Corneal alteration and pathogenesis in diabetes mellitus

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Abstract

The incidence of diabetes mellitus (DM) and its complications has increased considerably worldwide. Diabetic keratopathy is the major complication of the cornea characterized by delayed corneal wound healing, decreasing corneal epithelial sensitivity, and recurrent corneal ulcers. There is accumulating evidence that diabetic keratopathy is correlated with the hyperglycemic state. Different corneal components may produce different alterations under hyperglycemia. In addition, diabetic nerve alteration may become a novel biomarker of early-stage DM. Abnormalities of the corneal nerve plexus have been associated with diabetic inflammatory states. There is rapidly growing evidence based on investigations of diabetic corneal nerves through \textit{in vivo} confocal microscopy. Understanding the molecular pathogenesis caused by hyperglycemia may assist in the identification of novel biomarkers, as well as therapeutic targets for early treatment. This review mainly summarizes recent findings on corneal alteration and pathogenesis in DM.

KEYWORDS: diabetes mellitus; diabetic keratopathy; diabetic neuropathy; \textit{in vivo} confocal microscopy; advanced glycation end products

INTRODUCTION

With the rapid increase in the prevalence of diabetes mellitus (DM), diabetic ocular complications (i.e., diabetic keratopathy (DK), diabetic cataract, dry eye, and diabetic retinopathy [DR]) may lead to severe vision damage and blindness in adults worldwide \cite{1}. In recent years, DK has gained increasing attention. The main clinical manifestations include loss of corneal sensitivity, recurrent erosions of the corneal epithelium, dry eye, and neurotrophic corneal ulceration. The primary pathological manifestations include basement membrane abnormality, lacrimal functional unit (L FU) dysfunction, corneal neuropathy, and endothelial decompensation. In addition, diabetic neuropathy occurs even in the pre-diabetic states, and worsens with the development of DM. Loss of nerve innervation may result in the delay of corneal wound healing or neurotrophic ulceration. Persistent hyperglycemia triggers the expression of various cytokines, chemokines, and cell adhesion molecules (Figure 1). Over-expression of cytokines, chemokines, and other pro-inflammatory proteins and pro-apoptotic genes is a key contributor to developing DK \cite{2}. This review summarizes the current findings and knowledge regarding the corneal complications of DM (i.e., the morphology, pathophysiology, and cellular mechanism).
Figure 1 Schematic showing the pathogenesis of diabetic keratopathy. Hyperglycemia has distinct effects on different parts of the cornea, including advanced glycation end products, oxidative stress, diabetic neuropathy, inflammatory reaction, and immunocyte activation. These effects eventually lead to defective wound healing in the corneal epithelium, abnormalities of sub-basal and stromal nerves, and corneal stromal and endothelial dysfunction.

Abbreviations: NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells transcription factor; MMP, matrix metalloproteinase

DIABETIC CORNEAL NEUROPATHY
Diabetic corneal neuropathy is a potential visual impairment condition caused by damage to the trigeminal nerve under chronic hyperglycemia, and results in reduction or loss of corneal innervation. Diabetic corneal neuropathy is characterized by photophobia, ocular irritation, or pain. The majority of corneal symptoms are the result of damage to the small Aδ and C nerve fibers of the cornea [3]. The loss of corneal sensory innervation causes corneal epithelial breakdown, delayed wound healing, and subsequently progresses to corneal ulceration, melting, and perforation. However, those symptoms may not correlate with the severity of corneal neuropathy. A number of patients with diabetic corneal neuropathy often present without symptoms; this may be due to the decreased innervation of the cornea (Figure 2).
Figure 2 Transected view of the entire corneal nerve alterations. The epithelial innervation (yellow arrow) is supplied by two nerve networks, namely the limbal superficial nerve network and sub-conjunctiva nerve network (black arrows). Corneal stromal nerves originate from the sclera and branch into the epithelium (red arrow). Representative IVCM images are shown for A: corneal epithelial nerves; B: sub-basal nerves; C: corneal stromal nerves in patients with DM.

Abbreviations: IVCM, in vivo confocal microscopy; DM, diabetes mellitus

In-vivo confocal microscopy (IVCM) has revealed several significant findings in the epithelial nerve. The long nerve fiber bundles in the corneal sub-basal nerve plexus had significantly decreased in patients with DM and corneal sensitivity was negatively correlated with long nerve fiber length [4]. In addition, the corneal sub-basal nerves in diabetic patients showed pronounced thickening than those observed in control subjects [5]. Some studies showed that patients with DM had significantly decreased corneal sub-basal nerve fiber length and branch density [6]. Changes in nerve fibers correlated with the development of DR. Patients with proliferative DR showed significantly thicker, tortuous, and lower density nerve measurements than those without proliferative DR [7]. Kallinikos et al. reported that reduction of corneal sub-basal nerve tortuosity may predict the severity of somatic neuropathy in patients with DM [8]. Recent IVCM studies conducted by Deak et al. showed a significant reduction in corneal nerve fiber density in patients with DR [9].

Most studies are focused on the diabetic changes in corneal sub-basal nerves, with limited research focusing on the corneal stromal nerves. Patel et al. found that the mean stromal nerve thickness and the proportion of curved stromal nerves were significantly higher in patients with DM. Moreover, they confirmed that patients with proliferative retinopathy had thicker stromal nerves than patients with background retinopathy [10]. Nevertheless, the stromal nerve density can not calculated, because it has course obliquely in the corneal stroma and cannot be...
imaged through confocal microscopy. According to corneal immunofluorescence staining, stromal nerve fiber loops are one of the striking changes observed in corneal stromal nerves. Under hyperglycemia, the basement membrane may resist the stromal nerves entering the epithelium, leading to the occurrence of nerve fiber loops. Moreover, the alteration of the extracellular matrix in the diabetic corneal stroma may also result in the formation of nerve fiber loops [11].

Pathogenesis of diabetic corneal neuropathy
Multiple mechanisms, such as hyperglycemia-mediated inflammation, oxidative stress, and signal pathways, may play an important role in diabetic neuropathy. Advanced glycation end-products (AGEs) are reactive metabolites produced by the non-enzymatic glycosylation of sugar molecules, which are caused by hyperglycemia in DM [12]. Recent studies have demonstrated that the accumulation of AGEs may result in retinal diabetic neuropathy [13, 14]. AGEs and their receptors (RAGE) cause the formation of oxygen radicals and the release of pro-inflammatory cytokines [15]. Some studies have confirmed that poly (ADP-ribose) polymerase plays an important role in corneal neuropathy, which may trigger the mechanism of oxidative stress both in the diabetic rat and mouse model [16]. Chronic hyperglycemia can lead to the generation of reactive oxygen species (ROS), which results in mitochondrial damage [17]. Yagihashi et al. showed that mitochondrial damage in nerve fibers may lead to demyelination and conduction dysfunction [18]. In that study, immune mechanisms were suggested to play a prominent role in the progression of diabetic corneal neuropathy. The presence of immunocytes in the cornea can be observed via confocal microscopy. Studies have reported that the proportion of dendritic cells and Langerhans cells (LCs) was significantly increased in diabetic patients compared with control subjects. Furthermore, LC density was significantly increased in diabetic patients, and was significantly correlated with the severity of neuropathy [19]. The corneal nerve plexus plays an essential role in maintaining epithelial homeostasis and promoting wound healing through secretion of neuropeptides, growth factors, and cytokines. Chronic hyperglycemia may impair corneal nerve secretion of neuropeptides [20]. Notably, the ciliary neurotrophic factor (CNTF) may promote epithelial wound healing and nerve regeneration [21]. Interestingly, the proportion of dendritic cells is decreased in the diabetic cornea, which is the primary source of CNTF. As a systemic metabolic disease, DM may disrupt both the immune and neuroendocrine systems [22]. Recently, in the diabetic mice model, treatment with pigment epithelium-derived factor, docosahexaenoic acid, and ω-3 fatty acid was shown to promote epithelial wound healing and nerve regeneration [23].

CORNEAL EPITHELIUM ABNORMALITY
The corneal epithelium consists of cell layers and the basement membrane. The epithelium is an important barrier to the cornea, which can resist attacks from pathogens. However, diabetic patients are vulnerable to corneal epithelium dysfunctions, such as superficial puncture keratitis and epithelium erosion. Corneal epithelium abnormality is one of the most common and long-term complications of DM.

Corneal epithelial basal cells (CEBCs)
CEBCs are derived from the corneal stem cells at the limbus, and play an important role in forming the basement membrane. Under physiological conditions, CEBCs are presented as alternately dark and bright dense cluster
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polygonal cells, with a high reflective cell border and low reflective cytoplasm using IVCM. In DM, abnormal hyper-signals were detected at the interface between the epithelium and the anterior stroma. These abnormalities may reflect the accumulation of AGEs [24]. Qu et al. showed an increase in LCs and decrease in CEBCs in patients with type 2 DM [25].

There is a significant reduction of central corneal thickness (CCT) in severely diabetic rat models, indicating disruption of the normal homeostasis of the corneal epithelium [4]. However, this reduction was observed only in severe diabetic neuropathy [26]. Chang et al. revealed that changes in corneal epithelial parameters, including reduction of CEBC density, increased variability in cell size, and wider intracellular space were observed in patients with DM. In addition, they reported that reduction in the CEBC density was significantly correlated with nerve branch density and nerve fiber density [27]. Other studies using high-frequency ultrasound revealed changes occurring in the corneal epithelium during hyperglycemia, which can be useful for the early detection of damage to the corneal epithelium [28].

Alteration of innervation may be a major cause of CEBC decrease in patients with DM. As mentioned earlier in this review, corneal nerve fibers release multiple neuropeptides to maintain corneal epithelial homeostasis. Accumulating evidence suggests that neurotrophic factors, as pivotal regulatory molecules, play an important role in DK [29]. Nerve growth factor and CNTF may also reverse corneal pathologic alteration and accelerate corneal epithelial wound healing by attenuating apoptosis and inflammation in the diabetic cornea [30, 31]. Similarly, fibronectin-derived peptide (PHSRN) eye drop significantly facilitated the healing of corneal epithelial wounds in diabetic rats [32]. Other studies have shown that nerve growth factor promoted human corneal epithelial wound healing by stimulating phosphorylation of the Akt pathway. This finding suggests that the PI3K-Akt pathway is involved in corneal epithelial wound healing [33]. Akhtar et al. reported that Substance P – a neuropeptide mainly secreted by sensory nerve fibers – promoted diabetic corneal epithelial wound healing. This effect was exerted through the Substance P-neurokinin 1 receptor signal pathway by recovering the activation of Akt, epidermal growth factor receptor (EGFR), and silent mating type information regulation 2 homolog 1 (SIRT1), ameliorating the mitochondrial function, and increasing the ROS scavenging capacity [34]. In addition, a number of miRNAs showed a close relationship with the corneal wound healing process. For example, miR-204-5p mediated regulation of SIRT1 contributes to the delay of epithelial cell cycle traversal in DK [35]. Furthermore, overexpression of SIRT1 strongly promoted wound healing in Ins2 mice [36]. The miR-34c was found to repair diabetic corneal nerve endings [37]. Other animal studies revealed the detrimental effects of soluble epoxide hydrolase on the corneal epithelium, which may contribute to reduced corneal epithelial wound healing. Thus, pharmacologically targeting soluble epoxide hydrolase may be a potential therapy for DK [38].

**Corneal epithelial basement membrane**

Delayed epithelial wound healing and abnormal epithelial adhesion is attributed to alteration in the basement membrane by DM. Using transmission electron microscopy, Taylor et al. reported that the thickness of the corneal basement membrane was greater in diabetic patients [39]. However, Morishige et al. reported that the Z-scan may provide a light-scattering index (LSI), a quantitative parameter of the light reflectivity of tissues at the basement membrane. The LSI was significantly increased in diabetic patients; this parameter is relatively reproducible and correlated with the severity of diabetes. These results imply that measurement of the LSI may be a marker for the early detection of DM [40].
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Multiple mechanisms have been proposed to play a role in pathological alteration of the basement membrane in DM. Ljubimov et al. reported a reduction in the CEBC layer occupied by hemidesmosomes in the diabetic cornea [41]. Diminished expression of the components of the basement membrane (e.g., nidogen-1/entactin, laminins, and binding partner integrin α3β1) was observed in patients with DM [42]. These alterations may be attributable to abnormal basement membrane metabolism. Accumulating evidence has suggested that a number of matrix metalloproteinases (MMPs) play a pivotal role in corneal wound healing. In particular, the expression of MMP-10/stromelysin-2 is attributed to the proteolytic degradation of basement membrane components in DM [43, 44]. In addition, the expression of MMP-9 was enhanced in diabetic corneal epithelium wound healing models. It may also damage the type IV collagen and deteriorate its normal interaction with other proteins involved in cell attachment [45]. It is widely established that AGEs play an important role in diabetic epitheliopathy [46]. Ishida et al. were the first to detect elevated corneal autofluorescence in diabetic patients compared with healthy individuals [47]. The corneal autofluorescence was correlated with deposition of AGEs in the diabetic cornea. Accumulation of AGEs has been detected at the site of the corneal epithelium and the epithelial basement membrane in diabetic rats [48]. The AGEs are particularly distributed on the basement membrane laminin [49]. Furthermore, Sato et al. reported the corneal AGE autofluorescence corresponding to the severity of DR [35]. AGEs may induce apoptosis in human corneal epithelial cells through activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways and generation of ROS [50].

CORNEAL STROMA ABNORMALITY

DM may also cause alterations in the corneal stroma leading to corneal stroma disorder. DM may induce both structural and functional alterations in the corneal stroma, and these processes result in loss of corneal transparency and threaten the vision of the patients [51]. Studies showed that CCT increases in parallel with the severity of diabetic peripheral neuropathy due to an increase in stromal thickness, suggesting that the increase in CCT is an important clinical implication [52]. Using transmission electron microscopy, it was shown that the organization of the anterior stroma matrix was different in the diabetic cornea. In the center of diabetic corneas, although the structure of collagen lamellae was similar to that observed in the normal cornea, the basal epithelial lamina appeared thicker than that reported in the normal cornea. In the peripheral cornea, an abnormally tile-shaped collagen fibril appeared in the anterior epithelial basal lamina [24]. According to a long-term streptozotocin-induced diabetic monkey model, stroma changes affect the transparency of the cornea. Abnormal collagen fibril bundles with different thickness and variable spacing can be found in the corneal stroma, and AGE immune reactivity may also be observed in the corneal stroma. Importantly, AGE immune reactivity was detected throughout the corneal stroma, which may lead to collagen crosslinking and contribute to the stromal abnormality [53]. Additionally, keratocyte cell density in the posterior stroma was higher in young patients with type 1 DM, and the accumulation of ROS and several growth factors induces the proliferation and activation of keratocytes [54, 55]. However, Kalteniece et al. demonstrated that a reduction in keratocyte cell density, which was associated with damage to the corneal sub-basal nerve plexus [56]. Furthermore, treatment with an EGFR inhibitor may reverse corneal stroma abnormality by modulating the level of AGEs. In particular, it reverses the abnormality of the collagen fibrils and proteoglycans. This study suggests that the EGFR signal pathway contributes to the development of diabetes-induced corneal stroma remodeling [34]. The matrix metalloproteins (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) play a crucial role in the synthesis and degradation of the extracellular
matrix. DM destroys the delicate balance between MMPs and TIMPs; in DR corneas, MMP-3 and MMP-10 were upregulated, whereas TIMPs-4 was downregulated [57, 58] (Figure 3).

Figure 3 Schematic showing changes in the components of the stroma in diabetes mellitus. Abnormally aggregated collagen fibrils scattered in the corneal stroma. The accumulation of AGEs in the stroma causes abnormal cross-linking between the collagen fibers. Moreover, significantly higher keratocyte cell density was found in diabetes. The abnormal accumulation of AGEs, ROS, MMP, and some growth factors may result in the activation or proliferation of keratocytes.

Abbreviations: AGEs, advanced glycation end products; ROS, reactive oxygen species; MMP, matrix metalloproteinase

Schwarz et al. observed an increased biophysical adhesion strength of the endothelium-Descemet membrane complex in the diabetic cornea. The increased adhesive interface between the Descemet membrane and the underlying stroma may be associated with chronic hyperglycemia, and this study provided a novel direction for further investigations [59]. Moreover, using complete metabolism and liposome analysis, Priyadarsini et al. identified potential novel biomarkers in the corneal stroma (e.g., aminoacidic acid, pipecolic acid, and dihydroorotate). These potential biomarkers are significantly up-regulated in diabetic corneas, indicating that they may be involved in the corneal stroma response to a chronic hyperglycemic insult. Such biomarkers may be indicative of diabetes-induced stromal damage, allowing the prompt prediction of DM complications [60].

**CORNEAL ENDOTHELIUM ABNORMALITY**

DM also exerts a profound effect on the corneal endothelium. Changes in endothelial morphological parameters, such as endothelial cell density (ECD), hexagonality, and CCT have been reported in DM [48]. Liaboe et al. showed that patients with DM had a markedly lower mean ECD [61]. The coefficient of variation of the cell area was higher in the diabetic cornea. Although the lower percentage of hexagonal cells was not statistically significant, it may reflect the abnormality of the corneal endothelial recovery process [62-65]. Functional disturbances may lead to increased endothelial permeability and endothelial autofluorescence, which subsequently result in the impairment of cornea dehydration and lead to corneal swelling with increased CCT [66]. Moreover, the lower ECD was associated with a higher level of hemoglobin A1c [67], and ECD was significantly reduced in
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patients with DR [68]. Some studies showed a significant increase in CCT [62]; however, other studies did not [63, 64]. Moreover, the number of endothelial cells with polymegathism and pleomorphism was significantly higher among the eyes of diabetic patients [68]. Of note, polymegathism and pleomorphism are the independent quantitative parameters of endothelial cells under DM [69]. Beataet al. reported that ECD is decreased and CCT is increased in children and adolescents with DM, suggesting the duration of diabetes affects ECD and CCT [66]. The controversial results are likely due to differences in the duration of DM, age, and measurement methods.

The endothelium contains many immune and inflammatory factors, such as vascular endothelial growth factor, tumor necrosis factor-α, interleukin (IL), and MMP. These factors may also insult the corneal endothelium and lead to alterations in endothelial function and morphology, as well as changes at the molecular level. Of note, the function of the corneal endothelial barrier is impaired, and recovery of endothelial cells becomes slower and weaker [70, 71].

Hyperglycemia causes non-enzymatic glycosylation of proteins and abnormal accumulation of sorbitol. Accumulation of AGEs may cause a decrease in corneal endothelial cells with aging and disturbing endothelial cell metabolism [49, 72]. Other probable mechanisms of changes in the corneal endothelium include mitochondrial dysfunction, which results in the accumulation of ROS and mitochondrial injury [73, 74]. In addition, glycation of membrane adenosine triphosphatase may play a role in the disorders of oxygen metabolism [66].

The Descemet membrane is the basement membrane of the corneal endothelium, which plays a vital role in withstanding greater shear stresses from biological and mechanical pathogenic factors [75]. Using confocal microscopy, hyper-reflective and rod-shaped structures were detected in the peripheral Descemet membrane of the diabetic cornea; these structures have been identified as long-spacing collagen fibril. The abnormal secretion of long-spacing collagen fibril may also occur due to the deposition of AGEs [24]. However, confocal microscopy provided poorly contrasted images of these abnormalities and lacked specificity. At present, second harmonic generation (SHG) microscopy is a new imaging technique for the detection of collagen-rich tissues. SHG can overcome these disadvantages and SHG microscopy can show the deposition in the Descemet membrane [76]. Using electron microscopy and laser confocal microscopy, Akimoto et al. have also reported that the abnormal long-spacing collagen fibril bundles were frequently observed in the Descemet membrane of the diabetic rat model. Interestingly, several diabetic alterations in collagen-rich tissue (e.g., age-like changes) and the diabetic rat model showed an age-dependent increase in the density of long-spacing collagen. Moreover, the formation of long-spacing collagen may be suppressed by antidiabetic agents [77]. Thus, long-spacing collagen may be a new biomarker for measuring the effect of antidiabetic agents (Figure 4).
Figure 4 Schematic showing the pathogenesis of corneal endothelium and Descemet membrane in diabetes mellitus showing morphological and functional changes, including accumulation of AGEs, glycation of membrane ATPase, overproduction of ROS, and accumulation of sorbitol pathway products. A: Functional disturbances may lead to increased endothelial permeability, damage to cellular components, and stromal edema. B: Representative confocal microscopy image of low ECD and endothelial cells with polymegathism and pleomorphism in diabetic patients.

Abbreviations: ECD, endothelial cell density

CORNEAL LIMBAL STEM CELL ABNORMALITY

Corneal limbus is a narrow band of tissue that encircles the cornea. Under physiological conditions, corneal limbal epithelial stem cells (LESCs) give rise to progeny (transit amplifying cells), which differentiate into mature corneal epithelium during their radial migration towards the central cornea. The renewal of the corneal epithelium by LESCs may explain the clinically observed delays in diabetic wound healing.

Using the IVCM, the limbus of the cornea showed loss of the regular limbal epithelium, presence of intraepithelial cystic changes, and a mosaic pattern of cells of differing morphology. In addition, the more profound stroma of limbal palisades of Vogt showed irregularly arranged fibrous strands with scattered islands of basal limbal epithelial cells [78].

In DM, a reduction in the expression of LESC markers and slower wound healing in cultured diabetic LESCs have been observed, which may account for diabetic LESC dysfunction [79]. Overexpression of c-met, MMP-10, and cathepsin F gene in LESCs was shown to normalize wound healing, and increase diabetes-altered staining for putative markers of LESCs (i.e., ΔNp63α, ABCG2, keratins 15 and 17, and laminin γ3 chain) [80, 81]. Furthermore, treatment with insulin-like growth factor-1 exerts a preventive effect, which can protect against corneal damage in diabetes [82]. A study performed by Kulkami et al. identified miR-10b as one of the most abundant miRNAs in corneal limbal, which may control corneal epithelial homeostasis and stem cell functions.
TEAR FILM ABNORMALITY

The tear film is the primary interface between the ocular surface and the external environment, and plays pivotal roles in maintaining the morphological and functional integrity of the cornea. In addition, the lacrimal glands, lacrimal drainage system, and interconnecting innervation work together as the LFU.

DM is also associated with film abnormality and LFU insufficiency, which can deteriorate corneal components. Owing to the lack of tearing or abnormal tear dynamics, the diabetic patients are more prone to suffer from dry eye syndrome (DES) [84]. DES is very common in diabetic patients, especially in those with DR. DES is a potential visual impairment syndrome and can lead to superficial punctuate keratopathy, secondary bacterial infection, and even perforation. The decrease in lacrimal gland secretory function is the cardinal problem in DES [85].

Many mechanisms contribute to the onset and progression of the tear film abnormality in diabetic patients. Notably, chronic inflammation and peripheral neuropathy in diabetes play a vital role in DES. Chronic hyperglycemia is the main causative mechanism underlying the pathogenesis of tear film abnormality [86]. In addition, there was a significant elevation of inflammation or pre-inflammation markers in the tears and conjunctiva of diabetic patients, such as IL-1α, IL-1β, IL-6, and tumor necrosis factor-α [87]. As previously stated, MMP is an important mediator of inflammation in diabetes and contributes to tissue impairment. It was reported that elevated MMP-9 was significantly correlated with ocular surface inflammation [88]. In addition, the level of Substance P was significantly lower in the tears of diabetic patients. [89]. A recent study showed that the increasing level of metallic elements in the tears of patients with DM may be an indicator of ocular damage [90].

In addition, oxidative stress in the diabetic rat model leads to pathological alteration of the lacrimal gland acinar cells. An experimental study demonstrated that overexpression of SIRT1 in the diabetic dry eye model was evident for the DES oxidative stress mechanism [91]. Furthermore, chronic hyperglycemia may eventually lead to tearing film hyperosmolarity. Exposure of corneal structures, including the corneal epithelium and corneal limbus, to tear film hyperosmolarity leads to a cascade of inflammatory reactions [92]. Additionally, the elevated volume of the tear film of patients with DM may be attributed to tear film instability and rapid evaporation of tear, which lead to tear secretion in a reflex action. Usually, secretion of tears in patients with DM is reduced [93, 94]. Furthermore, tear film instability and tear film hyperosmolarity play significant roles in the vicious cycle of the diabetic tear film abnormality (Figure 5).
Figure 5 Schematic showing changes in components of the tear film in diabetes mellitus. As a result, the levels of tear proteins and neuropeptides (secreted by trigeminal sensory nerves on the cornea) in diabetics are often significantly lower than those reported in healthy individuals, whereas the levels of some inflammation factors are higher. The osmolarity of diabetic tears also increases. Regarding the tear fluid itself, the higher glucose concentration in tears alters the capability for corneal epithelial wound healing.

Lacrimal nerve fibers play a pivotal role in the maintenance of tear production and integrity of the LFU. Diabetic neuropathy may compromise the innervation of the LFU. Moreover, impairment of the LFU sensory nerve may also inhibit tear secretion associated with the reduced threshold of corneal sensitivity [95]. Interestingly, using confocal microscopy, the number of corneal sub-basal nerves was significantly correlated with Schirmer test values [96]. Such a phenomenon may indirectly reveal alterations in the corneal innervations in DES patients with diabetes. Furthermore, exposure to high levels of glucose is deleterious for human meibomian gland epithelial cells, and may help explain the importance of hyperglycemia for LFU in patients with DM (Figure 6) [97].
Figure 6 Schematic depiction of the key components of the LFU. The LFU consists of the lacrimal gland, conjunctival goblet cells, meibomian gland, as well as sensory and motor nerves. DM exerts distinct effects on different parts of the LFU, resulting in LFU dysfunction. Diabetic neuropathy may damage both corneal afferent fibers and efferent nerves. The concomitant inflammatory response with DM may also affect the meibomian gland, lacrimal gland, and conjunctival goblet cells.

Abbreviations: LFU, lacrimal functional unit; DM, diabetes mellitus

FUTURE PERSPECTIVES

The prevalence of DM has increased in recent years as a metabolic disease that can influence all structures of the eye. The clinical manifestations of DK are variable and mainly concern epithelial lesions, neuropathy, and tear film abnormalities. The molecular mechanisms responsible for DK remain to be elucidated. As summarized in this review, numerous underlying pathophysiologic mechanisms participated in changes to the cornea. Several novel and accurate methods have been developed to investigate alterations in the diabetic cornea. There is increasing research regarding the use of IVCM in corneal morphological alterations in diabetic patients. Therefore, such parameters may be noninvasive biomarkers for diabetic peripheral neuropathy. An improved understanding of both alterations and pathogenesis of the DK would be important for the optimal management of DM.
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