

Human Microbiota and Ophthalmic Disease

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The human ocular surface, consisting of the cornea and conjunctiva, is colonized by an expansive, diverse microbial community. Molecular-based methods, such as 16S rRNA[†] sequencing, has allowed for more comprehensive and precise identification of the species composition of the ocular surface microbiota compared to traditional culture-based methods. Evidence suggests that the normal microbiota plays a protective immunological role in preventing the proliferation of pathogenic species and thus, alterations in the homeostatic microbiome may be linked to ophthalmic pathologies. Further investigation of the ocular surface microbiome, as well as the microbiome of other areas of the body such as the oral mucosa and gut, and their role in the pathophysiology of diseases is a significant, emerging field of research, and may someday enable the development of novel probiotic approaches for the treatment and prevention of ophthalmic diseases.

INTRODUCTION

The Human Microbiome Project, launched in 2008 by the National Institutes of Health, has revealed a remarkably abundant and diverse community of microbial species that inhabit the human body [1]. Understanding the role of the trillions of bacteria, viruses, and fungi that comprise the human microbiota is critical to enhance our understanding of a variety of human diseases and their pathophysiology. Advances in the technology of next-generation sequencing and bioinformatics tools have facilitated the characterization of the human microbiome [2]. Discovery of various aspects of the human microbiota and its role in physiology and pathogenesis has revolutionized our approach in studying certain diseases and developing novel treatment modalities. The intestinal microbiota has been implicated in a variety of autoimmune and inflammatory diseases, including inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes [3-5].

While the Human Microbiome Project initially studied five main body areas – the gastrointestinal tract, the skin, the urogenital tract, the oral mucosa, and the nasal mucosa [6,11] – an emerging area of research is focusing on the eye and the microbiota of the ocular surface [7,14].

The fundamental question to explore is whether the ocular surface harbors a resident microbiota and if so, what species of microorganisms comprise it. Subsequently, we must examine how the microbial community is influenced by host and environmental factors. Finally, we can provide further clinical significance by investigating the potential associations between alterations of the microbiome and the pathogenesis of ophthalmic diseases. This study aims to systematically review and summarize evidence regarding the most up-to-date understanding of microbiota colonizing the ocular surface, as well as investigate the potential role of the human microbiota in ophthalmic disease.

THE EYE AND THE OCULAR SURFACE MICROBIOTA

Structurally, the eye is composed of an internal compartment – which consists of the anterior and posterior chambers, the iris, the lens, the vitreous cavity, the retina, the ciliary body, the choiroid, and intrinsic ocular muscles – and an external compartment – which consists of the conjunctiva, the cornea, the sclera, and the tear film (Figure 1). The internal compartment of the eye maintains a sterile environment, and is physically separated

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[†]Abbreviations: rRNA, Ribosomal RNA; PCR, Polymerase chain reaction; DNA, Deoxyribonucleic acid; IgA, Immunoglobulin A; CFU, Colony-forming unit; IVT, intravitreal; TMP-SMX, trimethoprim-sulfamethoxazole; CIE, corneal infiltrative events; BDNF, brain-derived neurotrophic factor.

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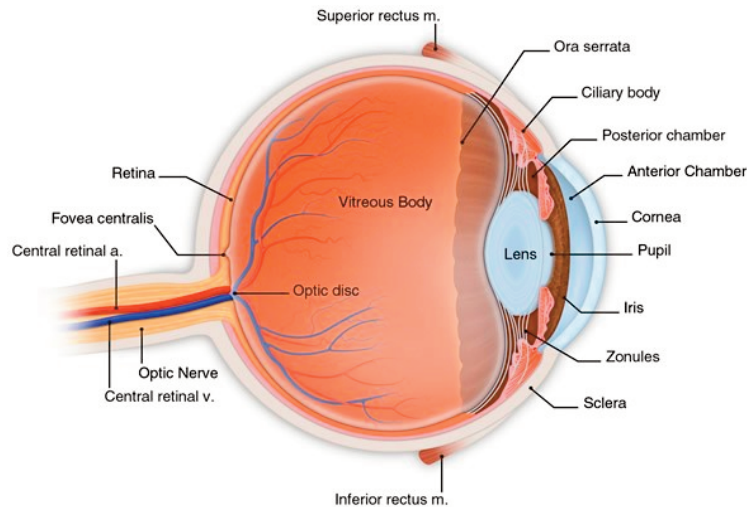


Figure 1. Anatomy of the eye
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from the immune system by the blood-retinal barrier. In contrast, the external compartment of the eye is exposed to microorganisms in the environment [8].

The ocular surface is comprised of the cornea and its overlying tissue, the conjunctiva. Thus, the ocular surface microbiota refers to the resident microorganisms that colonize the conjunctiva and the cornea and importantly, excludes the eyelid (microbes found in the eyelid are considered part of the skin microbiota, which is included in the five main research areas in the Human Microbiome Project). Direct exposure to the external environment means that the ocular surface is susceptible to a gamut of antigens and pathogens. In addition to serving as a physical barrier against the external environment, the ocular surface has a critical role in innate immunity [9]. The mucosal immune system protects the conjunctival and corneal epithelia via innate and adaptive defense mechanisms present in the tissue and tear film [10]. While the ocular surface epithelium is constantly in contact with commensal bacteria, the epithelial cells of the cornea and conjunctiva in a healthy individual do not undergo an inflammatory response. Studies have shown that ocular surface epithelial cells recognize and selectively respond to microbial components of ocular pathogenic bacteria by producing pro-inflammatory cytokines [9]. The lack of an inflammatory response to non-pathogenic bacteria suggests a unique innate immune response of the ocular surface epithelium that supports the colonization of a resident microbiota.

While a substantial amount of evidence strongly supports the existence of an ocular surface microbiota, it is worthwhile to note that analysis of the ocular microbiome is currently in its early stages. Willcox et. al. argues that determination of the ocular microbiome with molecular techniques has lagged in relation to analysis of the microbiome of other areas of the body, and a greater number of rigorous cross-sectional and longitudinal studies examining changes in the microbiota are necessary to maintain current hypotheses [7]. Turnbaugh et al., in 2007, questioned the existence of a stable core microbiome, proposing that there exist only transiently present organisms on

the ocular surface, as opposed to a resident microbiota [11]. In recent years however, investigation of the ocular microbiome using molecular techniques such as 16S rRNA gene analysis have identified, with greater precision, a wider range of homeostatic species that colonize the ocular surface, reinforcing the concept of a stable, unique ocular surface microbiota.

CHARACTERIZATION OF THE OCULAR MICROBIOTA

Previous analyses of the ocular surface microbiota, performed using microbiological culture techniques, reported a significantly different and less diverse profile than what has most recently been discovered using molecular techniques. The characterization of the ocular surface microbiota using culture-based methods was purported to be dominated by Gram-positive species, especially *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Propionibacterium* [12]. In addition, some Gram negative species, such as *Haemophilus* and *Neisseria*, as well as fungal isolates were cultured from the ocular surface of healthy human subjects [12,13]. A major disadvantage of culture-based techniques that may account for its inaccuracy in microbiome characterization is that species detection is significantly biased towards fast-growing microorganisms that can successfully be cultivated on standard media [14-16].

Genomics-based detection and identification of microbial species has exposed a significantly expanded diversity of ocular surface microbiota than what had been previously uncovered by culture-based methods. The first gene sequencing-based survey of the bacterial species found on the ocular surface, using 16S rRNA PCR, was conducted in 2011 by researchers at the Bascom Palmer Eye Institute. Deep sequencing of conjunctival DNA revealed an average of 221 species of bacteria per subject [14]. The bacteria were classified into five phyla and 59 distinct genera, with twelve genera being ubiquitous among all subjects in the analyzed cohort (Table 1). Of the five bacterial phyla, *Proteobacteria*, *Actinobacteria*,

Table 1. Composition of the ocular surface microbiota by phylum and genus, determined according to relative abundance of classified 16S rRNA gene reads [14].

Percentage of all sequences ^a (%)		
Phylum	<i>Proteobacteria</i>	64%
	<i>Acetivobacteria</i>	19.6%
	<i>Firmicutes</i>	3.9%
	Unclassified ^b	12.5%
Genus	<i>Pseudomonas</i>	18%
	<i>Bradyrhizobium</i>	12%
	<i>Propionibacterium</i>	11%
	<i>Acinetobacter</i>	9%
	<i>Corynebacterium</i>	8%
	<i>Brevundimonas</i>	4%
	<i>Staphylococcus</i>	2%
	<i>Aquabacterium</i>	2%
	<i>Sphingomonas</i>	0.5%
	<i>Streptococcus</i>	0.5%
	Other	2%
	Unclassified ^b	31%

^aDong et al. analyzed 115,003 sequences in total.

^bThe Ribosomal Database Project-II software was unable to classify 12.5% and 31% of sequences to the phylum and genus level, respectively. Unclassified bacteria are designated as novel phylotypes.

and *Firmicutes* accounted for more than 87 percent of all sequences; the remaining two phyla, *Cyanobacteria* and *Bacteroides*, were present in contamination-level quantities and were excluded from further analysis. The twelve common genera – *Pseudomonas*, *Propionibacterium*, *Bradyrhizobium*, *Corynebacterium*, *Acinetobacter*, *Brevundimonas*, *Staphylococci*, *Aquabacterium*, *Sphingomonas*, *Streptococcus*, *Streptophyta*, and *Methylobacterium* – comprised more than 96 percent of the classified microbiome [14]. The five most abundant genera out of the twelve ubiquitous genera identified by Dong et al. were *Pseudomonas*, *Bradyrhizobium*, *Propionibacterium*, *Acinetobacter*, and *Corynebacterium*, followed by *Brevundimonas*, *Staphylococcus*, *Aquabacterium*, *Sphingomonas*, and *Streptococcus*. As these twelve genera accounted for more than 96 percent of the total classified bacterial DNA sequences, the data strongly suggests that the healthy conjunctiva is colonized by a resident homeostatic microbiota. To date, the findings from this study remain the most comprehensive DNA sequencing-based characterization of bacterial diversity at the ocular surface.

Consistent with findings that genus composition of the microbiota varies at different layers in other areas of the body such as the human epidermis, the microbiota of the ocular surface appears to have vertical stratification of species composition [17]. Swabbing the ocular surface with light pressure yielded sequences of opportunistic and environmental species, such as *Rothia*, *Herbaspirillum*, *Leptothrichia*, and *Rhizobium*. These bacteria captured from the superficial layer likely represent transient species on the ocular surface. In contrast, using a “deep” swab, in

which dry cotton was applied with greater pressure, yielded an abundance of *Staphylococci*, *Corynebacteriae*, and *Proteobacteria* species, which localize to the mucosal layer and conjunctival epithelium [14]. Deep swabbing is thus necessary to obtain an accurate and comprehensive characterization of the diversity of the ocular surface microbiota.

HOST AND ENVIRONMENTAL FACTORS THAT MAY ALTER THE OCULAR SURFACE MICROBIOTA

The ocular surface microbiota can become altered by host factors, environmental insults, and disease states [18]. Disruption of the ocular surface may breach the innate immune system at the corneal and conjunctival epithelia and allow microbial ligands to trigger ocular inflammation [12,19]. Alterations in the microbiota of the ocular surface have been associated with conditions such as dry eye syndrome, contact lens wear, keratoprosthesis, antibiotics, and infection [12].

The tear film, which lubricates the ocular surface epithelia, contains antimicrobial compounds such as lysozyme, lactoferrin, immunoglobulin A (IgA), lipocalin, and complement [41]. Given the immunological role of tears in the defense against potential pathogens, it is reasonable to conjecture that certain situations that affect the tear film, such as dry eye syndrome and contact lens wear, may alter the ocular surface microbiota.

Dry eye syndrome has consistently been associated with inflammatory ocular surface conditions such as anterior blepharitis, keratitis, and ocular rosacea [20]. Gra-

ham et al. used both conventional culture and 16S rDNA PCR to compare the bacterial population of the ocular surface of normal and dry eye subjects, collecting conjunctival swab specimens from a cohort of patients with dry eyes as well as healthy control subjects over a three-month period. They found that certain bacteria species were found in samples from dry eye subjects only, including *Bacillus* sp. and *Klebsiella oxytoca*, in addition to an association between elevated bacterial count (CFU/swab) and the incidence of blepharitis [21]. Increasing bacterial count was correlated with a decrease in goblet cells among the subgroup of 27 subjects, consistent with previous studies that have demonstrated a depletion of goblet cells in other areas of the body after colonization by bacteria [22]. A reduction in goblet cells, which produce the mucins found on the ocular surface, results in a thinned tear film and diminishes the barrier to infiltration and colonization of the ocular surface by external pathogens [22]. The production of mucins on the ocular surface may pose resemblance to the production of fucosylated and sialylated glycoproteins in the intestinal tract [40]. Perhaps the human host secretes glycans and polysaccharides to promote the growth of certain microbial species in the ocular surface in an analogous manner to findings in the intestinal tract.

Alterations of the ocular surface microbiota in contact lens wear has also been studied. Larkin et al. examined the microbial colonization of the conjunctiva in contact lens wearers and compared them to control subjects. Their results included the finding that the ocular surface of contact lens wearers yielded higher bacterial counts than that of control subjects. However, the authors found no qualitative variation in the species of bacteria identified between the lens-wearing and control groups [23].

Studies of the bacterial microbiota colonizing the ocular surface of patients with Boston type 1 keratoprotheses (K-Pro) have found no quantitative difference in positive cultures from conjunctival swabs of K-Pro and control patients [24,25]. Qualitatively however, researchers found that samples from K-Pro patients grew not only coagulase-negative *Staphylococcus*, which was the singular species of bacteria to grow in the control group samples, but also a variety of other Gram-positive bacteria [24].

Ophthalmic antibiotics are used to treat and prevent a variety of infectious and inflammatory ocular conditions. The Antibiotic Resistance of Conjunctiva and Nasopharynx Evaluation (ARCANE) study, which aimed to determine the effects of repeated exposure of topical antibiotics on resistance patterns of the conjunctival microbiota, concluded that the repeated use of macrolide and fluoroquinolone ophthalmic antibiotics leads to a significant increase in Gram-positive species, particularly *Staphylococcus epidermidis*, isolated from culture [26]. Fontes et al. investigated the effect of orally administered trimetho-

prim-sulfamethoxazole (TMP-SMX) administration on the conjunctival microbiota in patients with HIV infection. Chronic administration of TMP-SMX was found to be associated with an altered conjunctival microbiota that contained a significantly greater percentage of antibiotic-resistant *Staphylococcus* species [27]. Yin et al. also discovered a shift in the conjunctival flora as a result of antibiotic use. Repeated use of a topical antibiotic, such as moxifloxacin, after intravitreal (IVT) injection was correlated with increased antibiotic resistance of the ocular surface microbiota [28].

Infectious pathologies have also been linked to shifts in the homeostatic ocular microbiome. An analysis of the microbial composition of the ocular surface of healthy eyes and eyes with bacterial keratitis, utilizing DNA sequencing, found that bacterial keratitis was associated with the depletion of homeostatic ocular microorganisms and the emergence of a pathological microbiome dominated by *Pseudomonas aeruginosa* [29]. Investigation of the conjunctival flora in patients with human immunodeficiency virus (HIV) infection compared to HIV-negative patients, however, concluded that there was no significant difference between the types and proportions of microbial organisms isolated from HIV-positive and HIV-negative eyes [30]. Analysis of the conjunctival flora of HIV patients who received antibiotic treatment with systemic clarithromycin demonstrated a significant decrease in the conjunctival flora [30].

ROLE OF THE MICROBIOME IN OCULAR DISEASE

Many diseases involving the ocular surface, such as dry eye syndrome, chronic follicular conjunctivitis, and various inflammatory eye diseases, appear to have idiopathic etiologies. Evidence indicating the existence of a resident ocular surface microbiota suggests that the normal microbiota plays a protective role in preventing proliferation of pathogenic species, and that ophthalmic pathologies are linked to alterations in the homeostatic microbiome.

Ocular Microbiota and Ophthalmic Diseases

Recent studies have investigated the link between alterations in the ocular surface microbiota and ophthalmic disease. Sankaridurg et al. conducted a study exploring the microbial colonization of soft contact lenses as a risk factor associated with corneal infiltrative events (CIE) and found that colonization of lenses with pathogenic bacteria, especially Gram-negative bacteria such as *Serratia marcescens* and *Haemophilus influenzae*, was significantly associated with CIE [31]. Lee et al. conducted DNA sequencing analysis of ocular surface samples from blepharitis patients and health controls and concluded that blepharitis may be induced by a change in the microbial

composition – namely, greater quantities of *Streptophyta*, *Corynebacterium*, and *Enhydrobacter* species [20].

As suggested by Dong et al., a number of potentially pathogenic bacteria may reside at the ocular surface [14]. Post-operative infectious endophthalmitis is a serious, vision-threatening complication of ocular surgery that involves inflammation of the ocular surface, the anterior and posterior compartments of the eye, as well as adjacent structures. Acute, post-operative endophthalmitis presumably occurs when the patient's own periocular bacteria enter the sterile intraocular compartments of the eye during surgery and cause diffuse infection and inflammation. Thus, pre-operative, intra-operative, and post-operative antibiotic prophylaxis are used to prevent acute endophthalmitis [32]. Consideration of the ocular surface microbiota may have two-fold significance in acute endophthalmitis. First, alterations in the microbiota can influence the species of microorganisms that colonize the ocular surface and heighten the risk for intraocular infection by pathogenic bacteria. Second, knowledge of the composition of both the core and transient microbiota of the ocular surface can aid in the determination of the most effective antibiotic prophylaxis to prevent post-operative acute endophthalmitis in patients [33].

Miller et al. evaluated the concept that infectious microorganisms play a role, perhaps as a cofactor to genetic and environmental risk factors, in ocular adnexal neoplasms. Several microorganisms may have a pathogenic role in ocular malignancies, including human papilloma virus in conjunctival papilloma and squamous cell carcinoma, HIV in conjunctival squamous cell carcinoma, Kaposi sarcoma-associated herpes virus in conjunctival Kaposi sarcoma, and *Helicobacter pylori*, *Chlamydia*, and hepatitis C virus in ocular adnexal mucosa-associated lymphoid tissue lymphomas [12]. Depletion or alteration in the commensal microbiota, due to antibiotics or other factors, may allow for colonization of the ocular surface by opportunistic pathogens, producing a greater risk for infection-associated ocular adnexal neoplasms [34].

Non-ocular Microbiota and Ophthalmic Diseases

Finally, we must consider the potential role of the general, non-ocular microbiome in ophthalmic disease. The pathophysiology of glaucoma involves local inflammatory responses. A study comparing the oral microbiome of patients with glaucoma and healthy controls found that patients with glaucoma had a higher quantity of bacterial organisms compared to controls [35]. The researchers of the study proposed that an increased bacterial count can lead to neurodegeneration of the optic nerve via activation of microglia in the retina and optic nerve; the altered commensal microbiome induces changes in cytokine signaling and complement activation [35]. Additionally, other glaucoma researchers have implicated the role of the human microbiome in modulating levels of brain-derived neurotrophic factor (BDNF), which has been shown to

have an effect on the survival of retinal ganglion cells in an animal model [36].

A number of recent studies have proposed a link between the intestinal microbiome and uveitis [37]. Uveitis, or intraocular inflammation, has various etiologies which include infectious pathologies as well as a number of immune-mediated conditions. Animal models of experimental autoimmune uveitis have demonstrated that administration of oral antibiotics that altered the intestinal microbiota resulted in a significant attenuation of the uveitis [38]. Presumably, an altered commensal flora led to an increase in regulatory T lymphocytes in lymphoid tissues as well as in the eye, leading to decreased inflammation. Another animal model study of experimental autoimmune uveitis found that oral antibiotics or a germ-free state significantly decreased the severity of uveitis [39].

CONCLUSION

Advances in next-generation sequencing and bioinformatics tools have revealed an expansive, diverse microbial community inhabiting the human cornea and conjunctiva. The most abundant genera in the microbiota of the ocular surface, identified using 16S rRNA sequencing, were *Pseudomonas*, *Bradyrhizobium*, *Propionibacterium*, *Acinetobacter*, and *Corynebacterium*. The ocular surface microbiota can be altered by a variety of host factors, environmental influences, and pathological states, including dry eye syndrome, contact lens wear, keratoprosthesis, antibiotics, and infection. Evidence strongly suggests that the homeostatic microbiome plays a protective role in preventing colonization of pathogenic species. Thus, disruption of the normal ocular surface microbiota may play a significant role as a cofactor in the pathogenesis of ophthalmic diseases, such as contact lens-associated corneal infiltrative events, blepharitis, and post-operative infectious endophthalmitis. Furthermore, recent studies suggest that the microbiome of other areas of the body are involved in the pathophysiology of certain ophthalmic diseases, such as the oral microbiome and glaucoma, as well as the intestinal microbiome and uveitis. Continued investigation of the ocular surface microbiome is necessary to enhance our understanding of the role of homeostatic microorganisms in ophthalmic diseases and inspire the development of novel, probiotic-based therapies for the prevention and treatment of ocular disease.

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