingly lost as the epidermis approached the inner lid border and the papillae became irregular or absent. The epithelium had a thin but clearly detectable granular layer and a stratum corneum consisting of flattened, enucleated squames (Table 1). In RLSM the cornified epithelial surface showed a bright hyper-reflective meshwork of cell borders (Fig. 2C). With a deeper plane of focus, the epithelial cells appeared darker, although the cells adjacent to the papillae remained brightly reflective. This corresponded to the more densely stained basal cell layer seen in histology.

Muco-cutaneous junction

On the free lid margin close to the inner lid border, the epithelial morphology changed abruptly because the granular cell layer was lost, along with the stratum corneum (Fig. 3A,B). In addition, the basement membrane and epithelium increased in thickness. In cross-sections where the orifice of a Meibomian gland was seen, it was typical for this change to occur at the proximal margin of the orifice. If no orifice was seen, the transition occurred further distally on the free lid margin. The cornified enucleated squames were replaced by thin squamous surface cells with a dense cytoplasm that still contained a nucleus with highly condensed chromatin. These features characterized the cells as para-keratinized (pk) cells. They formed a continuous pk layer that was initially only 2–3 cells thick. After a short distance of about 150–200 μm, the continuous pk surface layer was replaced by single discontinuous pk cells interspersed among ordinary squamous surface cells (squamous transition zone). Underneath the surface of the squamous transition zone, ordinary, rounder cells typically occurred. This transition zone was usually about the same width as the continuous pk zone. In the depth of the epithelium, the pk cells continued as a broad band down toward the basement membrane in a proximal direction (Fig. 3B). The basal epithelial cells were small, dark and tightly packed. Similar intensely stained basal cells continued underneath the lid wiper for varying distances. Compared to H&E stain, pk cells were more easily located in MG stain by an intense red colour (Fig. 4A–E). The level of the basement membrane underneath the MCJ was often lower than in the adjacent tissue (Figs 3 and 4) and formed a broad epithelial peg. This peg was lined by larger pointed papillae with increased vasculature including high endothelial venules (HEV) (Figs 3B and 4A).

By RLSM, the hyper-reflective meshwork of the cornified epidermis was seen to stop in an alignment through the middle of the orifices of the Meibomian glands. At an orifice, the epidermis reached further proximally and formed an epidermal cuff (Fig. 5). Close to the inner lid border, the epithelium appeared distinctly darker but had occasional spots with a less hyper-reflective meshwork. The pointed papillae including the vessels and intra-vascular cells were readily observed by RLSM.

Lid wiper

At the crest of the inner lid border, following the squamous transition zone, the epithelium assumed a distinctly different, conjunctival structure. The epithelium was composed of larger cuboidal and less dense typical conjunctival cells together with interspersed goblet cells, forming an elevation of multiple cell layers (typically 8–12, occasionally up to 15 layers).

The start of the lid wiper was defined by the occurrence of cells with a cuboidal shape at the epithelial surface (Figs 3B and 4A). Interspersed pk cells of different shapes (squamous to columnar) continued in decreasing number from the MCJ over the surface of the lid wiper (Fig. 4A–E) onto the tarsal conjunctiva. The number of pk cells was usually small, but varied in the different specimens.

Goblet cells regularly occurred within the lid wiper epithelium either as single cells or arranged into groups

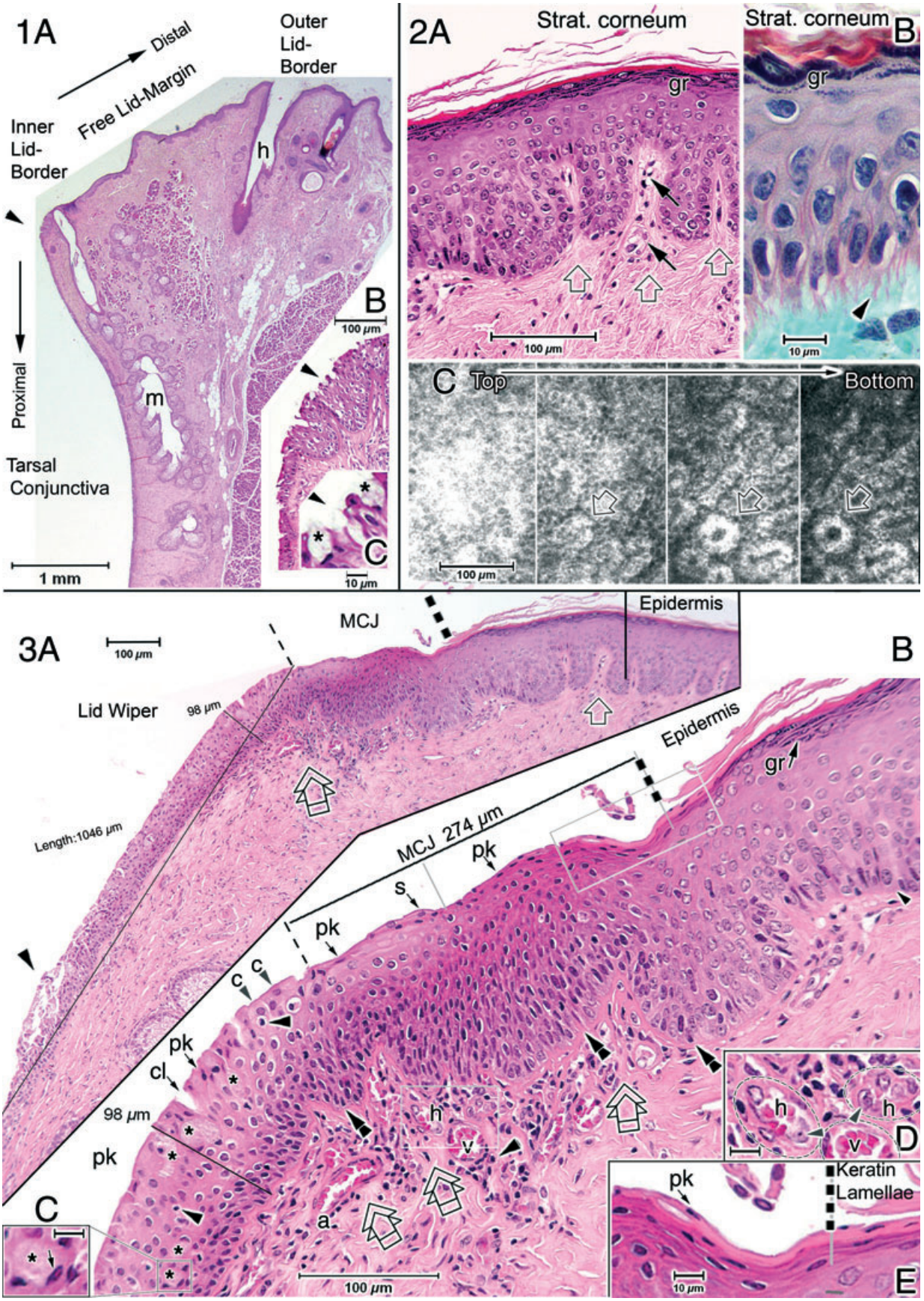


Table 1 Characteristics of the different zones at the human inner lid margin.

		MCJ/Marx line			
	Epidermis	Continuous para-keratinized	Squamous transition zone	Lid wiper	Subtarsal fold
Width		150–200 μm	100–150 μm	0.3–1.5 mm or more	
Thickness	50 μm	80–150 μm	80–150 μm	80–150 μm	30–40 μm
No. of cell layers	6–8	8–15	8–15	8–15	3–4
Cornified layer & Strat. granulosum	+	–	–	–	–
Surface cells	Squamous	Squamous	Squamous	Cuboidal (squamous to columnar)	Cuboidal – Columnar
Para-keratinized (pk) surface cells	–	Continuous	Discontinuous	++ interspersed	+ interspersed
Goblet cells	–	–	(+)	+++	++
Basement membrane thickness	+	+++	+++	+	+
Connective tissue papillae	+ roundish	+ large, pointed	++ large, pointed	(–)	–

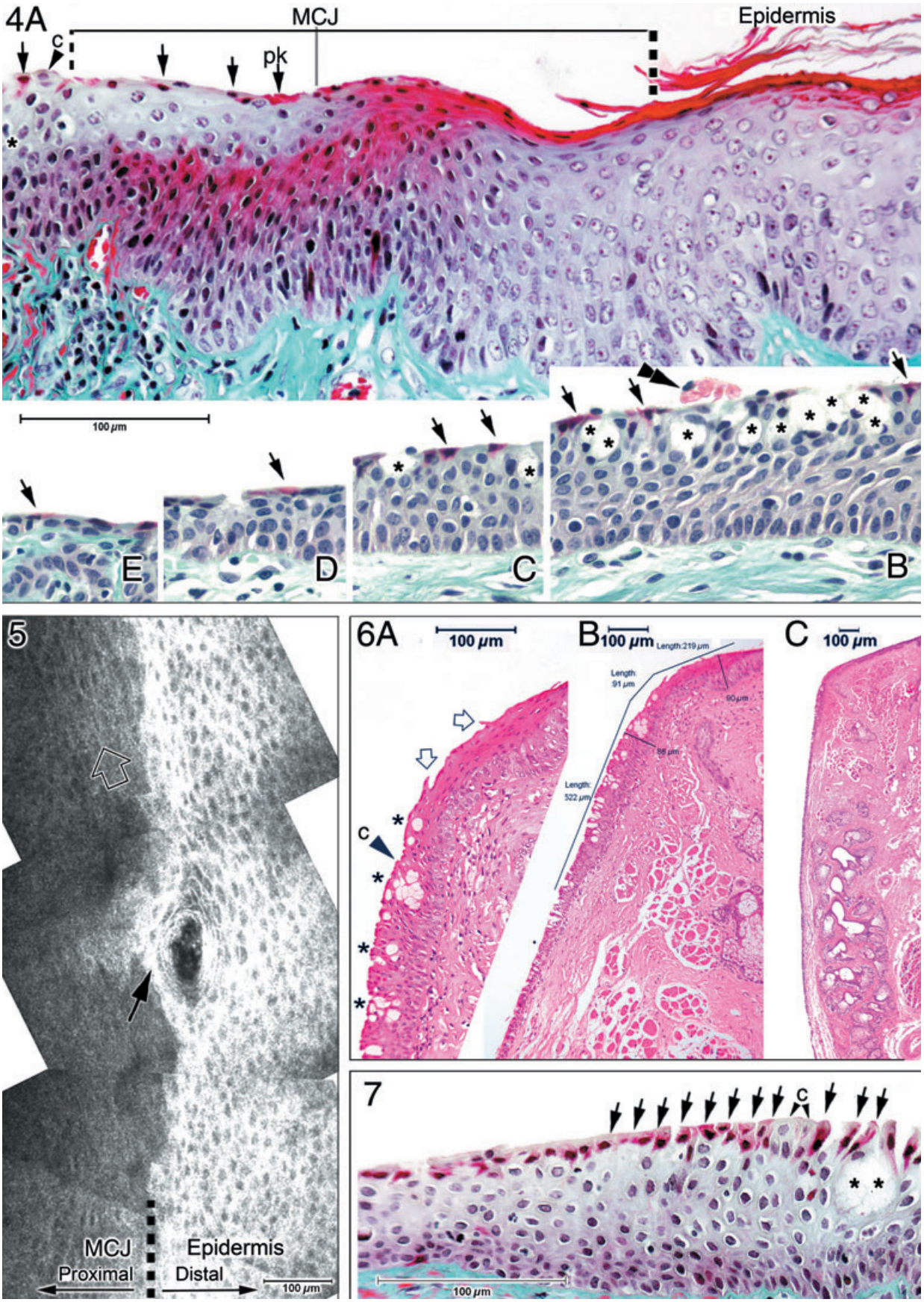
(Figs 3B and 4B–D). Some goblet cells were located in the superficial layers and opened onto the surface, whereas others occurred within the depths of the epithelium. In serial sections, clusters of deeper goblet cells were sometimes detectable arranged along cryptal infoldings [Stiedas clefts (Knop & Knop, 2002b)] that had a narrow opening at the surface of the epithelium. The number of goblet cells varied but they were a typical finding.

Typically, the lid wiper started on the crest of the inner lid border and therefore was the zone most closely apposed to the globe. The maximal height of the lid wiper epithelium (usually around 100 μm) occurred in its initial portion. Toward the tarsal conjunctiva, the epithelial height, i.e. thickness, gradually decreased over a distance of about 0.3–1.5 mm or more (Figs 3A and 4B–E). The lid wiper tended to be wider, i.e. longer, in more nasal and temporal positions along the lid margin compared to the centre of the eyelid. A zonal differentiation of the MCJ and lid wiper with similar characteristics was also observed in the margin of the lower lid (Fig. 6). In some specimens, cuboidal cells in the initial distal portion of the lid wiper epithelium were covered by ordinary squamous or pk cells. In a few specimens, pk cells formed the majority of surface cells (Fig. 7). However, in contrast to an ordinary squamous epithelium, the squamous surface cells in such areas did not gradually assume a roundish shape towards the deeper layers but

Fig. 1 Overview of a section through the center of an upper human eyelid margin. The epidermis extends over the roundish outer lid *border* onto the free lid *margin*. A ciliary hair follicle (h) and a Meibomian gland (m) are seen. The inner lid border (arrowhead at the crest) has a zone of increased epithelial thickness (as seen in higher magnification, B), which forms an elevation apposed to the globe. This zone is only 0.3 mm wide here and contains goblet cells (C, asterisks). H&E. Scale bar: 1 mm (A); 100 μm (B); 10 μm (C).

Fig. 2 Epidermal rete pegs line narrow dermal papillae (open arrows) that contain vessels (dark arrows). A distinct granular layer (gr in A,B) is covered by the stratum corneum. The denser basal cells have extensive basal processes into which epithelial filaments (arrowhead in B) terminate. RLSM *z*-scan through the epidermis shows a bright hyper-reflective meshwork of cell borders at the epithelial surface. Papillae have bright rings of basal epithelial cells with a dark core; the same papilla is marked by an open arrow in the sub-figures of (C). (A) H&E, bar = 100 μm; (B) MG stain, bar = 10 μm; (C) RLSM, bar = 100 μm).

Fig. 3 Higher enlargements of the inner lid border of an upper lid in a mid-temporal position (A–E). The narrow dermal papillae (open arrow in A) become irregular and stop. The epidermal cornified and granular (gr in B) layers stop abruptly (thick interrupted line in A,B,E). The MCJ forms a large epithelial peg lined by pointed papillae (double open arrows in A,B). The MCJ surface (here 274 μm wide) first has a zone of continuous pk cells for 150 μm (B, grey line), followed by a zone of discontinuous pk interspersed among ordinary squamous (s) cells. Small dense roundish basal cells continue underneath the initial part of the lid wiper. (B) At the start of the lid wiper (narrow interrupted line in A,B) a conjunctival epithelial structure with cuboidal (c in B) surface cells occurs. It reaches a maximal thickness here of 98 μm soon after its start. It gradually thins down, forms a slope, and extends for here about a 1000-μm width (A) until it transforms into that of the sub-tarsal fold; a preparation artefact is seen (A, arrowhead). The lid wiper is composed mainly of cuboidal cells, some columnar cells, and contains goblet cells (asterisks in B). Some interspersed pk cells of flat to columnar shape occur at the surface (B). Goblet cells with faint staining of granular content or a reticular meshwork and a flat basal nucleus are also located in the depth of the epithelium (asterisk and arrow on nucleus in enlarged detail, (C). A few intraepithelial lymphocytes (arrowheads in B) are seen, Occasional smaller clefts (cl in B) occur between epithelial cells. An increased number of lymphocytes (B, arrowhead) and vessels, including high endothelial venules (h) with brighter, roundish endothelial nuclei (arrowheads), ordinary venules (v), and arterioles (a) underneath the MCJ is better seen in higher magnification (D, vessels are encircled by dotted lines). In another magnification (E), pk cells are clearly identified. H&E stain. Scale bar: 100 μm (A,B); 10 μm (C–E).



were immediately followed by ordinary cuboidal cells and goblet cells.

In the initial part of the lid wiper, typically a few small, pointed connective tissue papillae followed the large ones underneath the MCJ. Further along the lid wiper, papillae were lost and the level of the basement membrane was more or less flat (Fig. 3A). An increased number of lymphocytes together with high endothelial venules (HEV) and other vessels typically occurred underneath the MCJ and the initial part of the lid wiper. This contributed to an increased cellularity and darker appearance of the inner lid border (Figs 3A,B and 4B).

The surface of the lid wiper showed a less uniform picture in RLSM as compared to the MCJ (Figs 8–13) and had dark and bright spots depending on differences in the reflectivity of the surface. The proximal border of the lid wiper at the beginning of the subtarsal fold was indicated in RLSM by the occurrence of many intensely bright spots that represented the leucocytes of the diffuse part of the physiological conjunctiva-associated lymphoid tissue (CALT) (Figs 10 and 12). In contrast, leucocytes were sparse in and underneath the lid wiper (Fig. 10). On the epithelial surface, there were bright tufts of mucus from goblet cells. Other bright or dark spots (Fig. 11A–D) presumably represented cell detritus, as also observed in histology (Fig. 4B). Occasionally, shallow, dark defects of different sizes were seen in the epithelium of the MCJ (Fig. 12) and lid wiper. The goblet cells, single or in clusters, were also detectable in RLSM. In *z*-scans they could be followed for some 10 μm in depth (Fig. 13A–D). The increasing width of the lid wiper zone in temporal and nasal locations of the lid margin was also verified in RLSM (compare Figs 10 and 12).

Discussion

MCJ/line of Marx

The present definition of the MCJ of the human lid margin is not precise. Sometimes the whole epithelial thickening, identified in the present study as the lid wiper, was regarded as the MCJ (Wirtschafter et al. 1999). Other authors indicated the MCJ as a simple narrow division line between epidermis and conjunctiva (Wolfram-Gabel & Sick, 2002; Foulks & Bron, 2003). The present study identifies the MCJ as a zone of pk cells that follows after the abrupt termination of the cornified epidermis. Wolff (1946) also observed squamous cells in this position but did not describe pk cells and indicated that the MCJ started directly at the level of the posterior border of the Meibomian orifices. In contrast, we have observed that the cornified epidermis typically extends proximal to the orifices and these are thus typically encircled by an epidermal cuff and still open within the epidermis.

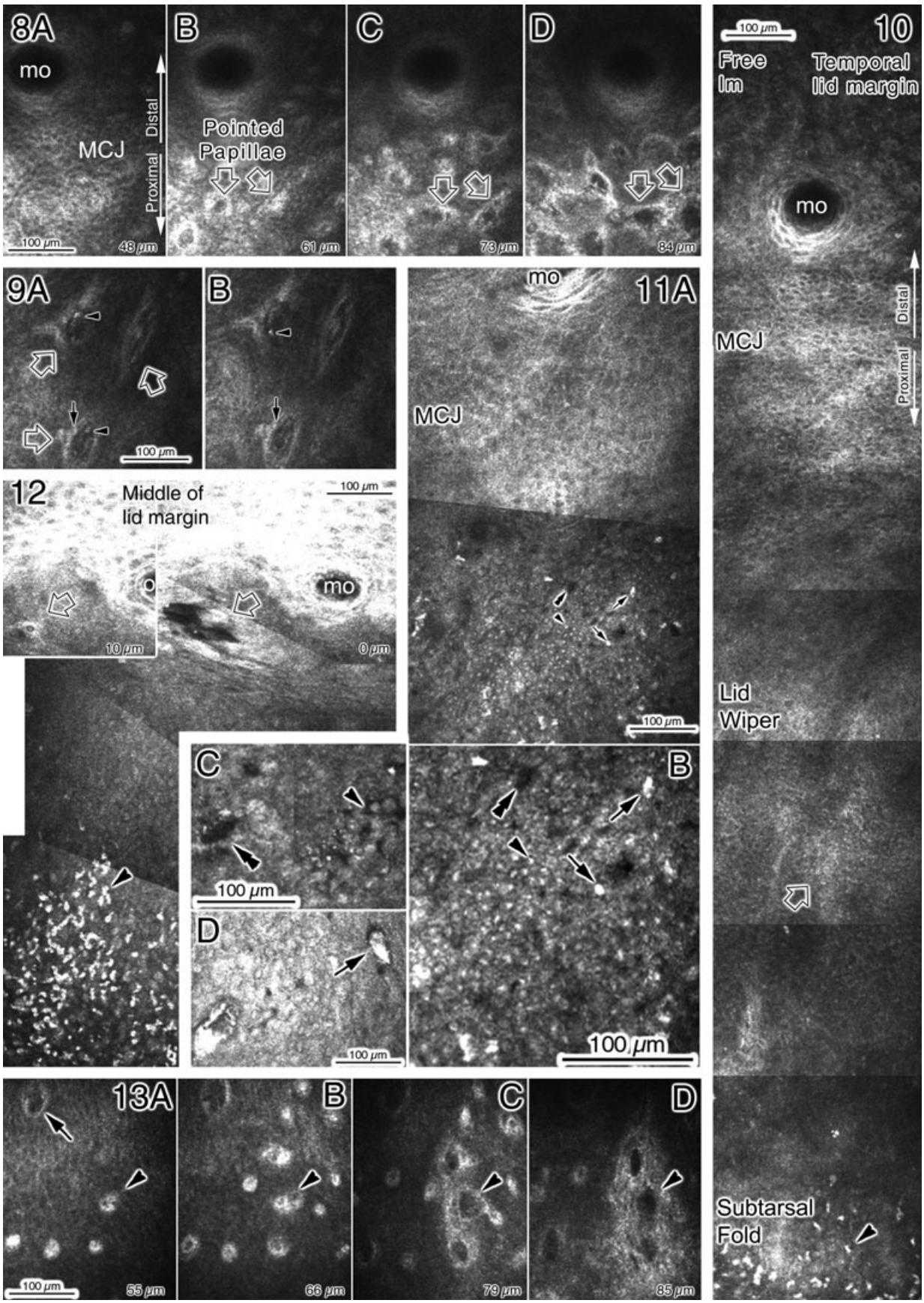
The observed histological morphology of the MCJ reflects precisely what was described and drawn by Marx in his original publication (Marx, 1924) as a 'fine red line' upon Rose Bengal vital staining. Marx's line occurred at the inner lid border between the 'palpebral conjunctiva and lid margin' and 'dispersed into single stained dots' towards the conjunctiva (Fig. 15A–C) (Korb, 2010; Pult et al. 2010). It can hence be concluded that the histo-morphological equivalent of the line of Marx is pk cells that take up several dyes more readily than the rest of the conjunctiva and epidermis. Masson–Goldner's trichrome stain is not specific for keratin but is based on electrostatic binding and on the texture of the tissue, as it consists of components of different size. It generally stains muscle cells, keratin and some other structures in a red colour (Böck, 1989). In the epithelium, MG stains those cells histologically identified as pk cells the most strongly, making them very obvious. Pk cells take up the red acidic fuchsin contained in the MG stain used in the present study, and as also used by Marx, much more readily than the rest of the epidermis and conjunctival epithelium. This is conceivably due to their high keratin content or to the dense meshwork in which the keratin filaments are arranged or due to other changes that occur during the differentiation preceding cornification (Presland & Dale, 2000; Alibardi, 2006).

Fig. 4 (A) Masson–Goldner's stain better displays pk cells by an intense red colour and hence the two subzones of the MCJ (continous and discontinuous pk) after termination of the epidermis (thick interrupted line). The first cuboidal cells (c) indicate the start (thin interrupted line) of the lid wiper. Single pk cells (arrows) continue in decreasing numbers in proximal direction along the lid wiper (B–C), the subtarsal fold (D) and further along the tarsal conjunctiva (E). Goblet cells (asterisks) are sometimes arranged into larger groups. Cell detritus, still containing a nucleus (B, double arrowhead) is seen on the surface in one place. (MG stain, bar for A–E = 100 μm).

Fig. 5 RLSM clearly shows the termination of the cornified epidermis as a distinct line (thick interrupted line) where the hyper-reflective meshwork stops along the middle of the orifices. At the opening of a Meibomian gland, the cornification goes around (arrow) the orifice. The MCJ epithelium is darker but still shows a less reflective surface meshwork of cell borders in places (open arrow). Scale bar: 100 μm.

Fig. 6 A lower lid margin is seen in different magnifications (A–C). At the sharp edge of the inner lid border (C), higher magnification (B) identifies the thickened (here 88-μm) lid wiper epithelium with bright spots of goblet cells. This ca.0.5-mm-wide lid wiper is preceded by a squamous transition zone of 90 μm and a continuous pk zone of about 200 μm in width. High magnification (A) clearly shows the goblet cells (asterisks), single and in clusters, located at the surface and inside the depth of the epithelium. The first cuboidal (c) cell defines the beginning of the lid wiper. A shallow defect (margins indicated by open arrows), equivalent to that seen in RLSM (Fig. 12), occurs at the surface of the MCJ. H&E stain. Scale bar: 100 μm (A–C).

Fig. 7 A lid wiper specimen that shows a large number of pk surface cells (arrows) with cuboidal to columnar shape may correspond to the vital staining observed in the clinical condition of lid wiper epitheliopathy. MG stain. Scale bar: 100 μm.



Because the pk layer of the MCJ was around 200–300 μm wide in histology it must be termed a 'zone'. This is in contrast to a 'line' although it may appear as a line upon unaided clinical inspection or by bio-microscopic magnification. At lower magnification, the single stained pk cells that occur proximal to the continuous layer may not be seen as part of the stained 'line'. This may explain why slit lamp bio-microscopic measurements tend to underestimate the width of Marx's line (Doughty et al. 2004; Yamaguchi et al. 2006).

The ocular MCJ resembles that of another MCJ of the head, i.e. the lip of the mouth [abstract TFOS conference (Knop & Knop, 2009; Knop et al. 2008b); Barrett et al. 2005; Riau et al. 2008] with its vermillion border and in particular its para-keratinized intermediate zone (Binnie & Lehner, 1970). The observed morphology of the basal cells in the ocular MCJ is consistent with that of stem cells and transient amplifying cells (Schlotzer-Schrehardt & Kruse, 2005) that have been described in this region (Pe'er et al. 1996; Wirtschafter et al. 1999; Liu et al. 2007). In ordinary stratified squamous epithelia, progenitor cells are distributed evenly along the basal cell layers (Ghazizadeh & Taichman, 2001; Fuchs & Nowak, 2008). In contrast, in the ocular MCJ region, progenitor cells appear enriched, as described in the rabbit (Wirtschafter et al. 1999), rat (Pe'er et al. 1996) and mouse (Riau et al. 2008), and contribute to the constant renewal of the conjunctival epithelium (Pe'er et al. 1996; Wirtschafter et al. 1999). The presence of similar cells in the human MCJ as observed here may indicate that this is also the case in the human.

An increased amount of conjunctival lymphocytes (Knop & Knop, 2000) as observed here is a general characteristic of epithelial transition zones in the body (Gray et al. 2005). It indicates that these areas are apparently more vulnerable and need increased immune protection. The lymphocytes at the ocular MCJ are a part of the eye-associated lymphoid tissue (EALT) (Knop & Knop, 2002a, 2005). EALT is the ocular branch of the physiological mucosal immune system and has important functions for ocular surface immune protection (Knop & Knop, 2000, 2010; Knop et al. 2008a).

The present study describes typical findings at the inner lid border but it must be noted that there is a certain natural variation (Norn, 1985; Hykin & Bron, 1992; Donald et al. 2003; Hirotani et al. 2003; Yamaguchi et al. 2006), which may increase with age (Hykin & Bron, 1992; Hirotani et al. 2003; Den et al. 2006). According to Hykin & Bron (1992), characteristics such as hyper-keratinization, squamous blepharitis and rounding of the posterior lid margin increase with age. Norn (1985) reported that the Meibomian orifices are more often displaced, leading to an irregularity of Marx's line, in patients over the age of 50 years. The orifices can occur closer to or more distant from the inner lid border and the cornification may already stop distal to the orifice. Accordingly, the MCJ can also exceptionally be found distal to the orifice, and the width of the marginal zones can vary. All of this was observed in the present study.

The lid wiper

On the basis of the morphology provided here, it can be stated that the epithelial thickening of the lid wiper is composed of a stratified cuboidal and partly columnar epithelium with goblet cells as depicted in a schematical drawing of the inner lid margin (Fig. 15). This epithelium hence represents the initial part of the conjunctiva and is not a part of the MCJ. Although the lid wiper showed a typical conjunctival structure, the change of the epithelial surface morphology at its beginning is often not as sharply defined

Fig. 8 RLSM. *z*-scan (A–D) through the epithelium around a Meibomian orifice (mo), the MCJ and the initial part of the lid wiper; depth in μm below the surface is indicated by small numbers in *z*-scans. The orifice is around 100 μm in diameter. In contrast to the roundish papillae in the distal region (compare Fig. 2C), the proximal papillae (open arrows in Figs 8 and 9) are irregular or longish and correspond to the pointed papillae seen in histology (compare Figs 3A,B and 4A).

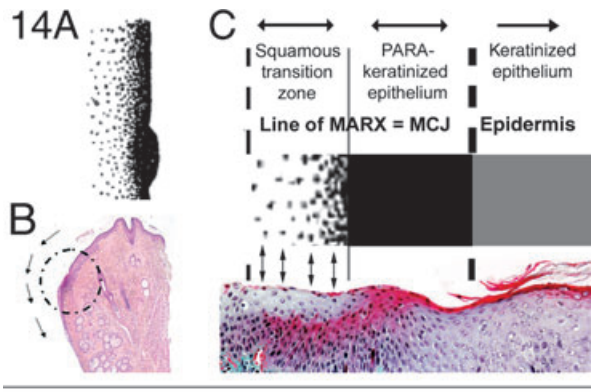
Fig. 9 The outline (arrow in Fig. 9A,B) of pointed papillae (open arrows in Fig. 9A) appears bright and their vessels contain bright moving particles that represent floating blood cells, in different positions in time lapse figures (arrowheads in Fig. 9A,B).

Fig. 10 Composite image of a temporal lower lid margin in mid-temporal position from the free lid margin (free lm) to the subtarsal fold. The free lid margin distal to the Meibomian orifice (mo) is met underneath the surface and hence shows no hyper-reflective meshwork (as seen in Fig. 12). A patchy, less reflective pattern is seen on the MCJ. The lid wiper with a width of about 1200 μm, is darker but shows patchy reflections in places (open arrow). Many bright intra- and subepithelial spots from leucocytes (arrowhead) characterize the start of the subtarsal fold.

Fig. 11 (A) The MCJ extends for about 250 μm proximal to a Meibomian orifice (mo). Enlargement (B) shows details of the lid wiper, such as small bright nuclei of the epithelial cells (arrowhead), a few bright particles on the surface (arrows) and occasional dark openings (double arrowhead). Further openings (C, double arrowhead) and small clefts between cells (C, arrowhead), equivalent to clefts seen in histology (Fig. 3), occur. Openings with whitish reflective content represent goblet cells (D, arrow, compare Figs 4B and 6A), which open onto the surface.

Fig. 12 In the middle (i.e. centre) of a lower lid margin a relatively narrow lid wiper ends when high numbers of bright cells indicate the beginning of the subtarsal fold. At the surface just proximal to a Meibomian orifice (mo) is a shallow epithelial defect (open arrow in Fig. 12 and inset), resembling that seen in Fig. 6A, of about 100 μm width and just 10 μm depth that appears closed when the focus plane goes 10 μm deeper (inset).

Fig. 13 In a *z*-scan through a lid wiper, goblet cells without an opening to the epithelial surface occur. They end at 55 μm (A) and start at 85 μm depth (D) where a diffuse bright layer indicates the basement membrane. Arrowheads indicate the same location in the different images. A cluster of surface goblet cells appears darker (A, arrow), conceivably because they have secreted their contents onto the surface. Scale bars in Figs 8–13: 100 μm (depth in μm below the surface is indicated by small numbers in *z*-scans).



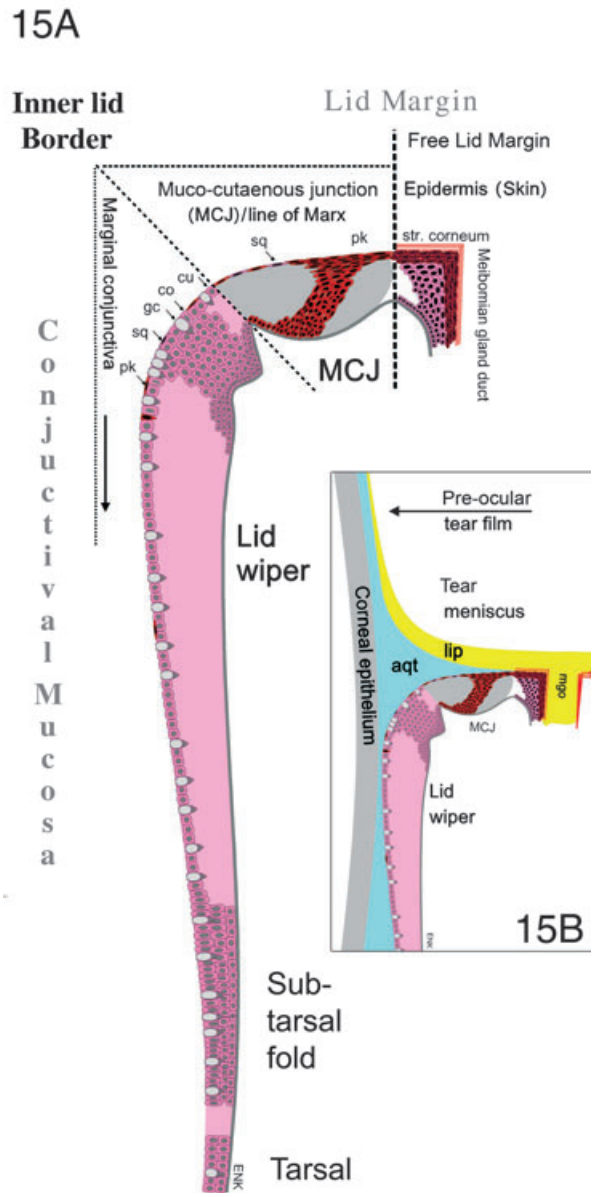


Fig. 14 The original drawing of the line of Marx (A) at the human inner lid border (B, circle) is in line with our finding of an abrupt start of the continuous para-keratinized (pk) zone proximal to the epidermis as well as with a following zone of discontinuous stained cells. If Marx's drawing is superimposed on the histology (C) it becomes clear that the stained dots represent pk cells (double-headed arrows) and that the line of Marx thus represents the surface of the MCJ.

Fig. 15 Schematic drawing summarizing the zones at the human inner eye lid border (compare with Fig. 3A,B). (A) The cornified (str. corneum) epidermis extends slightly proximal to the orifice of the Meibomian gland duct and then stops abruptly. The following muco-cutaneous junction (MCJ) consists of parakeratinized (pk) cells that form first a continuous and then a discontinuous zone with interspersed ordinary squamous (sq) cells. The MCJ surface is the equivalent of the vital stainable line of Marx (as explained in the legend to Fig. 14). On the crest of the inner lid border, the start of the epithelial lid wiper elevation is indicated by the cuboidal (cu) and occasional columnar (co) epithelium with goblet cells (gc), which has a conjunctival structure, although some ordinary squamous (sq) and pk cells are interspersed at the surface (compare with Fig. 3). The lid wiper is thicker at its start at the inner lid border and forms a slope towards the subtarsal fold. It hence constitutes an epithelial lip that is apposed to the globe and conceivably serves to distribute the thin pre-ocular tear film layer. (B) The proposed relation of the lid wiper to the surface of the globe (here cornea) and to the pre-ocular tear film. According to the findings of Norn (1966) it can be assumed that the line of Marx is covered by the aqueous tears (aqt) and is at the bottom of the tear meniscus. The Meibomian lipids (lip) from the orifice (mgo) are on top of the aqueous tears. This fits well with our observation of the location of the different epithelial zones at the inner lid border that suggests the direct apposition of the lid wiper to the ocular surface and a more outward position of the MCJ. Individual epithelial cells are in most parts depicted only at the epithelial surface.

as at the end of the epidermis. As the initial part of the lid wiper can still show considerable numbers of interspersed pk cells or patches of one very flat cell layer on top of cuboidal cells, the lid wiper in this respect can still have characteristics of a transition zone. Therefore the descriptions of Parsons (1904) and Ehlers (1965) of squamous cells at the surface are not totally inconsistent with our findings although they do not exactly match the epithelial structure as obtained here in high magnification. Also a different type of vascularization pattern distinguishes the lid wiper zone with the mucosal type, from the MCJ, which has a cutaneous type (Wolfram-Gabel & Sick, 2002). The cutaneous type shows a superficial network of papillary vessels that extend into the connective tissue papillae, whereas the mucosal type has a superficial flattened polygonal mesh of small vessels.

The identification of goblet cells in the lid wiper is clear in histology and based on well known characteristics such as their pale staining, the occasional presence of a faint reticular meshwork, and their basal, typically flat or indented nucleus. In RLSM, the identification of goblet cells is not as clear but it is still sufficiently conclusive because their morphology, size and location within the epithelium matched the findings observed in histology. Furthermore, it was occasionally observed that bright material at the epithelial surface was continuous with the content of the interior of such cells in RLSM and hence resembled the tufts of

mucus that can be observed in histology as originating from goblet cells.

The lid wiper represents an epithelial thickening that has incidentally been mentioned in the literature because it contributes to the formation of a relatively sharp angle of the inner lid border (this already occurs in the neonate; Wolfram-Gabel & Sick, 2002) compared to the rounder outer one (Parsons, 1904; Virchow, 1910; Bron et al. 1997). It was noticed early on that this area 'lies in contact with the globe' and it was speculated that 'the capillarity induced by this sharp angle of contact is of importance in the proper moistening of the surface of the eye' (Parsons, 1904). Others concluded that this 'bead gliding over the cornea' must be assumed to be a perfect 'wind screen wiper' (Ehlers, 1965) and recently it was termed the 'lid wiper' (Korb et al. 2002).

It had been reported that the region that extends onto the tarsal surface of the lid for about 1–2 mm consists of a non-cornified stratified squamous epithelium (Parsons, 1904; Ehlers, 1965). The extension of this zone roughly matches the extension of the lid wiper as observed in the present study, which further indicates that the same area is concerned, as investigated in the present study. The high variability in the reported width of the lid wiper can in the light of the present findings be explained by the differential width at different positions along the lid margin. The historic descriptions, however, do not match the histological findings of epithelial morphology obtained here in high magnification. We show that the epithelium proximal to the MCJ at the inner lid border that forms the epithelial elevation is no longer squamous, although squamous and pk cells, following those of the MCJ, are present, but it has a conjunctival structure consisting of cuboidal cells and contains goblet cells.

Functional considerations

Some functional considerations may follow from the morphological findings in the present study. They are necessarily speculative and represent conceptual thinking but are in line with the observed morphology and the available literature. It had been assumed that a squamous epithelium would provide a 'soft' surface 'gliding over the cornea' (Ehlers, 1965), which may be a reason for the occasional assumption the MCJ/line of Marx would be in contact with the cornea (Doughty et al. 2004; Shaw et al. 2009). The conjunctival lid wiper elevation would conceivably represent a softer structure. The cells are more loosely arranged (Rohen & Lütjen-Drecoll, 1991) than a squamous epithelium. It can be assumed that their higher water content allows a hydrodynamic resistance that is necessary to provide a close enough contact for the distribution of a very thin pre-ocular tear film (King-Smith et al. 2004). At the same time, this would avoid too much pressure, which could potentially injure the bulbar epithelium. In addition, the goblet cells typically observed in the lid wiper epithelium, right from the start, appear suitable as a 'built in' lubrication system due to the water-binding property of its mucins (Argueso & Gipson, 2001) at the lid wiper surface.

Connective tissue papillae, although of an unusual large and pointed type, are only found in the beginning of the lid wiper. This could indicate that the mechanical forces on the slope of the lid wiper are not particularly great and supports the assumption that it is most likely only the tip, i.e. the initial upper part, of the lid wiper that actually distributes the tears. Therefore, the restriction of connective tissue papillae to this initial part supports the identity and function of the lid wiper. This is further supported by mathematical models that appear to predict that the extension of the region that actually touches the surface of the globe may be rather small, probably about 100 μm wide (Jones et al. 2008). Ehlers' (1965) previous functional considerations also support the assumption that only the tip of the lid wiper is in contact with the globe. It is most likely that not the whole slope and certainly not the more distal subtarsal fold region is in contact with the globe because then 'blinking would be impeded if the lid was ''sucked'' closely against the eye' (Ehlers, 1965). Observations by Wolff (1946) that the tears are guided behind the lids are in line with this and Kessing's (1967) radiographical analysis further verified a retro-palpebral tear space behind the upper lid.

Our observations of the location of the different epithelial zones at the inner lid border suggesting the direct apposition of the lid wiper to the ocular surface and a more outward position of the MCJ also fit well with the observations of Norn (1966). He reported that the line of Marx is covered by aqueous tears and is the bottom of the tear meniscus, which appears to exclude the direct apposition of the MCJ/line of Marx to the globe.

Minor alterations of the lid wiper in normal lids, consisting of narrow gaps and holes between the surface cells, as occasionally observed in histology and in RLSM point to frictional forces. A majority of pk cells, as observed in MG staining at the surface of the lid wiper epithelium in some histological specimens, agree with the description of LWE by vital staining (Korb et al. 2002) in a minority of asymptomatic normal subjects and in a majority of symptomatic dry eye patients (Korb et al. 2005), with a six times greater incidence in dry eye patients (Korb et al. 2010). This clearly supports the proposed usefulness of LWE as an early indicator of tear film deficiencies preceding other conventional signs of dry eye. It also highlights the usefulness of confocal microscopy for *in vivo* investigation of the ocular surface on a cellular level.

Conclusion

This study presents in detail the histological and *in vivo* confocal morphology of the human eyelid margin, and in particular the lid wiper and MCJ. The line of Marx, the

morphological basis of which is unclear, was shown to consist of a zone of para-keratinized (pk) cells located immediately after the abrupt cessation of the epidermis and before, i.e. distal to, the crest of the inner lid border. Marx's line corresponds to the surface of the MCJ.

The prominent epithelial thickening of the lid wiper is distinct from the MCJ. The lid wiper is elevated and is the most prominent feature for apposition to the globe, and it is therefore suitable for spreading the thin pre-ocular tear film. A surprising finding of this study was that, counter-intuitively, the lid wiper is not composed of a stratified squamous epithelium but rather of a stratified cuboidal epithelium with a conjunctival structure with goblet cells. This suggests a specific method for the lubrication of the lid wiper which travels hundreds of metres daily over the bulbar surfaces, requiring a more sophisticated lubrication system than a simple exogenous lubrication.

As this epithelial lip-like structure of the inner lid border wipes the globe, and in particular the cornea, the term 'corneal wiper' (in analogy to the wind-screen wiper of a car) would seem to be a better nomenclature for its function than the more established term 'lid wiper'. However, as the lid wiper is an anatomical feature of the eyelid, it may best be described as a lid wiper that wipes the bulbar surface.

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Disclosure

Authors have nothing to declare.

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