Brewitt H (ed): Research Projects in Dry Eye Syndrome.
Dev Ophthalmol. Basel, Karger, 2010, vol 45, pp 108–122

The Lid Margin Is an Underestimated Structure for Preservation of Ocular Surface Health and Development of Dry Eye Disease

Erich Knop[a] · Donald R. Korb[b] · Caroline A. Blackie[b] · Nadja Knop[c]

[a]Research Laboratory, Department of Ophthalmology CVK, Charité – Universitätsmedizin Berlin, Berlin, Germany; [b]Korb Associates, Boston, Mass., USA; [c]Department of Cell Biology in Anatomy, Hannover Medical School, Hannover, Germany

Abstract

Purpose: The structure of the lid margin is insufficiently understood and defined, although it is of obvious importance in ocular surface integrity. **Methods:** The structure and function of the different zones of the lid margin are explained with a focus on dry eye disease. **Results:** The posterior lid margin, which is of particular significance for the integrity of the ocular surface, includes the meibomian glands that open within the cornified epidermis. Their obstructive dysfunction is a main cause of dry eye disease. The orifice is followed by the mucocutaneous junction, which extends from the abrupt termination of the epidermis to the crest of the inner lid border. The physiological vital stainable line of Marx represents its surface, and can be used e.g. as a diagnostic tool for the location and functionality of the meibomian gland orifices and lacrimal puncta. The marginal conjunctiva starts at the crest of the inner lid border and forms a thickened epithelial cushion. This is the point closest to the globe, and represents the zone that wipes the bulbar surface and distributes the thin preocular tear film. It is hence termed the 'lid wiper' and pathological alterations that result in a vital staining are a sensitive early indicator of dry eye disease. **Conclusions:** The margin of the eyelid is an important but currently underestimated structure in the maintenance of the preocular tear film and of the utmost importance for the preservation of ocular surface integrity and in the development of dry eye disease.

Introduction

The Lid Margin Is an Important but Currently Underestimated Structure

The margin of the eye lid is an essential structure in the maintenance of preocular tear film and of the utmost importance to the functional anatomy of the ocular surface [1] for the preservation of its integrity. Lid margin dysfunction is also an important factor in the development of dry eye disease [2–7].

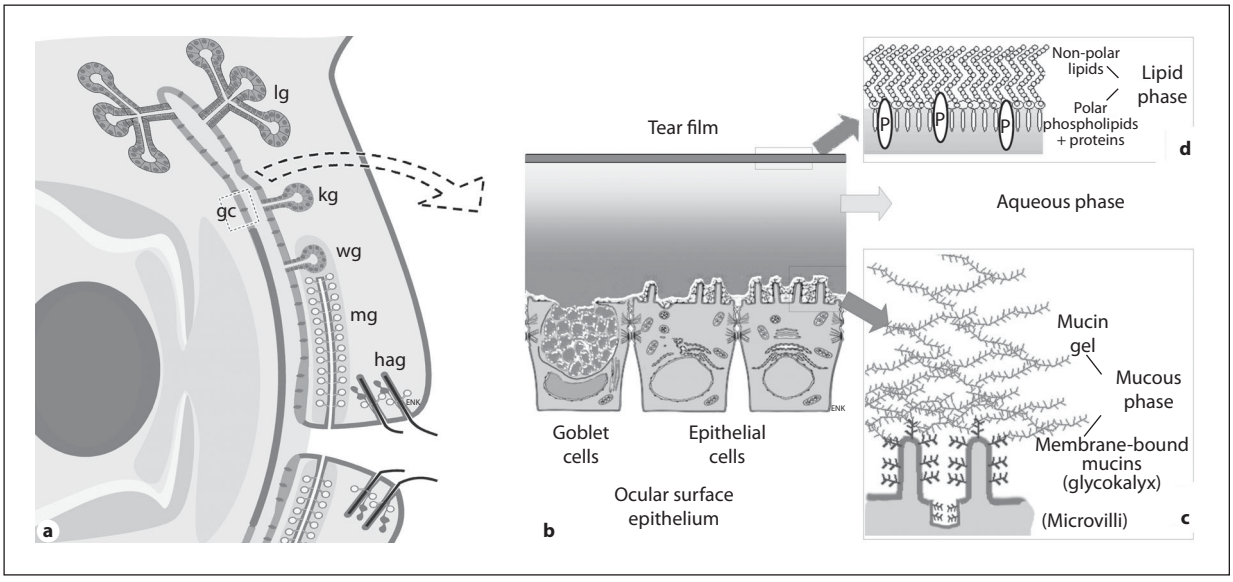

Fig. 1. Glands of the ocular surface and preocular tear film. **a** Several glands that are either located directly at the ocular surface or connected to it via their excretory duct contribute to the production of the tear film. The aqueous phase is produced by the main lacrimal gland (lg) that is located in the orbit and delivers its fluid via multiple excretory ducts into the conjunctival fornix, by the accessory lacrimal glands located in the orbital tissue (Krause glands, kg) and in the proximal part of the tarsus (Wolfring glands, wg). The mucin layer is produced by the goblet cells (gc) inside the conjunctival and by the conventional conjunctival epithelial cells; the lipid phase is produced by the large tarsal glands of Meibom (mg) inside the tarsal plates that deliver their oily secretion through a short excretory duct at the posterior lid margin onto the tear meniscus. At the anterior lid margin, there are also hair-associated glands (hag) of Zeis and Moll. **b** The tear film is principally thought to form 3 layers that mix to a certain degree. **c** The mucus phase consists of the membrane-bound glycocalyx of the ordinary epithelial cells of the ocular surface epithelia, located on their microvilli, and of the gel-forming mucins from the goblet cells. The gel-forming mucins increasingly mix and dilute within the overlying aqueous phase. This mucinous-aqueous phase constitutes the main part of the tear film and is provided with a superficial thin layer of lipids. **d** Within this, polar lipids and potentially proteins maintain the binding of the overlying non-polar lipids which can not directly mix with water. The exact conformation, composition and thickness of the tear film and its layers, particularly the lipid layer, is still under debate. Reproduced from E. Knop et al. [1] with permission from Springer.

The importance of the lid margin is apparently underestimated at present because research focused on ocular surface integrity and tears has usually been centered around the source of the tear fluid derived from the secretion of the ocular-surface-associated glands, i.e. the lacrimal gland, accessory lacrimal glands, goblet cells that are interspersed in the conjunctiva, and the meibomian glands inside the tarsal plates of the eyelids (fig. 1a). The latter contribute the superficial lipid layer to the tear film, but have received only limited attention clinically and scientifically. This has only recently begun to change with the recognition that alterations in the lipid phase [8] caused by meibomian gland dysfunction (in particular of the obstructive type [9]) are a main cause of dry eyes.

Another area of focus has been the equipment of the ocular surface epithelia with lubricative mucins that contribute to the adherence of aqueous tears [10] (fig. 1b, c) [11, 12]. This attention on mucins historically derives from early observations that seemed to show [13] that the corneal surface is non-wettable and could only be lubricated through coating with mucins [14]. Also the formation of dry spots, as an early sign of tear film deterioration and dry eye condition, was related to a local mucin deficiency [15].

Functions of the Lid Margin

The Lid Margin Preserves Ocular Surface Integrity

In contrast to these conventional considerations, the lid margin represents the 'other end' of the tears and appears to be equally important for ocular surface lubrication; it conceivably has several functions: (1) acting as a static dam, since the inner lid margin limits the tear lake (meniscus) and hence prevents the potential loss of tears from the ocular surface by spillage over the anterior lid border and guides the tear flow along the lid margin towards the lacrimal puncta [16, 17]; (2) distributing tears (posterior lid border) [18] in a way comparable with a windscreen wiper [19] because with every blink the movement of the lid margin guarantees the required thin expansion of the tear film in order to form a thin optically perfect tissue-air interface [4, 20–22]; (3) coating the thin tear film with oil of the tarsal meibomian glands (delivered anterior to the meniscus), and thus preventing evaporation of the aqueous phase [23–26]; (4) meibomian oil presumably prevents the skin lipids (found to induce tear film rupture [27]) entering the tear film.

Alterations in the Lid Margin Are Associated with Ocular Surface Disease

Clinically, it is well known that deterioration of the lid margin is often associated with ocular surface disease [28–34]. Surprisingly, however, the microanatomy of the different regions of the human lid margin has been poorly defined, which results in difficulties in describing normal morphology and its alterations in disease states. Clinically, the whole lid margin is usually addressed just as the 'margin' and subzones are not specifically differentiated (e.g. free *margin* versus anterior or posterior *border*).

If the mucocutaneous junction (MCJ) is considered, this is often thought to extend onto the tarsal side [35], and the nature and localization of the line of Marx have remained enigmatic for a long time. The zonal differentiation at the inner lid border is currently unclear with respect to the MCJ, the line of Marx and the lid wiper. Since this region is of the utmost importance for the continuous distribution and reformation of the preocular tear film with every blink, it conceivably has eminent implications for ocular surface health and integrity as well as for the development of dry eye disease. This also applies to the morphological changes that underlie the development of obstructive meibomian gland dysfunction, which appears to represent the single most frequent cause of dry eye disease [9].

The current knowledge and the remaining unresolved issues of lid margin anatomy and function that are relevant for understanding dry eye disease will be explained and discussed in this contribution.

Structures at the Lid Margin and Their Involvement in Dry Eye Disease

Anatomy and Pathology of the Meibomian Glands

The meibomian glands, also termed tarsal glands of Meibom (glandulae tarsales) because of their location inside the tarsal plates, are large sebaceous glands in the eyelids with no association to hairs. Pathological alterations in the meibomian glands, which are mainly summarized as 'meibomian gland dysfunction' (MGD) [36], are increasingly being recognized as a discrete disease entity [30, 37] and an important factor in evaporative dry eye disease [8, 9]. The meibomian glands are single glands that open at the posterior lid margin (fig. 2a) close to the termination of the cornified epidermis, but are usually still encircled by epidermis. The glands can be seen as white-to-yellowish structures underneath the conjunctiva throughout the length of the tarsal plates in the upper and lower lids when the lid is everted (fig. 2b) or visualized by transillumination. Numerous secretory acini are arranged around the long central duct and connected to it by small ductules (fig. 3c). Many of these separate glands are arranged in a sheet that almost completely fills the extension of the tarsal plates [38–41].

Meibomian Ductal System Shows Signs of Incipient Keratinization

The holocrine secretory acini of the meibomian glands have a longish-to-roundish shape and are completely filled with pale secretory cells, which are termed meibocytes [42] (fig. 3a). They produce and accumulate lipids inside the cell until they degenerate and release their entire cell contents which form the meibomian oil (meibum) [42]. The oil is released through the connecting ductule into the long central duct (fig. 2c), which are both lined by a 4-layer stratified squamous epithelium [41].

The terminal part of the central duct has a different structure and is formed by an ingrowth of the epidermis from the free lid margin; it must therefore be separately designated from the rest of the central duct as an excretory duct. The excretory duct is lined by epidermis that loses the cornification after about half a millimeter, by loss of the keratin lamellae and the granular layer, and transforms into the usual 4-layer stratified squamous epithelium of the ductal system. Around the terminal part of the gland (excretory duct, the terminal part of the central duct and terminal acini) close to the free lid margin, there are varying amounts of striated fibers of Riolan's muscle (fig. 2c, 3b, d) [41, 43, 44] which are split from the orbicularis muscle by the downgrowth of the cilia deep into the tarsal fold during embryological development [19].

Interestingly, recent investigations have shown that in fact the whole ductal epithelium of the normal human meibomian gland has preserved a certain degree of

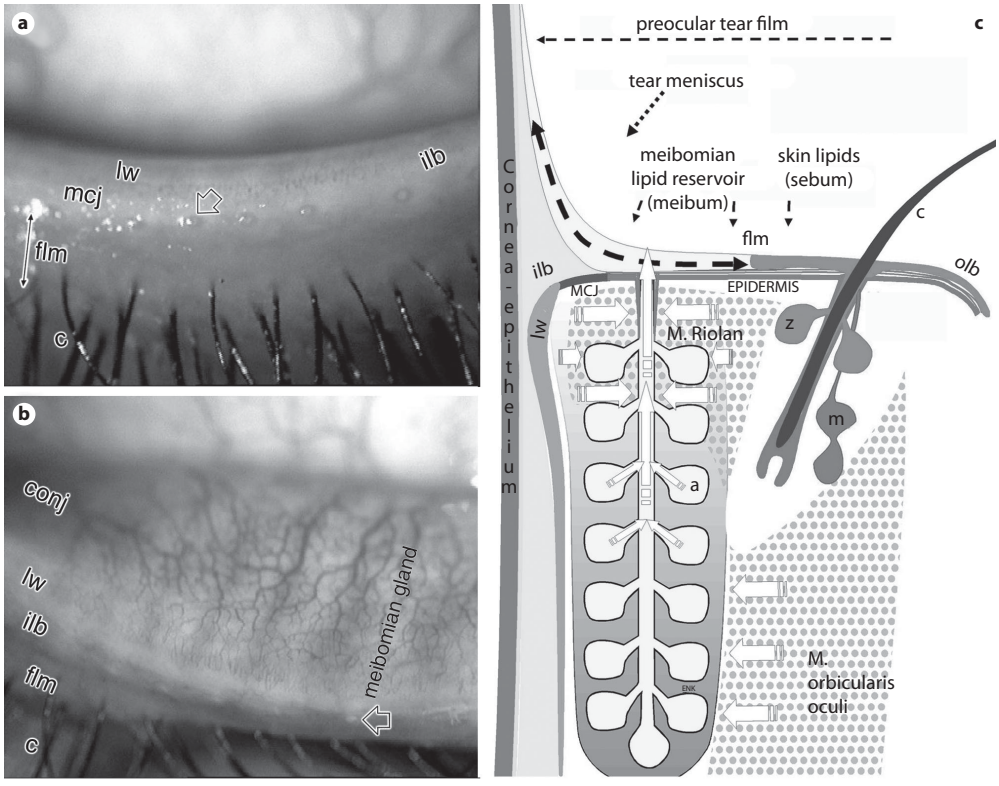

Fig. 2. Zones and structures of the human lid margin. The lid margin consists of different zones with differential structure as seen in photomicrographs that show a lid margin in a slightly (**a**) and in a strongly (**b**) everted position as well as in a schematic drawing (**c**). From distal to proximal, the cornified stratified squamous epithelium of the skin (epidermis) extends from the outer skin of the lid over the outer lid border (olb). It grows deep into the lid body to give rise to the ciliary hair follicles and their associated glands of Zeis (z) and Moll (ml). It covers the free lid margin (flm), grows deep again to provide the origin of the meibomian glands, and also typically encircles the orifices of the meibomian glands (open arrows). The MCJ is located proximal to the epidermal cuff around the orifices of the meibomian glands at the inner lid border (ilb) and is followed by the lid wiper (lw), which represents the initial thickening of the conjunctival mucosal epithelium and serves as a device to spread the tear fluid from the marginal tear meniscus into the thin preocular tear film during the upphase of every blink. The aqueous tear fluid is covered by a thin outer layer of lipids that are produced by the meibomian glands inside the tarsal plate of the lid. Lipids are produced by the numerous roundish holocrine sebaceous acini (a) in the gland periphery, then transported (small white arrows) through the connecting ductules into the long central duct and delivered through a short excretory duct and orifice onto the posterior lid margin. The driving forces for the delivery of meibum are the constant secretory force together with the mechanical action (large white arrows) of the orbicularis muscle (orb) and the muscle of Riolan at the posterior lid margin that occur with every blink. Reproduced from E. Knop et al. [37a, 37b] with permission from Springer.

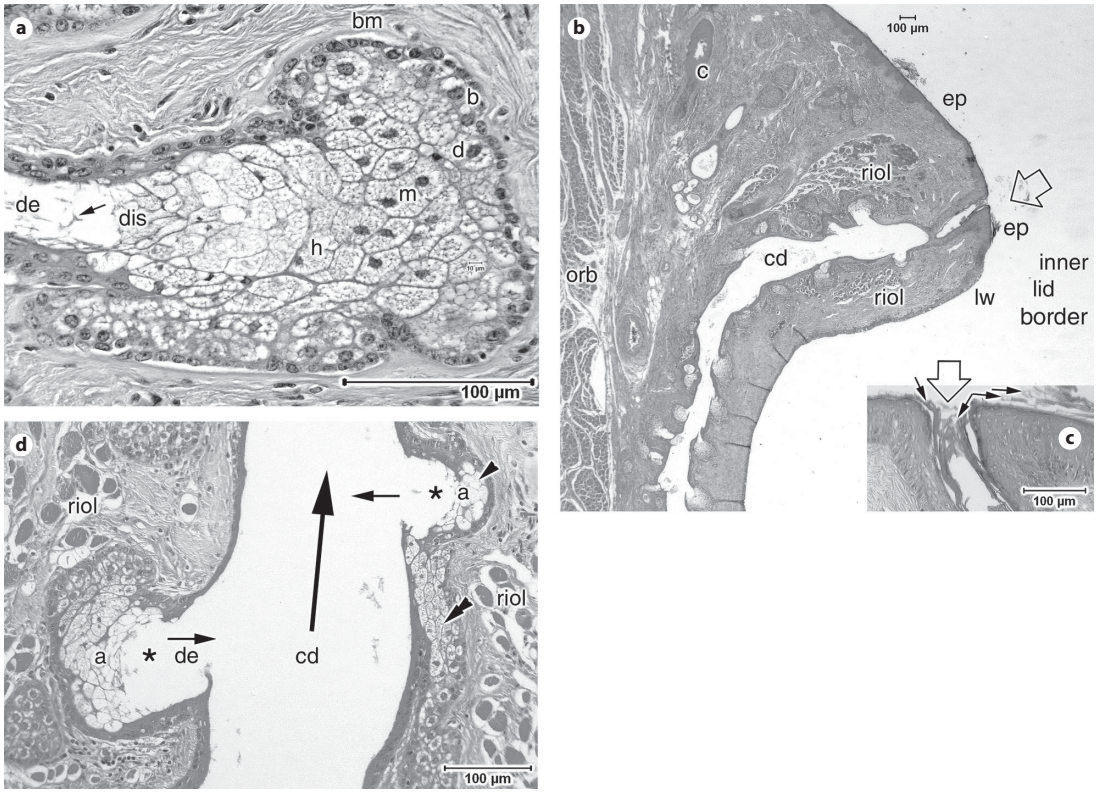

Fig. 3. Structure of the acini and ductal system of the meibomian gland. **a** Holocrine acinus of a normal meibomian gland filled with secretory cells (meibocytes) and surrounded by a basement membrane (bm). From the basal cells (b) at the periphery, differentiating (d), meibocytes start with the production and accumulation of lipids within droplets and move towards the acinus center leading to large mature (m) meibocytes. The very large hypermature (h) meibocytes, disintegrate (dis) and their entire cell contents form the oily secretory product (termed meibum) close to the connecting ductule (de). Remnants (arrow) of the meibocytes are still found inside the ductule. **b** An obstructed Meibomian gland shows signs of degeneration due to the increased internal pressure. The central duct (cd) is partly dilated and slightly undulated. **c** The orifice (open arrow) is obstructed by accumulations of cornified keratin lamellae (filled arrows). **d** Under higher magnification, the epithelium that lines the dilated central duct (cd) is seen to be compressed, the connecting ductules (de) are widened, the acini (a) are reduced in size and degenerated. Their secretory cells (meibocytes) are reduced in number to a few layers of bright cells in the periphery (arrowhead). Occasionally residual degenerated acini are embedded into the wall of the central duct (double arrowheads) HE.

incipient keratinization, as verified by the regular presence of keratohyalin granules in the luminal epithelial cell layer [45]. This can be explained by the similarity in structure and embryological development of the meibomian glands to the ciliary hair follicles [reviewed in 41], and it also explains why hyperkeratinization is a typical pathology that leads to MGD.

Hyperkeratinization Is a Major Cause of Obstructive MGD and Results in Degenerative Gland Dilatation and Atrophy

Obstructive MGD due to hyperkeratinization was first described in patients with only minimal or transient symptoms suggestive of ocular dryness who became clinically symptomatic due to a contact lens intolerance. Manual expression of their meibomian glands revealed clusters of desquamated hyperkeratotic epithelial cells embedded in a thickened meibum that had obstructed the orifices, and histology verified a dilatation of the central duct by such material [36]. After expression and removal of theses plugs, the tear film normalized and contact lens intolerance disappeared [46]. Later histological examinations of the meibomian glands from patients with symptomatic dry eye disease, which showed inspissation of orifices and expressible highly viscous secretion, verified obstruction of the excretory duct by increased keratinization. Inside the gland, this had resulted in dilatation of the ductal system as well as enlargement of acini with cystic degeneration, loss of secretory meibocytes (fig. 3b, d) and their replacement by a squamous metaplasia [47].

Obstructive MDG Results in Increased Evaporation and Dry Eye Symptoms

Obstruction of the meibomian glands leads to decreased delivery of their oil onto the posterior lid margin that results in: (1) a thinning of the tear film lipid layer; (2) tear film instability; (3) increased evaporation [48] of the aqueous phase leading to an evaporative dry eye condition with consequent hyperosmolarity of the remaining tears [49]. Hyperosmolarity [24] exerts stress on the ocular surface epithelia (cornea and conjunctiva) that results in an activation of the cells [50] with the release of inflammatory mediators. This contributes to the well-known signs and symptoms of a dry eye condition – such as stinging, burning and foreign body sensation – together with vital staining of the epithelial surface due to mechanical alteration from increased friction between the lids and bulbus, and is associated with reduced and unstable visual acuity [36, 51–53]. Continued activation of the ocular surface epithelia can lead to the onset of a self-enforcing inflammatory cascade that is modulated via a deregulation of the physiological mucosal immune system of the ocular surface, the eye-associated lymphoid tissue [54–56], see another contribution to this volume). This may, in advanced chronic stages, require an immunomodulatory therapeutic intervention [1, 57, 58].

Anatomy and Pathology of the MCJ of the Lid Margin

Currently, the definition of the MCJ of the human lid margin is not very precise. This is reflected by descriptions that apparently regard the whole epithelial thickening (now identified as the lid wiper [59–61]) as the MCJ [35, 62], whereas others have defined the MCJ as only a narrow division line between the cornified epidermis and the conjunctiva [30]. These different descriptions reflect the fact that only scant knowledge exists about this structure.

Line of Marx Is the Surface of the MCJ

Historical investigations have reported that the MCJ lies on the posterior part of the free lid margin at the level of the posterior rim of the meibomian orifices where the skin epidermis stops [63]. By in vivo confocal laser scanning microscopy of individuals around the fourth decade of life [61], it was seen that the epidermis ends sharply in about the midline through the orifices of the meibomian glands, but at an orifice this is completely encircled by an epidermal cuff. This is also a typical finding in histology of individuals aged in their mid-seventies [61], although sometimes the posterior rim of the meibomian gland orifice can already be covered by a non-cornified parakeratinized epithelium. Age dependent anterior movement of the line of Marx [64] and posterior movement of the orifices of the meibomian glands (retroplacement) [28] are shown as typical findings in aging. After the abrupt stop of the epidermis the MCJ starts and represents the transition zone between skin and mucous membrane.

In the same position, Marx [16] described a line that he observed in great detail after the application of several types of vital stains; this line is therefore known as the 'line of Marx' (fig. 4). Before him, Virchow [38] had also mentioned an 'admarginal zone' that was characterized by the intense uptake of pikrin and eosin stains. Marx reported that stained dots accumulate on the posterior lid margin, and these represent single or groups of surface cells, which condense towards the outside (i.e. to the skin) into a homogeneous thin line [16]. These basic findings have been supported by other authors [17, 19]. The functional significance of this vital staining line has remained a subject of speculation. Marx noted that this line: (1) has a relation to the outer margin of the tear meniscus; (2) may be caused by the interaction of the tears with the epithelium; (3) may serve to guide the tears along the lid margin to the lacrimal punctum. Ehlers [19] suggested it might be caused by friction during blinking, while Norn observed that it represented the bottom of the tear meniscus which argues against a direct contact with the globe and respective mechanical forces in this region.

Recent histological investigations have shown that the epithelium that follows after the abrupt stop of the keratin layers of the cornified epidermis is still stratified squamous, but the cytoplasm of the surface cells is very dense and still contains a nucleus which characterizes them as parakeratinized [61]. These cells stained very intensely with Masson-Goldners trichrome stain, which contains red acidic fuchsine, one of the stains already used by Marx (fig. 4). The parakeratinized cells of this continuous zone later disperse into single parakeratinized cells among ordinary brighter squamous cells. The reported results using Masson-Goldners trichrome stain on histological sections perfectly matched what had been described by Marx in his vital staining procedure. Therefore, it can be concluded that this narrow zone of parakeratinized cells located at the surface of the MCJ represents the histological equivalent of the vital staining line of Marx and the line of Marx is hence the surface of the ocular MCJ. On the crest of the inner lid border, the MCJ transforms into epithelium with a conjunctival structure composed of roundish cells of less density. This epithelial cushion forms the lid wiper (fig. 5).

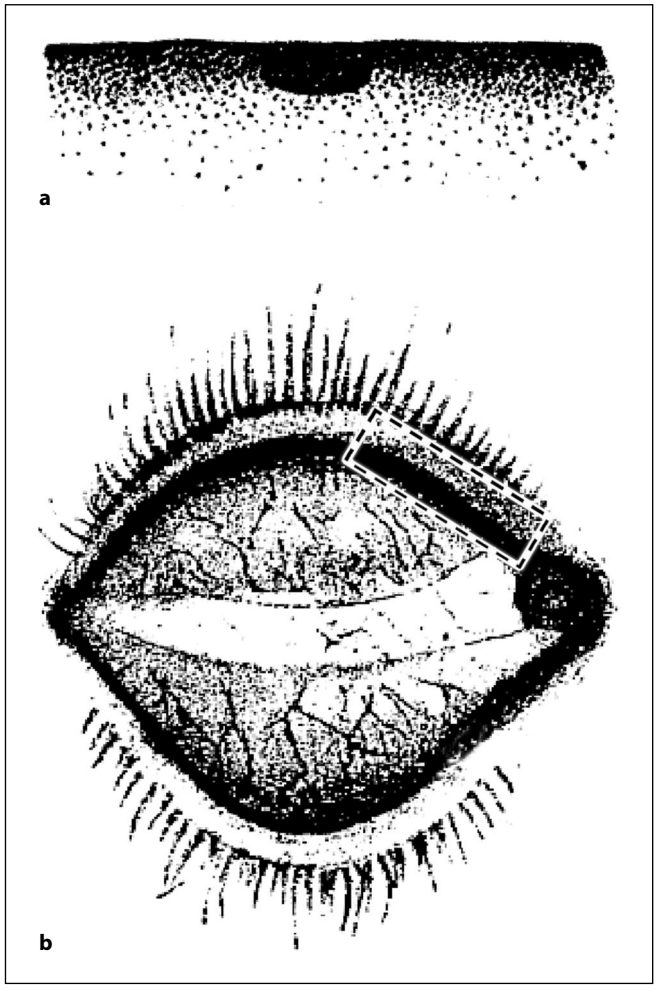

Fig. 4. Original drawing of the line of Marx. According to the original drawing by E. Marx, the vital staining line (dotted box in enlargement **a**) that he described in his paper in 1924 is located at the inner border of the eye lid 'between palpebral conjunctiva and lid margin' as indicated in **b**. It consists of single dots of vital-stained cells that increasingly accumulate from the conjunctival side (**a**, bottom) into a dense line towards the skin (**a**, top). The stained line also encircles the lacrimal punctum and extends into it, seen as a large dark spot in **a**. From Marx [16].

MCJ/Line of Marx Can Be Used as a Diagnostic Tool in Dry Eye Research

The line of Marx has meanwhile received increased interest because it can be easily stained (e.g. by the vital stains fluorescein, lissamine green and rose bengal) and its functional and pathological significance are under speculation [64, 65]. It was considered that this line may be the natural site of contact [65, 66] between the eyelid margin and the surfaces of the bulbus (conjunctiva and cornea). However, this appears to be unlikely due to several reasons: (1) as judged from the lid geometry, the line of Marx is too far outside, i.e. distal, on the posterior lid border to touch the globe – this is supported by the observation that it starts at the posterior margin of the meibomian gland orifices [17, 19, 64] and represents the bottom of the tear meniscus [17]; (2) this zone is too narrow; (3) it appears to be too rigid – as judged from its composition of parakeratinized cells [61] and compared to the conceivably soft cushion of the conjunctival

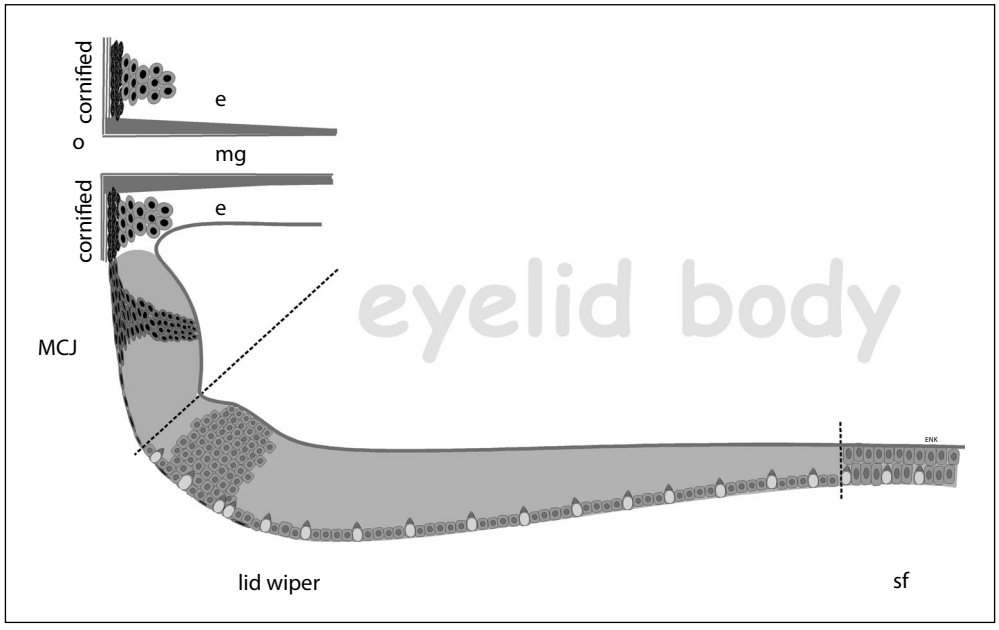

Fig. 5. Schematic drawing of the inner lid border including the meibomian gland orifice, MCJ and lid wiper, and related cell types. The different zones of the posterior lid border on the inside of the eyelid body consist of the opening (o) of the meibomian gland (mg), which is typically located still within the cornified epidermis (e). This is followed by the vital stainable 'line of Marx', which consists of flat parakeratinized surface cells and represents the surface of the MCJ. The MCJ ends at the crest of the inner lid border. At the crest (dotted line between MCJ and lid wiper), the stratified cubical epithelium of the lid wiper starts. It regularly contains goblet cells and varying numbers of flat and PK cells, continuing those that gradually disseminate from the line of Marx. The lid wiper forms an epithelial cushion and gradually decreases in thickness until it transforms into the epithelium of the subtarsal fold (sf).

structure of the more proximal lid wiper – to prevent destruction of the sensitive bulbar epithelia during the continuous travelling of the lid margin during the frequent physiological eye blinks.

The natural stainable line that occurs after application of vital stains was also assumed to indicate the functionality of the meibomian glands because these usually open in front (i.e. distal to it) on the free lid margin. This appears to have considerable clinical importance because meibomian oils are naturally delivered onto the tear meniscus [26] that starts around the posterior rim of the meibomian orifices and is located on the surface of the MCJ/line of Marx [17]. With increasing age, in blepharitis and in meibomian gland disease, however, the line of Marx moves in an anterior direction [64] and the orifices are also reported to move backwards (retroplacement) [28]. Both of these facts indicate a location of the orifice inside the tear meniscus, and consequently the delivery of meibomian oil into the tear meniscus rather than on top of it which conceivably results in an ineffective formation of the tear film lipid layer.

It is therefore not surprising that the location of the line of Marx was found to be strongly correlated with meibomian gland function. The observation of its location relative to the meibomian gland orifices is therefore suggested as a rapid and efficient clinical procedure at slit lamp examination for the assessment of meibomian gland function [64].

Anatomy and Pathology of the Lid Wiper

The MCJ on the posterior part of the free lid margin, behind the orifices of the meibomian glands, is followed on the crest of the inner lid border into the direction of the fornix by a stratified epithelium of a clearly conjunctival structure that represents the lid wiper (fig. 5). It contains cubical and even prismatic cells with a less dense cytoplasm and also, typically, goblet cells. This represents the start of the conjunctival mucosa, although some squamous and PK cells are still interspersed at the surface and continue those of the line of Marx, which again supports the original observations of Marx who reported that stained dots, recently verified as parakeratinized cells, fade out gradually to the conjunctival side.

The Lid Wiper Is the Device at the Inner Lid Border That Distributes the Thin Preocular Tear Film

The epithelium of this marginal conjunctival zone is stratified, has initially about 8–12 cell layers, and (further down to the proximal side in the direction of the fornix) the number of cell layers and the vertical height of the epithelium gradually decreases until it transforms into the epithelium of the subtarsal fold (fig. 5). This epithelium hence forms a thick cushion as judged by the cell shape and loose arrangement compared to the MCJ. The goblet cells, which are frequently arranged in groups, can provide a built-in lubrication system in order to decrease frictional forces between this epithelium and the bulbar surface, and further support that this is the zone which actually travels over the bulbar epithelium during every blink. Since this zone starts on the crest of the inner lid border and extends towards the tarsal side, it is also in a geometrically suitable location to be directly apposed to the globe, in contrast to the more distal zone of the line of Marx, i.e. the surface of the MCJ.

Due to its proposed function, this zone of the marginal conjunctiva is termed the 'lid wiper' [59, 60]. A thickening of the epithelium on the conjunctival side of the inner lid border was first reported by Virchow [38] and later by Ehlers [19]; the latter termed it a 'wind screen wiper' in analogy to the device of a car.

Lid Wiper Epitheliopathy Is a Sensitive Early Indicator of a Dry Eye

Even though this arrangement has immediate functional implications for the distribution of the tear film, until recently it had received limited attention. It gained interest when Korb et al. [60], who discovered pathological alterations in this area, termed

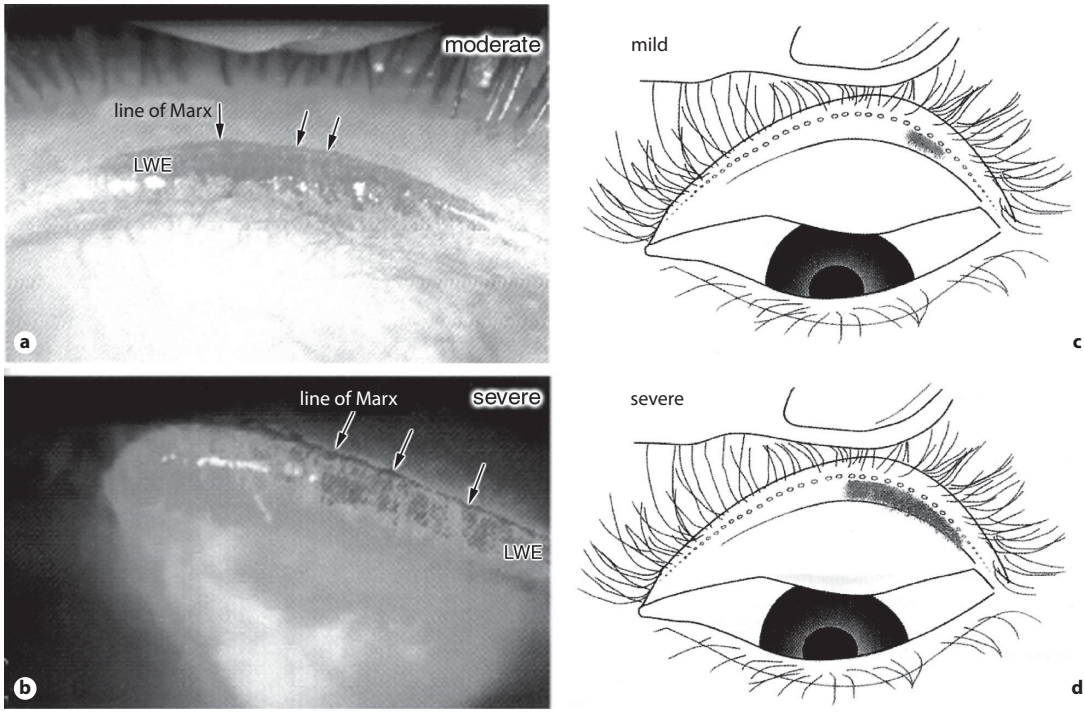

Fig. 6. Lid wiper epitheliopathy (LWE). In wetting deficiencies of contact lens wearers and in dry eye disease, the lid wiper is the first zone of the ocular surface that shows morphological alterations in the epithelium that are detectable in a vital staining, e.g. by rose bengal (**a**) or by fluorescein (**b**). LWE can be graded depending on the extension of staining (horizontal width along the lid margin and sagittal length proportional to the length of lid wiper zone) into mild (**c**), moderate (**a**) or severe stages (**b**, **d**) which reflects the severity of the wetting deficiency. Reproduced with permission from Korb et al. [59, 60].

it the 'lid wiper'; the pathological alterations in this zone were termed lid wiper epitheliopathy (LWE) [59, 60]. As shown in figure 6, LWE: (1) is an alteration in the epithelium of the portion of the marginal conjunctiva of the upper eyelid that wipes the ocular surface; (2) is diagnosed by vital staining; (3) is correlated to dry eye symptoms and disease [59, 60]; (4) occurs more frequently in patients with dry eye symptoms than in normal controls; (5) may be a sensitive early indicator of tear film instability and dry eye disease, as it may occur in either the presence or absence of conventional signs (Schirmer's test and tear film break-up time) [60].

Conclusion

In conclusion, the lid margin has an eminent and yet underestimated influence on the intact formation of the precorneal tear film, and hence also on the preservation of

ocular surface integrity and visual function. Consequently, pathological alterations in the lid margin also have a large and equally underestimated impact on the onset and course of dry eye disease, which have only recently begun to be unraveled. Similar to dry eye disease at the ocular surface proper (conjunctiva and cornea), the pathology at the lid margin also has, if undiagnosed and untreated, a tendency to self-propagate and to aggravate in vicious circles [67]. This concerns in particular a degenerative atrophic destruction of the meibomian gland structure inside the eyelids, but also contributes to corneal and conjunctival pathology. It is therefore of importance that the lid margin receives increased attention by the clinician and that a thorough investigation of the lid margin should be part of every investigation of a patient via the slit lamp, and in particular of patients of older age, before contact lens fitting and of those with incipient, or more advanced, symptoms of dry eye disease.

Acknowledgments

Supported by Deutsche Forschungsgemeinschaft (DFG KN317/11)

References

1 Knop E, Knop N, Brewitt H: Dry eye disease as a complex dysregulation of the functional anatomy of the ocular surface. New impulses to understanding dry eye disease (in German). Ophthalmologe 2003; 100:917–928.

2 Bron AJ, Tiffany JM: The contribution of meibomian disease to dry eye. Ocul Surf 2004;2:149–165.

3 Zierhut M, Dana MR, Stern ME, Sullivan DA: Immunology of the lacrimal gland and ocular tear film. Trends Immunol 2002;23:333–335.

4 King-Smith PE, Fink BA, Hill RM, Koelling KW, Tiffany JM: The thickness of the tear film. Curr Eye Res 2004;29:357–368.

5 Knop E, Knop N: New techniques in lacrimal gland research: the magic juice and how to drill for it. Opthalmic Res 2008;40:2–4.

6 Holly FJ: Tear film physiology. Am J Optom Physiol Opt 1980;57:252–257.

7 Rüfer F, Brewitt H: Das Trockene Auge. Klin Monatsbl Augenheilkd 2004;221:R51.-R70.

8 Heiligenhaus A, Koch JM, Kemper D, Kruse FE, Waubke TN: Therapy in tear film deficiencies (in German). Klin Monatsbl Augenheilkd 1994;204: 162–168.

9 Shimazaki J, Sakata M, Tsubota K: Ocular surface changes and discomfort in patients with meibomian gland dysfunction. Arch Ophthalmol 1995;113: 1266–1270.

10 Holly FJ, Lemp M: Wettability and wetting of corneal epithelium. Exp Eye Res 1971;11:239–250.

11 Argueso P, Gipson IK: Epithelial mucins of the ocular surface: structure, biosynthesis and function. Exp Eye Res 2001;73:281–289.

12 Jumblatt JE, Jumblatt MM: Detection and quantification of conjunctival mucins. Adv Exp Med Biol 1998;438:239–246.

13 Cope C, Dilly PN, Kaura R, Tiffany JM: Wettability of the corneal surface: a reappraisal. Curr Eye Res 1986;5:777–785.

14 Lemp M, Holly FJ, Shuzo I, Dohlman C: The precorneal tear film. 1. Factors in spreading and maintaining a continuous tear film over the corneal surface. Arch Ophthalmol 1970;83:89–94.

15 Holly FJ: Formation and rupture of the tear film. Exp Eye Res 1973;15:515–525.

16 Marx E: Über vitale Färbungen am Auge und an den Lidern. I. Über Anatomie, Physiologie und Pathologie des Augenlidrandes und der Tränenpunkte. Graefes Arch Clin Exp Ophthalmol 1924; 114:465–482.

17 Norn MS: Vital staining of the canaliculus lacrimalis and the palpebral border (Marx' line). Acta Ophthalmol (Copenh) 1966;44:948–959.

18 Parsons JH: Pathology of the Eye. London, Holder and Stoughton, 1904, vol 1: Histology, pp 2, 31.

19 Ehlers N: The precorneal film: biomicroscipical, histological and chemical investigations. Acta Ophthalmol Suppl 1965;81:1–134.
20 Korb DR, Baron DF, Herman JP, Finnemore VM, Exford JM, Hermosa JL, Leahy CD, Glonek T, Greiner JV: Tear film lipid layer thickness as a function of blinking. Cornea 1994;13:354–359.
21 Lemp MA: Precorneal fluid and blinking. Int Ophthalmol Clin 1981;21:55–66.
22 Tsubota K, Nakamori K: Effects of ocular surface area and blink rate on tear dynamics. Arch Ophthalmol 1995;113:155–158.
23 Mishima S, Maurice D: The oily layer of the tear film and evaporation from the corneal surface. Exp Eye Res 1961;1:39–45.
24 Gilbard JP, Farris RL: Tear osmolarity and ocular surface disease in keratoconjunctivitis sicca. Arch Ophthalmol 1979;97:1642–1646.
25 Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV: Meibomian gland dysfunction in chronic blepharitis. Cornea 1991;10:277–285.
26 Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW: Functional aspects of the tear film lipid layer. Exp Eye Res 2004;78:347–360.
27 McDonald JE: Surface phenomena of tear films. Trans Am Ophthalmol Soc 1968;66:905–939.
28 Hykin PG, Bron AJ: Age-related morphological changes in lid margin and meibomian gland anatomy. Cornea 1992;11:334–342.
29 Holbach LM: Diseases of the eyelid-conjunctival complex and corneal complications of lid disease. Curr Opin Ophthalmol 1995;6:39–43.
30 Foulks GN, Bron AJ: Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading. Ocul Surf 2003;1:107–126.
31 Di Pascuale MA, Espana EM, Liu DT, Kawakita T, Li W, Gao YY, Baradaran-Rafii A, Elizondo A, Raju VK, Tseng SC: Correlation of corneal complications with eyelid cicatricial pathologies in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis syndrome. Ophthalmology 2005;112:904–912.
32 McCulley JP, Shine WE: Eyelid disorders: the meibomian gland, blepharitis, and contact lenses. Eye Contact Lens 2003;29:S93–S95.
33 Den S, Shimizu K, Ikeda T, Tsubota K, Shimmura S, Shimazaki J: Association between meibomian gland changes and aging, sex, or tear function. Cornea 2006;25:651–655.
34 Hirotani Y, Yokoi N, Komuro A, Kinoshita S: Age-related changes in the mucocutaneous junction and the conjunctivochalasis in the lower lid margins (in Japanese). Nippon Ganka Gakkai Zasshi 2003;107:363–368.
35 Wirtschafter JD, Ketcham JM, Weinstock RJ, Tabesh T, McLoon LK: Mucocutaneous junction as the major source of replacement palpebral conjunctival epithelial cells. Invest Ophthalmol Vis Sci 1999;40:3138–3146.
36 Korb DR, Henriquez AS: Meibomian gland dysfunction and contact lens intolerance. J Am Optom Assoc 1980;51:243–251.
37a Knop E, Knop N, Brewitt H, Pleyer U, Rieck P, Seitz B, Schirra F: Meibomian Glands. III. Meibomian gland dysfunction (MGD) – plaidoyer for a discrete disease entity and as an important cause of dry eye (in German). Ophthalmologe 2009;106:966–979.
37b Knop E, Knop N, Schirra F: Meibomian glands. Part II: Physiology, characteristics, distribution and function of meibomian oil (in German). Ophthalmologe 2009;106:884–892.
38 Virchow H: Mikroskopische Anatomie der äusseren Augenhaut und des Lidapparates; in Saemisch T (ed): Graefe-Saemisch Handbuch der gesamten Augenheilkunde. Leipzig, Verlag W. Engelmann, 1910, vol 1, p 431.
39 Wolff E. Anatomy of the Eye and Orbit. London, Lewis and Co., 1954.
40 Duke-Elder S, Wybar KC: The Anatomy of the Visual System. London, Henry Kimpton, 1961, pp 577.
41 Knop N, Knop E: Meibomian glands. I. Anatomy, embryology and histology of the meibomian glands (in German). Ophthalmologe 2009;106:872–883.
42 Nicolaides N, Kaitaranta JK, Rawdah TN, Macy JI, Boswell FM, Smith RE: meibomian gland studies: comparison of steer and human lipids. Invest Ophthalmol Vis Sci 1981;20:522–536.
43 Bron AJ, Tripathi DM, Tripathi BJ: Wolff's Anatomy of the Eye and Orbit. London, Chapman & Hall Medical, 1997, p 231.
44 Kozak I, Bron AJ, Kucharova K, Kluchova D, Marsala M, Heichel CW, Tiffany JM: Morphologic and volumetric studies of the meibomian glands in elderly human eyelids. Cornea 2007;26:610–614.
45 Knop E, Knop N, Zhivov A, Kraak R, Korb DR, Greiner JV, Guthoff R: Keratinisation and mucocutaneous junction of the human lid margin in relation to the meibomian gland orifice. Invest Ophthal Vis Sci 2009;50:4833.
46 Henriquez AS, Korb DR: Meibomian glands and contact lens wear. Br J Ophthalmol 1981;65:108–111.
47 Gutgesell VJ, Stern GA, Hood CI: Histopathology of meibomian gland dysfunction. Am J Ophthalmol 1982;94:383–387.
48 Mathers WD: Ocular evaporation in meibomian gland dysfunction and dry eye. Ophthalmology 1993;100:347–351.
49 Gilbard JP, Gray KL, Rossi SR: A proposed mechanism for increased tear-film osmolarity in contact lens wearers. Am J Ophthalmol 1986;102:505–507.

50 Luo L, Li DQ, Corrales RM, Pflugfelder SC: Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. Eye Contact Lens 2005;31:186–193.

51 Gilbard JP, Rossi SR, Gray KL: A new rabbit model for keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci 1987;28:225–228.

52 McCulley JP, Sciallis GF: Meibomian keratoconjunctivitis. Am J Ophthalmol 1977;84:788–793.

53 Brewitt H, Kaercher T, Rüfer F: Dry eye and blepharitis (in German). Klin Monatsbl Augenheilkd 2008;225:R15–R36.

54 Knop E, Knop N: Eye-associated lymphoid tissue (EALT) is continuously spread throughout the ocular surface from the lacrimal gland to the lacrimal drainage system (in German). Ophthalmologe 2003; 100:929–942.

55 Knop E, Knop N: The role of eye-associated lymphoid tissue in corneal immune protection. J Anat 2005;206:271–285.

56 Knop E, Knop N: Conjunctiva immune surveillance; in Dartt DA, Edelhauser HF (eds): Encyclopedia of the Eye. Oxford, Elsevier, 2009.

57 Knop E, Knop N: Influence of the eye-associated lymphoid tissue (EALT) on inflammatory ocular surface disease. Ocul Surf 2005;3:S180–S186.

58 McDermott AM, Perez V, Huang AJ, Pflugfelder SC, Stern ME, Baudouin C, Beuerman RW, Burns AR, Calder VL, Calonge M, Chodosh J, Coster DJ, Dana R, Hazlett LD, Jones DB, Kim SK, Knop E, Li DQ, Mitchell BM, Niederkorn JY, Pearlman E, Wilhelmus KR, Kurie E: Pathways of corneal and ocular surface inflammation: a perspective from the Cullen symposium. Ocul Surf 2005;3:S131–S138.

59 Korb DR, Herman JP, Greiner JV, Scaffidi RC, Finnemore VM, Exford JM, Blackie CA, Douglass T: Lid wiper epitheliopathy and dry eye symptoms. Eye Contact Lens 2005;31:2–8.

60 Korb DR, Greiner JV, Herman JP, Hebert E, Finnemore VM, Exford JM, Glonek T, Olson MC: Lid-wiper epitheliopathy and dry-eye symptoms in contact lens wearers. CLAO J 2002;28:211–216.

61 Knop E, Knop N, Zhivov A, Kraak R, Korb D, Greiner JV, Guthoff R: 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.

62 Wolfram-Gabel R, Sick H: Microvascularization of the mucocutaneous junction of the eyelid in fetuses and neonates. Surg Radiol Anat 2002;24:97–101.

63 Wolff E: The mucocutaneous junction of the lid margin and the distribution of the tear fluid. Trans Ophthalmol Soc UK 1946;66:291–308.

64 Yamaguchi M, Kutsuna M, Uno T, Zheng X, Kodama T, Ohashi Y: Marx line: fluorescein staining line on the inner lid as indicator of meibomian gland function. Am J Ophthalmol 2006;141:669–675.

65 Doughty MJ, Naase T, Donald C, Hamilton L, Button NF: Visualisation of 'Marx's line' along the marginal eyelid conjunctiva of human subjects with lissamine green dye. Ophthalmic Physiol Opt 2004;24:1–7.

66 Shaw AJ, Collins MJ, Davis BA, Carney LG: Eyelid pressure: inferences from corneal topographic changes. Cornea 2009;28:181–188.

67 Knop E, Knop N: Meibomian glands. IV. Functional interactions in the pathogenesis of meibomian gland Dysfunction (MGD). Ophthalmologe 2009; 106:980–987.

PD Dr. Erich Knop
Research Laboratory of the Department of Ophthalmology CVK, Charité – Universitätsmedizin Berlin
Ziegelstrasse 5–9
DE–10117 Berlin (Germany)
E-Mail erich.knop@charite.de