The ROYAL COLLEGE of OPHTHALMOLOGISTS

REVIEW ARTICLE

The microbiome and the eye: a new era in ophthalmology

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The human microbiome has progressively been recognised for its role in various disease processes. In ophthalmology, complex interactions between the gut and distinct ocular microbiota within each structure and microenvironment of the eye has advanced our knowledge on the multi-directional relationships of these ecosystems. Increasingly, studies have shown that modulation of the microbiome can be achieved through faecal microbiota transplantation and synbiotics producing favourable outcomes for ophthalmic diseases. As ophthalmologists, we are obliged to educate our patients on measures to cultivate a healthy gut microbiome through a range of holistic measures. Further integrative studies combining microbial metagenomics, metatranscriptomics and metabolomics are necessary to fully characterise the human microbiome and enable targeted therapeutic interventions.

Eye (2025) 39:436–448; https://doi.org/10.1038/s41433-024-03517-z

INTRODUCTION

Human microbiome research began with microbial ecology studies historically and can be traced back to Antonie van Leeuwenhoek's (1632-1723) investigation of various microorganisms from water, mud and dental plaque samples [1]. His research revealed the presence of biofilms on the surface of these microorganisms composed of microbial cells enclosed in an extracellular polysaccharide matrix that allows attachment and detachment to their surrounding environment [1, 2]. The biofilm also creates a miniature ecosystem around the microorganism and plays an active role in gene transfer through exchange of extrachromosomal deoxyribonucleic acid (DNA) matter [2]. The complex interactions between the microorganism, its biofilm and their surrounding environment coupled with a continually evolving genome leads us back to the term 'microbiome' for which numerous definitions exist [1]. Essentially, it is a combination of the words 'micro' and 'biome'. 'Micro' refers to living microorganisms also known as microbiota composed of predominantly bacteria, but also viruses, bacteriophages, archae, fungi and protozoans within a well-defined habitat. Whilst 'biome' is the microbiotas structural elements including genetic material, metabolites and signalling molecules and environmental conditions which has been described as the microbes "theatre of activity" by Whipps and colleagues in 1988 [1, 3]. For the purposes of this review, we will be referring to bacterial microbes when discussing published research on the microbiome unless otherwise specified [4–8].

BACKGROUND

The majority of the estimated 10–100 trillion microbes in our body reside in the gut and specifically within the large intestine [4]. Over 90% of these belong to the phyla Firmicutes (gram-positive bacteria) and Bacteroidetes (gram-negative bacteria) followed by Proteobacteria (gram-negative bacteria), Actinobacteria (gram-positive bacteria) and Verrucomicrobia (gram-negative bacteria) [4]. The Firmicutes/Bacteroidetes (F/B) ratio is frequently used to indicate how healthy the gut microbiota is with a higher ratio seen in pathological states such as dry eye, experimental autoimmune uveitis (EAU), and age-related macular degeneration (AMD) mouse models to name a few [9–11].

The gut microbiota are responsible for producing short chain fatty acids (SCFAs) such as butyrate by the Firmicutes phylum and acetate and propionate by the Bacteroidetes phylum [12]. These SCFAs are produced through fermentation of fibre in the diet and play a significant role in regulating host metabolism, gut epithelial barrier function and immune regulation through their actions on cellular receptors such as G-protein coupled receptors (GPCR) and Toll-like receptors (TLR) [12, 13]. Furthermore, some gram-negative gut microbiota are also a source of endotoxins, for example lipopolysaccharides (LPS) which disrupt intestinal barrier by dysregulating tight junction proteins and contribute to the "leaky gut" phenomenon [14–16]. The shift in the gut microbiota away from a healthy to pathogenic state also known as dysbiosis incites chronic inflammation locally and in distant sites of the body [15–17]. This allows for translocation of microbes to places other than their normal territory, also known as atopobiosis [18]. During these processes, there is disruption to the balance between the anti-inflammatory regulatory T-cells (Tregs) and pro-inflammatory T-helper cells (Th cells) which include Th1, Th2 and Th17 subpopulations [13, 19]. The ensuing cytokine production causes activation of T-cells, B-cells and dendritic cells which travel into the lymphatic system [19]. Antigen presentation follows which stimulates autoreactive T and B cells causing target organ inflammation [19, 20]. The interactions between gut microbiota and the host's immune system with ensuing effects

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Received: 1 August 2024 Revised: 10 November 2024 Accepted: 26 November 2024
Published online: 19 December 2024

on the various organs forms the basis of the various gut-organ axes hypotheses [20, 21]. There is mounting evidence that gut dysbiosis is implicated in many ophthalmic pathologies via chronic, widespread inflammation. In addition, dysbiosis of the unique ocular microbiota may promote further ophthalmic disorders [22].

The purpose of this review is to summarise the current research on the relationship between the human microbiome and ophthalmic diseases to inform the presence of a gut-eye axis. The studies discussed have utilised 16S ribosomal (ribonucleic acid) rRNA gene sequencing technique for microbial analysis except where otherwise specified. The present manuscript will also explore the potential applications of pre- and pro-biotics as well as faecal microbiota transplant as potential treatment avenues in the future.

ADNEXAL MICROBIOME ANALYSIS

Lacrimal

Primary acquired nasolacrimal duct obstruction (PANDO) was coined by Linberg et al. [23]. to describe a clinical syndrome of lacrimal drainage disorders caused by inflammation or fibrosis without any precipitating cause. Since, a multitude of factors have been identified to contribute towards the aetiopathogenesis which include inflammation, vascular congestion, mucosal oedema, fibrosis, obstruction and stasis [24]. The role of microbial colonisation and recurrent infections as a trigger in the disease process has also been hypothesised [24]. Ali (2021) introduced the concept of a "lacriome" to define the collective microenvironments of the lacrimal drainage system. This approach allows for the molecular and cellular profiles within the lacrimal ecosystem to be studied in further detail as part of the process in understanding the disease aetiopathogenesis [25].

In a series of research publications, Ali et al. [26–30]. investigated the microbial metagenomics of the lacrimal sac in patients with PANDO who underwent endoscopic dacryocystorhinostomy (DCR) with silicone stenting as well as infective canaliculitis. In the first paper of this series, 10 intraoperative swabs of the lacrimal sac following full-length marsupialisation were obtained from 10 patients with PANDO undergoing endoscopic DCR [26]. Subsequent DNA analysis via metagenomic sequencing revealed the highest number of isolates to be of bacteria (mean 97.56%), followed by viruses (mean 0.29%), archaea (mean 0.04%), and others. In decreasing abundance, the five major phyla identified were Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria and Firmicutes. This was also seen in a separate group of 5 patients with infective canaliculitis who underwent non-incisional canalicular curettage [27]. The species in the PANDO patients were dominated by *Acinetobacter johnsonii, Porphyromonas catoniae, Escherichia coli*, and *Haemophilus influenzae* amongst others. These findings contrast with studies where culture-dependent methods were utilised which demonstrated *Staphylococci* as the most prevalent organism in patients with PANDO [31–33]. However, Curragh et al. (2020) utilised 16S rRNA gene sequencing and also found the lacrimal sac and the surrounding sinonasal microbiota to be dominated by *Staphylococci* and *Corynebacteria* [34]. The organisms prevalent in the 5 samples of canalicular concretions were *Fusobacterium nucelatum, Fusobacterium periodonticum, Parvimonas micra, Prevotella oris, Selonomonas noxia, Pseudopropionobacterium propoinicum, Campylobacter showae*, and *Streptococcus anginosus* [27]. *Actinomycetes israelii* was also isolated in each sample, however it was not the most abundant [27]. Contrarily, it is the most common organism to be isolated in canaliculitis cases where culture-dependent methods are used [35].

Silicone stent analysis following extubation, a month after endoscopic DCR surgery in the 10 PANDO patients discussed above identified 3 major phyla constituting of Proteobacteria, Firmicutes, and Actinobacteria [30]. The prevalent organisms include *Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium accolens, Dolosigranulum pigrum, Citrobacter koserii, Staphylococcus epidermidis, Escherichia coli*, and *Hemophilus influenza* [30]. The functional metagenomics of these patients revealed that *the* organisms most importantly carried out metabolism of carbohydrate, protein, ribonucleic acid (RNA), as well as also amino acids and their derivatives [28, 30]. Other functionalities include environmental, genetic and cellular processes. Additional functions of cell wall biogenesis, membrane transport, virulence, and defence mechanisms were also seen post-extubation and in the infective canaliculitis group [27, 30]. All 10 patients with PANDO who underwent endonasal DCR had successful surgical outcomes of epiphora resolution with anatomical and functional patency at the 6 month follow up [30].

Conversely, a prospective study of 10 lacrimal sac samples from patients with failed DCRs—endoscopic and external, requiring revision endoscopic DCR with mitomycin C and Crawford intubation were found to have different organisms from the PANDO cases [29]. These patients with previously failed DCRs had abundant *Stenotrophomonas maltophilia, Pseudomonas juntendi, Streptococcus pneumoniae, Acinetobacter ursingii, Citrobacter koseri*, and *Cutibacterium acnes*. The differences could be explained by influences of the sinonasal microbiome on the marsupialised lacrimal sac at the time of the primary surgery and post operative inflammation with alteration of local defences [29] There were no differences in the sampling techniques, geographical location and demographics between the failed DCR and PANDO groups.

Ali also studied the fungal microbiome (mycobiome) and virome in PANDO patients. The major phyla were Ascomycota and Basidiomycota, and the key genera were *Alternaria, Hyphopichia, Malassezia, Aspergillus* and *Epicoccum* [36]. Approximately 2–5% of the lacrimal sac virome was found to be contributed by the human endogenous retroviruses (HERV) which are considered to be commensals of a healthy human virome and play a role in overall immunity [37, 38]. The viruses isolated were mainly from the *Poxviridae* family with most the most abundant species identified as the BeAn 58058 virus seen in all 10 samples. This virus is rarely isolated from humans, with a handful of studies demonstrating its presence as a non-pathogenic organism in patients with respiratory illnesses such as chronic obstructive pulmonary disease (COPD), Coronavirus disease 2019 (COVID-19), and ocular adnexal extranodal marginal zone B-cell lymphoma [39–41]. Furthermore, the major components of any virome consist of phages which are viruses that infect, modify, replicate and regulate bacterial and archaea communities [42]. These phages are ubiquitous and have been found to play a significant role in microbiota homoeostasis [36].

The assessment of the lacriome in the various lacrimal pathologies discussed is a critical juncture in improving our understanding of the disease processes. Future therapies that target not only the organisms identified but their functionality could provide a novel approach towards addressing this group of diseases.

Meibomian gland dysfunction (MGD)

Meibomian gland dysfunction (MGD) has a pooled worldwide prevalence of 35.8%, affecting more men than women and more Asians than Caucasians [43]. It is defined as a "chronic, diffuse abnormality of the meibomian glands characterised by terminal duct obstruction with qualitative or quantitative changes in the glandular secretion" and is the commonest cause of evaporative DED [43–45]. Its aetiology can be subdivided into a hyposecretory component from atrophy of the glands or as a side effect of medications, an obstructive component consisting of ductal epithelium hypertrophy, and lastly a hypersecretory component through excess lipid production that is linked to other skin

conditions such as seborrhoeic dermatitis and rosacea [43, 46]. Certain risk factors also predispose towards developing MGD including age, history of atopy, skin conditions including acne and psoriasis, androgen deficiency, contact lens use and eyelid tattooing [43, 44]. The meibum is a lipid-rich secretion of the outermost layer of the tear film and acts to protect and nourish the ocular surface. In MGD, patients are often symptomatic from evaporative dry eye with or without reflex lacrimation, foreign body sensation, itching or burning sensation, crustiness or discharge [44]. The microbiota of the eyelids is predominantly populated by *Cutibacterium, Staphylococcus*, and to a lesser extent *Corynebacterium* genera [47]. Thus, it is unsurprising that some of the earliest studies on the microbial composition of meibomian glands isolated the same taxa, including from freshly expressed meibum of healthy individuals [47–49].

Suzuki et al. [50]. investigated the microbiome in meibum, the conjunctival-sac and eyelid skin of healthy subjects and found that younger patients between the ages of 30-35 had significantly more diversity in their meibum which was predominantly composed of *Cutibacterium acnes*. The meibum of patients in the older group aged between 60-70 years mainly consisted of *Corynebacterium* or *Neisseriaceae* species [50]. Whilst *Corynebacterium* species are recognised as non-infective commensals of the skin, mucous membranes, conjunctival sac, in addition to it being commonly found in soil, numerous case reports and series have implicated one of its strains - *C. macginleyi*, in ocular surface infection such as conjunctivitis, suture and contact lens related keratitis [51]. The patients were typically immunocompromised patients such as the elderly, diabetics, long-term topical steroid users, and patients with cornea epithelial damage [51] *Lactobacillus* species was absent from the bacterial microbiota composition of this study akin to the findings by Jiang et al. (2018) [52, 53]. However it was abundant in MGD patients from another study by Wang et al. (2021) [53]. These conflicting results highlight the complexity of the interactions within each individuals OSM and the differences in population-based factors as mentioned previously.

Zhao et al. [54]. assessed the meibum, eyelid skin and conjunctiva of 61 patients with MGD and 15 healthy individuals. The MGD patients were treatment naïve, had at least one symptom of dryness, foreign body sensation or burning, with at least two or more clinical signs including redness or thickening of the lid margin, telangiectasia, reduced, no or poor-quality secretions and gland obstruction [54]. Their severity was graded a score between 1 and 4 on a scale based on the International Workshop on Meibomian Gland Dysfunction [54, 55]. The meibum of patients affected with MGD was abundant with the pathogens *Campylobacter coli*, *Campylobacter jejuni* and *Enterococcus faecium species* which were absent from the healthy patients [54]. The microbiomes in the MGD meibum samples expressed genes for chemotaxis and immune invasion unlike that of healthy individuals [54].

Besides the analysis of the meibum microbiome in MGD, a significant element of morbidity in this subset of patients is evaporative DED. Ozkan et al. [56] excluded multiple intrinsic and extrinsic factors such as systemic drug use, blink and lid aperture abnormalities, the use of eye drops, and contact lens wear, thus leaving meibum deficiency to be evaluated in otherwise healthy participants [56]. They compared the OSM on the conjunctiva and eyelids of healthy controls against MGD patients with evaporative DED and combined evaporative and aqueous deficient patients [56]. Their results demonstrated increased levels of *Pseudomonas azotoformans, P. oleovorans* and *Caballeronia zhejiangensis* from the Proteobacteria phylum in the conjunctiva of the combined DED patients against the evaporative DED and healthy controls [56]. The evaporative DED had more abundant *Corynebacteria* species (belonging to the Actinobacteria phylum) from both the conjunctiva and eyelids [56]. This result is comparable to Song et al. [57]. who also demonstrated higher abundance of Actinobacteria in their aqueous deficient Sjogren syndrome cohort of patients as will be discussed in the dry eye section later. The composition of *Corynebacterium* species in healthy and diseased ocular surfaces remains inconclusive due to the opposing evidence in the literature yet these differences are considerable and must be appreciated in individuals with DED and MGD [26, 48]. Overall, the microbial composition in Ozkan et al.'s evaporative and combined DED patients was distinct from the healthy controls and this was also seen in other studies [58–60]. The association between MGD and an underlying atopic dermatitis component can be explained through the hygiene and biodiversity hypotheses. Urbanisation and pollution have been shown to adversely affect soil microbiota which then reduces the microbial transfer to humans living in their predominantly indoor environment [61, 62]. As a result, an individual's overall microbiota diversity is reduced. Thus arises a potential risk factor for developing immune-mediated diseases. Grönroos et al. [61]. demonstrated a temporary but significant increase in the skin microbial abundance, composition and diversity on the hands of 2 urban volunteers residing in Tampere, Finland when they were exposed to compost soil and plant-based materials as well as to moss contained within fabric pockets. Therefore, whilst a single exposure is unlikely to produce long-term effects, the concept that our skin microbiota can be advantageously modified via reintroduction of diverse flora from the soil warrants further study.

Two studies on atopic dermatitis have demonstrated the positive impact of a purified form of ammonia oxidising bacteria (AOB)- Nitrosomonas eutrophaD23 [63, 64] This bacterium is a common skin and soil commensal and is hypothesised to be depleted due to modern hygienic living conditions [61]. AOBs utilise ammonia from sweat to produce the antioxidants nitric oxide and nitrate. These two compounds are essential for wound healing, have anti-inflammatory properties, regulate keratinocyte proliferation and are microbicidal against skin pathogens [63, 65]. In the first study, topical application of the live biotherapeutic agent containing a purified state of the AOB, was found to effectively improve the major symptom of pruritus which was the primary end point of the study, in addition to the clinical signs of eczema area and severity with no adverse events [63]. In the second, the AOB's immune-modulatory properties were proven to suppress the production of Th2-mediated cytokine responses associated with IgE production [65]. Pretreatment with the AOB reduced the inflammatory response generated toward staphylococcal enterotoxin B (SEB) – both an inducer of Th2 response and contributor to atopic dermatitis pathophysiology [65]. Thus, it is evident that not only is the microbiome of the eyelid skin important but that topical agents are a potential viable treatment option for eyelid dermatitis. The use of these topical agents may potentially reduce the use of long-term systemic antibiotics such as tetracyclines for the management of MGD. Historically, the therapeutic effects of Dead Sea water immersion and mud have been proven to improve skin conditions such as psoriasis due to a synergistic effect of the various salts, minerals and ultraviolet radiation [66]. The acidity and hypersalinity of the water and mud only allows for a select group of viable organisms to survive. Whilst these have anti-microbiocidal actions, further studies are requested to establish their interaction with the human skin microbiome and whether this plays a more crucial role in ameliorating various skin conditions [67, 68].

ANTERIOR SEGMENT MICROBIOME ANALYSIS

Dry eye disease (DED)

Dry eye disease (DED) affects a large proportion of patients with varying severity and etiopathogenesis. It has been defined as a multifactorial disease with an inflammatory component that

shares common features with autoimmune disease [69]. DED is further classified into two categories: aqueous deficient and evaporative [45]. Aqueous deficiency is either a result of autoimmune disease, predominantly Sjogrens syndrome (SS) (and others including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis), or non-Sjogrens-related lacrimal disease and obstruction, systemic medications, and altered tear reflex [45, 70]. Evaporative DED is linked to intrinsic factors such as meibomian oil deficiency, lid aperture abnormalities, reduced blink rate and extrinsic factors including vitamin A deficiency, preservatives in eye drops, contact lens wear, and ocular surface disease [69, 70].

The ocular surface microbiome (OSM) is pauci-bacterial with approximately 1 bacterium per 17 human conjunctival cells in comparison to the gut where this ratio is 166-fold more [71]. Additionally, the OSM and gut microbiome are distinctly different from each other. However, the dysregulation of either of these can lead to DED. In this section, the aqueous deficiency type of DED is discussed as it dominates the research evidence in the literature.

Song et al. [57]. compared Sjögrens-associated dry eye (SSDE), non-Sjögrens associated dry eye (NSSDE) and normal controls (NC) who did not have evidence of dry eye on slit lamp examination. They found significantly less OSM diversity in the conjunctival sac of the dry eye groups [57]. At the phylum level, Actinobacteria were more abundant in SSDE than NSSDE ($P = 0.005$, SSDE vs. NSSDE, $P < 0.001$, SSDE vs. NC), followed by the NSSDE group ($P = 0.020$, NC vs. NSSDE) and NC group. Bacteroidetes were more abundant in the NC group than SSDE ($P = 0.011$), but there was no statistical difference between NC and NSSDE ($P = 0.110$) or NSSDE and SSDE ($P = 0.250$). There were predominantly more Proteobacteria in NSSDE than SSDE ($P = 0.014$) and NC but the relative abundance was similar between SSDE and NC ($P = 0.443$) [57]. At the genus level, *Bacillus* species were significantly more abundant in NC in comparison to the dry eye groups ($P < 0.001$, NC vs. NSSDE, $P < 0.001$, NC vs. SSDE), whilst *Acinetobacter* were more abundant in the dry eye groups than NC ($P < 0.001$ NC vs. NSSDE, $P = 0.001$ NC vs. SSDE). *Corynebacterium* were more abundant in SSDE followed by NSSDE and NC ($P = 0.076$ NC vs. NSSDE, $P = 0.003$ NC vs. SSDE) [57]. Qi et al. [72]. demonstrated similar findings at both phylum and genus level to Song et al. [57]. (Table 1) [72]. *Corynebacterium*, *Streptococcus* and *Prevotella* were most abundant in the autoimmune group at the genus level, whilst *Pelomonas* and *Herbaspirillum* were markedly higher in the non-autoimmune-related DED patients [72]. Gupta et al. [73]. however found reduced levels of *Corynebacterium* in their non-autoimmune DED group versus NC [73], whilst Kim et al. [74]. showed reduced *Corynebacterium* and Actinobacteria phylum in autoimmune DED [74] The differences observed in these studies are most likely related to population-based factors such as geographical location, diet and ethnicity – there were Chinese subjects in the Song et al. [57]. and Qi et al. [72]. studies, Indian subjects in Gupta et al. [73]. and South Korean subjects in Kim et al. [74]. The Chinese patients were also slightly younger on average with mean ages between 39 and 50, in contrast to mean ages of between 51 and 54 in the Indian and South Korean patients. All four studies were conducted in large urban cities where certain pollutants especially airborne may have impacted on the patients OSM.

Pal et al. [75]. analysed the bacterial microbiome in tears from healthy eyes with the use of sterile saline to enhance tear volume collection. The phylum Firmicutes, followed by Actinobacteria and Proteobacteria, dominated the samples and an identical distribution was seen in Sjögrens and non- Sjögrens aqueous deficiency dry eye [76]. The common genera of bacteria seen were *Lactobacillus, Bacillus, Corynebacterium, Staphylococcus* and *Cutibacterium*, amongst other less abundant ones [75]. From the 145 genera identified in the tear microbiome samples, 144 genera were seen in the conjunctival swab microbiome too, a noteworthy similarity [75]. The tear samples contained more *Lactobacillus* and *Bacillus*, whilst *Staphylococcus* and *Corynbacterium* were more abundant in the conjunctiva [75]. An identical sampling technique was adopted by Pal et al. [76]. to study the tear film microbiome in Sjögrens, non- Sjögrens aqueous deficiency and healthy eyes. The genera *Prevotella, Coriobacteriaceae_UCD-003, Enterococcus, Rhodobacter, Streptomyces, Ezakiella*, and *Microbacterium* were increased in patients with Sjögrens and non- Sjögrens aqueous deficiency compared to healthy eyes. *Prevotella* species in particular was especially higher in the former groups where it was observed to form multiple bacterial correlation networks with other genera within the group [76]. This suggests a possible association between *Prevotella*'s pro-inflammatory status with Sjögrens and non- Sjögrens aqueous deficiency. Additionally, the *Lactobacillus* and *Corynebacterium* genera were less abundant in the aqueous deficiency groups than the healthy eyes.

The relationship between the tear film's protein constituents (tear proteome) and OSM including the tear film, conjunctiva and eyelids is steadily being recognised as a contributing factor to the ocular surface-associated diseases. The Firmicutes phylum demonstrated a positive correlation with fatty acid binding proteins that regulate antimicrobial functions via Toll signalling pathways [77]. Furthermore, the antimicrobial humoral response of the tear proteome was positively correlated with the presence of genus *Agrobacterium* and vitamin B1 synthesis that plays a role in protection against oxidative damage. Other tear proteins detected from ocular surface microbes such as *Acinetobacter johnsonii* and *Cutibacterium acnes* were the arsenical resistance protein (ArsH) and helicase, respectively. These proteins are involved in mediating oxidative stress and bacterial DNA replication. It is evident that the taxonomical and functional profiling of the tear and OSM are vital in the immune defence system for ocular surface disease and may provide avenues for the development of future therapeutic targets.

Ocular surface dysbiosis in DED are generally comparable but not identical and this holds true also for gut dysbiosis. In patients with SS, the commonest finding is an increase in gut Bacteroidetes with a decrease in Firmicutes resulting in a reduced Firmicutes/Bacteroidetes (F/B) ratio [78]. The proportion of Actinobacteria was decreased whilst *Prevotella* was higher in SS patients compared to HC, leading to worse tear film break up time and reduced tear secretion in SS patients [78]. The phylum Actinobacteria is involved in regulating enteric pathogens in the gut and has a positive effect on tear film break up time [79]. Additionally, SS patients were found to have 50% reduction of the genus *Faecalibacterium* in their stool, which results in lowered production of butyrate. This SCFA is key to suppressing inflammation by promoting generation of tolerogenic dendritic cells (DCs) and regulatory T cells (Tregs) [78, 80]. There was an inverse relationship between gut microbiota diversity and the severity of ocular and systemic disease in SS patients, supporting the intestinal dysbiosis theory in these patients [80]. Mice given a cocktail of oral antibiotics had greater goblet cell loss and worse dry eye when subjected to desiccating stress versus controls, and an increase in pathogenic strains in their stool with higher levels of *Enterobacter*, *Escherichia*, *Shigella*, and *Pseudomonas*, as well as a reduction in the beneficial genus *Faecalibacterium* [18].

Further evidence from animal studies has shown a worse phenotype of Sjögren-like lacrimal keratoconjunctivitis in germ-free (GF) mice in comparison to conventional C57BL/6 J mice [81]. Increased cornea barrier disruption and reduced goblet cell density were reversed when faecal microbiota were transplanted from the conventional to GF mice, thus suggesting that commensal gut bacteria have a role in the homoeostasis of the ocular surface [81]. Zaheer et al. [82]. demonstrated reversal of lymphocytic infiltration of the lacrimal gland in GF CD25 knock-

Table 1. Conjunctival ocular surface microbiome analysis in dry eye disease (DED).

Author, Year	Analysis	Taxa			Implication
		Classes	Increase	Decrease	
Song [57]	SSDE	Phylum	*Actinobacteria, Proteobacteria*	*Bacteroidetes*	Worse Schirmer test and cornea staining
		Genus	*Acinetobacter & Corynebacterium*	*Bacillus*	Increase ocular surface inflammation, reduction in synthesis of lipopeptides which improve tear film stability
	non-SSDE	Phylum	*Proteobacteria*	*Bacteroidetes*	Worse Schirmer test and cornea staining
		Genus	*Acinetobacter*	*Bacillus*	Reduction in synthesis of lipopeptides which improve tear film stability
Qi [72]	autoimmune DED	Phylum	*Actinobacteria, Bacteroidetes, Firmicutes*	*Proteobacteria*	as for SSDE Phyla (see above)
		Genus	*Acinetobacter, Corynebacterium, Prevotella, Streptococcus*	*Herbaspirillum, Pelomonas*	Increase pro-inflammatory bacteria activate macrophage function and drive autoimmunity
	non-autoimmune DED	Phylum	*Proteobacteria*	*Actinobacteria, Bacteroidetes, Firmicutes*	Worse Schirmer test and cornea staining
		Genus	*Herbaspirillum, Pelomonas*	*Acinetobacter, Corynebacterium, Prevotella, Streptococcus*	Earlier first break of tear breakup time, lipid layer deficiency, higher meibomian gland drop out
Gupta [73]	non-autoimmune DED	Genus	*Streptococcus*	*Corynebacterium*	Decreased mucin production, loss of goblet cell function, and increase susceptibility to opportunistic pathogens
Kim [74]	autoimmune DED	Genus	*Acinetobacter, Neisseriaceae, Staphylococcus*,	*Corynebacterium, Actinobacteria*	Decreased ability to form protective biofilm and increase susceptibility to opportunistic pathogens
	non-autoimmune DED	Genus	*Corynebacterium, Streptococcus*	*Acinetobacter, Neisseriaceae, Staphylococcus*,	Decreased neutrophil recruitment and release of antimicrobial agents into tears

out mice following faecal transplant from healthy mice [82]. Therefore, if recolonisation is able to reverse inflammatory change then this is a potential avenue for future therapeutic roles in the field of DED [81]. Regardless, a note of caution must be applied when extrapolating results from animal studies as there are distinct differences in their gut microbiomes from humans.

Intraocular fluid and aqueous humour

The aqueous humour once thought to be sterile is in reality host to its own distinct microbial community [83]. Deng et al. [83] retrieved the aqueous humour fluid from 1000 healthy eyes at the start of routine cataract surgery and detected *Propionibacterium acnes* (now renamed *Cutibacterium acnes*) on polymerase chain reaction (PCR) assay in 71.4% of the eyes. Other microbes identified include *Enterococcus faecalis* and *Staphylococcus epidermis* in anaerobic cultures to a much lesser extent [83]. They applied robust processes to eliminate any risks of false positives throughout their experiments by using negative controls in the form of 0.9% sodium chloride, sampling the conjunctiva, eyelid skin and plasma for comparison to the aqueous humour, and performing metagenomic analysis to sequence the full-length genome of *Cutibacterium acnes* [83]. All of the results strongly suggest a distinct signature intraocular microbiota of the aqueous humour from the other sampled sites, free from contamination from the reagents or environment, with absence of DNA in the negative controls thus reinforcing the initial findings of a true community of microbes within the aqueous humour [83].

INTERMEDIATE AND POSTERIOR SEGMENT MICROBIOME ANALYSIS

In this section the relationship between gut dysbiosis and ocular disease in humans and mice is explored (Table 2).

Uveitis

Uveitis is the umbrella term for a group of heterogenous inflammation subtypes within the eye involving the uveal tissue consisting of the iris, ciliary body and choroid plus their surrounding structures such as the vitreous, retina, and optic disc. It can be categorised according to the site of involvement e.g. anterior, intermediate, posterior; and its aetiology of infectious, and non-infectious (autoimmune). Typically, autoimmune uveitis is associated with systemic autoimmune disease or autoimmune disease localised to the eye with sight-threating implications through the involvement of the neuroretina—a T-cell driven process [10]. Whilst there are various recognised factors influencing the development of autoimmune uveitis, including genetic factors, the role of gut dysbiosis as an emerging environmental factor is gaining attention.

Nakamura et al. [84]. utilised the conventional model of inducible T-lymphocyte mediated uveitis via immunisation with interphotoreceptor binding protein (IRBP) known as experimental autoimmune uveitis (EAU) in B10.RII mice, which are highly susceptible to the protein [84]. A week before immunisation, one cohort of the mice were administered oral antibiotics (ampicillin, metronizadole, neomycin, vancomycin) [84]. This was associated with a significantly reduced mean uveitis severity score and overall microbial load including the major bacterial phylums of Firmicutes and Bacteroidetes in the gut of these animals compared to controls that were not pre-treated [84]. Single use of metronizadole or vancomycin also offered the same protection as broad-spectrum cover. Furthermore, the oral antibiotic group had reduced inflammatory cytokines, effector T-cell lymphocytes, and retinal vascular disruption with a significant increase in both the gut and retinal Tregs, suggesting that gut microbiome alterations can offer protective effects in distant sites [84].

Heissigerova et al. [85]. conducted a similar EAU study and pre-treated their conventionally housed mice (CV) with combined broad-spectrum antibiotics (metronizadole and ciprofloxacin) a week prior to and on the day of immunisation [85]. They also induced EAU in GF and CV mice without antibiotic pre-treatment. Their results demonstrated less inflammation in the GF and CV mice pre-treated with antibiotics from a week prior to immunisation, as seen by reduced T cell infiltration in the retina and reduced Th1 and Th17-type T cells in the eye draining lymph nodes [85]. Therefore, they concluded that the presence of microbiota plays a role in susceptibility to EAU, and treatment with antibiotics may be considered as an adjunctive therapy for uveitis. Nonetheless, Heissigerova and Nakamura's studies have only been able to demonstrate the effectiveness of antibiotics in reducing the severity of the inflammatory response in their murine models rather than preventing it altogether. Both suggest that there may be potentially uveitogenic gut microbiota which can be manipulated through oral antibiotic use. However, the impact of these antibiotics on other commensals and the downstream effects on other pathogenic processes in the body has not been studied and remains unknown.

In contrast, natural triggers of the disease were evaluated in a spontaneous autoimmune uveitis model using T cell receptor (TCR) transgenic R161H mice that have a high frequency of autoreactive T cells [10]. They were given identical oral antibiotics as the EAU model described above from before birth which depleted their commensal microbiota and subsequently attenuated the development of spontaneous disease significantly which was also seen in a separate cohort of GF mice [10] Both groups of mice were found to have decreased Th17 cells in their intestinal lamina propria which supported the theory that commensal microbiota contributes to spontaneous uveitis via antigenic mimicry by their action on retina specific TCR in the gut, independent of the endogenous IRBP antigen [10]. Additionally, commensal gut microbiota and their SCFA metabolites can induce and recruit Tregs as a beneficial adjuvant effect [10, 84, 86].

The two models of autoimmune uveitis discussed suggest that underlying gut dysbiosis facilitates migration and translocation of microbial antigens and activated immune cells to peripheral and immune privileged sites such as the retina [87]. This hints at the presence of an immune-driven gut-retina axis.

Primary open angle glaucoma (POAG)

Glaucoma is a disease affecting the retinal ganglion cells in the neural retina and their axons in the optic nerve resulting in an irreversible optic neuropathy with the only known modifiable risk factor being elevated intraocular pressure (IOP). Yet, even with normalised IOP there is progressive loss of retinal ganglion cells thus indicating that other mechanisms of damage besides pressure mediated are at play [88, 89]. Risk factors include advancing age, systemic diseases such as diabetes, hypo/hypertension, hyperlipidaemia, thyroid disease, obstructive sleep apnoea, autoimmune factors, mitochondrial dysfunction, and genetic mutations. The earliest hint of the role for gut microbiota in the pathogenesis of glaucoma was first described by Kountouras et al. [90]. when gram negative *H. pylori* bacteria was isolated in patients with POAG [90]. Multiple studies have attempted to investigate the link with conflicting and inconsistent findings until a meta-analysis conducted to understand this relationship better showed a statistically significant association between *H. pylori* with POAG and normal tension glaucoma (NTG) [91]. Several theories have been suggested including oxidative stress induced by bacterial metabolites and the cross-reactivity of *H. pylori* antibodies with ciliary body epithelial antigens. Interference of the apoptotic process within the trabecular meshwork is another presumption, however the exact mechanisms remain unclear [92].

Further studies have sought to evaluate the contribution of the gut microbiota in patients with POAG. A rat model demonstrated significantly reduced caecal bacteria composition in glaucomatous rats compared to the controls, with a *raised* Firmicutes/

Table 2. Gut dysbiosis (measured by faecal microbiota) in a range of ocular diseases in humans and mice.

Author, Year	Analysis	Taxa			Interpretation
		Classes	Increase	Decrease	
Moon [78]	SS	Phylum	*Bacteroidetes*	*Actinobacteria, Frimicutes*	Decreased regulation of enteric pathogen virulence
		Genus	*Prevotella*	*Faecalibacterium*	Worst tear film break up time, reduced tear secretion, lowered production of butyrate
Gong [94]	POAG	Family	*Enterobacteriaceae, Prevotellaceae*	(-)	Neuronal inflammation, secretion of pro-inflammatory cytokines
		Genus	(-)	*Megamonas*	Increase in mitochondrial dysfunction
		Species	*Escherichia coli*	*Bacteroides plebeius*	Increase pro-inflammatory bacteria associated with LPS release and subsequent complement activation
Andriessen [11]	nAMD	Phylum	*Firmicutes, Proteobacteria*	*Bacteroidetes*	Increase intestinal permeability and subsequent pro-inflammatory bacteria leading to increase formation of CNV
Rowan [101]	nAMD	Phylum	*Firmicutes*	*Bacteroidetes*	Increase intestinal permeability and subsequent pro-inflammatory bacteria leading to increase formation of CNV
Zinkernagel [102]	nAMD	Family	*Oscillospiraceae*		Increase intestinal permeability
		Species	*Eubacterium ventriosum, ruminococcus torques*	*Bacteroides eggerthii*	Increase in pro-inflammatory cytokines
Das [113]	DR (NPDR & PDR)	Phylum	*Firmicutes, Proteobacteria*	*Actinobacteria, Bacteroidetes*	Increased association with obesity and insulin resistance
		Genus	*Cloacibacillus, Escherichia, Enterobacter, Shigella*	*Bifidobacterium, Faecalibacterium, Lachnospira, Lactobacillus, Roseburia*	Increase in pro-inflammatory and decrease in anti-inflammatory and SCFA producing bacteria thus compromising gut wall integrity and increased susceptibility to insulin resistance
	T2DM without DR	Phylum	*Bacteroidetes, Firmicutes*	*Proteobacteria*	Increase in inflammatory injuries of endothelium through release of LPS
		Genus	*Escherichia, Enterobacter, Methanobrevibacter and Treponema*	*Anaerostipes, Blautia, Coprococcus, Lachnospira, Phascolarctobacterium, Roseburia*	Increase in pro-inflammatory and decrease in anti-inflammatory and SCFA producing bacteria
Huang [117]	DR	Phylum	*Bacteroidetes*	*Firmicutes*	Increase in inflammatory injuries of endothelium through release of LPS
	DR & T2DM without DR	Genus	*Bifidobacterium, Lactobacillus*	*Clostridium, Escherichia, Eubacterium_hallii, Faecalibacterium, Shigella*	Increase in pro-inflammatory and decrease in anti-inflammatory and SCFA producing bacteria

Bacteroidetes ratio, and increase in the Verrucomicrobia phylum and certain genera such as *Romboutsia*, *Akkermansia*, and *Bacteroides*, all of which were associated with loss of retinal ganglion cells [93]. The levels of glutathione—an antioxidant crucially involved in cellular reactive oxygenation species generation and elimination - were reduced in the blood of glaucoma rats [93]. Glutathione was found to be negatively correlated with *Romboutsia* and the Firmicutes/Bacteroidetes ratio but positively correlated with retinal ganglion cells, suggesting a link between oxidative stress related alterations in glaucoma pathogenesis [93]. Similar findings were noted in a human study that showed a distinct gut microbial profile in POAG patients versus controls [94]. Thirty POAG and 30 non-POAG healthy Chinese patients from the same city and diet preferences (and age-matched to 5 years and sex-matched) were included in the study. The POAG patients had moderate severity of the disease on average with a mean visual field mean defect of $-7.62 +/- 5.12$ dB and cup to disc ratio of >0.6 in at least 1 eye. The control group did not have any current or prior history of raised IOP, nor the use of any IOP lowering meds with cup to disc ratio of <0.4 in both eyes and the absence of any intraocular pathology as well as family history of glaucoma. Their early morning stool sample revealed POAG patients had increased levels of *Prevotella*, *Enterobacteriacae*, and *Escherichia coli*, all of which are implicated in neuronal inflammation and secretion of pro-inflammatory cytokines [94].

A Japanese study on patients with normal tension glaucoma (NTG) identified a link between polymorphisms in the toll-like receptor 4 (TLR4) involved in mediating immune responses to heat shock proteins (HSP) and bacterial LPS, and the risk of developing the disease [95]. Astafurov et al. [96]. observed an IOP-independent upregulation of TLR4 signalling, microglial and complement system activation from peripheral subcutaneous administration of LPS in mouse models, resulting in enhanced axonal degeneration and neuronal loss [96]. Chen et al. [88]. demonstrated transient elevation of IOP activated T-cell responses to HSP—which have previously been detected in human and animal models of glaucoma, resulting in T cell infiltration into the retina of mice leading to prolonged retinal neurodegeneration thereafter [88]. GF mice did not demonstrate HSP related T cell responses or glaucomatous neurodegeneration following induced elevation in IOP either indicating that the commensal microbiota is linked in the development of the disease coupled with an autoimmune mechanism that is T cell driven [88].

The future of glaucoma research in the context of the human microbiome is likely to revolve around the interaction between pivotal molecular pathways and their influence on mediating the gut microbiota. One example has been the discovery of several hub key genes in POAG patients: a transcription factor - nuclear factor kappa B (NF-κB1), a pro-inflammatory cytokine—interleukin 8 (IL8), and a pattern recognition receptor—TLR9, which have demonstrated the ability to regulate gut macrophage activity and simultaneously impacting disease progression [89]. Although the exact mechanisms are not fully understood yet, these molecules and their downstream immune modulatory actions may be potential targets for future research into gut dysbiosis and glaucoma [89].

Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a leading cause of vision loss in the over 60 age group in the western world. It is characterised by macula damage from hyaline deposits referred to as drusen between the retinal pigment epithelium (RPE) and Bruch's membrane. Accumulation of drusen leads to progressive RPE dysfunction and photoreceptor cell damage. Ultimately, these changes either produce geographic atrophy in the macula which is seen in the "dry" type or develop choroidal neovascularisation (CNV) membrane in the "wet" type also known as neovascular AMD (nAMD) [97–99]. Whilst studies have identified risk factors associated with the disease such as increasing age, smoking, dietary intake, genetic predisposition, complement system dysregulation, and chronic low-grade inflammation, there is emerging evidence that the gut microbiome also contributes to the complex interactions in the pathogenesis of AMD [11, 100].

Rowan et al. [101]. studied the effects of high-glycaemic (HG) and low-glycaemic (LG) index diet over a 12 month period on otherwise healthy wild-type C57BL/6 J mice. At the end of the study, those fed a high-glycaemic (HG) index diet were found to have gut microbiota enriched with the Firmicutes phyla and *Clostridia* class which was related to advanced retinal damage in the form of photoreceptor cell damage and retina pigment epithelium abnormalities that highly resemble human dry AMD. The mice fed on a low-glycaemic (LG) index diet had predominantly *Bacteroidales* order (belonging to the *Bacteroidia* class and Bacteroidetes phylum) and *Erysipelotrichi* classes associated with protection from development of retinal lesions seen in dry AMD [101]. Switching from HG to LG diets was shown to slow and reverse the development of lipofuscin granules and lipid droplets synonymous with dry AMD by altering gut microbiota and their metabolite production which protect against the disease [101]. The gut microbial metabolite serotonin, sampled via the plasma of these mice, was shown to have an inverse relationship with the frequency of dry AMD development in the LG and HG to LG groups [101].

Andriessen et al. [11]. found that mice on a high fat diet had raised *Proteobacteria* species -a sign of gut dysbiosis, F/B ratio from baseline, and an increase in CNV formation with localisation of microglia and macrophages within the lesions, consistent with findings from human studies [11]. These mice had a threefold increase in intestinal permeability which allows for translocation of bacterial by-products such as LPS and higher pro-inflammatory cytokines such as IL-1β, IL-6, tumour necrosis factor (TNF)-α, IL-17A, IFN-γ and vascular endothelial growth factor (VEGF)-A driving the CNV process [11, 101]. Administration of oral neomycin, however, was able to restore the Firmicutes/Bacteroidetes ratio and improve their glucose tolerance, confirming effective modulation of gut microbes. Similarly, caecal microbiota transplantation from regular to high fat diet mice restored the healthy equilibrium of the Firmicutes/Bacteroidetes ratio and reduced CNV formation [11].

Zinkernagel et al. [102] subsequently sequenced the gut metagenomes of AMD patients and controls in order to identify whether the composition and functional diversity of the gut microbiome is linked to the disease process and progression [102]. Their results revealed that AMD patients had enrichment of *Oscillibacter species, Anaerotruncus species, Eubacterium ventriosum*, and *Ruminococcus torques*, all of which are associated with increased gut permeability and elevated inflammatory cytokines [103–105]. These patients also demonstrated up-regulation of L-alanine fermentation as seen in various retinopathies, glutamate degradation that decreases neurotransmission in the retina, and increased arginine biosynthesis that is associated with progressive chorioretinal atrophy [106–108]. Moreover, the gut microbiome of the controls was enriched with *Bacteroides eggerthii*. This microbe has a significant role in fermenting dietary carbohydrates to provide SCFAs [102].

The relationship between the complement system and gut microbiome was assessed in a human and mouse study [109]. Mice with C3-deficiency had raised F/B ratio similar to the animals in the high-fat and HG diet described earlier [109]. AMD patients' gut microbiomes were enriched with genes of aberrant purine signalling pathways involved in immune dysregulation plus the class *Negativicutes* belonging to the phylum Firmicutes was more abundant in this subset of patients and was positively correlated with complement factor H 3 (CFH3) [109].

It is clear that gut microbiota are responsive to dietary changes with consequent effects on the AMD disease process. The Age-Related Eye Disease Studies (AREDS and AREDS2) demonstrated

the ability to reduce the risk of AMD progression in patients with an oral supplementation consisting of vitamin C and E, copper, zinc, lutein and zeaxanthin [110]. Yet, the bioavailability and absorption of these components depends upon the gut microbiome composition of individuals [102]. For instance, zinc absorption has been shown to be influenced by competition between the gut microbiome of the host [111]. It is undeniable that gut microbiome provide a link between nutrition and the development of AMD and further research to address preventative measures in this respect such as the AREDS studies would be beneficial for reducing the disease burden [102].

Diabetic retinopathy (DR)

The incidence of Type 2 diabetes mellitus (T2DM) is increasing concurrent with the growing obesity pandemic however Type 1 diabetes mellitus (T1DM) – which has been widely attributed to immunogenetic factors in susceptible individuals, is also demonstrating an upward trend [112]. As global life expectancy continues to rise, and age of onset of both type 1 and type 2 diabetes continues to fall, this increased incidence will translate into greater prevalence of this chronic disease and its inevitably associated microvascular complications such as diabetic retinopathy (DR). The underlying mechanisms of both non-proliferative (NPDR) and proliferative (PDR) diabetic retinopathy are well known to be due to chronic hyperglycaemia mediated micro-angiopathy, inflammation and more recently the role of gut microbiome.

Das et al. [113]. compared the gut microbiome of patients with DR (NPDR and PDR) with T2DM patients without DR and healthy controls [113]. At the phylum level, Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria were the most abundant in all three groups at similar proportions with Bacteroidetes and Actinobacteria found significantly less in DR patients than healthy controls [113]. At the genus level, T2DM patients had increase in pro-inflammatory bacteria such as *Escherichia, Enterobacter, Methanobrevibacter* and *Treponema* with a reduction in anti-inflammatory bacteria including *Roseburia, Lachnospira, Coprococcus, Phascolarctobacterium, Blautia*, and *Anaerostipes* against healthy controls [113]. DR patients also had increase in pro-inflammatory *Escherichia, Enterobacter, Cloacibacillus, Shigella* and a reduction in anti-inflammatory *Faecalibacterium, Roseburia, Lachnospira, Bifidobacterium*, and the probiotic *Lactobacillus* in comparison to healthy controls [113]. *Roseburia* and *Faecalibacterium* are the dominant SCFAs-producing organisms and promote gut integrity by reducing bacterial translocation and positively correlated with pancreatic beta cell immunity from destruction and insulin sensitivity [114, 115]. The pathogenic bacterium *Escherichia* expresses extracellular fibres which are a form of bacterial amyloid and together with their viral phages can trigger autoimmunity in vulnerable individuals particularly children toward developing T1DM [116]. Their abundance in T2DM and DR suggests a probable pathway of both diabetic and retinopathy disease progression [116].

Contrarily, Huang et al. [117]. demonstrated that DR patients had significantly higher abundances of the phylum Bacteroidetes than HC and T2DM patients without DR [117] As a gram-negative bacterium, Bacteroidetes cell walls are rich in LPS known to trigger inflammatory injuries of the endothelium. Furthermore, their results demonstrated that both T2DM patients without DR and DR patients had increased anti-inflammatory *Bifidobacterium* and *Lactobacillus* with reduced pro-inflammatory *Escherichia* and *Shigella* in comparison to healthy controls [117].

The observations of these two studies alone highlight the incongruity across the literature in profiling the gut microbiome amongst diabetic, non-diabetic and DR patients. The various reasons for this include differences in diet, ethnicities, and geographical factors and confounders such as diabetic medication. Whilst it is difficult to prove the causal relationship between the differences seen in the gut microbiota composition amongst all the published research, dysbiosis is clearly present within the diabetic population and has an impact on DR.

TREATMENTS

Faecal microbiota transplantation (FMT)

FMT was first performed in 1958 and over half a century later it has been approved by the Food and Drug Administration (FDA) in the US for the prevention of recurrence of *Clostridiodes difficile* infections (CDI) in adults following antibiotic treatment for recurrent CDI [118]. This therapy also known as stool transplant involves the transfer of healthy donor faecal microbes into another individual to reverse the dysbiotic state, reduce inflammation and improve the gut epithelial barrier.

Within ophthalmology, half of the patients with aqueous deficient immune-mediated DED reported subjective improvement in their symptoms at 3 months following healthy donor FMT but the results were not statistically significant especially pertaining to dry eye signs [119]. Nevertheless, there were no adverse effects seen in this small study. Two other studies of FMT from conventional to germ-free mice restored goblet cell density and cornea barrier function [81, 82].

In an AMD model, FMT from older to younger mice showed increased retinal inflammation via higher levels of C3 expression in the retina associated with worsening extracellular deposits in the outer retina and this was reversed when FMT was transferred from younger to the older mice group [120]. The underlying mechanisms are thought to be linked to upregulation of inflammatory cytokine signalling on exposure to bacterial toxins in the gut such as LPS resulting in accumulation of complement proteins at retinal membranes [120].

FMT undertaken from human participants with Behcets disease (BD) and Vogt Koyanagi Harada(VKH) into EAU mice resulted in more severe intraocular inflammation suggesting a role of intestinal microbiota in uveitis [121, 122].

Limited studies have taken place in humans however and further research into the safety profiling and optimal dosing of FMT is needed as well as considering confounders such as diet, environmental and genetic factors. OpenBiome—a non-profit stool bank, is one such example where microbiome research is centred on investigating FMT for conditions including recurrent *Clostridium difficile* infections and malnutrition [123]. The organisation is also focused on microbiome conservation especially pertaining to underrepresented human microbiome diversity from outside the United States and Western Europe as the majority of research in this field is also centred amongst these populations.

Probiotics & prebiotics

Probiotics as defined by the World Health Organization (WHO) are "live microorganisms which when administered in adequate amounts, confer a health benefit upon the host" [124]. They consist of non-pathogenic bacterial and yeast populations such as *Lactobacillus* and *Bifidobacterium* that produce lactic and acetic acid which have been shown to have inhibitory effects on Gram negative bacteria plus *Saccharomyces* which produce SCFAs, to name a few [125–127]. Prebiotics are substrates that are selectively utilised by host microorganisms conferring a health benefit and are typically non-digestible carbohydrates such as fructo-, oligo-, and trans-galacto-oligosaccharides which are fermented by gut microbiota to produce SCFA [128, 129].

Pro- and prebiotics when utilised together they are known as synbiotics [130]. They are both available via oral food supplementation and naturally through food sources such as yoghurts, kefir, sauerkraut, whole grains, bananas, onions, garlic and soybeans though this is not an exhaustive list.

In aqueous deficiency DED, consumption of synbiotics can reduce symptoms as assessed by the OSDI (Ocular surface disease index) in otherwise healthy participants with dry eye symptoms

however there was no improvement in the clinical signs observed at the 1-month mark at the end of the study [131]. Several other human studies on oral probiotics have shown significantly improved subjective symptoms and objective measures of aqueous deficiency DED through increase tear secretion and TFBUT [132–134]. However, these patients had mild disease and none with immune mediated DED. A murine EAU and dry eye model investigated the effects of oral administration of IRT5 - a probiotic cocktail consisting of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus reuteni*, *Bifidobacterium bifidium* and *Streptococcus thermophilus*, on intraocular and surface inflammation. The results revealed attenuation of inflammation in the outer nuclear layers, lower ocular staining score and higher tear secretion in the treated mice [134–136]. The underlying mechanism of action remains unknown but postulated to be related to interactions with gut associated lymphoid tissue (GALT) and immune modulation by reducing production of pathogenic cytokines (IL-17, INF-Y, TNF alpha) and increasing Treg cells [135–137]. Topical applications of the prebiotic resveratrol delivered to the ocular surface through sustained release via a contact lens in a rabbit study demonstrated reduced growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* with anti-inflammatory properties [138]. This exciting discovery though still in infancy stages could have considerable beneficial impact on CL wearers through limiting the risk of microbial keratitis and improving the ocular surface health.

Six patients with vernal keratoconjunctivitis were treated with *Lactobacillus acidophilus* containing probiotic drops known for their anti-inflammatory properties for a month [139]. At the end of the study, a significant improvement in patients clinical signs and symptoms of photophobia, tearing, conjunctival hyperaemia and chemosis [139].

A prospective comparative pilot study of 26 children with chalazions demonstrated the group given an oral probiotics combination therapy in addition to medical treatment of lid hygiene, warm compression and combined dexamethasone with tobramycin ointment demonstrated significantly shorter time taken for resolution of their symptoms with an average of 28 days against 54 days in the non-probiotic group [140]. There were no complications and no recurrence in either group during the follow up period.

Mice fed heat-killed *Lactobacillus paracasei* KW3110, a lactic acid producing bacteria that can suppress inflammation led to restoration of the F/B ratio towards a healthy state and decreased ganglion cell loss in the retina [141]. Pre-treatment with live probiotic *Escherichia coli* Nissle 1917 reduced the severity of EAU but had no effect when it was started a fortnight after the induction of uveitis proving that prevention is possible and can be successful [141, 142].

These are several examples of the wide breadth of evidence in the pro- and pre-biotics field. Nevertheless, all these results have to be carefully interpreted and not necessarily generalisable within a population. There are risks associated with probiotic supplements especially in patients with weakened immune systems including infection both localised or disseminated sepsis, bowel ischaemia from increase oxygen demand of the higher density population of microbes, allergic reactions, and the potential for antibiotic resistance through horizontal gene transfer between the non-pathogenic strains to the opportunistic pathogens [143]. Whilst these findings of pro- and pre-biotic use are promising, the research is still in its infancy and more in vivo human experiments are needed.

CONCLUSION

The abundance of scientific literature discussed hitherto on the bi-directional relationship between gut and specific organ microbiota such as the eye clearly cements the role of our microbiome in health and disease. These observations must be acknowledged and taken into consideration in the routine management of patients [144–148].

Our role as ophthalmologists is not only to provide direct care of the organ we specialise in, but also to consider the holistic factors underlying health and disease, including the gut microbiome. Thus, our patients should be advised as to how they can improve their eye health through taking steps to look after gut health and the immune system in a variety of manners. This includes dietary and lifestyle measures to include increasing dietary fibre and intake of a variety of plants totalling over 30 each week, 'eat the rainbow' for polyphenols, incorporating fermented foods whilst avoiding ultra-processed foods, reducing exposure to pollution and chemicals (including household chemicals and cosmetics), as well as increasing physical activity [149–157]. Probiotics in the form of capsules, pills, and liquid preparations may also benefit the gut microbiome, though evidence is mixed about mechanisms of action and efficacy, and effects are likely to be minimal compared to increasing dietary fibre intake [158]. Where specific information is not available about which strains are beneficial for a given health condition, the best choice of probiotic is one with multiple strains in a high dose. Patients should also be aware that antibiotic use can have a detrimental effect on the gut microbiome [159, 160]. Whilst antibiotics are often necessary, estimates suggest that roughly half of prescribed antibiotics are unnecessary, which contributes to the development of antibiotic resistance in both the patient and the community, as well as causing collateral damage to the microbiome and, by extension, the immune system [160]. Targeted advice around the microbiome and specific foods to consume and avoid is not yet possible, and can be counter-productive, as the broader message is one of reducing inflammation through plant-based variety and a decrease in the proportion of the diet coming from animal foods and refined plant foods [149–157, 161].

There is no single signature microbiome representing either health or disease due to variation in ethnicity, diet, location, sex, age, and many other factors. The microbiota contains much redundancy, with overlap between species, gene sharing, and constant flux, meaning that the characterisation of species or strains present is less informative than the functionality of the microbiome as a community. Metabolomics will continue to reveal far more about the functions and interactions between microbial species, as well as between the microbiome and its human host [162]. This will lead to therapeutic interventions that are much more targeted where necessary.

We anticipate that future ocular microbiome studies will continue to develop on exacting microbiota profiles and the molecular mechanisms to firmly establish the critical associations between gut and eye health. Trial designs must be refined to reflect our evolving understanding of the ocular microbiome to improve the interpretation of variability across studies. This will allow a more sophisticated analysis of the published data and inevitably translate into potential for more research on customised interventions specifically targeting the microbiome for the management of ocular disease.

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AUTHOR CONTRIBUTIONS

SK: Investigation, writing—original draft preparation, review and editing. AC: Writing—review and editing. BP: Conceptualization, Investigation, Resources, Writing—review and editing. RM: Conceptualization, Investigation, Resources, Supervision.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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