Impact of Microbiome on Ocular Health

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ABSTRACT The ocular surface is continuously exposed to the environment and, therefore, it is surprising that it harbors only few commensals with low degree of diversity. This unique aspect of the ocular surface physiology prompts the question whether there are core ocular commensal communities and how they affect ocular immunity. The purpose of this review is to provide an overview of what is known about the ocular surface commensals in health and disease and what we would like to learn in the near future. In addition, we discuss how microbiota at sites other than the eye may influence ocular immune responses. The information discussed in the review has been gathered using PubMed searches for literature published from January 1982 to December 2015.

KEY WORDS contact lenses, IgA, microbiota, ocular surface

I. Is there a core ocular commensal microbiota?

One of the most exciting discoveries made in the 21st century is undoubtedly the discovery of how microbiome affects human health.[1] We now know that an average human body harbors as many microbial species as human cells.[2] Many studies have linked microbiome to cancer, obesity, asthma, artherosclerosis, and diabetes, illustrating the significance of gut microbiota in health and disease.[3-6] The initial studies of gut microbiota were followed by investigations describing core microbiota species at different sites such as skin, urinary tract, and oral mucosal surfaces.[7,8] Therefore, the question that naturally arises is "What are the characteristics of the healthy ocular microbiota and how do they change during disease?"

Typically, microbiota is defined as *microbial species that are present in the majority of the tested individuals at a particular location*. Unlike any other body site, the healthy conjunctiva, lid margins, and tears have remarkably fewer microbial species than what has been reported for other mucosal sites, such as the oral mucosal surface. The most frequently identified species from the conjunctival surfaces in healthy humans are the *Coagulase Negative Staphylococcus sp* (*CNS sp*), which include *Staphylococcus epidermidis*. They are typically isolated from 20-80% of conjunctival swabs and from 30-100% of swabs from the lid margin areas. Among the less frequently present microbial species are *Propionibacterium sp* (*P. acnes*), *Corynebacterium sp*, *S. aureus*, *Streptococcus sp*, *Micrococcus sp*, *Baccilus sp*, and *Lactobacillus sp*. Unlike the above-mentioned gram-positives, the gram-negatives are less frequently detected on the healthy ocular surface. These include *P. aeruginosa*, *Enterobacter sp*, *E. coli*, *Proteus sp*, and *Acinetobacter sp*.[9-14] The data are mostly based on experiments where moistened ocular cotton swabs were used to sample the ocular tissues and aliquots were allowed to grow on selective agar-based media. What is striking across these studies is the huge variability in the number of samples showing positive bacterial growth, ranging from 16% to 89% (for *CNS sp*), for it also reveals that a significant number of the ocular swabs contain non-expanding in vitro microbial growth. Only a few studies reported the actual numbers of colony-forming units (**cfu**) measured per individual swab seeding. For example, Ermis et al reported that 80% of the seeded samples showed microbial growth and only 17% of them had more than one microbial species, thus demonstrating that the sustainable in vitro ocular microbiota is not significantly diverse.[15] This is in agreement with another study that measured 17-64 cfu per conjunctival swab in 10% of the swabs, 5-16 cfu/swab in 15 % of the swabs, and as few as 0-4 cfu/swab in 75% of the swabs, illustrating the measurable but infrequent bacterial presence in the conjunctival tissues.[16] In contrast, lid swabs yielded a high number of cfu: 101-1000 cfu/swab in 3% of the cases, 11-100 cfu/swab in

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38% of the swabs, and finally 0-10 cfu/swab in 59% of the swabs.[17] Cumulatively, these experiments demonstrated that there is a limited abundance of the in vitro sustainable microbial species at the ocular surfaces, including the lid margin areas, which is remarkably different from what is present in the oral mucosa or saliva.[18-21] Consistently, 100% of the swabs taken from the oral mucosa and saliva yielded bacterial presence and contained 10^7-10^8 cfu/ml of sustainable bacterial sp.[22]

The advent of the deep sequencing technique allowed an improved and significantly higher-resolution method for detection of microbial species. In particular, ocular microbiota revealed 12 genera that could be viewed as constituting the core of the conjunctival microbiome. These included *Pseudomonas sp* (20% of the detected genera), *Propionibacterium* (20%), *Bradyrhizobium* (16%), *Corynebacteria* (15%), *Acinetobacter* (12%), *Brevundomonas* (5%), *Staphylococcus* (4%), *Aquabacterium* (2%), *Sphingomonas* (1%), and *Streptococcus* (1%).[23] In these experiments, it was surprising to see high numbers of *Pseudomonas sp*, because it did not correlate with the data originating from the probing of microbiota using culturing techniques. The elevated presence of *P. aeruginosa* may have been skewed by the increased abundance of these microorganisms in one of the four tested individuals. This differs from the findings reported by Graham et al.[11] In the latter study, 16S ribosomal based sequencing of 57 samples from healthy subject's conjunctiva demonstrated presence of *CNS sp, Baccilus sp, Rhodococcus sp, Corynebacterium sp, Propionebacterium sp, Klebsiella sp,* and *Ervinia sp*. The differences between the two studies may also partly be a consequence of using cloned 16S fragments for sequencing leading to overrepresentation of detected species. Consistently, six genera, including *Corynebacterium*, *Streptococcus*, *Propionibacterium*, *Staphylococcus*, *Bacillus*, and *Ralstonia,* were reported present in more than 80% of the surveyed normal healthy conjunctiva in the study cohort from Gambia.[24] This cohort did not reveal high relative abundance of *Pseudomonas, Bradyrhizobium*, and *Acinetobacter* as previously reported.[23,24]

In contrast to the low bacterial abundance and diversity detected in the prior studies, Shin et al showed that the conjunctival alpha diversity was significantly higher than that of the skin under the eye,[25] suggestive of a more complex commensal repertoire. There was higher abundance of *Haemophilus, Streptococcus, Staphylococcus*, and *Corynebacterium sp* in the conjunctiva when compared to the skin of the eye, supportive of the concept of the ocular commensal signature.

Clearly, these findings suggest that the conjunctival commensal repertoire includes *Haemophilus, Streptococcus, Staphylococcus*, *Propionibacterium,* and *Corynebacterium,*[11,23,25,26] justifying the need to resolve the lack of correlation between the significant diversity of the commensal community at the conjunctival surfaces detected by deep sequencing and the very limited diversity of bacterial species grown in culture. While there may be recognizable limitations in the culturing protocols, similar experiments using skin, lid margin, or oral mucosal swabs yielded a remarkably higher number of detectable commensal organisms, suggesting that the ocular microbiota is not relatively more abundant. It is also important to address whether bacteria actively colonize the ocular surfaces and replicate there or are only transiently introduced. A potential approach would be to employ transcriptome-based analysis of commensal communities in reconstitution experiments with germ-free mice exposed to different ocular commensals.

One of the limitations of the 16S sequencing approach is the inability to identify down to bacterial species level. Therefore, it is important to utilize alternative approaches, such as transcriptome-based analysis or culturing methods, to characterize the commensals. While a comprehensive, longitudinal quantification of the ocular microbiota is yet to be recognized, especially in the context of disease states, future studies of the ocular microbial communities should not be limited solely to the identification of bacterial species, and the potential presence of viriome should be considered.

One important consideration often overlooked in experiments like those described above is how the microbial presence changes with sex and aging. The majority of studies conducted did not evaluate sex or age-specific differences (cited above) with the exception of those reported in references 24 and 25. Expectedly, age-related changes in the composition of the ocular microbiota were observed among the individuals in the Gambian cohort[24] with the diversity of the detected species changing from children (<10 years old) to adults. These observations are consistent with the age-dependent maturation of the immune system, which may define to some extent the composition of the micriobiota. As the strength of the immune system gradually declines with aging, further changes in the microbial communities of the eye are expected in the elderly. Evidence to support this inference comes from experiments with young and aged mice, where a significant trend of increase with age in the number of sustainable in vitro conjunctival species was observed.[27]

A number of unresolved questions remain. Are the detected sequences representing live bacterial species versus dead bacterial debri? Is the ocular surface colonized