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Diabetic complications in the cornea

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Abstract

Diabetic corneal alterations, such as delayed epithelial wound healing, edema, recurrent erosions, neuropathy/loss of sensitivity, and tear film changes are frequent but underdiagnosed complications of both type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus. The disease affects corneal epithelium, corneal nerves, tear film, and to a lesser extent, endothelium, and also conjunctiva. These abnormalities may appear or become exacerbated following trauma, as well as various surgeries including retinal, cataract or refractive. The focus of the review is on mechanisms of diabetic corneal abnormalities, available animal, tissue and organ culture models, and emerging treatments. Changes of basement membrane structure and wound healing rates, the role of various proteinases, advanced glycation end products (AGEs), abnormal growth and motility factors (including opioid, epidermal, and hepatocyte growth factors) are analyzed. Experimental therapeutics under development, including topical naltrexone, insulin, inhibitors of aldose reductase and AGEs, as well as emerging gene and cell therapies are discussed in detail.

Keywords

diabetic cornea; keratopathy neuropathy; gene therapy; naltrexone; insulin; corneal epithelium; limbal stem cell; dry eye; growth factors; proteinases

1. Introduction

Diabetes mellitus is currently the leading cause of legal blindness in the working age adults worldwide. Diabetic retinopathy (DR) is a major and the most severe ocular complication of diabetes mellitus (Aiello et al., 1998). DR is the leading cause of new blindness in persons 25 to 74 years of age in the United States, accounting for about 8,000 new blindness cases each year (Aiello et al., 1998; Negi and Vernon, 2003). Patients with insulin-dependent diabetes (type 1, IDDM) will develop DR in more than 90% of cases, and proliferative DR

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(PDR), in up to 50% of cases after 20 years of disease; patients with non-insulin-dependent diabetes (type 2, NIDDM) also frequently develop DR (Aiello et al., 1998; Negi and Vernon, 2003; Klein, 2008). Overall, 43% of type-I and 60% of type-II diabetics lose vision within 5 years of the onset of PDR.

Diabetic eye disease is typically considered as a retinal microvascular disorder and is thus generally called diabetic retinopathy. Nonproliferative DR (NPDR) is associated with retinal ischemia, pericyte loss, capillary closure, retinal hemorrhages, microaneurysms, and macular edema. PDR is associated with intravitreal hemorrhages, optic disc or peripheral neovascularization, preretinal fibrovascular membranes and vitreoretinal traction with retinal detachments (Yam and Kwok, 2007). DR is mainly treated with tight blood glucose control that is more effective in IDDM and laser surgery [panretinal laser photocoagulation (PRP)], (Henricsson et al., 1997; Aiello et al., 1998; Davis et al., 1998; Negi and Vernon, 2003; Yam and Kwok, 2007). Eyes with nonclearing vitreous hemorrhage are treated with vitrectomy followed by PRP (Aiello et al., 1998). DR and/or PDR progression may be slowed down by treatment with steroids, somatostatin analogs, and antagonists of vascular endothelial growth factor (Jonas, 2007; Ljubimov et al., 2008; Dhoot and Avery, 2016). Results from animal models suggest that combination therapy may be the most promising future approach to PDR treatment (Jo et al., 2006; Kramerov et al., 2006; Dorrell et al., 2007).

Given its major impact on vision, retinal disease in diabetes remains the primary concern of physicians. At the same time, other parts of the eye (e.g., iris, lens, optic nerve) also suffer from diabetic complications, although they are encountered in less than 30% of diabetic patients (Ducrey, 1996; Nakamura et al., 2005; Blum et al., 2007; Helbig, 2007; Murtha and Cavallerano, 2007). Corneal problems seem to be more frequent in diabetics affecting up to 70% of examined diabetic patients (Schultz et al., 1981; Didenko et al., 1999; Abdelkader et al., 2011; Vieira-Potter et al., 2016). However, these changes are rarely diagnosed (Herse, 1988; Wylegała et al., 2006). Major attention is paid to retina despite the proposed inclusion of ocular surface assessment during eye examination in diabetic patients (Ben Osman et al., 1995; Aiello et al., 1998; DeMill et al., 2016). Currently, 18% of corneas transplanted in the United States come from diabetic donors (Lass et al., 2015), which may not be beneficial for recipients due to lasting epigenetic changes in these corneas. The present review will attempt at bridging this gap and presenting a comprehensive analysis of corneal diabetic complications. Earlier reviews on diabetic corneal disease focused primarily on clinical problems (Sánchez-Thorin, 1998; Cisarik-Fredenburg, 2001; Kaji, 2005). More recently, appropriate attention was given to mechanisms of disease and available therapies (Bikbova et al., 2012; Calvo-Maroto et al., 2014; Misra et al., 2016; Vieira-Potter et al., 2016; Shih et al., 2017). This review provides recent updates in this rapidly developing field and analyzes in more detail the latest therapeutic candidates. Emphasis is also given to molecular events and mechanisms underlying this disease, which need to be understood in more detail to develop new effective treatments (Karamichos, 2015).

2. General manifestations of diabetes in the cornea

Clinically observed corneal diabetic alterations include increased corneal thickness, epithelial defects, epithelial fragility and recurrent erosions, ulcers, edema, superficial

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punctate keratitis, delayed and incomplete wound repair, endothelial changes, and neuropathy exemplified by reduced corneal sensitivity (Herse, 1988; Cavallerano, 1992; Saini and Khandalavla, 1995; Sánchez-Thorin, 1998; Malik et al., 2003; Negi and Vernon, 2003; Saito et al., 2003; Gekka et al., 2004; Wylegała et al., 2006; Su et al., 2008; Módis et al., 2010; Bikbova et al., 2012; Vieira-Potter et al., 2016; Shih et al., 2017). Diabetic corneal neuropathy, corneal autofluorescence [possibly due to the accumulation of advanced glycation end products (AGEs)], and epithelial fragility are all augmented in patients with DR (Chang et al., 1995; Janiec et al., 1995; Saini and Mittal, 1996a; Van Schaik et al., 1999; Bikbova et al., 2012; DeMill et al., 2016; Calvo-Maroto et al., 2016). Diabetics often have low tear secretion and dry eye syndrome (Inoue et al., 2001; Yoon et al., 2004; Cousen et al., 2007; Manaviat et al., 2008; Beckman, 2014). Similar to diabetic retina, diabetic corneas are also affected by dyslipidemia with increased content of sphingosines and ceramides (Priyadarsini et al., 2015; 2016a). Major changes in human and animal diabetic corneas are listed in Table 1.

2.1. Epithelial abnormalities

Diabetic epithelial problems are often summarily referred to as diabetic keratopathy emphasizing the major impact on corneal epithelium. Signs of diabetic keratopathy include epithelial fragility, defects and recurrent erosions, non-healing ulcers, corneal edema due to altered epithelial barrier function, superficial punctate keratitis, abnormally slow and often incomplete wound healing, lower cell density especially in the basal layer, and increased susceptibility to injury (Ben Osman et al., 1995; Saini and Khandalavla, 1995; Ohashi, 1997; Sánchez-Thorin, 1997; Inoue et al., 2001; Cavallerano, 2002; Gekka et al., 2004; Quadrado et al., 2006; Wylegała et al., 2006; Szalai et al., 2016; Vieira-Potter et al., 2016). The data on the prevalence of diabetic keratopathy depending on the type of diabetes remain inconsistent and need to be revisited (Schultz et al., 1981; Didenko et al., 1999). Women with poor glycemic control have a higher diabetic keratopathy incidence, especially with IDDM (up to 90%) (Schultz et al., 1981).

It has been long suggested that diabetic keratopathy is a sign of peripheral neuropathy (Schultz et al., 1983). This idea was substantiated by later findings of frequent association between corneal epithelial changes and manifestations of diabetic neuropathy (Didenko et al., 1999; Chang et al., 2006; Mocan et al., 2006; Bikbova et al., 2016). At the same time, neurotrophic keratopathy is reportedly a rare event in diabetics (Lockwood et al., 2006). Moreover, direct structural and functional changes in the corneal epithelium were observed in diabetic patients and animals, which may not be related to neuropathy unless in severe disease (Rosenberg et al., 2000; Saghizadeh et al., 2001a; 2005; 2011; Quadrado et al., 2006; Chikama et al., 2007). Further, high glucose treatment mimicking the diabetic hyperglycemia has a fast and direct impact on the normal corneal epithelial cells and the epithelium of organ-cultured corneas decreasing cell adhesion and slowing down wound healing (Fujita et al., 2003; Tomomatsu et al., 2009; Xu et al., 2009; Yin and Yu, 2010). Additional studies should elucidate the degree and molecular mechanisms of the relationship between diabetic keratopathy and neuropathy.

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2.2. Corneal nerve changes

Decreased corneal sensitivity in diabetic patients was already recognized in the 1970's (Nielsen, 1978). Since then, the first findings were corroborated many times. It is now well established that corneal sensitivity decrease is very common in diabetic patients and animals. Its degree correlates with the disease severity (Rosenberg et al., 2000; Saito et al., 2003; Cousen et al., 2007; Tavakoli et al., 2007; De Cillà et al., 2009; Zhivov et al., 2013), although the age factor should also be taken into account (Murphy et al., 2004). This symptom is related to abnormalities of corneal nerve structure and function in diabetes. Detailed examination including the use of *in vivo* confocal microscopy has revealed abnormalities in nerve fiber density, length and branch density, as well as increased nerve tortuosity and thickness in human diabetic corneas (Rosenberg et al., 2000; Malik et al., 2003; Kallinikos et al., 2004; Mocan et al., 2006; De Cillà et al., 2009; Szalai et al., 2016). These alterations may be worsened after laser photocoagulation in PDR (De Cillà et al., 2009). In both diabetic patients and animal models, the most severe reduction in nerve fiber and branch density occur in the sub-basal nerve plexus close to the corneal epithelium, possibly explaining the correlation between diabetic keratopathy and corneal neuropathy (De Cillà et al., 2009; He and Bazan, 2012; Wang et al., 2012; Zhivov et al., 2013; Cai et al., 2014; Davidson et al., 2014; Stem et al., 2014). Upon corneal epithelial wounding, severed subbasal nerves regenerate significantly slower in diabetic than in non-diabetic animals (Wang et al., 2012; Gao et al., 2016). The sub-basal nerve alterations in diabetic mice are accompanied by abnormalities of dendritic cells that may serve neurotrophic functions (Leppin et al., 2014; Gao et al., 2016). Several studies have documented corneal neuropathy early in diabetes, before the development of DR (Zhivov et al., 2013; Papanas and Ziegler, 2013; Petropoulos et al., 2015; Szalai et al., 2016). Moreover, in rat models, corneal nerve damage occurred not only in animals with NIDDM but also in those that were obese but non-diabetic, suggesting that corneal neuropathy may develop even before the onset of hyperglycemia (Davidson et al., 2014) and calling for therapeutic interventions in pre-diabetes. Along with keratopathy, diabetic neuropathy is considered a hallmark of diabetes in the cornea and an important factor for non-invasive diagnostics (Saini and Mittal, 1996a; Saito et al., 2003; Tavakoli et al., 2007; Cruzat et al., 2017).

2.3. Stromal changes

To date, there are only a few studies of the corneal stroma in diabetics. In patients with NIDDM corneal stroma acquires abnormal collagen fibril bundles of variable thickness (Rehany et al., 2000b). In monkeys with induced IDDM similar stromal bundles were found (Zou et al., 2012). Importantly, the diabetic corneal stroma accumulates AGEs, which may lead to collagen crosslinking and could contribute to increased central corneal thickness (Sady et al., 1995). This accumulation may also underlie changes in type IV collagen expression, impaired cell adhesion, and increased keratocyte apoptosis observed in mice with NIDDM and in rats with IDDM (Watanabe et al., 2002; Kim et al., 2011). In diabetic rats, stromal edema was also reported (Gül et al., 2008). The thickness and tortuosity of stromal nerves appears to be increased in diabetic patients (Mocan et al., 2006). Two matrix metalloproteinases (MMP), MMP-3 and MMP-10 were found to be upregulated in the stroma of human diabetic but not keratoconic corneas (Saghizadeh et al., 2001a), which may contribute to altered stromal maintenance and remodeling.

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2.4. Corneal endothelial abnormalities

Several studies have evaluated the morphology, number and function of corneal endothelium in diabetic patients. Endothelial cell morphology is reportedly changed in diabetics with increased pleomorphism and variability of cell area (Matsuda et al., 1990; Weston et al., 1995; Larsson et al., 1996; Roszkowska et al., 1999; Shenoy et al., 2009; Módis et al., 2010; El-Agamy and Alsubaie, 2017). Some data indicate no change in cell density (Matsuda et al., 1990; Larsson et al., 1996), whereas more recent results show decreased *in vivo* endothelial cell counts in corneas of patients with IDDM and NIDDM (Roszkowska et al., 1999; Inoue et al., 2002; Shenoy et al., 2009; Liaboe et al., 2017; El-Agamy and Alsubaie, 2017). A similar controversy surrounds endothelial function: some studies found that it was decreased compared to non-diabetic corneas in terms of deswelling (Saini and Mittal, 1996b), but others failed to confirm this finding (Weston et al., 1995; Larsson et al., 1996). Although most studies did not specifically address the issue of endothelial loss severity or the presence of DR, endothelial diabetic changes seem to be rather minor overall. However, diabetes carries increased risk of endothelial complications following corneal surgery.

2.5. Conjunctival involvement

Abnormalities of conjunctiva are common in diabetic patients (Yoon et al., 2004; Gunay et al., 2016). Problems specifically related to conjunctiva include microvascular alterations partly similar to the retinal ones, including loss of capillaries, macrovascular dilation, uneven vessel distribution, and low numbers of goblet cells (Cheung et al., 2001; Yoon et al., 2004; Owen et al., 2005; To et al., 2011). Anti-angiogenic ranibizumab was effective in ameliorating conjunctival sensitivity in diabetics (Örnek et al., 2015). Diabetic conjunctiva with dry eye also shows increased proinflammatory cytokines (Zhang et al., 2016). Interestingly, conjunctival flora appears to be abnormal in diabetic patients (Martins et al., 2004; Bilen et al., 2007). These patients have a significantly higher percentage of positive cultures than non-diabetic patients. The pattern of predominant bacterial strains (mostly *Staphylococcus*) also differs from control subjects with Gram-negative bacteria being more common (Bilen et al., 2007; Adam et al., 2015). Resistance of these strains to penicillin, streptomycin and tetracycline increases the risk of postoperative endophthalmitis in diabetic patients because the conjunctiva may not be adequately sterilized before surgery. Vancomycin should be used in these cases because of lack of resistance to its action (Bilen et al., 2007).

2.6. Tear film changes

Tear film plays a significant role in corneal health and immune protection. It is mainly produced by the lacrimal gland that shows signs of inflammation, hyperglycemia-related oxidative stress, and accumulates AGEs in human and animal diabetes (Alves et al., 2008; Módulo et al., 2009). As a result, tear secretion in diabetics is often significantly lower than normal and dry eye incidence increases (Dogru et al., 2001; Inoue et al., 2001; Yoon et al., 2004; Cousen et al., 2007; Manaviat et al., 2008; Beckman, 2014). Osmolarity of diabetic tears also increases (Beckman, 2014). In diabetic patients, decreased stability of tear film correlates with neuropathy, poor glucose control, and reduced density of conjunctival goblet cells that secrete tear mucins (Dogru et al., 2001; Yoon et al., 2004). The severity of

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keratoconjunctivitis sicca was found to correlate with the severity of DR (Nepp et al., 2000; Manaviat et al., 2008; Lv et al., 2014). Overall, diabetic tear film abnormalities and dry eye are serious clinical problems and may also contribute to diabetic keratopathy (Liu et al., 2015).

2.7. Biomechanical abnormalities

Corneal diabetic alterations include increased central thickness, altered BM composition, abnormal collagen structure in the stroma and accumulation of AGEs that may cause collagen crosslinking (Ljubimov et al., 1998; Rehany et al., 2000b; Zou et al., 2012; El-Agamy and Alsubaie, 2017). Therefore, corneal biomechanics may be also altered in diabetics. This issue has been explored in several human studies measuring corneal resistance factor (CRF; related to elasticity and overall resistance of the cornea) and corneal hysteresis (CH; related to viscoelastic properties and indicating biomechanical integrity) using ocular response analyzer. These studies also established an increase in corneal thickness associated with diabetes (Kotecha et al., 2010; Scheler et al., 2012). The reported changes are inconsistent. One study found a decrease in CH (lower viscosity) and no change in CRF (Sahin et al., 2009), but two other studies reported an increase in both parameters (Kotecha et al., 2010; Scheler et al., 2012). The discrepancy may arise from the fact that the first study did not measure the glycation levels (as glycated hemoglobin Hb1Ac). In contrast, two other studies showed a significant correlation between Hb1Ac values and biomechanical parameters. The data suggest that biomechanical properties of the cornea are altered more with poorer diabetes control manifested by high Hb1Ac. Although corneal biomechanical changes in diabetes seem to correlate with altered metabolism, their contribution to pathophysiology remains to be established. Overall, it appears that diabetic corneas become progressively more viscoelastic and more resistant to pressure, possibly due to increasing protein glycation and crosslinking. This view is consistent with protective effect of NIDDM on keratoconus, a thinning corneal degeneration, characterized by corneal protrusion and low biomechanical parameters (Seiler et al., 2000).

2.8. Surgical problems

Structural and functional abnormalities in diabetic corneas contribute to an increased risk of surgical complications. Even with improved surgical techniques individuals with diabetes account for 80% of cases of corneal complications after cataract and refractive surgery, vitrectomy for non-clearing vitreous hemorrhage, and PRP (Foulks et al., 1979; Chung et al., 1988; Hiraoka et al., 2001; Chiambo et al., 2004; Dogru et al., 2004; Wylegała et al., 2006; Chen et al., 2009; Bikbova et al., 2012; Vieira-Potter et al., 2016). In some patients, diabetic keratopathy may develop following ocular surgery (Sakamoto et al., 2004; Chen et al., 2009). These problems arise as a consequence of epithelial debridement usually performed during both procedures to improve intraoperative visualization (Schulze et al., 2006; Chen et al., 2009). During debridement subbasal nerve endings are severed and regrow slower than normal. Combined insult on epithelial cells and subbasal nerves underlies slow wound healing and temporary reduction (up to six months) in corneal sensation postoperatively (Chen et al., 2009; Mahgoub and Macky, 2014). For this reason, additional treatment, e.g., autologous serum has been used in these patients to accelerate the wound healing process (Schulze et al., 2006). Cataract surgery may bring about an increase in corneal thickness and

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in endothelial cell loss compared to non-diabetic patients (Langwińska-Wośko et al., 2004; Morikubo et al., 2004; Lee et al., 2005; Hugod et al., 2011; Yang et al., 2011; Dhasmana et al., 2014). Corneal edema after phacoemulsification during cataract surgery is also more persistent in diabetics (Tsaousis et al., 2015) who are also at higher risk of graft failure in Descemet's membrane keratoplasty (Greiner et al., 2014).

Refractive surgery including photorefractive keratotomy and LASIK is more risky in diabetics because of recurrent erosions and persistent epithelial defects, epithelial downgrowth, keratitis, poor wound healing, and increased possibility of infections (Costin, 2001: Fraunfelder and Rich, 2002; Jabbur et al., 2004). LASIK in some cases can aggravate DR (Ghanbari and Ahmadieh, 2003). This procedure could be recommended only to patients with well-controlled diabetes and without diagnosed DR (Halkiadakis et al., 2005; Cobo-Soriano et al., 2006). However, some ophthalmologists continue to caution against performing refractive surgery in all diabetic patients (Mohammadpour, 2007).

3. Molecular alterations and disease markers of diabetes in the cornea

Despite clinical relevance, there is still no comprehensive picture of the molecular mechanisms of diabetic corneal disease. Because of its clear involvement, the corneal epithelium has been the main focus of such studies. In this section, major findings will be described, mostly related to the epithelial wound healing and stem cells, and various factors that may play a role in the development of diabetic keratopathy. The list of components altered in human and animal diabetic corneas is provided in Table 2. In this section the data on IDDM and NIDDM are discussed together, as no significant differences in corneal molecular alterations between two types are currently known.

3.1. Cell adhesion and basement membrane

It has been long recognized that diabetic corneal changes involved abnormal interactions of corneal epithelium with altered epithelial basement membrane (BM). These abnormalities include increased BM fragility, decreased number of hemidesmosomes, altered epithelial adhesion, and delayed BM reassembly after wounding (Hatchell et al., 1983; Tabatabay et al., 1988; Azar et al., 1989a,b; 1992; Sato et al., 1999; Gül et al., 2008). In human diabetic epithelial BM distinct reduction in immunostaining was further found for its major components, chains of laminin-511/laminin-10 ($\alpha5\beta1\gamma1$), nidogen-1/entactin, and $\alpha_3\beta_1$ laminin-binding integrin, which was more pronounced in corneas of patients with DR (Ljubimov et al., 1998; Fujita et al., 2003). Laminin $\gamma3$ chain and fibronectin were also decreased in the diabetic limbal epithelial BM (Saghizadeh et al., 2011). These data are consistent with reduced corneal epithelial cell adhesion in high glucose condition (Lu et al., 2006). Diabetic BM fragility leads to easy removal of cells with BM by manual debridement, which does not happen in normal corneas. For this reason, wounding of diabetic corneal epithelium should be done by chemical cell removal, e.g., with n-heptanol (Hatchell et al., 1985; Kabosova et al., 2003). Our subsequent studies found no changes in gene expression for BM components and $\alpha_3\beta_1$ integrin. At the same time, increased expression and activity of MMP-10 and cathepsin F was observed in diabetic corneas, suggesting that BM and integrin changes were possibly due to protein degradation

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(Saghizadeh et al., 2001a; 2005). The degradation hypothesis was fully supported by experiments on boosting or silencing the expression of these proteinases by adenovirus (AV)-delivered full-length constructs or shRNA, respectively. Increasing MMP-10 and cathepsin F expression in normal corneas led to the discontinuity of immunostaining for chains of laminin-511 and laminin-332, nidogen-1, nidogen-2, and $\alpha_3\beta_1$ integrin (Saghizadeh et al., 2010a), whereas proteinase silencing resulted in the normalization of BM and integrin patterns (Saghizadeh et al., 2013; 2014; Ljubimov and Saghizadeh, 2015; Kramerov et al., 2016). The available evidence suggests that epithelial BM and cell adhesion abnormalities may result from degradative processes in the diabetic cornea and may negatively impact epithelial wound healing.

The second corneal BM, the Descemet's membrane, has been much less studied in diabetic conditions. Data obtained in rats with either IDDM or NIDDM show that diabetic Descemet's membrane contains unusual wide-spaced collagen bundles and more than normal long-spacing collagen fibrils (Rehany et al., 2000a; Akimoto et al., 2008). These data have been corroborated in corneas from patients with NIDDM (Rehany et al., 2000b). The authors suggest that collagen bundle appearance may be a result of excessive collagen glycation, although metabolic abnormalities of diabetic endothelial cells cannot be ruled out.

3.2. Proteinases

Proteolytic enzymes (MMPs, plasminogen activators, cathepsins, etc.) play a major role in embryonic development, blood coagulation, wound healing and tissue remodeling. As diabetic corneas have slow and incomplete epithelial wound healing, and BM fragility and fragmentation, it is possible that proteinases are involved in these alterations. In fact, corneal epithelial cells in high glucose and rat diabetic corneal epithelium showed enhanced MMP activity during wound healing (Takahashi et al., 2000). Using gene microarrays, immunostaining, and zymography, our group has shown that *ex vivo* human diabetic corneas upregulated the expression of MMP-10 in the epithelium and keratocytes, MMP-3 in the keratocytes, and cathepsin F in the epithelium (Saghizadeh et al., 2001a; 2005). These changes were not seen in keratoconus or bullous keratopathy corneas, suggesting specific changes induced by diabetes. Some other MMPs (1, 2, 7, 11, 13) were downregulated, but several cathepsins, and plasminogen activators remained unchanged according to gene microarray analysis (Saghizadeh et al., 2005). Specific proteinase upregulation in diabetic corneas has functional implications, as shRNA-mediated downregulation of MMP-10 and cathepsin F in organ-cultured diabetic corneas accelerated wound healing and restored altered epithelial BM and integrin patterns (Saghizadeh et al., 2013; 2014; Kramerov et al., 2016). Mouse diabetic corneas were recently found to have abnormal regulation of Serpine-1/plasminogen activator inhibitor-1 in epithelial wound healing. Serpine-1 levels were lower than normal during healing, and its addition accelerated the healing process (Sun et al., 2015). Thus, specific proteinases may be promising targets for therapy aiming at preventing or alleviating symptoms of diabetic corneal epitheliopathy.

3.3. Growth factors and signaling molecules

Growth factors and cytokines are powerful regulators of cell behavior and tissue remodeling including wound healing. Specific growth factors have altered levels in diabetic tissues, and

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new treatments aimed at blocking their abnormal expression are being successfully tested in clinical trials (Aiello and Hata, 1999; Caldwell et al., 2003; Aiello et al., 2004; Amoaku et al., 2015). Since growth factors and cytokines affect corneal cell behavior and wound healing (Wilson et al., 1999; Imanishi et al., 2000; Klenkler and Sheardown, 2004; Ljubimov and Saghizadeh, 2015), they may play a role in diabetic keratopathy. Altered expression of growth factors/cytokines may contribute to BM changes, decreased cell adhesion, epithelial fragility, abnormal wound healing, and subbasal nerve loss typical for diabetic corneas. Some better-studied growth factor systems with relevance to diabetic corneal disease are discussed below.

3.3.1. Opioid growth factor system—Opioid growth factor (OGF, or [Met^5]-enkephalin) and its ζ receptor (OGFR) is an important system mediating corneal homeostasis, and acting as a negative regulator of epithelial proliferation and wound healing (Sassani et al., 2016). Elevated levels of OGF are found in plasma of diabetic patients and in the obese diabetic (*db/db*) mice (McLaughlin et al., 2010). OGF-OGFR system is expressed in the corneal cells, and its systemic or topical blockade by opioid antagonists enhances corneal epithelial wound healing in rats and rabbits with IDDM. This effect was also reported for non-diabetic animals and organ cultures of normal human corneas (McLaughlin et al., 2010). Wound healing acceleration was accompanied by increased cell proliferation, which is thought to be the main mechanism behind the antagonist effect.

3.3.2. Insulin-like growth factor-1 (IGF-1)—IGF-1 is important for cell growth and metabolism. In corneal epithelial cells it acts through insulin receptor, and IGF receptors. IGF-1 and its receptors are found in human corneal keratocytes and epithelial cells (Li and Tseng, 1995), and mediate cell migration, proliferation, and survival (Lee et al., 2006; Yanai et al., 2006). In *ex vivo* human diabetic corneas IGF-1 expression is significantly elevated, especially in the epithelium (Saghizadeh et al., 2001b). In corneas of diabetic rats IGF-1 synergizes with a neuropeptide substance P in accelerating epithelial wound healing (Nakamura et al., 2003). IGF-1 can bind to several proteins (IGFBPs) that modulate the bioavailability of extracellular IGF-1, preventing its activation of the IGF-1R and function (Wu et al., 2012). IGFBP3 is elevated in diabetic tears and immortalized corneal epithelial cells in hyperglycemic conditions. Moreover, *in vitro* phosphorylation of IGF-1R was blocked in the presence of IGFBP3 and IGF-1 when given to corneal cells at a high diabetic but not at a low normal ratio (Wu et al., 2012). It appears that elevated levels of IGF-1 alone in diabetic corneas may not be beneficial for corneal wound healing and epithelial maintenance due to the interference by elevated tear IGFBP3. Another related key metabolic regulator present in tears is insulin but its changes in diabetes have not been explored.

3.3.3. Epidermal growth factor (EGF)—EGF signals through its receptor (EGFR1/ErbB1, or EGFR) to activate intracellular pathways comprising downstream protein kinase effectors, phosphatidylinositol-3-kinase (PI3K) – Akt kinase axis, and extracellular regulated kinase (ERK) (Zhang and Akhtar, 1997; Xu et al., 2009; Xu and Yu, 2011; Funari et al., 2013; Winkler et al., 2014). This pathway is critical for cell migration and proliferation, and is a major mediator of corneal epithelial wound healing (Zieske et al., 2000; Nakamura et al., 2001; Ljubimov and Saghizadeh, 2015). In corneas of rats with IDDM phosphorylation

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of EGFR and its signaling intermediates Akt and ERK was diminished, as well as downstream apoptotic BAD signaling pathways. These abnormalities were associated with a significant delay in corneal epithelial wound healing compared to non-diabetic animals (Xu and Yu, 2011). Similar changes were observed in corneal epithelial cells and organ-cultured porcine and human corneas cultured in high glucose (Xu et al., 2009). The relationship of EGFR signaling with diabetic corneal wound healing was further tested by manipulating levels of diabetes-increased proteinases in normal and diabetic organ-cultured human corneas. Overexpression of MMP-10 and cathepsin F in normal corneas decreased phospho-EGFR and phospho-Akt expression, whereas phospho-ERK and phospho-p38 remained unchanged. This was accompanied by slower epithelial wound healing (Saghizadeh et al., 2010a). Conversely, silencing of these proteinases in diabetic corneas increased phospho-EGFR and phospho-Akt expression and accelerated wound healing (Saghizadeh et al., 2013; 2014). Overall, EGFR signaling is downregulated in the diabetic corneas, which may negatively impact epithelial wound healing.

3.3.4. Hepatocyte growth factor (HGF)—HGF in the cornea is mostly produced by the epithelial cells, whereas its receptor, c-met proto-oncogene is made by stromal cells, but also by the epithelium. *HGF* and *c-met* mRNAs are found in all three major cell types of the human cornea (Wilson et al., 1993), suggesting the existence of autocrine loops in corneal endothelium and epithelium. HGF is involved in cell proliferation, migration, and apoptosis (Wilson et al., 1993; Kakazu et al. 2004; Saghizadeh et al., 2010b; 2011). During corneal epithelial wound healing HGF is upregulated in both keratocytes and epithelial cells (Li et al., 1996; Kakazu et al., 2008; Saghizadeh et al., 2010b). Using gene microarray analysis validated by immunostaining, RT-PCR, and functional assays, we have documented upregulation of HGF in donor human diabetic corneas. At the same time, these corneas showed downregulation of c-met receptor (Ljubimov et al., 2005). Upregulation of c-met in organ-cultured human diabetic corneas led to marked normalization of diabetic marker patterns and wound healing time. Analysis of signaling intermediates identified p38 mitogen-activated protein kinase as the major downstream effector of the HGF–c-met pathway (Sharma et al., 2003; Saghizadeh et al., 2010b). These data support the hypothesis that the alterations of HGF–c-met system contribute to abnormalities observed in diabetic corneas (Saghizadeh et al., 2005; 2010b; 2011; 2014; Kramerov et al., 2016).

3.3.5. Thymosin β_4—$T\beta_4$ is a member of the β-thymosin family of conserved 5 kDa peptides. They interact with G-actin and are actin-sequestering proteins (Dedova et al., 2006). $T\beta_4$ has many other biological activities, and is one of the mediators of cell migration, angiogenesis, inflammation, and tissue regeneration. $T\beta_4$ promotes corneal re-epithelialization during healing, and inhibits apoptosis both *in vitro* and *in vivo*. One of the main mechanisms of $T\beta_4$ action is thought to be blocking of tumor necrosis factor-α (TNF-α) induced inflammation and related NF-κB activation (Sosne et al., 2010). $T\beta_4$ also upregulates epithelial BM component laminin-332 (laminin-5), which mediates epithelial migration, as well as diabetes-downregulated MMP-1 (Sosne et al., 2004; 2007; 2010). In the cornea, $T\beta_4$ is mostly found in epithelial cells. Gene microarray analysis and immunohistochemistry revealed significant decrease of $T\beta_4$ in human diabetic corneas (Saghizadeh et al., 2005), which may be related to slow wound closure by epithelial cells

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and increased cell death. $T\beta_4$ thus appears to be a promising target for therapy aimed at restoring normal wound healing and reducing apoptosis in the diabetic cornea.

3.3.6. Other factors altered in diabetic corneas—Several more factors have been identified as altered in diabetic corneas and appear to contribute to diabetic complications.

Diabetic corneas have significant epithelial stem cell abnormalities manifested by decreased expression of a number of putative stem cell markers (Saghizadeh et al., 2011; Ueno et al., 2014). These alterations may contribute to slow wound healing, as therapeutic restoration of the expression patterns was accompanied by normalized wound healing in organ cultured diabetic corneas and stem cell-enriched limbal epithelial cultures (Saghizadeh et al., 2011; 2013; 2014; Kramerov et al., 2016).

Recent studies have revealed altered purinergic signaling in diabetic corneal wound healing. Activation of this signaling is thought to be one of the first events in the wound healing process (Ljubimov and Saghizadeh, 2015). In diabetic corneas of humans, rats and mice ATP-activated P2X7 receptor is increased (Mankus et al., 2011). This could contribute to slow epithelial wound healing in these tissues (Minns and Trinkaus-Randall, 2016).

Genome-wide transcriptional analysis of healing corneal epithelium in rats with IDDM and NIDDM, and mice with IDDM recently revealed downregulation of TGF-β3, an anti-fibrotic TGF-β isoform. The administration of TGF-β3 accelerated wound closure in diabetic animals via SMAD and PI3K-Akt pathways and upregulation of Serpine1 (Bettahi et al., 2014), suggesting its possible translational use in diabetic patients.

Corneal epithelium of diabetic patients, and high glucose-exposed cultured epithelial cells have increased immunoreactivity for ephrin-A1, a member of cell-cell signaling family and a negative mediator of cell migration. It is a ligand for tyrosine kinase EphA2 receptor, mutations of which are associated with cataract formation (Kaplan et al., 2012). In cultured corneal epithelial cells, ephrin-A1 activation of EphA2 restricted cell migration while suppressing activation of ERK1/2 and Akt kinases (Kaplan et al., 2012). The data suggest that ephrin-A1/EphA2 as an inhibitor of cell migration may be a mediator of slow diabetic epithelial wound healing in the cornea.

The advent of genome-wide transcriptome sequencing may soon bring about new markers of diabetic corneas that would facilitate the development of new therapeutics against diabetic corneal disease.

3.4. Advanced glycation end products (AGEs)

Hyperglycemia in diabetics causes non-enzymatic glycosylation of proteins leading to the formation of AGEs that accumulate in diabetic tissues (Zou et al., 2012; Madonna et al., 2017). AGEs interact with their receptors (RAGEs) triggering intracellular signaling including NF-κB activation and formation of reactive oxygen species (ROS) (Kim et al., 2011; Shi et al., 2013a;b). Their accumulation in the diabetic corneal BMs and stroma may be responsible for clinically observed increased autofluorescence (Kaji et al., 2000; McDermott et al., 2003; Calvo-Maroto et al., 2016). In cultured corneal

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telomeraseimmortalized epithelial cells AGEs cause delayed wound healing and increased cell apoptosis in a ROS- and RAGE-dependent manner (Shi et al., 2013a;b). This was accompanied by activation of NADPH oxidase generating ROS, as well as JNK and p38 kinase pathways. AGEs may cause stromal collagen crosslinking altering corneal biomechanics (Sady et al., 1995).

AGEs may affect epithelial and keratocyte attachment to the diabetic corneal extracellular matrix. Corneal epithelial cells poorly attach to glycated type I collagen, fibronectin, and laminin (McDermott et al., 2003). Glycated laminin and fibronectin mimicking diabetic Descemet's membrane only poorly support corneal endothelial cell adhesion (Kaji et al., 2000; 2001). Thus, increased corneal oxidative stress through RAGE signaling and direct adhesive protein modifications by AGEs may exacerbate diabetic abnormalities.

3.5. Polyol pathway

The polyol pathway is known to be activated in diabetes, whereby excess glucose is converted to sorbitol by aldose reductases, e.g., AKR1B1 and AKR1B10 (Huang et al., 2010). Accumulation of sorbitol pathway products is important for diabetic cataract formation (Huang et al., 2010) but its consequences are less studied in the cornea. Inhibitor studies in galactosemic rats showed normalization of delayed epithelial wound healing by aldose reductase inhibitor treatment (Datiles et al., 1983), which may have translational significance.

3.6. MicroRNA

MicroRNAs are powerful biological regulators with mostly negative effects on gene expression. These effects are complex due to the action of each miRNA on more than one cellular target. Using miRNA arrays, our group has identified a number of miRNA differentially expressed in diabetic human corneas (Funari et al., 2013). Two miRNAs, h-miR-146a and h-miR-424 were upregulated in diabetic corneas and retarded corneal epithelial wound healing both in cultured cells and organ-cultured corneas, whereas their specific inhibitors accelerated healing (Funari et al., 2013; Winkler et al., 2014). MiR-146a inhibition was accompanied by increased expression of phospho-EGFR, phospho-p38, and a diabetic marker, integrin $\alpha_3\beta_1$ (Winkler et al., 2014). Western analysis of cells treated either by mir-146a or its inhibitor identified EGFR as a target for this miRNA, compatible with its effects on wound healing (Funari et al., 2013). Recently, miR-204-5p increase in diabetic corneas was observed in the Akita mouse model of IDDM (Gao et al., 2015). In the cornea, this miRNA suppresses it target, silent mating type information regulation 2 homolog 1 (SIRT1). In limbal epithelial cell line grown in high glucose miR-204-5p was increased and SIRT1 inhibition occurred. Downregulation of miR-204-5p by a specific inhibitor restored cell growth. In Akita mice, subconjunctival injections of miR-204-5p inhibitor promoted wound healing with upregulation of SIRT1 and cyclin D1 (Gao et al., 2015). Although studies of miRNA changes in diabetic corneas have just begun, they have already identified possible candidates responsible for corneal abnormalities and amenable for therapeutic interventions.

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4. *In vitro* systems for studies of diabetes in the cornea

Corneal cell cultures in normal (5 mM) and high (25-30 mM) glucose with mannitol control for osmolarity have been widely used to mimic diabetic alterations, in order to examine molecular mechanisms of wound healing and test certain drug candidates. Most studies dealt with epithelial cells including SV40 T antigen-transformed (now mostly abandoned), and telomerase-immortalized cells that are closer to the corneal epithelium (Lu et al., 2006; Xu et al., 2009; Yin and Yu 2010; Wang et al., 2014; Yang et al., 2014; Gao et al., 2015; Ljubimov and Saghizadeh, 2015). It should be borne in mind that immortalized cells are closer to the epithelial progenitors than to differentiated epithelial cells of the central corneas, which may be a drawback. Stem cell-enriched human primary limbal epithelial cultures have also been used to study wound healing.

These cultures preserve slower healing and alterations in marker patterns similar to the *in vivo* corneas (Kramerov et al., 2015), possibly due to stable epigenetic changes typical for tissues of long-term diabetics (Kowluru et al., 2015; Chen et al., 2016). Limbal cultures may be more suitable for diabetes research than immortalized cell lines derived from normal cells and put in a transient high glucose environment. Stromal diabetic cells have not been studied in detail yet. Recently, a culture system using diabetic donor corneal stromal cells for making 3D diabetic stromal models was recently introduced and revealed significant alterations in these cells (Priyadarsini et al., 2016b).

A better model is represented by whole corneal organ culture that even allows for a successful corneal transplantation (Devasahayam et al., 2016). Using one of these models where the corneas are kept at the air-liquid interface and maintained at normal corneal temperature of around 35°C, we have successfully shown that organ-cultured corneas from DM and DR patients preserved the delayed wound healing and abnormal BM structure, proteinase and growth factor patterns that are seen in intact corneas from such patients (Kabosova et al., 2003; Ljubimov and Saghizadeh, 2015; Kramerov et al., 2016). Diabetic changes persist in organ cultures for more than a month as a maximum culture period, possibly due to the epigenetic phenomenon of diabetic memory (Kowluru et al., 2015; Chen et al., 2016). These cultures allowed us to work out successful gene therapy alleviating some traits of diabetic keratopathy such as slow wound healing (Ljubimov and Saghizadeh, 2015; Kramerov et al., 2016). Corneal organ cultures are thus suitable not only to study diabetes-related changes (with a known drawback of lack of innervation and blood flow), but also to test possible drugs aimed at ameliorating signs of diabetic corneal disease (Kramerov et al., 2016).

5. Animal models

Models of IDDM and NIDDM have been established in the rabbit, mouse, rat, guinea pig, cat, dog, pig, and monkey. All models mimic fairly early signs of human diabetic ocular disease. However, diabetic animals only develop background DR but do not show signs of PDR, and have no macula (except for non-human primates) to develop macular edema seen in many diabetic patients (Stitt et al., 2016). At the same time, some corneal symptoms in these models are similar to humans, which allows studying mechanisms of slow epithelial

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wound healing and reduced innervation (Chicama et al., 2007; Yin et al., 2011; Wang et al., 2012; Byun et al., 2015; Sassani et al., 2016). However, some traits of diabetes in the cornea of animals differ from human disease. Delayed epithelial wound healing was seen in rats but not rabbits (Hatchell et al., 1983; Jiang et al., 1996; Nakamura et al., 2003). However, diabetic rabbits as humans have degradative BM changes as in human corneas, but diabetic rats and mice show increased BM staining for laminin (Hatchell et al., 1983; Jiang et al., 1996; Nakamura et al., 2003; Watanabe et al., 2002). A mouse IGF-1 transgenic model develops retinal signs of diabetes but no corneal changes (Ruberte et al., 2004). Overall, animal models of diabetic keratopathy should be well characterized for a particular diabetic symptom or a group of symptoms to study and the data compared to the human situation. A necessity for comparative studies of both animal and human material has been emphasized (Eisma et al., 2015).

6. Treatment possibilities

Diabetic keratopathy and diabetic neuropathy treatment remains largely symptomatic (Cavallerano, 1992; Abdelkader et al., 2011). Several treatment modalities have been developed and tested in recent years, but many of them are currently at the experimental stage. The most promising approaches are discussed below and summarized in Table 3. It should be noted that the emerging drugs with the exception of naltrexone have not been tested in both types of diabetes, which may be needed in the future if their potential molecular differences are identified.

6.1. Insulin

Insulin appears an obvious drug to use against diabetes and its complications (both IDDM and severe cases of NIDDM). Local insulin implants can accelerate corneal wound healing in rats with IDDM (Zagon et al., 2007). In IDDM mice, insulin eye drops were able to prevent the loss of subbasal corneal nerves (Chen et al., 2013). Recently, insulin was shown to restore diabetes-altered circadian rhythm in the corneal epithelium, which may be a mechanism of its beneficial action (Song et al., 2016). In a clinical setting, insulin eye drops were successfully used in 14 diabetic patients who had corneal epithelial debridement before vitreoretinal surgeries. With this treatment, corneas re-epithelialized significantly faster than without it (Bastion and Ling, 2013). These data suggest that insulin treatment may be used to treat corneal defects in diabetics. IGF-1 that may utilize insulin receptor for signaling has been tested for prevention of diabetic keratopathy in NIDDM mice (*db/db*). Retrobulbar injections of recombinant IGF-1 were able to prevent decreased expression of epithelial stem cell markers in diabetic mice, as well as increase subbasal nerve density (Ueno et al., 2014).

As a note of caution, the efficacy of insulin or IGF-1 treatment may depend on whether the epigenetic diabetic changes are mild severe. It should also be noted, that in normal corneas insulin does not accelerate wound healing (Abdelkader et al., 211). Moreover, in diabetic organ-cultured corneas wound healing is still compromised compared to normal corneas despite the presence of high insulin concentration in the medium (Kabosova et al., 2003). Finally, DR may increase initially after insulin treatment. This may be a consequence of upregulation of IGF-1 that activates the production of vascular endothelial growth factor and

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exacerbates retinopathy (Smith et al., 1999). More data including biodistribution and examination of retinal effects are thus needed before insulin or IGF-1 local therapy can be recommended as a treatment for diabetic keratopathy.

6.2. Naltrexone

Naltrexone is an opioid antagonist and an inhibitor of OGF-OGFR interaction (see 3.3.1). It is safe and approved for clinical treatment of alcohol and opioid dependence. It has been convincingly shown by Zagon's group to normalize corneal epithelial wound healing, tear secretion, and corneal surface sensitivity in diabetic animals of different species (McLaughlin et al., 2010; Zagon et al., 2014; Sassani et al., 2016). This was established in several animal species with both IDDM and NIDDM (Sassani et al., 2016). In contrast to many wound healing promoting agents that increase epithelial cell motility, naltrexone apparently lifts a restriction on cell proliferation imposed by OGF (McLaughlin et al., 2010). The effects of naltrexone on corneal nerves could be mediated by mechanisms other than OGFR silencing (Hota et al., 2016). The availability of naltrexone eye drops makes it possible to introduce it to clinical practice for diabetic keratopathy in the near future.

6.3. Aldose reductase inhibitors

Studies in galactose-fed rats have shown that they develop corneal abnormalities similar to those found in diabetic animals. Treatment with aldose reductase inhibitor (ARI) ranirestat ameliorated corneal wound healing, and normalized the expression of previously described diabetic markers (Saghizadeh et al., 2001a) MMP-10 and integrin α_3 (Takamura et al., 2013). Another ARI, CT-112, reportedly reversed abnormal epithelial morphology and reduced corneal sensitivity in some diabetic patients (Hosotani et al., 1995). However, a randomized placebo-controlled clinical trial with topical CT-112 did not find differences in corneal sensitivity between placebo and ARI groups, although improvement of corneal barrier function in ARI-treated group was observed both at four and eight weeks of treatment (Nakahara et al., 2005). Overall, it is still unclear whether ARI should be clinically used for diabetic keratopathy treatment.

6.4. Other pharmacological agents

$T\beta_4$ as a wound healing promoting and anti-inflammatory agent was tested in diabetic patients with $T\beta_4$ neurotrophic keratopathy in a small clinical trial (Dunn et al., 2010). Treatment with $T\beta_4$ eye drops resulted in a clinically significant reduction of non-healing epithelial defects in all four patients without noticeable side effects. $T\beta_4$ thus appears a promising agent to treat diabetic keratopathy (Sosne et al., 2016).

Autologous serum increases corneal wound healing in patients with long-term diabetes (Schulze et al., 2006), but it is unclear what factors in serum contribute to the effect. It is thought that adhesive (fibronectin), mitogenic (EGF), and motogenic (transforming growth factor-β and EGF) may be responsible (Pflugfelder, 2006).

Antidiabetic agents nateglinide and glibenclamide were shown to suppress Descemet's membrane changes in NIDDM Goto-Kakizaki rats (Akimoto et al., 2008). It remains to be established whether they would normalize other symptoms of diabetic keratopathy. Several

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treatments have also been recently reported to ameliorate some symptoms of diabetes in the cornea using animal models. These include 1,5-isoquinolinediol [poly(ADP-ribose) polymerase inhibitor] that increased corneal sensitivity and epithelial healing in diabetic rats (Byun et al., 2015); ciliary neurotrophic factor (downregulated in the diabetic cornea) that activated corneal epithelial stem cells, increased nerve density, and promoted epithelial healing through the activation of STAT3 in diabetic mice (Zhou et al., 2015); topical nerve growth factor that reduced apoptosis and inflammation in corneas of diabetic rats (Park et al., 2016); interleukin-1 receptor antagonist that increased epithelial wound healing, sensory reinnervation, and reduced apoptosis in diabetic mouse corneas (Yan et al., 2016); substance P that produced similar effects in diabetic mice and high glucose-treated corneal epithelial cells through neurokinin-1 receptor (Yang et al., 2014); curcumin that promoted wound healing and recovery of corneal sensation when used in nanomicelles with intranasal delivery; it may work through reduction of reactive oxygen species (Guo et al., 2016); and topically delivered mitogenic protein lacritin fused with elastin-like polypeptide–based nanoparticles that enhanced corneal epithelial wound healing in NOD diabetic mice (Wang et al., 2014). Although promising, most of these agents need to be studied in more detail including pharmacology and side effects. It is also possible that the best treatment results would be eventually achieved by a combination of several agents (Davidson et al., 2015). Due to superior tissue penetration, nanoformulations may increase the effect.

6.5. Gene therapy

Gene therapy has been successfully used for different tissues including the cornea. Viruses are mostly used in these studies because they are very efficient gene delivery vehicles (Ljubimov and Saghizadeh, 2015). Despite lasting effects and high efficiency, viruses may induce immune reactions, inflammation, and uncontrolled virus integration into the host genome. New recombinant adenoviruses, adeno-associated viruses, and lentiviruses do not elicit serious immune responses, and transfect non-dividing cells. However, safety concerns remain, including noted toxicity for stem cells. Viral gene therapy has been applied by our group for normalization of organ-cultured human corneas from long-term diabetic donors using AV as delivery vehicle (Kramerov et al., 2016). AV is internalized by the corneal epithelium through caveolae pathway, which can be activated by phosphodiesterase-5 inhibitors (e.g., Viagra) to boost virus uptake (Saghizadeh et al., 2010a). Targets for therapy were selected from previous gene microarray and MMP studies and included c-met, MMP-10 and cathepsin F (Saghizadeh et al., 2001a; 2005). C-met was downregulated in diabetic corneas and gene therapy with full-length c-met cDNA was aimed at restoring its levels. Proteinases were upregulated in the diabetic corneas, and their expression was silenced by shRNA technology. Increased expression of c-met using AV vector normalized the expression of diabetic BM and integrin markers, accelerated epithelial wound closure, and restored the expression of putative stem cell markers in the limbus (Saghizadeh et al., 2010b; 2011; 2014). The main signaling pathway of c-met action involves p38 (Saghizadeh et al., 2010b). Similar albeit weaker effects were observed with proteinase silencing, which was accompanied by the activation of the EGFR-Akt pathway (Saghizadeh et al., 2013; 2014). The strongest effect was seen with combination therapy using all three AVs. In this case, both p38 and EGFR-Akt pathways were activated, which may explain the enhanced effect (Saghizadeh et al., 2013; 2014). Interestingly, transduction of stem cell-harboring

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limbal compartment only was sufficient to normalize various epithelial alterations in diabetic corneas (Saghizadeh et al., 2014).

Gene silencing can be also achieved by microRNas that are important post-transcriptional gene expression regulators. Saghizadeh et al. uncovered a subset of miRNAs that were increased in the diabetic human corneas (Funari et al., 2013). They further showed that miR-146a increased in diabetic cornea was able to retard epithelial wound healing by suppressing EGFR, and respective miRNA inhibitor (antagomir) accelerated healing in organ-cultured diabetic corneas by activating EGFR signaling and restored the expression of some diabetic and stem cell markers (Funari et al., 2013; Winkler et al., 2014). Despite this success, it should be noted that most miRNAs act on more than one target and are not coordinately changed in different diabetic tissues. Therefore, their use for therapy should be thoroughly validated including the delivery route and biodistribution. Overall, gene therapy appears to be a promising tool for the restoration of diabetic corneal abnormalities and should be further developed for future clinical use.

6.6. Stem cells

Human corneal epithelium is rapidly renewed from the limbus, with limbal epithelial stem cells (LESC), or slow-cycling cells, being the source. LESC (1-3.5% of all cells) also actively participate in epithelial wound healing (Ljubimov and Saghizadeh, 2015). Cultured limbal epithelium is enriched in LESC and is often used for transplantation to patients in an autologous or allogeneic manner with considerable success (Holland, 2015). We have shown previously that LESC in diabetic corneas appear to be dysfunctional because the expression of a number of putative markers is significantly reduced (Saghizadeh et al., 2011). Specific gene therapy of limbal compartment harboring LESC was able to restore the marker expression levels and accelerate healing of large epithelial wounds (Saghizadeh et al., 2014). In severe cases of diabetes and with pronounced neuropathy, LESC may become seriously dysfunctional (Ueno et al., 2012; Zhou et al., 2015), and a transplantation of healthy cells may become an option (Kramerov and Ljubimov, 2016). Normal LESC may be obtained from donors, especially in cases of bilateral stem cell deficiency. Another source of cells in such severe cases may be hematopoietic stem cells (HSC) obtained from cord blood. In experiments with diabetic rats, injection of human HSC ameliorated corneal epithelial changes (Zickri et al., 2012). It remains to be established whether it was a direct or indirect effect.

If a diabetic patient's own LESC are available, they may be propagated in culture (Kramerov et al., 2015) and normalized using gene therapy. Correction of c-met, MMP-10, and cathepsin F levels in diabetic limbal epithelial cultures by AV-driven gene transfer was able to accelerate scratch wound healing and increase putative LESC marker expression (Kramerov et al., 2017, ARVO). This therapy was also effective when the virus was substituted by a cell-targeted nanopolymer (Kramerov et al., 2016, ARVO). Another way of normalizing diabetic corneal epithelial progenitor cells would be to generate autologous induced pluripotent stem cells and differentiate them to limbal cells for transplantation. Although these studies have just begun, they may yield in the near future promising

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candidates for autologous cell therapy effective against diabetic keratopathy. Similar strategies could be also used to normalize stromal and endothelial diabetic corneal cells.

7. Conclusions and perspectives

Diabetic corneal disease is a significant clinical problem affecting over a half of diabetic population. However, it is not receiving proper attention from physicians and remains underdiagnosed and underestimated. Clinical manifestations are variable and mainly concern epithelial problems (the most salient being slow and incomplete wound healing) neuropathy (loss of corneal sensation and possibility of developing neurotrophic ulcers), and tear film. A number of molecular alterations have been documented in the diabetic corneas including basement membrane changes, dysfunctional stem cells, altered profiles of growth factors, cytokines, and miRNAs, and accumulation of AGEs. The mainstream therapy remains symptomatic, although some promising therapeutic candidates have recently emerged. Topical insulin, and an FDA-approved opioid antagonist naltrexone appear to be ready for clinical use due to their safety and efficacy. They were also shown to alleviate symptoms of both neuropathy and keratopathy, which is an obvious advantage. Other treatments including autologous serum, thymosin β_4 and some growth factors are still being examined for efficacy. A drawback in preclinical testing of new drugs is the predominant use of animals with IDDM (Table 3), although the majority of human diabetics have NIDDM. For this reason, the use of human corneal organ cultures may be advantageous, as they seem to reflect the ratio between diabetes types more correctly. Another unresolved issue is a potential difference in corneal changes depending on the type of diabetes, which should be examined in future studies. New promising approaches involving gene, nano, and cell therapy, are currently at fairly early preclinical stages. The development of new biological treatments that would counteract both keratopathy and neuropathy in the diabetic cornea is much needed to alleviate consequences of ulcerations, as well as of laser treatment for DR and refractive surgeries. It may be anticipated that therapy for major abnormalities of corneal epithelium, nerves, and tear film would require a combination of several drugs for most efficacy, similar to modern cancer treatment.

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References

Abdelkader H, Patel DV, McGhee CNj, Alany RG. New therapeutic approaches in the treatment of diabetic keratopathy: a review. Clin Exp Ophthalmol. 2011; 39:259–270. [PubMed: 20973888]

Adam M, BalcI M, Bayhan HA, İnkaya AÇ, Uyar M, Gürdal C. Conjunctival flora in diabetic and nondiabetic individuals. Turk J Ophthalmol. 2015; 45:193–196. [PubMed: 27800231]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL III, Klein R. Diabetic retinopathy. Diabetes Care. 1998; 21:143–156. [PubMed: 9538986]

Aiello LP, Hata Y. Molecular mechanisms of growth factor action in diabetic retinopathy. Curr Opin Endocrinol Diabetes. 1999; 6:146–156.

Aiello LP, Cahill MT, Cavallerano JD. Growth factors and protein kinase C as novel therapies for the medical management diabetic retinopathy. Eye. 2004; 18:117–125. [PubMed: 14762400]

Akimoto Y, Sawada H, Ohara-Imaizumi M, Nagamatsu S, Kawakami H. Change in long-spacing collagen in Descemet's membrane of diabetic Goto-Kakizaki rats and its suppression by antidiabetic agents. Exp Diabetes Res. 2008; 2008:818341. [PubMed: 18779868]

Alves Mde C, Carvalheira JB, Módulo CM, Rocha EM. Tear film and ocular surface changes in diabetes mellitus. Arq Bras Oftalmol. 2008; 71(6 Suppl):96–103. [PubMed: 19274419]

Amoaku WM, Saker S, Stewart EA. A review of therapies for diabetic macular oedema and rationale for combination therapy. Eye (Lond). 2015; 29:1115–1130. [PubMed: 26113500]

Azar DT, Gipson IK. Repair of the corneal epithelial adhesion structures following keratectomy wounds in diabetic rabbits. Acta Ophthalmol Suppl. 1989a; 192:72–79. [PubMed: 2554660]

Azar DT, Spurr-Michaud SJ, Tisdale AS, Gipson IK. Decreased penetration of anchoring fibrils into the diabetic stroma. A morphometric analysis Arch Ophthalmol. 1989b; 107:1520–1523. [PubMed: 2803103]

Azar DT, Spurr-Michaud SJ, Tisdale AS, Gipson IK. Altered epithelial-basement membrane interactions in diabetic corneas. Arch Ophthalmol. 1992; 110:537–540. [PubMed: 1532888]

Bastion ML, Ling KP. Topical insulin for healing of diabetic epithelial defects?: A retrospective review of corneal debridement during vitreoretinal surgery in Malaysian patients. Med J Malaysia. 2013; 68:208–216. [PubMed: 23749008]

Beckman KA. Characterization of dry eye disease in diabetic patients versus nondiabetic patients. Cornea. 2014; 33:851–854. [PubMed: 24915011]

Ben Osman N, Jeddi A, Sebai L, Zghal I, Kaoueche M, Gaigi S, Ayed S. [The cornea of diabetics] (in French). J Fr Ophtalmol. 1995; 18:120–123. [PubMed: 7738303]

Bettahi I, Sun H, Gao N, Wang F, Mi X, Chen W, Liu Z, Yu FS. Genome-wide transcriptional analysis of differentially expressed genes in diabetic, healing corneal epithelial cells: hyperglycemia-suppressed TGFβ3 expression contributes to the delay of epithelial wound healing in diabetic corneas. Diabetes. 2014; 63:715–727. [PubMed: 24306208]

Bikbova G, Oshitari T, Baba T, Yamamoto S. Neuronal changes in the diabetic cornea: perspectives for neuroprotection. Biomed Res Int. 2016; 2016:5140823. [PubMed: 28044131]

Bikbova G, Oshitari T, Tawada A, Yamamoto S. Corneal changes in diabetes mellitus. Curr Diabetes Rev. 2012; 8:294–302. [PubMed: 22587515]

Bilen H, Ates O, Astam N, Uslu H, Akcay G, Baykal O. Conjunctival flora in patients with type 1 or type 2 diabetes mellitus. Adv Ther. 2007; 24:1028–1035. [PubMed: 18029329]

Blum M, Kloos C, Müller N, Mandecka A, Berner R, Bertram B, Müller UA. [Prevalence of diabetic retinopathy. Check-up program of a public health insurance company in Germany 2002-2004] (in German). Ophthalmologe. 2007; 104:499–500. 502–4. [PubMed: 17457588]

Byun YS, Kang B, Yoo YS, Joo CK. Poly(ADP-ribose) polymerase inhibition improves corneal epithelial innervation and wound healing in diabetic rats. Invest Ophthalmol Vis Sci. 2015; 56:1948–1955. [PubMed: 25711635]

Cai D, Zhu M, Petroll WM, Koppaka V, Robertson DM. The impact of type 1 diabetes mellitus on corneal epithelial nerve morphology and the corneal epithelium. Am J Pathol. 2014; 184:2662–2670. [PubMed: 25102563]

Caldwell RB, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, Caldwell RW. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Diabetes Metab Res Rev. 2003; 19:442–455. [PubMed: 14648803]

Calvo-Maroto AM, Perez-Cambrodí RJ, Albarán-Diego C, Pons A, Cerviño A. Optical quality of the diabetic eye: a review. Eye (Lond). 2014; 28:1271–1280. [PubMed: 25125072]

Calvo-Maroto AM, Perez-Cambrodi RJ, Garcia-Lazaro S, Ferrer-Blasco T, Cerviño A. Ocular autofluorescence in diabetes mellitus. A review J Diabetes. 2016; 8:619–628. [PubMed: 27147470]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Cavallerano J. Ocular manifestations of diabetes mellitus. Optom Clin. 1992; 2:93–116.

Chang SW, Hsu HC, Hu FR, Chen MS. Corneal autofluorescence and epithelial barrier function in diabetic patients. Ophthalmic Res. 1995; 27:74–79. [PubMed: 8538986]

Chen DK, Frizzi KE, Guernsey LS, Ladt K, Mizisin AP, Calcutt NA. Repeated monitoring of corneal nerves by confocal microscopy as an index of peripheral neuropathy in type-1 diabetic rodents and the effects of topical insulin. J Peripher Nerv Syst. 2013; 18:306–315. [PubMed: 24147903]

Chen WL, Lin CT, Ko PS, Yeh PT, Kuan YH, Hu FR, Yang CM. In vivo confocal microscopic findings of corneal wound healing after corneal epithelial debridement in diabetic vitrectomy. Ophthalmology. 2009; 116:1038–1047. [PubMed: 19394702]

Chen Z, Miao F, Paterson AD, Lachin JM, Zhang L, Schones DE, Wu X, Wang J, Tompkins JD, Genuth S, Braffett BH, Riggs AD, DCCT/EDIC Research Group. Natarajan R. Epigenomic profiling reveals an association between persistence of DNA methylation and metabolic memory in the DCCT/EDIC type 1 diabetes cohort. Proc Natl Acad Sci U S A. 2016; 113:E3002–E3011. [PubMed: 27162351]

Cheung AT, Ramanujam S, Greer DA, Kumagai LF, Aoki TT. Microvascular abnormalities in the bulbar conjunctiva of patients with type 2 diabetes mellitus. Endocr Pract. 2001; 7:358–363. [PubMed: 11585371]

Chiambo S, Baílez Fidalgo C, Pastor Jimeno JC, Coco Martín RM, Rodríguez de la Rúa Franch E, De la Fuente Salinero MA, Herreras Cantalapiedra JM. [Corneal epithelial complications after vitrectomy: a retrospective study] (in Spanish). Arch Soc Esp Oftalmol. 2004; 79:155–161. [PubMed: 15124071]

Chikama T, Wakuta M, Liu Y, Nishida T. Deviated mechanism of wound healing in diabetic corneas. Cornea. 2007; 26(9 Suppl 1):S75–S81. [PubMed: 17881921]

Chung H, Tolentino FI, Cajita VN, Acosta J, Refojo MF. Reevaluation of corneal complications after closed vitrectomy. Arch Ophthalmol. 1988; 106:916–919. [PubMed: 3390054]

Cisarik-Fredenburg P. Discoveries in research on diabetic keratopathy. Optometry. 2001; 72:691–704. [PubMed: 12363257]

Cobo-Soriano R, Beltrán J, Baviera J. LASIK outcome in patients with underlying systemic contraindications: a preliminary study. Ophthalmology. 2006; 113:1118.e1–e8. [PubMed: 16647130]

Costin JA. Is laser vision correction safe in patients with diabetes? Cleve Clin J Med. 2001; 68:385. [PubMed: 11352317]

Cousen P, Cackett P, Bennett H, Swa K, Dhillon B. Tear production and corneal sensitivity in diabetes. J Diabetes Complications. 2007; 21:371–373. [PubMed: 17967709]

Cruzat A, Qazi Y, Hamrah P. In vivo confocal microscopy of corneal nerves in health and disease. Ocul Surf. 2017; 15:15–47. [PubMed: 27771327]

Datiles MB, Kador PF, Fukui HN, Hu TS, Kinoshita JH. Corneal reepithelialization in galactosemic rats. Invest Ophthalmol Vis Sci. 1983; 24:563–569. [PubMed: 6841002]

Davidson EP, Coppey LJ, Kardon RH, Yorek MA. Differences and similarities in development of corneal nerve damage and peripheral neuropathy and in diet-induced obesity and type 2 diabetic rats. Invest Ophthalmol Vis Sci. 2014; 55:1222–1230. [PubMed: 24519423]

Davidson EP, Holmes A, Coppey LJ, Yorek MA. Effect of combination therapy consisting of enalapril, α-lipoic acid, and menhaden oil on diabetic neuropathy in a high fat/low dose streptozotocin treated rat. Eur J Pharmacol. 2015; 765:258–267. [PubMed: 26291662]

De Cillà S, Ranno S, Carini E, Fogagnolo P, Ceresara G, Orzalesi N, Rossetti L. Corneal subbasal nerves changes in patients with diabetic retinopathy: an in vivo confocal study. Invest Ophthalmol Vis Sci. 2009; 50:5155–5158. [PubMed: 19553615]

Dedova IV, Nikolaeva OP, Safer D, De La Cruz EM, dos Remedios CG. Thymosin β4 induces a conformational change in actin monomers. Biophys J. 2006; 90:985–992. [PubMed: 16272441]

DeMill DL, Hussain M, Pop-Busui R, Shtein RM. Ocular surface disease in patients with diabetic peripheral neuropathy. Br J Ophthalmol. 2016; 100:924–928. [PubMed: 26500330]

Devasahayam R, Georges P, Hodge C, Treloggen J, Cooper S, Petsoglou C, Sutton G, Zhu M. Implementation of organ culture storage of donor corneas: a 3 year study of its impact on the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

corneal transplant wait list at the Lions New South Wales Eye Bank. Cell Tissue Bank. 2016; 17:377–385. [PubMed: 27142497]

Dhasmana R, Singh IP, Nagpal RC. Corneal changes in diabetic patients after manual small incision cataract surgery. J Clin Diagn Res. 2014; 8:VC03–VC06. [PubMed: 24959498]

Dhoot DS, Avery RL. Vascular endothelial growth factor inhibitors for diabetic retinopathy. Curr Diab Rep. 2016; 16:122. [PubMed: 27766582]

Didenko TN, Smoliakova GP, Sorokin EL, Egorov VV. [Clinical and pathogenetic features of neurotrophic corneal disorders in diabetes] (in Russian). Vestn Oftalmol. 1999; 115:7–11.

Dogru M, Kaderli B, Gelisken O, Yücel A, Avci R, Goto E, Shimmura S, Shimazaki J, Tsubota K. Ocular surface changes with applanation contact lens and coupling fluid use after argon laser photocoagulation in noninsulin-dependent diabetes mellitus. Am J Ophthalmol. 2004; 138:381–388. [PubMed: 15364219]

Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmology. 2001; 108:586–592. [PubMed: 11237914]

Dorrell MI, Aguilar E, Scheppke L, Barnett FH, Friedlander M. Combination angiostatic therapy completely inhibits ocular and tumor angiogenesis. Proc Natl Acad Sci USA. 2007; 104:967–972. [PubMed: 17210921]

Ducrey N. [Ocular lesions due to diabetes] (in French). Schweiz Med Wochenschr. 1996; 126:1610–1612. [PubMed: 8927966]

Dunn SP, Heidemann DG, Chow CY, Crockford D, Turjman N, Angel J, Allan CB, Sosne G. Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta4. Arch Ophthalmol. 2010; 128:636–638. [PubMed: 20457990]

Eisma JH, Dulle JE, Fort PE. Current knowledge on diabetic retinopathy from human donor tissues. World J Diabetes. 2015; 6:312–320. [PubMed: 25789112]

El-Agamy A, Alsubaie S. Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus. Clin Ophthalmol. 2017; 11:481–486. [PubMed: 28280298]

Foulks GN, Thoft RA, Perry HD, Tolentino FI. Factors related to corneal epithelial complications after closed vitrectomy in diabetics. Arch Ophthalmol. 1979; 97:1076–1078. [PubMed: 444136]

Fraunfelder FW, Rich LF. Laser-assisted in situ keratomileusis complications in diabetes mellitus. Cornea. 2002; 21:246–248. [PubMed: 11917170]

Fujita H, Morita I, Takase H, Ohno-Matsui K, Mochizuki M. Prolonged exposure to high glucose impaired cellular behavior of normal human corneal epithelial cells. Curr Eye Res. 2003; 27:197–203. [PubMed: 14562170]

Funari VA, Winkler M, Brown J, Dimitrijevich SD, Ljubimov AV, Saghizadeh M. Differentially expressed wound healing-related microRNAs in the human diabetic cornea. PLoS One. 2013; 8:e84425. [PubMed: 24376808]

Gao J, Wang Y, Zhao X, Chen P, Xie L. MicroRNA-204-5p-mediated regulation of SIRT1 contributes to the delay of epithelial cell cycle traversal in diabetic corneas. Invest Ophthalmol Vis Sci. 2015; 56:1493–1504. [PubMed: 25613939]

Gao N, Yan C, Lee P, Sun H, Yu F. Dendritic cell dysfunction and diabetic sensory neuropathy in the cornea. J Clin Invest. 2016; 126:1998–2011. [PubMed: 27064280]

Gekka M, Miyata K, Nagai Y, Nemoto S, Sameshima T, Tanabe T, Maruoka S, Nakahara M, Kato S, Amano S. Corneal epithelial barrier function in diabetic patients. Cornea. 2004; 23:35–37. [PubMed: 14701955]

Ghanbari H, Ahmadieh H. Aggravation of prolidferative diabetic retinopathy after laser in situ keratomileusis. J Cataract Refract Surg. 2003; 29:2232–2233. [PubMed: 14670438]

Greiner MA, Rixen JJ, Wagoner MD, Schmidt GA, Stoeger CG, Straiko MD, Zimmerman MB, Kitzmann AS, Goins KM. Diabetes mellitus increases risk of unsuccessful graft preparation in Descemet membrane endothelial keratoplasty: a multicenter study. Cornea. 2014; 33:1129–1133. [PubMed: 25222000]

Gül M, Emre S, Eşrefoğlu M, Vard N. Protective effects of melatonin and aminoguanidine on the cornea in streptozotocin-induced diabetic rats. Cornea. 2008; 27:795–801. [PubMed: 18650666]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gunay M, Celik G, Yildiz E, Bardak H, Koc N, Kirmizibekmez H, Gunay BO, Yesiltepe Mutlu RG. Ocular surface characteristics in diabetic children. Curr Eye Res. 2016; 41:1526–1531. [PubMed: 27159168]

Halkiadakis I, Belfair N, Gimbel HV. Laser in situ keratomileusis in patients with diabetes. J Cataract Refract Surg. 2005; 31:1895–1898. [PubMed: 16338557]

Hatchell DL, Magolan JJ Jr, Besson MJ, Goldman AI, Pederson HJ, Schultz KJ. Damage to the epithelial basement membrane in the corneas of diabetic rabbits. Arch Ophthalmol. 1983; 101:469–471. [PubMed: 6830506]

Hatchell DL, Ubels JL, Stekiel T, Hatchell MC. Corneal epithelial wound healing in normal and diabetic rabbits treated with tretinoin. Arch Ophthalmol. 1985; 103:98–100. [PubMed: 3977683]

He J, Bazan HE. Mapping the nerve architecture of diabetic human corneas. Ophthalmology. 2012; 119:956–964. [PubMed: 22325488]

Helbig H. Surgery for diabetic retinopathy. Ophthalmologica. 2007; 221:103–111. [PubMed: 17380064]

Henricsson M, Nilsson A, Janzon L, Groop L. The effect of glycemic control and the introduction of insulin therapy on retinopathy in non-insulin dependent diabetes mellitus. Diabet Med. 1997; 14:123–131. [PubMed: 9047089]

Herse PR. A review of manifestations of diabetes mellitus in the anterior eye and cornea. Am J Optom Physiol Opt. 1988; 65:224–230. [PubMed: 3284372]

Hiraoka M, Amano S, Oshika T, Kato S, Hori S. Factors contributing to corneal complications after vitrectomy in diabetic patients. Jpn J Ophthalmol. 2001; 45:492–495. [PubMed: 11583671]

Holland EJ. Management of limbal stem cell deficiency: a historical perspective, past, present, and future. Cornea. 2015; 34(Suppl 10):S9–S15. [PubMed: 26203759]

Hosotani H, Ohashi Y, Yamada M, Tsubota K. Reversal of abnormal corneal epithelial cell morphologic characteristics and reduced corneal sensitivity in diabetic patients by aldose reductase inhibitor, CT-112. Am J Ophthalmol. 1995; 119:288–294. [PubMed: 7872388]

Hota D, Srinivasan A, Dutta P, Bhansali A, Chakrabarti A. Off-label, low-dose naltrexone for refractory painful diabetic neuropathy. Pain Med. 2016; 17:790–791. [PubMed: 26814245]

Huang SP, Palla S, Ruzycki P, Varma RA, Harter T, Reddy GB, Petrash JM. Aldo-keto reductases in the eye. J Ophthalmol. 2010; 2010:521204. [PubMed: 20628518]

Hugod M, Storr-Paulsen A, Norregaard JC, Nicolini J, Larsen AB, Thulesen J. Corneal endothelial cell changes associated with cataract surgery in patients with type 2 diabetes mellitus. Cornea. 2011; 30:749–753. [PubMed: 21317781]

Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: Importance in wound healing and maintenance of transparency of the cornea. Prog Ret Eye Res. 2000; 19:113–129.

Inoue K, Kato S, Inoue Y, Amano S, Oshika T. The corneal endothelium and thickness in type II diabetes mellitus. Jpn J Ophthalmol. 2002; 46:65–69. [PubMed: 11853716]

Inoue K, Kato S, Ohara C, Numaga J, Amano S, Oshika T. Ocular and systemic factors relevant to diabetic keratoepitheliopathy. Cornea. 2001; 20:798–801. [PubMed: 11685054]

Jabbur NS, Chicani CF, Kuo IC, O'Brien TP. Risk factors in interface epithelialization after laser in situ keratomileusis. J Refract Surg. 2004; 20:343–348. [PubMed: 15307396]

Janiec S, Rzendkowski M, Bolek S. The relation between corneal autofluorescence, endothelial cell count and severity of the diabetic retinopathy. Int Ophthalmol. 1994-1995; 18:205–209.

Jo N, Mailhos C, Ju M, Cheung E, Bradley J, Nishijima K, Robinson GS, Adamis AP, Shima DT. Inhibition of platelet-derived growth factor B signaling enhances the efficacy of anti-vascular endothelial growth factor therapy in multiple models of ocular neovascularization. Am J Pathol. 2006; 168:2036–2053. [PubMed: 16723717]

Jonas JB. Intravitreal triamcinolone acetonide for diabetic retinopathy. Dev Ophthalmol. 2007; 39:96–110. [PubMed: 17245081]

Kaji Y. Prevention of diabetic keratopathy. Br J Ophthalmol. 2005; 89:254–255. [PubMed: 15722297]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Kaji Y, Amano S, Usui T, Suzuki K, Tanaka S, Oshika T, Nagai R, Horiuchi S. Advanced glycation end products in Descemet's membrane and their effect on corneal endothelial cell. Curr Eye Res. 2001; 23:469–477. [PubMed: 12045898]

Kaji Y, Usui T, Oshika T, Matsubara M, Yamashita H, Araie M, Murata T, Ishibashi T, Nagai R, Horiuchi S, Amano S. Advanced glycation end products in diabetic corneas. Invest Ophthalmol Vis Sci. 2000; 41:362–368. [PubMed: 10670463]

Kakazu A, Chandrasekher G, Bazan HE. HGF protects corneal epithelial cells from apoptosis by the PI-3K/Akt-1/Bad- but not the ERK1/2-mediated signaling pathway. Invest Ophthalmol Vis Sci. 2004; 45:3485–3492. [PubMed: 15452053]

Kakazu A, Sharma G, Bazan HE. Association of protein tyrosine phosphatases (PTPs)-1B with c-Met receptor and modulation of corneal epithelial wound healing. Invest Ophthalmol Vis Sci. 2008; 49:2927–2935. [PubMed: 18579758]

Kallinikos P, Berhanu M, O'Donnell C, Boulton AJ, Efron N, Malik RA. Corneal nerve tortuosity in diabetic patients with neuropathy. Invest Ophthalmol Vis Sci. 2004; 45:418–422. [PubMed: 14744880]

Kaplan N, Fatima A, Peng H, Bryar PJ, Lavker RM, Getsios S. EphA2/Ephrin-A1 signaling complexes restrict corneal epithelial cell migration. Invest Ophthalmol Vis Sci. 2012; 53:936–945. [PubMed: 22247486]

Karamichos D. Corneal diabetes: where to next? J Clin Res Ophthalmol. 2015; 2:065–066.

Kim J, Kim CS, Sohn E, Jeong IH, Kim H, Kim JS. Involvement of advanced glycation end products, oxidative stress and nuclear factor-kappaB in the development of diabetic keratopathy. Graefes Arch Clin Exp Ophthalmol. 2011; 249:529–536. [PubMed: 21104267]

Klein, R. The Epidemiology of Diabetic Retinopathy. In: Duh, E., editor. Contemporary Diabetes: Diabetic Retinopathy. Humana Press; Totowa: 2008. p. 67-107.

Klenkler B, Sheardown H. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Exp Eye Res. 2004; 79:677–688. [PubMed: 15500826]

Kotecha A, Oddone F, Sinapis C, Elsheikh A, Sinapis D, Sinapis A, Garway-Heath DF. Corneal biomechanical characteristics in patients with diabetes mellitus. J Cataract Refract Surg. 2010; 36:1822–1828. [PubMed: 21029887]

Kowluru RA, Kowluru A, Mishra M, Kumar B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. Prog Retin Eye Res. 2015; 48:40–61. [PubMed: 25975734]

Kramerov AA, Ljubimov AV. Stem cell therapies in the treatment of diabetic retinopathy and keratopathy. Exp Biol Med (Maywood). 2016; 241:559–568. [PubMed: 26454200]

Kramerov AA, Saghizadeh M, Ljubimov AV. Adenoviral gene therapy for diabetic keratopathy: effects on wound healing and stem cell marker expression in human organ-cultured corneas and limbal epithelial cells. J Vis Exp. 2016; 7:e54058.

Kramerov AA, Saghizadeh M, Maguen E, Rabinowitz YS, Ljubimov AV. Persistence of reduced expression of putative stem cell markers and slow wound healing in cultured diabetic limbal epithelial cells. Mol Vis. 2015; 21:1357–1367. [PubMed: 26788028]

Kramerov AA, Saghizadeh M, Pan H, Kabosova A, Montenarh M, Ahmed K, Penn JS, Chan CK, Hinton DR, Grant MB, Ljubimov AV. Expression of protein kinase CK2 in astroglial cells of normal and neovascularized retina. Am J Pathol. 2006; 168:1722–1736. [PubMed: 16651637]

Lang GE. Pharmacological treatment of diabetic retinopathy. Ophthalmologica. 2007; 221:112–117. [PubMed: 17380065]

Langwińska-Wośko E, Chociszewska-Nitka A, Zielińska E, Hapunik A. [Evaluation of corneal endothelium following cataract surgery in diabetic patients] (in Polish). Klin Oczna. 2004; 106:28–30. [PubMed: 15218760]

Larsson LI, Bourne WM, Pach JM, Brubaker RF. Structure and function of the corneal endothelium in diabetes mellitus type I and type II. Arch Ophthalmol. 1996; 114:9–14. [PubMed: 8540858]

Lass JH, Riddlesworth TD, Gal RL, Kollman C, Benetz BA, Price FW Jr, Sugar A, Terry MA, Soper M, Beck RW. Cornea Donor Study Research Group. The effect of donor diabetes history on graft failure and endothelial cell density 10 years after penetrating keratoplasty. Ophthalmology. 2015; 122:448–56. [PubMed: 25439611]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Lee HK, Lee JH, Kim M, Kariya Y, Miyazaki K, Kim EK. Insulin-like growth factor-1 induces migration and expression of laminin-5 in cultured human corneal epithelial cells. Invest Ophthalmol Vis Sci. 2006; 47:873–882. [PubMed: 16505019]

Lee JS, Lee JE, Choi HY, Oum BS, Cho BM. Corneal endothelial cell change after phacoemulsification relative to the severity of diabetic retinopathy. J Cataract Refract Surg. 2005; 31:742–749. [PubMed: 15899451]

Leppin K, Behrendt AK, Reichard M, Stachs O, Guthoff RF, Baltrusch S, Eule JC, Vollmar B. Diabetes mellitus leads to accumulation of dendritic cells and nerve fiber damage of the subbasal nerve plexus in the cornea. Invest Ophthalmol Vis Sci. 2014; 55:3603–3615. [PubMed: 24781935]

Li Q, Weng J, Mohan RR, Bennett GL, Schwall R, Wang ZF, Tabor K, Kim J, Hargrave S, Cuevas KH, Wilson SE. Hepatocyte growth factor and hepatocyte growth factor receptor in the lacrimal gland, tears, and cornea. Invest Ophthalmol Vis Sci. 1996; 37:727–739. [PubMed: 8603858]

Li DQ, Tseng SC. Three patterns of cytokine expression potentially involved in epithelial-fibroblast interactions of human ocular surface. J Cell Physiol. 1995; 163:61–79. [PubMed: 7896901]

Liaboe CA, Aldrich BT, Carter PC, Skeie JM, Burckart KA, Schmidt GA, Reed CR, Zimmerman MB, Greiner MA. Assessing the impact of diabetes mellitus on donor corneal endothelial cell density. Cornea Mar. 2017; 16 Epub ahead of print. doi: 10.1097/ICO.0000000000001174

Liu H, Sheng M, Liu Y, Wang P, Chen Y, Chen L, Wang W, Li B. Expression of SIRT1 and oxidative stress in diabetic dry eye. Int J Clin Exp Pathol. 2015; 8:7644–7653. [PubMed: 26261685]

Ljubimov AV, Saghizadeh M. Progress in corneal wound healing. Prog Retin Eye Res. 2015; 49:17–45. [PubMed: 26197361]

Ljubimov, AV., Boulton, M., Caballero, S., Grant, MB. Antagonism of the growth hormone axis as a therapeutic strategy for diabetic retinopathy. In: Duh, E., editor. Contemporary Diabetes: Diabetic Retinopathy. Humana Press; Totowa, NJ: 2008. p. 449-463.

Ljubimov AV, Burgeson RE, Butkowski RJ, Couchman JR, Zardi L, Ninomiya Y, Sado Y, Huang Z, Nesburn AB, Kenney MC. Basement membrane abnormalities in human eyes with diabetic retinopathy. J Histochem Cytochem. 1996; 44:1469–1479. [PubMed: 8985139]

Lockwood A, Hope-Ross M, Chell P. Neurotrophic keratopathy and diabetes mellitus. Eye. 2006; 20:837–839. [PubMed: 16215544]

Lu W, Ebihara N, Miyazaki K, Murakami A. Reduced expression of laminin-5 in corneal epithelial cells under high glucose condition. Cornea. 2006; 25:61–67. [PubMed: 16331044]

Lv H, Li A, Zhang X, Xu M, Qiao Y, Zhang J, Yu L. Meta-analysis and review on the changes of tear function and corneal sensitivity in diabetic patients. Acta Ophthalmol. 2014; 92:e96–e104. [PubMed: 23782539]

Madonna R, Balistreri CR, Geng YJ, De Caterina R. Diabetic microangiopathy: Pathogenetic insights and novel therapeutic approaches. Vascul Pharmacol. 2017; 90:1–7. [PubMed: 28137665]

Mahgoub MM, Macky TA. Changes in corneal sensation following 20 and 23 G vitrectomy in diabetic and non-diabetic patients. Eye (Lond). 2014; 28:1286–1291. [PubMed: 25033900]

Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ. Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia. 2003; 46:683–688. [PubMed: 12739016]

Manaviat MR, Rashidi M, Afkhami-Ardekani M, Shoja MR. Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients. BMC Ophthalmology. 2008; 8:10. [PubMed: 18513455]

Mankus C, Rich C, Minns M, Trinkaus-Randall V. Corneal epithelium expresses a variant of $P2X_7$ receptor in health and disease. PLoS One. 2011; 6:e28541. [PubMed: 22163032]

Martins EN, Alvarenga LS, Höfling-Lima AL, Freitas D, Zorat-Yu MC, Farah ME, Mannis MJ. Aerobic bacterial conjunctival flora in diabetic patients. Cornea. 2004; 23:136–142. [PubMed: 15075882]

Matsuda M, Ohguro N, Ishimoto I, Fukuda M. Relationship of corneal endothelial morphology to diabetic retinopathy, duration of diabetes and glycemic control. Jpn J Ophthalmol. 1990; 34:53–56. [PubMed: 2362374]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

McDermott AM, Xiao TL, Kern TS, Murphy CJ. Non-enzymatic glycation in corneas from normal and diabetic donors and its effects on epithelial cell attachment in vitro. Optometry. 2003; 74:443–452. [PubMed: 12877277]

McLaughlin PJ, Sassani JW, Klocek MS, Zagon IS. Diabetic keratopathy and treatment by modulation of the opioid growth factor (OGF)-OGF receptor (OGFr) axis with naltrexone: a review. Brain Res Bull. 2010; 81:236–247. [PubMed: 19683562]

Minns MS, Trinkaus-Randall V. Purinergic signaling in corneal wound healing: a tale of 2 receptors. J Ocul Pharmacol Ther. 2016; 32:498–503. [PubMed: 27643999]

Misra SL, Braatvedt GD, Patel DV. Impact of diabetes mellitus on the ocular surface: a review. Clin Exp Ophthalmol. 2016; 44:278–288. [PubMed: 26661628]

Mocan MC, Durukan I, Irkec M, Orhan M. Morphologic alterations of both the stromal and subbasal nerves in the corneas of patients with diabetes. Cornea. 2006; 25:769–773. [PubMed: 17068451]

Módis, LJr, Szalai, E., Kertész, K., Kemény-Beke, A., Kettesy, B., Berta, A. Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II. Histol Histopathol. 2010; 25:1531–1537. [PubMed: 20886433]

Módulo CM, Jorge AG, Dias AC, Braz AM, Bertazolli-Filho R, Jordão AA Jr, Marchini JS, Rocha EM. Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats. Endocrine. 2009; 36:161–168. [PubMed: 19551521]

Mohammadpour M. LASIK and systemic contraindications. Ophthalmology. 2007; 114:1032–1033. [PubMed: 17467541]

Morikubo S, Takamura Y, Kubo E, Tsuzuki S, Akagi Y. Corneal changes after small-incision cataract surgery in patients with diabetes mellitus. Arch Ophthalmol. 2004; 122:966–969. [PubMed: 15249359]

Murphy PJ, Patel S, Kong N, Ryder RE, Marshall J. Noninvasive assessment of corneal sensitivity in young and elderly diabetic and nondiabetic subjects. Invest Ophthalmol Vis Sci. 2010; 45:1737–1742.

Murtha T, Cavallerano J. The management of diabetic eye disease in the setting of cataract surgery. Curr Opin Ophthalmol. 2007; 18:13–18. [PubMed: 17159441]

Nakahara M, Miyata K, Otani S, Miyai T, Nejima R, Yamagami S, Amano S. A randomised, placebo controlled clinical trial of the aldose reductase inhibitor CT-112 as management of corneal epithelial disorders in diabetic patients. Br J Ophthalmol. 1995; 89:266–268.

Nakamura M, Kanamori A, Negi A. Diabetes mellitus as a risk factor for glaucomatous optic neuropathy. Ophthalmologica. 2005; 219:1–10. [PubMed: 15627820]

Nakamura M, Kawahara M, Morishige N, Chikama T, Nakata K, Nishida T. Promotion of corneal epithelial wound healing in diabetic rats by the combination of a substance P-derived peptide (FGLM-NH2) and insulin-like growth factor-1. Diabetologia. 2003; 46:839–842. [PubMed: 12764579]

Nakamura Y, Sotozono C, Kinoshita S. The epidermal growth factor receptor (EGFR): role in corneal wound healing and homeostasis. Exp Eye Res. 2001; 72:511–517. [PubMed: 11311043]

Negi A, Vernon SA. An overview of the eye in diabetes. J R Soc Med. 2003; 96:266–272. [PubMed: 12782689]

Nepp J, Abela C, Derbolav A, Wedrich A. Is there a correlation between the severity of diabetic retinopathy and keratoconjunctivitis sicca? Cornea. 2000; 19:487–491. [PubMed: 10928764]

Nielsen NV. Corneal sensitivity and vibratory perception in diabetes mellitus. Acta Ophthalmol (Copenh). 1978; 56:406–411. [PubMed: 581131]

Ohashi Y. [Diabetic keratopathy] (in Japanese). Nippon Ganka Gakkai Zasshi. 1997; 101:105–110. [PubMed: 9124089]

Örnek N, Örnek K, Erbahçeci İE. Corneal and conjunctival sensitivity changes following intravitreal ranibizumab injection in diabetic retinopathy. J Ocul Pharmacol Ther. 2015; 31:37–42. [PubMed: 25244301]

Owen CG, Newsom RSB, Rudnicka AR, Ellis TJ, Woodward EG. Vascular response of the bulbar conjunctiva to diabetes and elevated blood pressure. Ophthalmology. 2005; 112:1801–1808. [PubMed: 16111757]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Papanas N, Ziegler D. Corneal confocal microscopy: a new technique for early detection of diabetic neuropathy. Curr Diab Rep. 2013; 13:488–499. [PubMed: 23666893]

Park JH, Kang SS, Kim JY, Tchah H. Nerve growth factor attenuates apoptosis and inflammation in the diabetic cornea. Invest Ophthalmol Vis Sci. 2016; 57:6767–6775. [PubMed: 27978558]

Petropoulos IN, Green P, Chan AW, Alam U, Fadavi H, Marshall A, Asghar O, Efron N, Tavakoli M, Malik RA. Corneal confocal microscopy detects neuropathy in patients with type 1 diabetes without retinopathy or microalbuminuria. PLoS One. 2015; 10:e0123517. [PubMed: 25853247]

Priyadarsini S, McKay TB, Sarker-Nag A, Allegood J, Chalfant C, Ma JX, Karamichos D. Complete metabolome and lipidome analysis reveals novel biomarkers in the human diabetic corneal stroma. Exp Eye Res. 2016a; 153:90–100. [PubMed: 27742548]

Priyadarsini S, Sarker-Nag A, Allegood J, Chalfant C, Karamichos D. Description of the sphingolipid content and subspecies in the diabetic cornea. Curr Eye Res. 2015; 40:1204–1210. [PubMed: 25426847]

Priyadarsini S, Sarker-Nag A, Rowsey TG, Ma JX, Karamichos D. Establishment of a 3D *in vitro* model to accelerate the development of human therapies against corneal diabetes. PLoS One. 2016b; 11:e0168845. [PubMed: 28005998]

Quadrado MJ, Popper M, Morgado AM, Murta JN, Van Best JA. Diabetes and corneal cell densities in humans by in vivo confocal microscopy. Cornea. 2006; 25:761–768. [PubMed: 17068450]

Rehany U, Ishii Y, Lahav M, Rumelt S. Collagen pleomorphism in Descemet's membrane of streptozotocin-induced diabetic rats: an electron microscopy study. Cornea. 2000a; 19:390–392. [PubMed: 10832705]

Rehany U, Ishii Y, Lahav M, Rumelt S. Ultrastructural changes in corneas of diabetic patients: an electron-microscopy study. Cornea. 2000b; 19:534–538. [PubMed: 10928773]

Rosenberg ME, Tervo TM, Immonen IJ, Muller LJ, Gronhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. Invest Ophthalmol Vis Sci. 2000; 41:2915–2921. [PubMed: 10967045]

Roszkowska AM, Tringali CG, Colosi P, Squeri CA, Ferreri G. Corneal endothelium evaluation in type I and type II diabetes mellitus. Ophthalmologica. 1999; 213:258–261. [PubMed: 10420110]

Ruberte J, Ayuso E, Navarro M, Carretero A, Nacher V, Haurigot V, George M, Llombart C, Casellas A, Costa C, Bosch A, Bosch F. Increased ocular levels of IGF-1 in transgenic mice lead to diabetes-like eye disease. J Clin Invest. 2004; 113:1149–1157. [PubMed: 15085194]

Sady C, Khosrof S, Nagaraj R. Advanced Maillard reaction and crosslinking of corneal collagen in diabetes. Biochem Biophys Res Commun. 1995; 214:793–797. [PubMed: 7575546]

Saghizadeh M, Brown DJ, Castellon R, Chwa M, Huang GH, Ljubimova JY, Rosenberg S, Spirin KS, Stolitenko RB, Adachi W, Kinoshita S, Murphy G, Windsor LJ, Kenney MC, Ljubimov AV. Overexpression of matrix metalloproteinase-10 and matrix metalloproteinase-3 in human diabetic corneas: a possible mechanism of basement membrane and integrin alterations. Am J Pathol. 2001a; 158:723–734. [PubMed: 11159210]

Saghizadeh M, Chwa M, Aoki A, Lin B, Pirouzmanesh A, Brown DJ, Ljubimov AV, Kenney MC. Altered expression of growth factors and cytokines in keratoconus, bullous keratopathy and diabetic human corneas. Exp Eye Res. 2001b; 73:179–189. [PubMed: 11446768]

Saghizadeh M, Dib CM, Brunken WJ, Ljubimov AV. Normalization of wound healing and stem cell marker patterns in organ-cultured human diabetic corneas by gene therapy of limbal cells. Exp Eye Res. 2014; 129:66–73. [PubMed: 25446319]

Saghizadeh M, Epifantseva I, Hemmati DM, Ghiam CA, Brunken WJ, Ljubimov AV. Enhanced wound healing, kinase and stem cell marker expression in diabetic organ-cultured human corneas upon MMP-10 and cathepsin F gene silencing. Invest Ophthalmol Vis Sci. 2013; 54:8172–8180. [PubMed: 24255036]

Saghizadeh M, Kramerov AA, Tajbakhsh J, Aoki AM, Wang C, Chai NN, Ljubimova JY, Sasaki T, Sosne G, Carlson MR, Nelson SF, Ljubimov AV. Proteinase and growth factor alterations revealed by gene microarray analysis of human diabetic corneas. Invest Ophthalmol Vis Sci. 2005; 46:3604–3615. [PubMed: 16186340]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Saghizadeh M, Kramerov AA, Yaghoobzadeh Y, Hu J, Ljubimova JY, Black KL, Castro MG, Ljubimov AV. Adenovirus-driven overexpression of proteinases in organ-cultured normal human corneas leads to diabetic-like changes. Brain Res Bull. 2010a; 81:262–272. [PubMed: 19828126]

Saghizadeh M, Kramerov AA, Yu FS, Castro MG, Ljubimov AV. Normalization of wound healing and diabetic markers in organ cultured human diabetic corneas by adenoviral delivery of *c-Met* gene. Invest Ophthalmol Vis Sci. 2010b; 51:1970–1980. [PubMed: 19933191]

Saghizadeh M, Soleymani S, Harounian A, Bhakta B, Troyanovsky SM, Brunken WJ, Pellegrini G, Ljubimov AV. Alterations of epithelial stem cell marker patterns in human diabetic corneas and effects of *c-met* gene therapy. Mol Vis. 2011; 17:2177–2190. [PubMed: 21866211]

Sahin A, Bayer A, Ozge G, Mumcuoğlu T. Corneal biomechanical changes in diabetes mellitus and their influence on intraocular pressure measurements. Invest Ophthalmol Vis Sci. 2009; 50:4597–4604. [PubMed: 19443722]

Saini JS, Khandalavla B. Corneal epithelial fragility in diabetes mellitus. Can J Ophthalmol. 1995; 30:142–146. [PubMed: 7627899]

Saini JS, Mittal S. Graded corneal sensitivity for screening of diabetic retinopathy. Indian J Ophthalmol. 1996a; 44:219–223. [PubMed: 9251266]

Saini JS, Mittal S. In vivo assessment of corneal endothelial function in diabetes mellitus. Arch Ophthalmol. 1996b; 114:649–653. [PubMed: 8639073]

Saito J, Enoki M, Hara M, Morishige N, Chikama T, Nishida T. Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy. Cornea. 2003; 22:15–18. [PubMed: 12502941]

Sakamoto A, Sasaki H, Kitagawa K. Successful treatment of diabetic keratopathy with punctal occlusion. Acta Ophthalmol Scand. 2004; 82:115–117. [PubMed: 14738499]

Sánchez-Thorin JC. The cornea in diabetes mellitus. Int Ophthalmol Clin. 1998; 38:19–36. [PubMed: 9604736]

Sassani JW, Mc Laughlin PJ, Zagon IS. The Yin and Yang of the opioid growth regulatory system: focus on diabetes-The Lorenz E. Zimmerman Tribute Lecture. J Diabetes Res. 2016; 2016:9703729. [PubMed: 27703986]

Sato N, Nakamura M, Chikama T, Nishida T. Abnormal deposition of laminin and type IV collagen at corneal epithelial basement membrane during wound healing in diabetic rats. Jpn J Ophthalmol. 1999; 43:343–347. [PubMed: 10580654]

Scheler A, Spoeri E, Boehm AG. Effect of diabetes mellitus on corneal biomechanics and measurement of intraocular pressure. Acta Ophthalmol. 2012; 90:e447–e451. [PubMed: 22691299]

Schultz RO, Peters MA, Sobocinski K, Nassif K, Schultz KJ. Diabetic keratopathy as a manifestation of peripheral neuropathy. Am J Ophthalmol. 1983; 96:368–371. [PubMed: 6311017]

Schultz RO, Van Horn DL, Peters MA, Klewin KM, Schutten WH. Diabetic keratopathy. Trans Am Ophthalmol Soc. 1981; 79:180–199. [PubMed: 7342400]

Schulze SD, Sekundo W, Kroll P. Autologous serum for the treatment of corneal epithelial abrasions in diabetic patients undergoing vitrectomy. Am J Ophthalmol. 2006; 142:207–211. [PubMed: 16876497]

Seiler T, Huhle S, Spoeri E, Kunath E. Manifest diabetes and keratoconus: a retrospective case-control study. Graefes Arch Clin Exp Ophthalmol. 2000; 238:822–825. [PubMed: 11127568]

Shenoy R, Khandekar R, Bialasiewicz A, Al Muniri A. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. Eur J Ophthalmol. 2009; 19:369–375. [PubMed: 19396780]

Shi L, Chen H, Yu X, Wu X. Advanced glycation end products delay corneal epithelial wound healing through reactive oxygen species generation. Mol Cell Biochem. 2013a; 383:253–259. [PubMed: 23955437]

Shi L, Yu X, Yang H, Wu X. Advanced glycation end products induce human corneal epithelial cells apoptosis through generation of reactive oxygen species and activation of JNK and p38 MAPK pathways. PLoS One. 2013b; 8:e66781. [PubMed: 23776698]

Shih KC, Lam KS, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. Nutr Diabetes. 2017; 7:e251. [PubMed: 28319106]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Smith LE, Shen W, Perruzzi C, Soker S, Kinose F, Xu X, Robinson G, Driver S, Bischoff J, Zhang B, Schaeffer JM, Senger DR. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. Nat Med. 1999; 5:1390–1395. [PubMed: 10581081]

Song F, Xue Y, Dong D, Liu J, Fu T, Xiao C, Wang H, Lin C, Liu P, Zhong J, Yang Y, Wang Z, Pan H, Chen J, Li Y, Cai D, Li Z. Insulin restores an altered corneal epithelium circadian rhythm in mice with streptozotocin-induced type 1 diabetes. Sci Rep. 2016; 6:32871. [PubMed: 27611469]

Sosne G, Qiu P, Kurpakus-Wheater M, Matthew H. Thymosin β4 and corneal wound healing: visions of the future. Ann N Y Acad Sci. 2010; 1194:190–198. [PubMed: 20536468]

Sosne G, Qiu P, Kurpakus-Wheater M. Thymosin β-4 and the eye: I can see clearly now the pain is gone. Ann N Y Acad Sci. 2007; 1112:114–122. [PubMed: 17495249]

Sosne G, Rimmer D, Kleinman HK, Ousler G. Thymosin beta 4: a potential novel therapy for neurotrophic keratopathy, dry eye, and ocular surface diseases. Vitam Horm. 2016; 102:277–306. [PubMed: 27450739]

Sosne G, Xu L, Prach L, Mrock LK, Kleinman HK, Letterio JJ, Hazlett LD, Kurpakus-Wheater M. Thymosin beta 4 stimulates laminin-5 production independent of TGF-beta. Exp Cell Res. 2004; 293:175–183. [PubMed: 14729067]

Stem MS, Hussain M, Lentz SI, Raval N, Gardner TW, Pop-Busui R, Shtein RM. Differential reduction of corneal nerve fiber length in patients with type 1 or type 1 diabetes mellitus. J Diabetes Complications. 2014; 28:658–661. [PubMed: 25044236]

Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res. 2016; 51:156–86. [PubMed: 26297071]

Su DH, Wong TY, Wong WL, Saw SM, Tan DT, Shen SY, Loon SC, Foster PJ, Aung T. Singapore Malay Eye Study Group. Diabetes, hyperglycemia, and central corneal thickness: the Singapore Malay Eye Study. Ophthalmology. 2008; 115:964–968. [PubMed: 17964654]

Szalai E, Deák E, Módis L Jr, Németh G, Berta A, Nagy A, Felszeghy E, Káposzta R, Malik RA, Csutak A. Early corneal cellular and nerve fiber pathology in young patients with type 1 diabetes mellitus identified using corneal confocal microscopy. Invest Ophthalmol Vis Sci. 2016; 57:853–858. [PubMed: 26943147]

Tabatabay CA, Bumbacher M, Baumgartner B, Leuenberger PM. Reduced number of hemidesmosomes in the corneal epithelium of diabetics with proliferative vitreoretinopathy. Graefe's Arch Clin Exp Ophthalmol. 1988; 226:389–392. [PubMed: 3169590]

Takahashi H, Akiba K, Noguchi T, Ohmura T, Takahashi R, Ezure Y, Ohara K, Zieske JD. Matrix metalloproteinase activity is enhanced during corneal wound repair in high glucose condition. Curr Eye Res. 2000; 21:608–615. [PubMed: 11148597]

Takamura Y, Matsumoto T, Tomomatsu T, Matsumura T, Takihara Y, Inatani M. Aldose reductase inhibitor counteracts the enhanced expression of matrix metalloproteinase-10 and improves corneal wound healing in galactose-fed rats. Mol Vis. 2013; 19:2477–2486. [PubMed: 24339723]

Tavakoli M, Kallinikos PA, Efron N, Boulton AJ, Malik RA. Corneal sensitivity is reduced and relates to the severity of neuropathy in patients with diabetes. Diabetes Care. 2007; 30:1895–1897. [PubMed: 17372147]

To WJ, Telander DG, Lloyd ME, Chen PC, Cheung AT. Correlation of conjunctival microangiopathy with retinopathy in type-2 diabetes mellitus (T2DM) patients. Clin Hemorheol Microcirc. 2011; 47:131–141. [PubMed: 21339633]

Tomomatsu T, Takamura Y, Kubo E, Akagi Y. Aldose reductase inhibitor counteracts the attenuated adhesion of human corneal epithelial cells induced by high glucose through modulation of MMP-10 expression. Diabetes Res Clin Pract. 2009; 86:16–23. [PubMed: 19682763]

Tsaousis KT, Panagiotou DZ, Kostopoulou E, Vlatsios V, Stampouli D. Corneal oedema after phacoemulsification in the early postoperative period: A qualitative comparative case-control study between diabetics and non-diabetics. Ann Med Surg (Lond). 2015; 5:67–71. [PubMed: 26865977]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ueno H, Hattori T, Kumagai Y, Suzuki N, Ueno S, Takagi H. Alterations in the corneal nerve and stem/progenitor cells in diabetes: preventive effects of insulin-like growth factor-1 treatment. Int J Endocrinol. 2014; 2014:312401. [PubMed: 24696681]

Van Schaik HJ, Benitez del Castillo JM, Caubergh MJ, Gobert A, Leite E, Moldow B, Rosas V, Van Best JA. Evaluation of diabetic retinopathy by fluorophotometry European concerted action on ocular fluorometry. Int Ophthalmol. 1998-1999; 22:97–104.

Vieira-Potter VJ, Karamichos D, Lee DJ. Ocular complications of diabetes and therapeutic approaches. Biomed Res Int. 2016; 2016:3801570. [PubMed: 27119078]

Wang F, Gao N, Yin J, Yu FS. Reduced innervation and delayed reinnervation after epithelial wounding in type 2 diabetic Goto-Kakizaki rats. Am J Pathol. 2012; 181:2058–2066. [PubMed: 23063510]

Wang W, Despanie J, Shi P, Edman-Woolcott MC, Lin YA, Cui H, Heur JM, Fini ME, Hamm-Alvarez SF, MacKay JA. Lacritin-mediated regeneration of the corneal epithelia by protein polymer nanoparticles. J Mater Chem B Mater Biol Med. 2014; 2:8131–8141. [PubMed: 25530855]

Watanabe H, Katakami C, Miyata S, Negi A. Corneal disorders in KKAy mouse: a type 2 diabetes model. Jpn J Ophthalmol. 2002; 46:130–139. [PubMed: 12062217]

Weston BC, Bourne WM, Polse KA, Hodge DO. Corneal hydration control in diabetes mellitus. Invest Ophthalmol Vis Sci. 1995; 36:586–595. [PubMed: 7890490]

Wilson SE, Liu JJ, Mohan RR. Stromal-epithelial interactions in the cornea. Prog Retin Eye Res. 1999; 18:293–309. [PubMed: 10192515]

Wilson SE, Walker JW, Chwang EL, He YG. Hepatocyte growth factor, keratinocyte growth factor, their receptors, fibroblast growth factor receptor-2, and the cells of the cornea. Invest Ophthalmol Vis Sci. 1993; 34:2544–2561. [PubMed: 8392040]

Winkler MA, Dib C, Ljubimov AV, Saghizadeh M. Targeting miR-146a to treat delayed wound healing in human diabetic organ-cultured corneas. PLoS One. 2014; 9:e114692. [PubMed: 25490205]

Wu YC, Buckner BR, Zhu M, Cavanagh HD, Robertson DM. Elevated IGFBP3 levels in diabetic tears: a negative regulator of IGF-1 signaling in the corneal epithelium. Ocul Surf. 2012; 10:100–107. [PubMed: 22482470]

Wylegała E, Moćko L, Woyna-Orlewicz A, Teper S, Orzhechowska-Wylegała B. [Diabetic complications within ocular surface] (in Polish). Pol Merkur Lekarski. 2006; 21:495–497. [PubMed: 17345849]

Xu K, Yu FS. Impaired epithelial wound healing and EGFR signaling pathways in the corneas of diabetic rats. Invest Ophthalmol Vis Sci. 2011; 52:3301–3308. [PubMed: 21330660]

Xu KP, Li Y, Ljubimov AV, Yu FX. High glucose suppresses EGFRPI3K-AKT signaling pathway and attenuates corneal epithelial wound healing. Diabetes. 2009; 58:1077–1085. [PubMed: 19188434]

Yam JC, Kwok AK. Update on the treatment of diabetic retinopathy. Hong Kong Med J. 2007; 13:46–60. [PubMed: 17277392]

Yan C, Gao N, Sun H, Yin J, Lee P, Zhou L, Fan X, Yu FS. Targeting imbalance between IL-1β and IL-1 receptor antagonist ameliorates delayed epithelium wound healing in diabetic mouse corneas. Am J Pathol. 2016; 186:1466–1480. [PubMed: 27109611]

Yanai R, Yamada N, Inui M, Nishida T. Correlation of proliferative and anti-apoptotic effects of HGF, insulin, IGF-1, IGF-2, and EGF in SV40-transformed human corneal epithelial cells. Exp Eye Res. 2006; 83:76–83. [PubMed: 16530761]

Yang L, Di G, Qi X, Qu M, Wang Y, Duan H, Danielson P, Xie L, Zhou Q. Substance P promotes diabetic corneal epithelial wound healing through molecular mechanisms mediated via the neurokinin-1 receptor. Diabetes. 2014; 63:4262–4274. [PubMed: 25008176]

Yang R, Sha X, Zeng M, Tan Y, Zheng Y, Fan F. The influence of phacoemulsification on corneal endothelial cells at varying blood glucose levels. Eye Sci. 2011; 26:91–95. [PubMed: 21692206]

Yin J, Yu FS. LL-37 via EGFR transactivation to promote high glucose-attenuated epithelial wound healing in organ-cultured corneas. Invest Ophthalmol Vis Sci. 2010; 51:1891–1897. [PubMed: 19797203]

Yin J, Huang J, Chen C, Gao N, Wang F, Yu FS. Corneal complications in streptozocin-induced type I diabetic rats. Invest Ophthalmol Vis Sci. 2011; 52:6589–6596. [PubMed: 21715347]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Yoon KC, Im SK, Seo MS. Changes of tear film and ocular surface in diabetes mellitus. Korean J Ophthalmol. 2004; 18:168–174. [PubMed: 15635831]

Zagon IS, Sassani JW, Carroll MA, McLaughlin PJ. Topical application of naltrexone facilitates reepithelialization of the cornea in diabetic rabbits. Brain Res Bull. 2010; 81:248–255. [PubMed: 19853024]

Zagon IS, Sassani JW, Immonen JA, McLaughlin PJ. Ocular surface abnormalities related to type 2 diabetes are reversed by the opioid antagonist naltrexone. Clin Exp Ophthalmol. 2014; 42:159–168. [PubMed: 23777539]

Zhang C, Xi L, Zhao S, Wei R, Huang Y, Yang R, Su L, Liu X. Interleukin-1β and tumour necrosis factor-α levels in conjunctiva of diabetic patients with symptomatic moderate dry eye: case-control study. BMJ Open. 2016; 6:e010979.

Zhang Y, Akhtar RA. Epidermal growth factor stimulation of phosphatidylinositol 3-kinase during wound closure in rabbit corneal epithelial cells. Invest Ophthalmol Vis Sci. 1997; 38:1139–1148. [PubMed: 9152233]

Zhivov A, Winter K, Hovakimyan M, Peschel S, Harder V, Schober HC, Kundt G, Baltrusch S, Guthoff RF, Stachs O. Imaging and quantification of subbasal nerve plexus in healthy volunteers and diabetic patients with or without retinopathy. PLoS One. 2013; 8:e52157. [PubMed: 23341892]

Zhou Q, Chen P, Di G, Zhang Y, Wang Y, Qi X, Duan H, Xie L. Ciliary neurotrophic factor promotes the activation of corneal epithelial stem/progenitor cells and accelerates corneal epithelial wound healing. Stem Cells. 2015; 33:1566–1576. [PubMed: 25546438]

Zickri MB, Ahmad NA, Maadawi ZM, Mohamady YK, Metwally HG. Effect of stem cell therapy on induced diabetic keratopathy in albino rat. Int J Stem Cells. 2012; 5:57–64. [PubMed: 24298355]

Zieske JD, Takahashi H, Hutcheon AEK, Dalbone AC. Activation of epidermal growth factor receptor during corneal epithelial migration. Invest Ophthalmol Vis Sci. 2000; 41:1346–1355. [PubMed: 10798649]

Zou C, Wang S, Huang F, Zhang YA. Advanced glycation end products and ultrastructural changes in corneas of long-term streptozotocin-induced diabetic monkeys. Cornea. 2012; 31:1455–1459. [PubMed: 22695699]

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Table 1

Manifestations of corneal diabetes

Abnormality	Manifestation	Reference
Neuropathy (nerves)	Decreased sensitivity	Saini and Mittal, 1996a
	Decreased subbasal nerve fiber and branch density	Rosenberg et al., 2000
	Delayed nerve regeneration after injury	Gao et al., 2016
	Increased stromal nerve thickness and tortuosity	Mocan et al., 2006
Keratopathy (epithelium)	Delayed wound healing	Chen et al., 2009
	Compromised barrier function	Chang et al., 1995
	Persistent epithelial defects	Herse, 1988
	Recurrent erosions	Herse, 1988
	Epithelial fragility	Saini and Khandalavla, 1995
	Edema	Cisarik-Fredenburg, 2001
	Ulceration	Herse, 1988
	Low cell density	Szalai et al., 2016
	Stem cell dysfunction	Saghizadeh et al., 2011
	Increased autofluorescence	Chang et al., 1995
Immune cell alterations	Dendritic cell accumulation	Leppin et al., 2014
Stromal changes	Abnormal collagen bundles	Zou et al., 2012
	Stromal edema	Gül et al., 2008
Endothelial changes	Decreased cell density	Szalai et al., 2016
	Cell pleomorphism	Matsuda et al., 1990
Tear film changes	Low tear secretion	Cousen et al., 2007
	Increased tear osmolarity	Beckman, 2004
Biomechanics problems	Increased corneal response factor	Kotecha et al., 2010

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Table 2
Molecular alterations in diabetic corneas

Protein/pathway/structure	System/species	Effect	Reference
Hemidesmosomes	Human	Reduced in the epithelium	Tabatabay et al., 1988
Laminins and nidogen-1	Human	Reduced in the epithelial BM	Ljubimov et al., 1998
$\alpha_3\beta_1$ Integrin	Human	Reduced in the epithelium	Ljubimov et al., 1998
AGEs	Human	Accumulate in the epithelial BM and stroma	Kaji et al., 2000
Abnormal collagen bundles	Human, Rat	Appear in the Descemet's membrane	Rehany et al., 2000a;b
MMP-3	Human	Accumulates in the stroma	Saghizadeh et al., 2001
MMP-10	Human	Accumulates in the epithelium and stroma	Saghizadeh et al., 2001a
Cathepsin F	Human	Accumulates in the epithelium	Saghizadeh et al., 2005
IGF-I	Human	Accumulates in the epithelium	Saghizadeh et al., 2001b
IGFBP3	Human	Accumulates in the epithelial cells and tears	Wu et al., 2012
EGFR	Human, Rat, Pig	Decreased phosphorylation and signaling through Akt in the epithelium	Xu and Yu, 2011
Akt	Human, Rat	Decreased phosphorylation in the epithelium	Xu et al., 2009
HGF	Human	Increased in the epithelium	Saghizadeh et al., 2005
c-Met (HGF receptor)	Human	Decreased in the epithelium	Saghizadeh et al., 2005
Thymosin β_4	Human	Decreased in the epithelium	Saghizadeh et al., 2005
FGF-3/FGFR3	Human	Decreased gene expression	Saghizadeh et al., 2005
TIMP-4	Human	Decreased gene expression	Saghizadeh et al., 2005
P2X7 receptor	Human, Mouse, Rat	Increased in the epithelium	Mankus et al., 2011
TGF-β3	Rat	Decreased gene expression	Bettahi et al., 2014
Ephrin-A1	Human	Increased in the epithelium	Kaplan et al., 2012
Putative stem cell markers ABCG2, N-cadherin, K15, K17, K19, ΔNp63α, β_1 integrin	Human	Decreased in the limbal epithelium	Saghizadeh et al., 2011
MiR-146a	Human	Increased in the epithelium	Funari et al., 2013
MiR-204-5p	Mouse	Increased in the epithelium	Gao et al., 2015

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Table 3
Potential new treatments for diabetic corneal disease

Treatment	System	Effect	Reference
Thymosin β_4 eye drops	Diabetic patients	Reduction of chronic non-healing epithelial defects	Dunn et al., 2010
Autologous serum drops	Diabetic patients	Increase of corneal wound healing	Schulze et al., 2006
Topical CT-112 (ARI)	Diabetic patients	Improvement of corneal barrier function but not corneal sensitivity	Nakahara et al., 2005
Insulin eye drops	Diabetic patients	Faster re-epithelialization after debridement for vitreoretinal surgeries	Bastion and Ling, 2013
Insulin eye drops	Mouse IDDM	Prevention of subbasal nerve loss	Chen et al., 2013
Insulin implants	Rat IDDM	Accelerated wound healing	Zagon et al., 2007
Topical ranirestat (ARI)	Rat galactosemia	Faster epithelial wound healing; normalization of MMP-10 and integrin α_3 expression	Takamura et al., 2013
IGF-I injections	Mouse NIDDM	Prevention of epithelial stem cell marker loss and increase in subbasal nerve density	Ueno et al., 2014
Naltrexone (OGFR antagonist) eye drops	Mouse, Rat IDDM and NIDDM	Normalization of corneal epithelial wound healing, tear secretion, and corneal sensitivity	Sassani et al., 2016
Antidiabetics nateglinide and glibenclamide	Rat NIDDM	Suppression of Descemet's membrane changes	Akimoto et al., 2008
Ciliary neurotrophic factor	Mouse IDDM	Activation of LESC, increased nerve density, and promotion of epithelial healing	Zhou et al., 2015
PARP inhibitor	Rat IDDM	Alleviation of delayed epithelial healing and decreased corneal sensitivity	Byun et al., 2015
Topical nerve growth factor	Rat IDDM	Reduction of apoptosis and inflammation	Park et al., 2016
IL-1 receptor antagonist	Mouse IDDM	Faster epithelial wound healing and sensory reinnervation, reduction in apoptosis, increase of Akt signaling	Yan et al., 2016
Substance P	Mouse IDDM	Faster epithelial wound healing and reinnervation, reactivation of EGFR/Akt signaling	Yang et al., 2014
Topical lacritin	NOD mice IDDM	Faster epithelial wound healing	Wang et al., 2014
Intranasal curcumin in nanomicelles	Mouse IDDM	Promotion of wound healing and recovery of corneal sensation	Guo et al., 2016
Gene therapy with AV	Human diabetic organ-cultured corneas	Faster epithelial wound healing, normalization of diabetic and LESC markers upon overexpression of c-met and silencing of MMP-10 and cathepsin F	Ljubimov and Saghizadeh, 2015
Mir-146a inhibition	Human diabetic organ-cultured corneas	Faster epithelial wound healing, increase of EGFR and epithelial integrin $\alpha_3\beta_1$	Winkler et al., 2014

ARI, aldose reductase inhibitor; PARP, poly(ADP-ribose) polymerase; NOD, non-obese diabetic; AV, adenovirus