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Considerations in Understanding the Ocular Surface Microbiome

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One of the great advances in early-21st-century medicine has been progress in understanding the human microbiota. Many body sites, including the gut, mouth, and skin, are normally colonized by a large and diverse consortium of bacteria, fungi, and viruses. The term microbiota is used to describe the community of microbes that inhabit a particular site, and the term microbiome for their collective genomes. The advent of cultivation-independent techniques of microbial identification such as polymerase chain reaction and 16S ribosomal DNA sequencing has provided a much more detailed picture of the human bacterial microbial consortium than was available through traditional culture techniques. These new methodologies promise both to reveal uncultivable microbes that previously would remain undetected and to provide a much more complete picture of the microbial community structure associated with different niches of the human body. We now know these organisms are far more extensive than previously appreciated—the average human has more than 10 *trillion* microbes in the gut alone, more than 10 times the number of human cells in the body.¹ The microbiota underlies more pathophysiology than was previously appreciated. *Clostridium difficile* colitis, irritable bowel syndrome, and inflammatory bowel disease are all thought to involve dysregulation of the gut microbiome.² The successful treatment of *C difficile* colitis with fecal microbiota transplantation provides strong evidence for the potential of therapeutic manipulation of gut microbes. Additional studies have linked the microbiota to risks of colon cancer, obesity, diabetes, and, most recently, other inflammatory diseases including rheumatoid arthritis, potentially by altering large-scale host-immune relationships.^{3,4}

In ophthalmology, the question naturally arises as to whether or not the ocular surface, like other mucous membrane surfaces, has a resident microbiota, and, if so, what role it plays in ocular surface physiology. This question has provoked much debate over many years. The initial description of culture results from normal conjunctiva was published in this journal by Keilty 84 years ago in 1930⁵; he found 43% of conjunctival cultures from normal subjects to be “sterile.” Perkins and associates studied the culturable microbiota of the ocular surface in normal and infected conjunctiva using more modern culture techniques.⁶ This group found

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bacterial isolates in 87 of 96 control eyes, with the primary organisms being coagulase-negative *Staphylococcus* and *Propionibacterium acnes*. More recently, molecular surveys of the ocular surface using 16S ribosomal DNA amplification and sequencing have revealed a much broader potential ocular surface microbiome, with scores of genera consistently found among samples.^{7,8} These studies, and many similar, have given rise to 2 views. In the first, microbial DNA and organisms may be isolated sporadically from the ocular surface, but without the implication that they are stable colonizers. Rather, their fate is to be killed or removed from the eye. By contrast, others suggest that, similar to other mucosal sites, there is a normal consortium of microbes that colonize the ocular surface.⁸

The same questions arise with viruses and fungi. Herpes simplex, for example, has been found by polymerase chain reaction (PCR) to be shed into human tears in approximately one third of volunteers.⁹ Similarly, hepatitis B virus has been found by PCR to be shed in the tears of about 30% of carriers and to be found repeatedly in about half of these.¹⁰ Fungal DNA, detected by 28S PCR, has been detected in about 30% of children,¹¹ although it is cultured only infrequently from normal conjunctiva. But does molecular detection, using techniques capable of detecting fewer than 10 genomes, correspond to a true community?

The importance of this question is significant. A number of ocular surface disorders—including dry eye syndrome, episcleritis, chronic follicular conjunctivitis, pterygium, and Thygeson's disease, to name only a few—remain essentially idiopathic. All have inflammatory components. By analogy to the situation in the intestine, it is conceivable that dysregulation of an ocular surface microbiotic community (even a very small one, by release of specific toxins or triggering of a large immune response) could trigger or contribute to any or all of these conditions. Moreover, similar to the example of *C difficile* colitis cited above, it may be possible to intervene in ocular surface disease by introducing appropriate commensal microbes.

While we believe that the question of the existence and potential makeup of the ocular surface microbiome has yet to be definitively answered, we would like to suggest a framework by which it might be addressed. It is important that all life forms, including bacteria, fungi, viruses, and bacteriophage, be included in a complete description of this microbiome. *A true ocular surface microbiota would be characterized by the persistence of a stable consortium of viable microorganisms on the ocular surface over time.* We would further anticipate that disease states of the ocular surface would alter this microbiome. For example, in patients with atopic dermatitis colonization and infection with *Staphylococcus aureus* on the skin and the ocular surface has been demonstrated and hypothesized to be related to deficits in innate immune responses.¹²

We suggest the following technical framework for characterizing the ocular surface microbiome molecularly. First, it is essential that ocular microbiome researchers directly address the problem of DNA contamination in describing and reporting their studies. Since culture studies have demonstrated that bacteria are many orders of magnitude less abundant on the ocular surface than on other mucosal surfaces,^{13,14} such as the oral cavity, contaminating organisms and/or DNA are more problematic in studying the ocular surface. Simply put, when studying the ocular surface microbiome, the *noise* from contaminants may

more easily overwhelm the *signal* of the actual microbiome. For this reason, study of the ocular surface demands extreme care to exclude contamination. Reporting of the prevalence of microbial DNA detected, normalized to a common unit such as ocular swabs or human DNA content, will also aid in the interpretation of such investigations. Using quantitative PCR (qPCR), accurate counts of the numbers of genomes of bacteria and viruses recovered can be made.¹⁵ Second, researchers should attempt to distinguish between DNA recovered from the ocular surface, which is related to viable and nonviable organisms, potentially by demonstration of mRNA. Since the ocular surface is known to be highly antimicrobial, DNA recovered there may simply represent microbes that have succumbed to its defense rather than a community of colonizing organisms. Thus, to understand the relevance of detecting the DNA of a particular organism, it is critical to know if it came from a viable population. Third, it will be important to demonstrate whether any proposed microbial communities are stable over time in both health and disease. The study of the stability of ocular microbiota is important, as it addresses the question of whether identified microbes are part of a permanent community or just a transient, possibly nonviable visitor to the ocular surface. Finally, data from ocular microbiome studies must be used to generate testable hypotheses about the role of such microbes in disease and health, which are evaluated in model systems. Reporting the presence of particular microbes alone will not add much to our understanding of the ocular surface unless it generates hypotheses that are testable. The advent of high-throughput DNA sequencing has brought a second golden age of microbiology. It is essential in ophthalmology that appropriate rigor be used in applying these techniques to the study of the ocular surface.

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