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REVIEW ARTICLE

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Ocular surface microbiota dysbiosis contributes to the high prevalence of dry eye disease in diabetic patients

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ABSTRACT

People with diabetes mellitus (DM) are at an increased risk for developing dry eye disease (DED). However, the mechanisms underlying this phenomenon remain unclear. Recent studies have found that the ocular surface microbiota (OSM) differs significantly between patients with DED and healthy people, suggesting that OSM dysbiosis may contribute to the pathogenesis of DED. This hypothesis provides a new possible explanation for why diabetic patients have a higher prevalence of DED than healthy people. The high-glucose environment and the subsequent pathological changes on the ocular surface can cause OSM dysbiosis. The unbalanced microbiota then promotes ocular surface inflammation and alters tear composition, which disturbs the homeostasis of the ocular surface. This "high glucose-OSM dysbiosis" pathway in the pathogenesis of DED with DM (DM-DED) is discussed in this review.

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1. Introduction

The TFOS DEWS II Report defines dry eye disease (DED) as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play aetiological roles" (Craig et al. 2017). DM is a risk factor for DED (Stapleton et al. 2017). There were approximately 451 million people with DM worldwide in 2017 (Cho et al. 2018), and approximately 54.3% of diabetic patients suffer from DED (Manaviat et al. 2008). We previously reported that the prevalence of DED in diabetic patients was significantly higher than that in healthy individuals among the Chinese Han population (Wang et al. 2019). This result was consistent with those of other studies (Seifart and Strempel 1994; Kaiserman et al. 2005). The number of patients with DM-DED may further increase along with the increase in the number of diabetic patients, causing a global public health issue.

Several mechanisms contribute to the loss of tear film homeostasis, among which ocular surface inflammation is the core (Wei and Asbell 2014). Other possible mechanisms include the following: high levels of glucose in tears induce epithelial cell apoptosis, the accumulation of advanced glycation end products (AGEs) damages the ocular surface, and corneal neuropathy leads to infrequent blinking and reduced secretion by glands (Shih et al. 2017). The OSM refers to the community of microorganisms that colonize the ocular surface, among which bacteria constitute the majority (Aragona et al. 2021). Currently, the role of OSM dysbiosis in the pathogenesis of DED has attracted worldwide attention. Many studies have found that the composition of the OSM differs between patients with DED and healthy people, indicating that OSM dysbiosis contributes to the pathogenesis of DED (Graham et al. 2007; Dong et al. 2019; Li et al. 2019; Kittipibul et al. 2020; Willis et al. 2020; Zhao et al. 2020; Andersson et al. 2021; Zhang et al. 2021). This manuscript reviews the OSM changes in DED, especially in DM-DED, as well as DED resulting in an altered OSM. Furthermore, it elucidates how DM affects the OSM, as well as how OSM dysbiosis contributes to the pathogenesis of DM-DED, aiming to provide new ideas for the treatment of DM-DED.

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2. Characteristics of the OSM in DED

2.1. Diversity of the OSM in DED

Diversity indicates the range of different kinds of species present in a specific environment (Aragona et al. 2021). It is usually evaluated according to alpha diversity, which indicates the number of species in a specific environment, and beta diversity, which indicates the dissimilarity of species composition among different communities (Aragona et al. 2021). Both the alpha and beta diversity of the OSM are different between patients with DED and healthy people.

Previous studies have shown different results regarding the alpha diversity of the OSM in patients with DED. On the one hand, an abundance of research has demonstrated higher alpha diversity in patients with DED (Li, Gong, et al. 2019; Shimizu et al. 2019; Kittipibul et al. 2020; Willis et al. 2020). Shimizu believes that the higher alpha diversity in DED may be attributed to ocular surface epithelial damage and the reduction in the levels of mucins and antibacterial substances in tears, making it difficult to capture and eliminate microbes (Shimizu et al. 2019). On the other hand, however, some studies have found that the alpha diversity in patients with DED is similar to or lower than that in healthy people (De Paiva et al. 2016; Dong et al. 2019; Andersson et al. 2021; Liang et al. 2021). Such distinct results may be explained by the following. First, the ocular surface is characterized by a low microbial density environment (Ozkan and Willcox 2019). Additionally, the sample sizes of patients with DED are small, ranging from 15 to 47 cases (Dong et al. 2019; Li, Gong, et al. 2019; Willis et al. 2020; Liang et al. 2021). Finally, OSM changes with many factors, ranging from demographic factors to various sampling techniques (Aragona et al. 2021). While OSM is dictated by age, sex, race, and environment, the sampling site, pressure, and specific ocular surface location also have a certain influence on the accurate identification of the microbiota composition (Dong et al. 2011; Zhou et al. 2014; Wen et al. 2017; Ozkan et al. 2019; Deng et al. 2020). However, the use of topical anaesthetics has little effect on most ocular surface bacteria except for *Cutibacterium* (Delbeke et al. 2022). Regarding the beta diversity of the OSM, most studies have demonstrated that there is a significant difference between patients with DED and healthy people (Graham et al. 2007; Dong et al. 2019; Li et al. 2019; Kittipibul et al. 2020; Willis et al. 2020; Zhao et al. 2020; Andersson et al. 2021; Zhang et al. 2021), suggesting an altered OSM in DED.

2.2. Composition of the OSM in DED

The bacterial taxonomy in patients with DED varies among previous studies, as presented in Table 1. At the phylum level, the dominant bacteria in DED and healthy people are similar, mainly comprising *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*. The order of their relative abundance from largest to smallest is *Proteobacteria* (34.8 ~ 47.62%), *Firmicutes* (17.2 ~ 31.84%), and *Bacteroidetes* (2.21 ~ 16.54%), although the relative abundance of *Actinobacteria* (6.24 ~ 34.17%) has shown great variation among previous studies (Dong et al. 2019; Li, Gong, et al. 2019; Kittipibul et al. 2020; Zhang et al. 2021). Previous studies have agreed that the relative abundance of specific bacterial phyla is different between patients with DED and healthy people. However, the alterations were different. For example, Dong showed a higher relative abundance of *Proteobacteria* in patients with DED than in healthy people (Dong et al. 2019), while Li found the opposite result (Li, Gong, et al. 2019). At the genus level, the dominant bacteria in the DED group were similar to those in the control group in the same study but varied among different studies (Dong et al. 2019; Li, Gong, et al. 2019; Kittipibul et al. 2020; Zhang et al. 2021). *Corynebacterium*, a commensal on the ocular surface (Willcox 2013), is always the dominant genus in DED, as presented in Table 1. It can cause infection in DM or serious DED (Aoki et al. 2021), indicating its potential pathogenicity when the ocular circumstances change. *Staphylococcus* is enriched in patients with DED compared with healthy people (Dong et al. 2019; Kittipibul et al. 2020). Andersson found that the relative abundance of *Staphylococcus* negatively correlated with the ocular surface disease index (OSDI) (Andersson et al. 2021), and Dong found that it was positively correlated with meibo-scores (Dong et al. 2019). This finding suggests that *Staphylococcus* may play a crucial role in the pathogenesis of DED. Notably, the same genus may play different roles in the pathogenesis of DED with or without other diseases, as Qi reported in 2021(Qi et al. 2021). They explain this result by the following: (1) the bacterial abundance determines its function in a complex community, and there was a statistically significant difference in the relative abundance of the genera between the two groups; and (2) the function of the same bacteria may be altered in different immune states (Qi et al. 2021). Therefore, a comprehensive review is needed when analysing the bacterial role in the pathogenesis of DED.

2.3. Diversity and composition of the OSM in DM-DED

To our knowledge, only one article has previously reported the characteristics of OSM in patients with DM-DED. We found that the alpha diversity was higher and the beta diversity was significantly different in patients with DM-

Table 1. Studies comparing the OSM between patients with DED and healthy people using 16S rRNA gene sequencing.

Source	Country	Group (number)	Characteristic of DED	Age (years)	Sample collection	Dominant bacteria and their relative abundance in DED		Microbiota changes in DED compared with healthy people	
						Phylum	Genus	Phylum	Genus
Zhang et al. (2021)	China	DED ($n = 37$) Control ($n = 22$)	DM-DED	DED: Male: 59.4 ± 3.5; Female: 62.5 ± 4.8 Control: Male: 56.8 ± 3.4; Female: 53.9 ± 4.5	Schirmer test strips were placed at the outer one-third of the temporal canthus of each eye for 5 min without anaesthesia	*Proteobacteria* 44.90% *Firmicutes* 31.84% *Actinobacteria* 9.31% *Bacteroidetes* 4.55%	*Ochrobactrum* 22.47% *Corynebacterium* 10.96% *Bacillus* 9.53% *Cupriavidus* 5.49% *Lactococcus* 4.07% *Lactobacillus* 3.65%	↑: *Firmicutes*, *Bacteroidetes*	↑: *Amycolatopsis*
Li, Gong, et al. (2019)	China	DED ($n = 35$) Control ($n = 54$)	DED	DED: 57 ± 14 Control: 52 ± 16	After topical anaesthesia, sterile dry cotton swabs were used to wipe the upper and lower palpebral, caruncle, and fornix conjunctiva of a random eye	*Proteobacteria* 47.62% *Firmicutes* 17.20% *Bacteroidetes* 16.54% *Actinobacteria* 6.24%	*Pseudomonas* 11.49% *Acinetobacter* 7.79% *Bacillus* 7.10% *Chryseobacterium* 2.25% *Corynebacterium* 2.61%	↑: *Bacteroidetes* ↓: *Proteobacteria*	↓: *Pseudomonas*
Dong et al. (2019)	China	DED ($n = 47$) Control ($n = 42$)	MGD	DED: 57.53 ± 15.10 Control: 62.76 ± 9.73	After topical anaesthesia, sterile dry cotton swabs were used to wipe the upper and lower conjunctival sac and eyelid margins of a random eye two times	*Actinobacteria* 34.17% *Firmicutes* 31.70% *Proteobacteria* 27.46% *Bacteroidetes* 2.21%	*Staphylococcus* 20.71% *Corynebacterium* 20.22% *Propionibacterium* 9.29% *Sphingomonas* 5.73% *Snodgrassella* 4.17% *Streptococcus* 2.80%	↑: *Proteobacteria*, *Firmicutes* ↓: *Actinobacteria*	↑: *Sphingomonas*, *Staphylococcus* ↓: *Corynebacterium*
Kittipibul et al. (2020)	Thailand	DED ($n = 20$) Control ($n = 20$)	SJS	DDE: 44.5 (20 ~ 77) Control: 44.2 (24 ~ 77)	After topical anaesthesia, sterile cotton swabs were applied from the medial to the lateral part of inferior fornix of the conjunctival sac of the worse eye three times without touching the eyelids or symblepharon area	*Proteobacteria* 34.8% *Firmicutes* 23.8% *Bacteroidetes* 13.1% *Tenericutes* 11.9% *Actinobacteria* 9.8%	NA	NA	↑: *Acinetobacter*, *Bacillus*, *Bacteroides*, *Lactobacillus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*

MGD: meibomian gland dysfunction; SJS: Stevens-Johnson syndrome; ↑: increased relative abundance; ↓: decreased relative abundance.

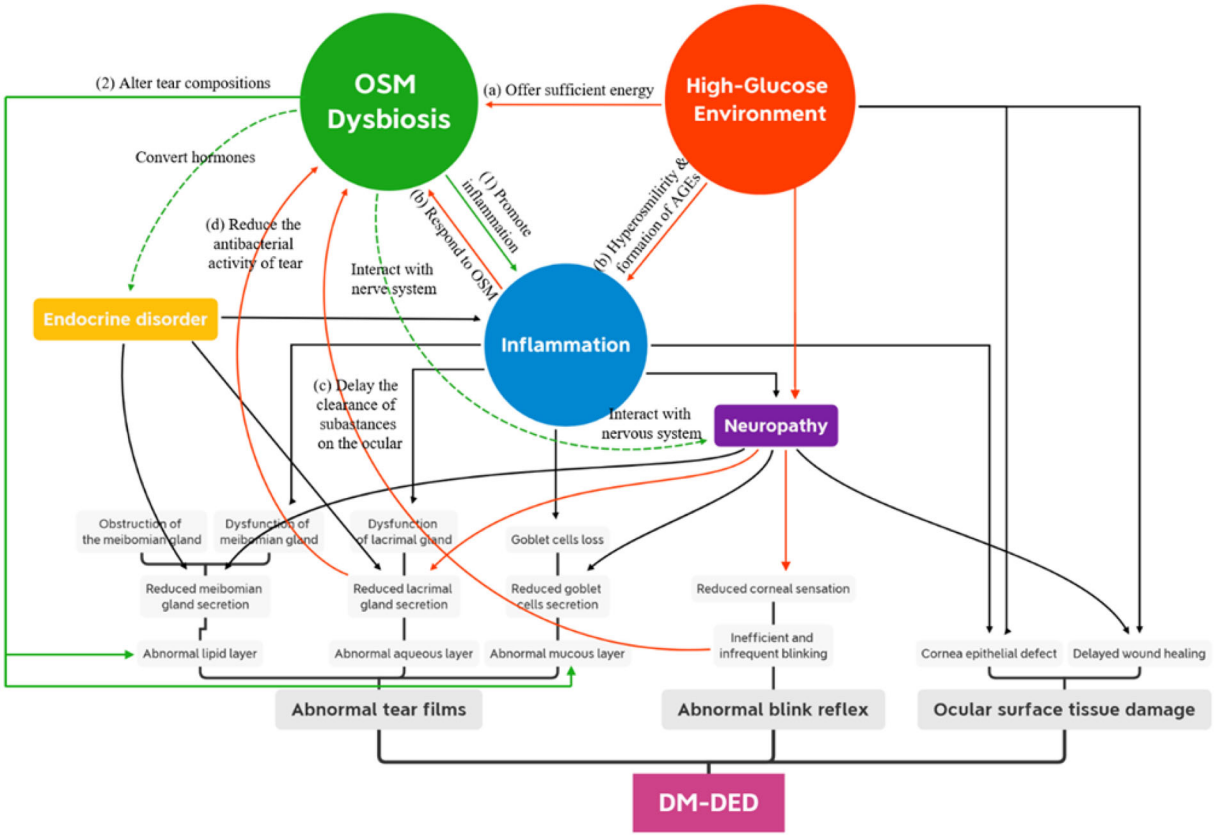

Figure 1. The "high glucose-OSM dysbiosis" pathway in the pathogenesis of DM-DED. Red lines refer to how a high-glucose environment leads to OSM dysbiosis, while green solid lines refer to how OSM dysbiosis contributes to the pathogenesis of DM-DED. Green dotted lines refer to the possible mechanisms that remain to be investigated. The high-glucose ocular surface environment leads to OSM dysbiosis in DM-DED through multiple pathways. (a) High glucose content offers sufficient energy for microbial growth. (b) Hyperosmolarity and the formation of AGEs induce ocular inflammation, which leads to massive microbial death. (c) Reduced blinking frequency delays the clearance of substances on the ocular surface. (d) Lacrimal gland dysfunction causes the reduced antibacterial activity of tears. Then, OSM dysbiosis (1) promotes ocular surface inflammation and (2) alters tear composition, which contributes to the pathogenesis of DM-DED. In addition, the OSM may also influence the homeostasis of the ocular surface by interacting with the ocular surface nervous system and converting hormones.

DED compared with those in healthy people and non-diabetic patients with DED (DED-only) (Zhang et al. 2021). In addition, the composition of the OSM varied between DM-DED and DED-only patients. At the phylum level, the DM-DED group showed a lower relative abundance of *Proteobacteria* and a higher relative abundance of *Actinobacteria* and *Bacteroidetes* than the DED-only group (Zhang et al. 2021). At the genus level, the DM-DED group showed a higher relative abundance of *Corynebacterium* and a lower relative abundance of *Ochrobactrum*, *Bacillus*, and *Cupriavidus* than the DED-only group (Zhang et al. 2021). These results are quite different from the abovementioned changes in the OSM in DED-only patients. Hence, we believe that the change in the OSM in patients with DM-DED may be unique, which contributes to the high prevalence of DED in the diabetic population.

3. How DM leads to OSM dysbiosis

Previous studies have demonstrated distinct OSMs between diabetic patients and healthy people (Li, Yi, et al. 2019; Zhu et al. 2021). Such OSM dysbiosis might be attributed to the high-glucose ocular surface environment in diabetic patients. A proposed model for the pathophysiology of DM-induced OSM dysbiosis is presented in Figure 1.

First, a high glucose level provides an ideal environment for microbial growth, which may explain the high diversity of the OSM in diabetic patients (Li, Yi, et al. 2019; Zhu et al. 2021). Second, high glucose contents increase tear osmolarity and promote the formation of AGEs. Hyperosmolarity and the accumulation of AGEs can activate immune cells and induce ocular surface inflammation (Shi et al. 2013; Shih et al. 2017; Clayton 2018). The active immune system then responds to the OSM, and an enhanced immune response may cause massive

microbial death, leading to OSM dysbiosis. Third, inflammation can damage corneal nerve fibres (Leppin et al. 2014), and AGEs can reduce the blood supply to neurons by damaging capillaries (Goldin et al. 2006). In addition, the levels of neurotrophic factors such as serum nerve growth factor and sphingolipids are reduced in DM (Kim et al. 2009; Priyadarsini et al. 2015). These induce corneal neuropathy and subsequently decrease corneal sensation. As a result, on the one hand, blinking frequency is reduced, delaying the clearance of substances on the ocular surface (Zhang et al. 2017). It is beneficial for the OSM to colonize and grow on the ocular surface. On the other hand, lacrimal gland secretion, including that of antibacterial substances, is reduced, impairing the antibacterial ability of tear film (Zhang et al. 2017).

Through the above mechanisms, the composition of the OSM in diabetic patients is altered, manifested as a higher relative abundance of *Bacteroidetes* (Li, Yi, et al. 2019; Zhu et al. 2021) and a lower relative abundance of *Proteobacteria* (Li, Yi, et al. 2019). This may explain the increased *Bacteroidetes* and decreased *Proteobacteria* abundances in DM-DED compared with DED-only (Zhang et al. 2021). *Bacteroidetes* can degrade a wide range of complex carbohydrates by carbohydrate-active enzymes (McKee et al. 2021). This activity may enable *Bacteroidetes* dominance in a high-glucose environment. The relationship between high glucose content and the relative abundance change of bacteria needs to be further studied.

4. How OSM dysbiosis contributes to the pathogenesis of DM-DED

4.1. The OSM promotes ocular surface inflammation

Ocular surface inflammation is the core mechanism of DED (Wei and Asbell 2014). It damages the epithelium, glands, and nerves, resulting in the reduction of tear production and blinking frequency, as well as altering tear composition (Clayton 2018). Under normal conditions, the ocular surface coexists with the OSM. However, the mechanisms supporting OSM-ocular surface coexistence are disrupted in patients with DM-DED, triggering an immune response to the OSM. In addition, bacteria that can promote the secretion of inflammatory factors are enriched in patients with DM-DED, aggravating ocular surface inflammation.

4.1.1. Disruption of the OSM-ocular surface coexistence

The physical–chemical barrier and immune tolerance are the main mechanisms supporting the long-term coexistence between the OSM and the ocular surface. The physical–chemical barrier limits the direct contact between the OSM and the ocular surface. Immune tolerance maintains low immune responses to commensals. However, the ocular surface tissue damage and diminished immunomodulatory function in DM-DED disrupt the coexistence mechanisms. As a result, the OSM contacts the ocular surface directly, triggering an inflammatory response.

The physical barrier of the ocular surface is composed of intact and tightly arranged epithelial cells and tight junctions between cells (Na et al. 2015), which prevent the invasion of the ocular tissue by the OSM. The chemical barrier includes antibacterial substances such as lysozyme and secretory immunoglobulin A, and mucins are mainly secreted by goblet cells (Na et al. 2015). These proteins can inhibit microbial growth, neutralize toxins, and eliminate pathogens. However, in DED, an abundance of immune cells infiltrate the ocular surface, among which Th1 and Th17 cells secrete proinflammatory cytokines represented by IFN-γ and IL-17, respectively (De Paiva et al. 2009). These cytokines trigger damage to the physical–chemical barriers, including epithelial squamous metaplasia, epithelial tight junction destruction, goblet cell dysfunction and loss, and depletion of antibacterial components in tears (Zhang et al. 2017). Due to the breakdown of the physical–chemical barrier, the OSM contacts the ocular surface directly and initiates an immune response, exacerbating inflammation.

Immunologic tolerance refers to the capacity of the immune system to modulate the response to specific antigens from commensals (Aragona et al. 2021). It comprises non-specific tolerance mediated by pattern recognition receptors and specific tolerance mediated by regulatory immune cells. Both types of immunologic tolerance are impaired in patients with DM-DED. In terms of non-specific tolerance, Toll-like receptor (TLR) 2 and TLR-4 are expressed by epithelial cells at only the intracellular level and cannot bind to their corresponding antigens (Ueta et al. 2004). However, translocation of cytoplasmic TLR4 was found in DED (Lee et al. 2012). This translocation enables corneal epithelial cells to recognize the lipopolysaccharide (LPS) of gram-negative bacteria and initiate an immune response to the OSM. In terms of specific tolerance, dendritic cells (DCs) and regulatory T cells (Tregs) are essential for the formation of immune tolerance to specific antigens. DCs facilitate the differentiation of naive T cells into Tregs through TGF-β and IL-10. However, the expression of these cytokines is downregulated, and Tregs are diminished in DED (Perez et al. 2020). As a result, specific immunologic tolerance fails to develop. Moreover, goblet cells

can promote the formation of specific immunologic tolerance. Goblet cells express TGF-β2 and alter the DC phenotype to a tolerogenic type by downregulating DC expression of MHC class II and the costimulatory molecules CD80, CD86, and CD40 (Contreras-Ruiz and Masli 2015). Goblet cells can also secrete retinoic acid, which suppresses CD86 expression and IL-12 production by myeloid cells, suggesting their function in maintaining immunologic tolerance (Xiao et al. 2018). Barbosa found that ocular surface antigens binding to mucins pass through goblet cell-associated passages (GAPs) and are taken up by CD11b + F4/80+ cells adjacent to goblet cells (Barbosa et al. 2017). It has been demonstrated that intestinal GAPs can deliver antigens to tolerogenic DCs to induce immunologic tolerance (McDole et al. 2012). Conjunctival goblet cells may have a similar function. However, the loss of goblet cells in DED may disturb the formation of immunologic tolerance (Zhang et al. 2017). Due to the impaired immunologic tolerance in DED, the immune system indiscriminately attacks the OSM, aggravating ocular surface inflammation.

There are other possible mechanisms for OSM-ocular surface coexistence. LPS-binding protein (LBP) and CD14 complement the LPS receptor complex and can modulate ocular innate immunity (Blais et al. 2005). This may explain Ueta's finding that corneal epithelial cells did not produce IL-6 and IL-8 even in the presence of LPS in the cytoplasm (Ueta and Kinoshita 2010). Given the high serum LBP content in DM (Sato et al. 2014), tears from patients with DM-DED may contain high levels of LBP, triggering an immune response to the OSM. The expression of the complementary proteins in DM-DED should be investigated in further studies.

4.1.2. The OSM modulates the expression of inflammatory factors

The expression of various inflammatory factors is upregulated in DM-DED, including IL-1β, IL-6, IL-8, IL-17, IFN-γ, TNF-α, and matrix metalloproteinases (MMPs) (Roda et al. 2020). MMPs are multidomain calcium and zinc ion-dependent, proteolytic enzyme family (Shoari et al. 2021). MMP-9 is the most well-studied in DED. In addition to disrupting the ocular surface barrier by cleaving the tight junction proteins, MMPs cleave pro-cytokines and aggravate inflammation in DED (Lanza et al. 2016; Perez et al. 2020). The activated proinflammatory cytokines, in turn, upregulate the expression of MMPs, thus initiating a vicious cycle of inflammation (Lanza et al. 2016). These factors activate immune cells and further amplify the inflammatory response, which disturbs the homeostasis of the ocular surface. Pathogens can promote inflammation by producing toxins and superantigens. Several commensals on the ocular surface can also modulate the expression of inflammatory factors. The enrichment of such bacteria may play a part in DM-DED pathogenesis.

Staphylococcus aureus, a well-known pathogen, increases in abundance in DED (Albietz and Lenton 2006), although its relative abundance is low in healthy individuals (Willcox 2013). It can secrete various toxins ranging from α-toxin to leukocidins. The toxins can not only cause cytolysis but also promote the production of inflammatory factors and the pyrin-containing nod-like receptor protein 3 (NLRP3) inflammasome at sublytic concentrations (Tam and Torres 2019). NLRP3 contributes to the maturation of IL-1β and IL-18 and causes cell pyroptosis followed by the release of a large number of inflammatory factors (Perez et al. 2020). Moreover, *S. aureus* can produce T-cell and B-cell superantigens, which induce an intense immune response (Tam and Torres 2019). Notably, *S. aureus* expression of the virulence factors is regulated by the quorum sensing system (Yarwood and Schlievert 2003). *Staphylococcus aureus* secretes toxins only when the population reaches a critical density.

Coagulase-negative staphylococcus (CNSs) are the most abundant bacteria on the ocular surface in both patients with DED and healthy people (Graham et al. 2007; Willcox 2013). Kugadas found that placing CNSs on the ocular surface of germ-free mice upregulated the expression of IL-1β, which improves ocular resistance to *Pseudomonas aeruginosa* infection (Kugadas et al. 2016). However, IL-1β plays a key role in ocular surface inflammation in DM-DED. It can induce DC maturation and cell pyroptosis. It also promotes the expression of ICAM-1, which has a major role in the migration of immune cells and the interactions between DCs and T cells (Yerramothu et al. 2018; Perez et al. 2020; Periman et al. 2020). Upregulation of IL-1β expression, attributed to the increased abundance of CNSs in DED, can amplify the inflammatory response.

Corynebacterium is an ocular surface commensal (Willcox 2013) and the dominant genus in DED (Dong et al. 2019; Kittipibul et al. 2020; Andersson et al. 2021; Zhang et al. 2021). St Leger found that *Corynebacterium mastitidis* elicited IL-17 production in γδT cells (St Leger et al. 2017). IL-17 is the core cytokine in DED pathogenesis and is positively related to the OSDI in DED (Liu et al. 2017). IL-17 can promote the production of inflammatory mediators such as IL-1, IL-6, IL-8, TNF-α, and MMPs (De Paiva et al. 2009). It also recruits monocytes and neutrophils to inflammatory sites (De Paiva et al. 2009; Garbutcheon-Singh et al. 2019; Perez et al.

2020), increases B-cell proliferation (Subbarayal et al. 2016) and diminishes Treg activity (Chauhan et al. 2009). The increased relative abundance of *Corynebacterium* in DM-DED (Zhang et al. 2021) can further promote the expression of IL-17. Moreover, trehalose dimycolates, mycolic acid-containing glycolipids, from *Corynebacterium* can activate macrophages and promote TNF-α secretion (Burkovski 2018). Given that pathogenicity is positively correlated with the chain length of trehalose dimycolates (Burkovski 2018), the enrichment of *Corynebacterium* with long-chain mycolic acids may contribute to DM-DED pathogenesis. To prove these two hypotheses, the composition of the OSM in DM-DED at the species level is needed, as well as the immune-modulating functions of other *Corynebacterium* species.

4.2. The OSM alters tear composition

The tear film is composed of an outermost lipid layer, a middle aqueous tear, and an innermost mucous layer (Zhang et al. 2017). Each layer plays a significant role in maintaining the homeostasis of the tear film. Commensals can affect tear compositions by utilizing substances from tears and secreting enzymes and metabolites. Therefore, OSM dysbiosis in DM-DED may alter tear composition. As a result, the homeostasis of the tear film is disturbed, and dry eye symptoms ultimately arise.

The lipid layer comprises distinct lipids, including cholesteryl esters, wax esters, triglycerides, phospholipids, etc (Lam et al. 2011). Lipids can reduce surface tension and retard tear evaporation (Zhang et al. 2017). Hence, they are essential for the homeostasis of the tear film. Zhao found that the genes involved in bacterial lipid metabolism were upregulated in MGD meibum (Zhao et al. 2020), which aggravated the inadequacy of lipids in the tear film. This can be attributed to the enrichment of bacteria that can utilize lipids. For instance, *Staphylococcus epidermidis* can secrete cholesterol esterase and fatty wax esterase (Zhao et al. 2020), and *S. aureus* can secrete phospholipases, lipases, and fatty acid-modifying enzymes (Tam and Torres 2019). In addition, *Corynebacterium macginleyi* and *Corynebacterium accolens*, the predominant species among *Corynebacterium* on the ocular surface (Hoshi et al. 2020), are lipophilic bacteria (Riegel et al. 1995), and *C. accolens* can hydrolyze triolein, releasing oleic acid (Bomar et al. 2016).

The mucous layer is composed of mucins produced by goblet cells. They can reduce the surface tension and evenly spread the tear film on the ocular surface (Zhang et al. 2017). The OSM can degrade mucins (Berry et al. 2002). On the positive side, the OSM contributes to the turnover of mucins and maintains the antibacterial ability of the tear film. On the negative side, however, the overproliferation of mucin-degrading bacteria may destroy the composition of the tear film. However, the key genera or species involved in degrading ocular mucins have not been identified thus far. Moreover, Pickering found that the relative abundance of *Corynebacterium* positively correlated with the expression of mucins (MUC1/MUC4) (Pickering et al. 2019). This finding reveals that bacteria such as *Corynebacterium* may also play a role in regulating the production of mucins.

4.3. Other possible mechanisms

Peripheral neuropathy is one of the pathogenic mechanisms of DM-DED. It results in infrequent blinking and reduced gland secretion, which eventually induces tear film instability (Shih et al. 2017; Zhang et al. 2017). Previous studies have reported that bacteria can interact with the nervous system by stimulating neurons directly (Kunze et al. 2009; Mao et al. 2013) or secreting neurotransmitters (Strandwitz 2018). Furthermore, inflammatory factors can regulate the activity of the nervous system. IL-2 can bind directly to the delta opioid receptor in peripheral nerve cells (Wang et al. 1996). IL-1β can inhibit neurally mediated lacrimal gland secretion (Zoukhri et al. 2002). The OSM may regulate neural function indirectly by promoting the expression of such cytokines.

Androgen exerts an anti-inflammatory role via the induction of the synthesis of TGF-β and reduction in TNF-α and IL-1β levels (Zhang et al. 2017). It also promotes the secretion of the lacrimal and meibomian glands (Wang and Deng 2021). Ridlon showed the high potential of *Clostridium scindens* to convert glucocorticoids into androgens (Ridlon et al. 2013). Bacteria in the OSM with similar functions may play a role in DM-DED.

5. Conclusions

The low-density microbial environment on the ocular surface is the chief difficulty for OSM research. A range of studies has demonstrated the composition changes of the OSM in DED, suggesting the role of OSM dysbiosis in DED pathogenesis. However, to our knowledge, only one study was on the OSM in DM-DED. Given the high glucose content in tears, people with DM-DED may have a unique OSM composition, which appears to contribute to the high prevalence of DED in diabetic patients. To confirm the unique composition, there is a

great need for more studies on the OSM in DM-DED. Multiple-centre clinical trials may be helpful due to their large sample size and more reliable and representative results. Although molecular biology techniques offer insight into the OSM composition, the data may contain contamination. To identify the bacteria that truly play a part in DM-DED pathogenesis, further studies should examine the correlation between bacterial abundance and ocular surface parameters. Furthermore, a longitudinal comparison of OSM composition in DM-DED would be helpful. Long-term follow-up with OSM sampling at regular intervals is recommended for diabetic patients. By analyzing the dynamic OSM changes during the course of DM-DED, we can identify the key microorganisms in DM-DED pathogenesis.

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