National Eye Institute Anterior Segment Initiative Symposium

Ocular Surface Microbiome-Best Practices for Low Biomass Research

Executive Summary

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Abstract

Enhanced understanding of the ocular surface microbiome may provide insights into ocular surface health and disease. On August 2, 2021, the National Eye Institute hosted an online symposium to facilitate discussion on the technical issues associated with studying the microbiome in low biomass tissues, specifically the ocular surface. Characterizing microorganisms in a low biomass niche is challenging due to the high signal-to-noise ratio resulting from contamination. The data presented suggest that while the ocular surface is not sterile, it is "paucibacterial." Sessions addressed specimen collection and processing, methods for characterizing the ocular surface microbiome, best practices for generating reproducible data, and approaches for validating microbes. Overall, the scientific community concurred that the most important aspect of sequencing the microbiome in low biomass tissues is quality control (QC) for each step of the sample collection, processing, and analysis pipeline, including rigorous use of both positive and negative controls.

Introduction

The National Eye Institute (NEI) Anterior Segment Initiative (ASI) was launched in 2019 to address challenges in understanding and treating disorders of the anterior segment of the eye. The scientific community and the National Advisory Eye Council identified the interaction of the human ocular microbiome together with the immune system as an area that is not well understood and of potential import. In response, the NEI hosted a symposium to discuss challenges to characterizing the ocular microbiome and its role in promoting or preventing ocular diseases.

The microbiome is defined as a community of microbial organisms that reside in a specific host niche. We recognize today that there are certain sites on the human body that have diverse microbiomes such as the gut and mucous membranes. Organisms within these sites are known to have correlations with many diseases, either directly as in colitis and dermatitis or indirectly by establishing the overall inflammatory tone linked to many diseases such as diabetes or Sjogren's disease. Many ocular diseases with high morbidity are inherently infectious including blepharitis, bacterial conjunctivitis, microbial keratitis, herpetic keratitis, endophthalmitis, and adenoviral keratoconjunctivitis, while others such as dry eye disease are inflammatory and may have a microbial component. It is reasonable to hypothesize that the normal ocular surface has mechanisms to protect it against a dysregulated microbiome that might predispose someone to these morbid conditions. Tears continually wash out microbes that enter the ocular surface and contain lysozyme and antimicrobial peptides such as defensins, which have antibacterial actions and work to prevent invasion and infection. Because of these mechanisms, the ocular surface may be unique among mucous membranes in the body in hosting a limited and unique microbiome.

Dr. Russell Van Gelder opened the meeting with a brief review of the history of the ocular microbiome beginning with a review of historical culturing experiments. The ocular microbiome was first studied over 70 years ago, with early attempts at characterizing this surface querying whether the relatively few detected bacteria were resident or transient. Nolan reported that 60% of cultures were sterile and the other 40% contained upper respiratory tract flora like *Staphylococcus pyogenes* and *Pneumococci* (Nolan, 1967). Wilson and Dowell published in 1978 that the conjunctival sac contains, at most, small numbers of culturable anaerobic, aerobic, or facultatively anaerobic bacteria (McNatt et al., 1978).

Contradicting this expectation of near sterility, Dong and coworkers published a paper suggested that the human conjunctiva contains a rich population of bacteria (Dong et al., 2011). Following up on Dong's work, Dr. Van Gelder highlighted his research using both 16S metagenomics and the deep DNA sequencing method BRiSK (biome representational in silico karyotyping) to classify organisms in the conjunctiva. His group found that 80% of the ocular samples grew organisms on culture; however, only four groups of organisms (*Staphylococcus, Streptococcus, Corynebactiera,* and *Propionibacteria*) accounted for more than 90% of the species that grew. 16S identified scores of operational taxonomic units (OTUs). However, quantitative PCR revealed that the ratio of bacteria/host DNA in the conjunctiva was about 1:50, compared to the ~100:1 ratio seen in skin and buccal mucosa. This meant that the total number of bacterial genomes per swab was on the order of 50, strongly suggesting that the majority of detected OTUs were artefactual (Doan et al., 2016a). Note, BRiSK is a technique that is agnostic to DNA and can detect all DNA-based life forms including viruses and fungi. BRiSK confirmed bacterial culture results regarding the makeup of the bacterial microbiome, but it unexpectedly found resident viruses – particularly torque teno virus and Merkel cell polyoma virus – on substantial numbers of samples, suggesting there is a resident ocular surface virome in many individuals. Dr. Van Gelder also noted that bacteriophage is detected in abundance on the ocular surface, and its presence is of unclear clinical import.

Other labs studying the ocular microbiome have also reported similar inconsistencies. For example, Dr. Thuy Doan reported that in her lab a large proportion of healthy people (92%) had no *cultures* grow when samples were taken from their intraocular fluids for culturing in a CLIA-certified lab, yet for all the samples, *sequence* reads could be obtained that matched many organisms (Doan et al., 2016b). This leads to the question, how should the discrepancy between the low culture yield be reconciled with the frequently reported sequencing of a variety of microbes?

While sequencing methods have been extremely effective in characterizing microbial communities in large biomass samples such as the gut, data inconsistencies appear to be a common problem in low microbial niches. Data inconsistencies have been observed in the skin, nasopharynx, and the placenta. Contamination has a much larger impact when working with lower biomass samples because in high biomass samples the legitimate microbial population overwhelms contamination. Dr. Gordon Smith highlighted this issue in his paper on the reagent microbiome (de Goffau et al., 2018). Important questions that the field should address include: What should the standards of evidence be for characterizing the ocular surface microbiome? Which techniques are appropriate? What are the essential controls? What are best practices for verification? Is imaging required? Is

RNA-based PCR required to detect viable organisms? What can be learned from experiments in other parts of the body that also have relatively low levels of bacteria? What studies should be done to establish the healthy and diseased microbiome of the eye? What collaborations would be helpful?

Overview of the NEI symposium

Sessions

The symposium was co-chaired by Drs. Karen Nelson and Russell Van Gelder and was a widely attended event with over 220 participants, representing 25 countries and wide-ranging scientific disciplines. The meeting comprised four sessions with presentations addressing key topics as provided below along with ample time for group discussion.

Session I	Emerging Evidence: The Case for or Against the Existence of Microbiota on the Ocular Surface and Ocular Microbial Defense Mechanisms That May Play a Role
	Panel moderator: Dr. Mark Willcox
Session II	Lessons Learned: Experience from Studies of Other Low Biomass Niches
	Panel moderator: Dr. Heidi Kong
Session III	Best Practices: How Can Ocular Surface Sample Collection and Processing Protocols Be Standardized to Minimize Artifacts and Contaminants (e.g., DNA) and Generate Reproducible Data?
	Panel moderator: Dr. Joseph F. Petrosino
Session IV	Approaches to Validating Microbes
	Panel moderator: Dr. Sinem Beyhan

Key topics

Model Systems

Dr. Mark Willcox reviewed his data on the microbiome of the eyelid margin and the conjunctival surface in humans, guinea pig, mice and rabbits, and showed that periocular skin has a diverse microbiome compared to the eyelid margin or ocular surface, while conjunctive tissue has the lowest abundance and diversity of microbes. Diversity of the microbiome appeared to decline with age with no difference between males and females (Ozkan et al., 2019).

During this analysis, Dr. Wilcox found that people have different communities of microbes or a different “bar code”, although the stability of these communities is not clear, and needs further investigation. Compared with guinea pigs and rabbits, mice showed the greatest similarity in bacterial composition to humans in conjunctival tissue, suggesting mice would be a better animal model (Ozkan et al., 2021).

In addition to the components of a microbial community, it is important to understand the geographic distribution of bacteria. Dr. Michael Zegans discussed biofilm formation, which is a well-recognized mechanism by which particular microbial communities may assemble by attaching to a surface. Biofilm formation is also known to occur on contact lenses and scleral buckles. Infectious crystalline keratopathy is an example of a biofilm infection (Zegans et al., 2016; Visick et al., 2021). The presence of biofilms creates challenges for sampling as adjacent micro-regions could support different communities.

Another key question concerns the existence of resident vs. transient microbes. Dr. Anthony St. Leger posed the question, what makes a strain of bacteria a transient passenger versus a colonizer? Using comparative genomics on isolates obtained from the ocular surface of human patients and mice, his group found that more than 160 genes could be responsible for the ability to colonize eyes; however, given 90% of those genes code for hypothetical proteins, some of these may be artefactual.

During discussion, Dr. Zegans commented that the ocular microbiome may not be a site that supports a resident community. Rather, bacteria from the eyelid may be repopulating the ocular surface. Dr. St. Leger stated that his group tried to colonize the eye in mice with bacteria from other sources including the skin; however, only specific *Corynebacteria* stayed in the eye. Some species seemed to have had the ability to colonize the eye, but others did not (St. Leger et al., 2017).

In aggregate, the data presented support the notion that at any given time, a small population of bacteria are found on the ocular surface. Whether this is a continuously renewed selection of adjacent skin bacteria, or a self-propagating stable niche remains unclear.

Contamination

The relative ubiquity of bacteria combined with the very high sensitivity of PCR creates opportunity for false-positive 16S results when metagenomic sequencing is applied to low biomass samples. Dr. Joseph Petrosino pointed out that variability in collection methods could contribute to contamination issues. Dr. Heidi Kong noted that reducing the risk of contaminants entering the experimental system must occur at all steps in the analysis process. That includes adding negative controls for every sample, and at every time point, and they should be processed alongside the experimental samples. She recommended collecting samples using noninvasive swabs rather than scrapes or punch biopsies as well as sterile saline, which tends to attract lower microbial debris compared to lysis buffers, which have a mild surfactant.

Setting up and maintaining the pipeline with frequent QC is critical. Dr. Kong also recommends using mock communities for testing and validating the system. The concept of use of both positive and negative controls in all experiments was a recurrent theme among presenters. Regarding positive controls, it is important that the organisms that are put into the pipeline are the only sequences coming out, when applied to otherwise negative control samples. Extraction is another important consideration. Different methods such as bead-beating, robot processing, or hand extraction are commonly used. Dr. Kong stated that in her experience, hand extraction works best to maintain the level of microbial biomass as compared to kit-robot processing which usually results in a decrease in the amount of DNA over time. There is also an issue of kit contamination.

Dr. Kelly Nichols presented techniques for sampling the eyelid margin and Meibomian gland orifice and noted that disease status can impact choice of sampling technique (e.g., dry eye decreases tear quantity) and stated that standardizing collection protocols would improve reproducibility (Postnikoff et al., 2020). Dr. Petrosino queried whether bacterial load had been associated with ocular surface characteristics or with disease states. Dr. Greg Gloor responded that bacterial load, while underappreciated and understudied, is probably at least as important as bacterial composition in health and disease.

The consensus of the group was that strict adherence to pipelines, segregation of experimental areas to minimize carry-over contamination, and meticulous sourcing of reagents are critical to minimizing risk of false-positive results.

Controls

Inconsistent or inadequate use of controls was a topic of significant concern. Dr. Christopher Dupont noted that using sterile water and mock samples are important and should yield very few reads, otherwise you'll have to question the experimental validity. It was noted that while negative controls are important, positive controls are equally important and raised the question, what is a good spike-in or positive control? Dr. Smith suggested spiking a sample with a single type of bacteria since using whole bacteria provides a closer simulation of reality than extracted DNA, since different species have different types of cell walls and can lyse differently, Dr. Chrysi Sergaki recommended using both a complex community of about 20 strains as well as extracted DNA. Dr. Dupont said he uses *bacillus* DNA as a spike-in control for quantitation and extraction in studies of the nasopharynx since it's not normally found in nasopharyngeal samples.

What can we learn from other low biomass environments such as the skin, nasopharynx and placenta? Dr. Heidi Kong listed important considerations for sample collection, including the use of antibiotics or topical medications, the anatomical site where swabbing occurs, and importance of timing. For example, when studying the skin microbiome, only subjects who haven't bathed for at least 24 hours are swabbed. Collecting negative controls during sampling is an important step, and frequent quality control of the research pipeline is necessary to prevent or address contamination (Kong et al., 2012; Oh et al, 2014). Dr. Kristi Hoffman also emphasized the importance of controls including negative controls for collection tools and solutions and for reaction chemistry as well as positive controls for extraction and library prep—such as a mock community of cells or DNA.

The group recognized the need for the inclusion of rigorous positive and negative controls in all experiments. Dr. Van Gelder also pointed out that qPCR to determine bacterial load provides an internal control for normalizing the number of operational taxonomic units expected from analysis.

Standards

The United Kingdom's National Institute of Biological Standards and Control (NIBSC) plays a leading role in assuring the quality of biological medicines and diagnostics. Dr. Chrysi Sergaki, one of NIBSC's senior investigators, highlighted the need for a global set of standards for the microbiome field, given the variability and unreliability across microbiome studies of the same niche. Sequences detected rely on the DNA extraction methods, sequencing techniques such as

shotgun or 16S rRNA, and the bioinformatic pipelines. The NIBSC is leading 18 World Health Organization (WHO)-endorsed projects to establish reference reagents for microbiome analysis of 6 body sites including the gut, vagina, mouth, lung, nasopharynx and skin. Dr. Sergaki and colleagues developed mock DNA communities for different microbiome sites using reference strains. She also developed four key reporting measures: sensitivity, relative abundance of false positives, diversity of observed species, and similarity between known composition and observed composition. Acceptable ranges are currently being set for each of these measures (Amos et al., 2020). Dr. Sergaki advised the vision community to utilize the WHO reference reagents developed for other sites until specific standards for the ocular surface can be developed.

Sequencing

Amplicon-based sequencing and deep sequencing techniques such as shotgun metagenomics are platforms used to evaluate bacterial diversity and detect microbial abundance in various environments. Dr. Kristi Hoffman noted that non-targeted whole genome sequencing is more prone to host contamination and recommended a targeted approach—such as amplicon-based sequencing of 16S rRNA. Dr. Heidi Kong said that it is critical to compare the reads from the negative controls to the experimental samples. Very few reads in the negative control will give you confidence that the results are indeed from the experimental sample.

Dr. Christopher Dupont described a microbiome enrichment method that he has developed for characterizing low biomass niches. He concluded that 1) enrichment by CpG methylated DNA depletion allows for cost-effective metagenomic profiling of low biomass samples, 2) low input library preparation methods circumvent the need for amplification, and 3) deeper microbiome coverage allows for multiple types of assembly-based metagenomic analyses (Williams et al., 2020).

Informatics approaches for data analysis

Getting samples free of contaminants is extremely difficult. Dr. Greg Gloor noted that any large-scale sequencing is probabilistic sampling. While it's common to assume that sampling is random, sequencing methods may undercount rare strains and skew the proportions of bacteria detected. The solution to unreliable correlations is to use log ratios to look for linear log-ratio relationships. To counteract the fact that low-count features are under-represented, one can sequence more deeply, focus on high abundance species (i.e., ignore or filter out low-count features), or acknowledge the uncertainty of low-count features. None of these methods are perfect, Dr. Gloor said, and no single feature can be interpreted in isolation.

Dr. Gloor also suggested specific analytical tools to work with compositional biplots of log-ratio transformed data, to work with networks, to study differential abundance, and to use classification to study the difference between samples. He concluded that while sequencing leads to potentially misleading compositional data, there are ways to address this problem (Gloor, 2017).

Dr. Kristi Hoffman stated that there are computer programs that can be used to mitigate DNA contamination in microbial samples such as the Decontam R package, a tool that is used for identifying contaminants in marker-gene and metagenomics data. She also noted data processing considerations for amplicon-based sequencing. For low biomass samples such as the ocular surface she recommended denoising which takes advantage of error profiles to resolve sequencing data

into exact sequence features over clustering which groups sequences at predefined identity thresholds.

Validating microbes

DNA from dead bacteria is indistinguishable from DNA from live microbes. To validate sequencing results, it is important to show that the bacteria detected are alive, and not dead or debris. To date, no cultivation technique is capable of selectively isolating and growing all bacterial species; however, organoid-based models are gaining in popularity for studying host-microbiota interactions. Dr. Sinem Beyhan described her *in vitro* microfluidic organ-on-a-chip models which combine fluid physics with 3D cell cultures to study gut-microbe interactions. She plans to introduce commensal and pathogenic microbes to the flow system to study the qualitative and quantitative changes in microbes and host cells (Radtke et al., 2010; Barker, 2014). Aspirationally, the ability to develop organoid microbiome cocultures would allow study of the features supporting specific communities.

Dr. Timothy Blenkinsop developed corneal and conjunctival epithelium cells/organoids as a model for testing the microbiome on the human ocular surface ectoderm. His group exposed the outer surface of the organoids to air and inoculated the exposed surface with microbes from human tears. What grows is currently being characterized by sequencing. In addition, Dr. Blenkinsop generated a whole eye organoid model where concentric rings develop containing different cell types and used this model to demonstrate that it can be infected with SARS-CoV-2 (Eriksen et al., 2021).

Another method of detecting microorganisms is fluorescent *in situ* hybridization (FISH). Dr. Jessica Mark-Welch studied the biogeography of the oral microbiome and reported using FISH in combination with combinatorial labeling, to enable her lab to visually distinguish different taxa (Mark-Welch et al., 2019; Mark-Welsh et al., 2016; Wilbert et al., 2020).

Dr. Suzanne Fleiszig in collaboration with Dr. Carolyn Bertozzi developed click chemistry to label live bacteria on corneal epithelial cells. In a healthy mouse, the researchers found no metabolically active bacteria on the cornea, but numerous live bacteria were observed on the conjunctiva. Next, Dr. Fleiszig's group used a method to specifically label *Actinobacteria*, a class of filamentous bacteria that are found on conjunctiva. This group also demonstrated the use of 16S rRNA FISH to detect bacteria on cornea and contact lenses (Wan et al., 2018; Kamariza et al., 2018).

Click chemistry and other probes only label metabolically active bacteria but will not identify microbes that don't synthesize the specific click chemistry target. 16S RNA FISH labels RNA, which is short-lived, and indicative of living cells, however, this method is problematic for detecting bacteria in conjunctiva due to low sensitivity. For bacteria that are not very metabolically active but still alive, these methods will not work as well. Stains can be helpful if they label microbes and not the host. Antibodies can also be used for labeling but may not bind across related strains because of their specificity. Dr. Fleiszig suggested 16S DNA FISH could be used to detect genes but would not necessarily signal the presence of bacterial bodies. Validation by visualization remains challenging.

Moving forward: Charting a course for rigorous study of the ocular surface microbiome

Based on the discussions and participation, the scientific community is very interested in understanding how microbial communities on the ocular surface and periocular region contribute to health and disease. There are a number of conditions that are morbid and that we do not fully understand their underlying pathogenesis, such as blepharitis, dry eye disease, and chronic ocular pain – all of which could be caused or exacerbated by continued insults from microbes. In addition, it is possible that commensals contribute to autoimmune diseases such as Sjogren's syndrome, Grave's disease, and some forms of uveitis.

Characterizing the microbiome in a paucibacterial space is fraught with potential challenges. Dr. Gordon Smith's story about the placental microbiome is a cautionary tale. The presence of an indigenous placental microbiome was first described in 2014; however, the plausibility of the observations came into doubt with the reported presence of cyanobacteria among the types of microbes observed. Such a signal likely reflects contamination. In further examination of the placenta, Dr. Smith concluded that the only true signal was extremely rare *Streptococcus agalactiae* while other species previously identified reflected contamination (Aagaard et al., 2014). Dr. Smith admonishes that, "the lesson learned is that one has to have a degree of paranoia when processing samples to ensure accuracy."

"Reproducibility is what you want to achieve with standards," said Dr. Chrysi Sergaki. If the community can agree to use certain standards, then you can compare clinical samples with other studies. If for instance, your standard has 20 strains, but your sequencing platform yields 150 microorganisms in an otherwise sterile spike sample, one can identify the false-positive signals and potentially exclude them from analysis. A large part of the discussion focused on the use of proper controls to generate reliable data. Negative controls are important; however, when sequencing organism in a low biomass niche, positive controls are as crucial for determining valid reads. The development of standards for spiking both bacteria and DNA in the samples would be tremendously powerful in helping distinguish absolute levels of bacterial load rather than relative levels.

Dr. Van Gelder encouraged consideration and adoption of standards for collecting and reporting certain types of data. For example, should researchers use separate equipment and streams for low biomass analysis? Desirable reporting standards and standards for developing assays will make it easier to compare among studies and identify when experiments fail.

Dr. Karen Nelson gave the meeting wrap-up, echoing the challenges of generating reproducible data working in a paucibacterial space. These include sample contamination, reagent batch effects, differences in sequencing pipelines, and analytic techniques. Careful attention to processes and standards is needed in order to generate consistent data. More work is needed to standardize protocols for sample collection, selecting standards, DNA extraction methods, sequencing platforms, and informatics approaches for data analysis. Dr. Nelson also recommended addressing the adequacy of study sample sizes. Are the cohorts large enough to include variability? What about parameters such as age, sex, and timing of sample collection? What model systems should be used to validate live bacteria?

Once sequences are identified, we must determine if they represent bacteria that are metabolically active. What model systems should be used to validate microbes? Dr. Fleiszig's presentation suggests there are ways to achieve a gold standard for microbiome research—seeing live bacteria via metabolically specific methods such as click chemistry, which is achievable in animal models. An aspirational goal would be to achieve the same standard in humans. However, just counting the numbers of microbes without looking at their structures may not tell the whole story. Drs. Jessica Mark-Welsh and Michael Zegans highlighted the importance of understanding the structures of communities of microbes, i.e., the microenvironment and how they form biofilms. Dr. Welch noted that spatial organization is important not only for how bacteria relate to each other but also how they relate to the host surface.

Numerous challenges for the microbiome field persist. Most of the microbiome results generated to date are relative abundance data and is semi quantitative; however, absolute quantitation is important as we move forward to understand the function of these microbes. The question of quiescent microbiota is another important consideration. Many imaging studies require replicating bacteria to image the microbes and therefore may underestimate the biomass. Transitioning from association studies to understanding the pathophysiology still remains a challenge.

The symposium achieved its goals. The NIH obtained current and expert information and recommendations for moving ocular surface microbiome research forward. Workshop participants shared research ideas and developed contacts for future collaboration. This executive summary is intended to extend the symposium insights and discussions to a broader base of interested researchers. Ultimately, the NEI anticipates that rigorous research in the field will allow for a better understanding of ocular surface disease mechanisms and point to novel interventional targets.

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Meeting Website

https://event.capconcorp.com/wp/iosm/

Acknowledgments

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Dr. St. Leger is an assistant professor at the University of Pittsburgh. His laboratory focuses on understanding how the microbiome may influence susceptibility to ocular surface disease. When he was a postdoctoral fellow in the laboratory of Dr. Rachel Caspi, he was able to identify *Corynebacterium mastitidis* as an immunologically relevant component of the murine ocular microbiome. His laboratory is now interested in identifying the microbial factors that allow bacteria to colonize the eye and the microbial factors responsible for generating mucosal immunity at the ocular surface.

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Dr. Dupont is an associate professor in the genomic medicine, environment & sustainability, and synthetic biology groups at JCVI. He studies a variety of human microbiome niches where low biomass and high amounts of host DNA provide unique technical challenges.

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Dr. Gloor is a professor and chair of the Department of Biochemistry at the Schulich School of Medicine and Dentistry. He has studied microbiomes and their analysis for over a decade. He uses and develops wet lab and computational tools to examine 16S rRNA gene composition, gene content and expression of mixed population samples (meta-genomes and meta-transcriptomes), and metabolomic analysis of clinical samples.

Chrysi Sergaki, Ph.D.
Interim Head of the Microbiome Section
National Institute for Biological Standards and Control
Dr. Sergaki joined the National Institute for Biological Standards and Control (NIBSC), UK, as a microbiome scientist after completing her Ph.D. in 2019 at the University of Warwick, UK, where she studied microbial interactions in fungi and bacteria and their impact on the host. At NIBSC, she leads research projects on the gut microbiome, the development of whole cell standards for the microbiome field, as well as a large international collaborative study to establish the first WHO reference reagent for NGS analysis of microbiome samples. Dr. Sergaki recently became as the Interim Head of the Microbiome section at NIBSC, leading the microbiome research and standardization projects within the Institute.

Gordon C. Smith, M.D., Ph.D., D.Sc.
Professor
Department of Obstetrics and Gynecology
University of Cambridge
Dr. Smith is chair of obstetrics and gynecology, University of Cambridge, and a consultant in Maternal-Fetal Medicine at the Rosie Hospital, Cambridge, UK. He has M.D., Ph.D., and D.Sc. degrees from Glasgow University. He held Wellcome Trust research training fellowships at Glasgow University, UK (1992-93), and Cornell University, USA (1996-1999). He is currently a Wellcome Investigator (2020-2025) and theme lead for Women's Health and Pediatrics in the National Institute for Health Research Cambridge Biomedical Research Centre. He was elected a Fellow of the UK Academy of Medical Sciences in 2010. His current research is focused on placentally-related complications of human pregnancy, addressing mechanisms and prediction of disease.

Jessica L. Mark Welch, Ph.D.

Associate Scientist
Bay Paul Center for Comparative Molecular Biology and Evolution
Marine Biological Laboratory
Dr. Welch is an associate scientist at the Marine Biological Laboratory in Woods Hole. She investigates the structure and organization of microbial communities in the human mouth as well as in the gut and in marine organisms, using a combination of fluorescence spectral imaging microscopy and metagenomic sequence analysis.

Heidi H. Kong, M.D., MHSc
Head
Cutaneous Microbiome and Inflammation Section
Senior Investigator
Dermatology Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
Dr. Kong is Senior Investigator and Head of the Cutaneous Microbiome and Inflammation Section, Dermatology Branch, NIAMS, NIH. Her research has advanced our understanding of the microbial communities of human skin. Dr. Kong combines translational research expertise with advanced genomic technologies to study skin microbes to gain insights into the pathogenesis of skin diseases and to potentially identify therapeutics for patients. Dr. Kong's ongoing work includes investigations in the microbiome of inflammatory skin diseases including atopic dermatitis and in patients with primary immunodeficiency syndromes.

Joseph F. Petrosino, Ph.D.
Professor and Chair
Department of Molecular Virology and Microbiology
Director
Alkek Center for Metagenomics and Microbiome Research
Human Genome Sequencing Center
Dan L. Duncan Cancer Center
Baylor College of Medicine
Dr. Petrosino is a professor, Kyle and Josephine Morrow Endowed Chair in Molecular Virology, and chair of the Department of Molecular Virology and Microbiology at Baylor College of Medicine. He also directs the Alkek Center for Metagenomics and Microbiome Research (Alkek CMMR) and holds joint appointments in Baylor's renowned Human Genome Sequencing Center, Dan L Duncan Comprehensive Cancer Center, and the Department of Ophthalmology. Dr. Petrosino's laboratory investigates how the human microbiome influences host health and disease with the goal of introducing new methods to diagnose and treat a variety of human diseases. He has authored or co-authored more than 200 original papers on these efforts. In addition, Dr. Petrosino launched Diversigen in 2015, a Baylor startup that provides microbiome analytics services and diagnostic insights to pharmaceutical companies, which was purchased by OraSure Technologies in November of 2019. He is also chief scientific officer for another Baylor microbiome-based startup, Anizome, which launched in 2018 with the goal of delivering microbiome-based therapies to companion animals.

Karen E. Nelson, Ph.D.

Chief Scientific Officer
Thermo Fisher Scientific
Dr. Nelson received her Ph.D. from Cornell and is an elected member of the National Academy of Sciences and a fellow of the American Academy of Microbiology. Dr. Nelson has authored or co-authored more than 220 peer reviewed publications and is currently editor-in-chief of the journals Microbial Ecology and PNAS Nexus. Dr. Nelson is a pioneer of the human microbiome field.

Kelly K. Nichols, O.D., MPH, Ph.D., FAAO
Dean
School of Optometry
University of Alabama at Birmingham
Dr. Nichols received her doctor of optometry degree from the University of California, Berkeley, completed a residency in ocular disease at Omni Eye Specialists of Colorado, and earned her MPH degree in biostatistics and Ph.D. degree in vision science at Ohio State University. In 2014, Dr. Nichols was named Dean of the University of Alabama at Birmingham School of Optometry. Dr. Nicholas is co-director of the Ocular Surface Institute at the Clinical Eye Research Facility. Dr. Nichols has served on each of the TFOS steering committees (DEWS, DEWS II, Contact Lens Discomfort, and MGD workshops). A leading expert in dry eye disease, Dr. Nichols is or has been on the editorial boards of the journals Optometry and Vision Science, and The Ocular Surface, and is extensively published with over 105 papers. Dr. Nichols has previous (PI) and current (Co-I) NEI, NIH funding in the area of dry eye and MGD. She has participated in conducting numerous clinical trials and research studies over the last 25 years. Dr. Nichols is a board member of the research advocacy group National Alliance for Eye and Vision Research (NAEVR)/Alliance for Eye and Vision Research (AEVR) and is President of the Association of Schools and Colleges of Optometry.

Kristi L. Hoffman, Ph.D., MPH
Assistant Professor
Department of Molecular Virology and Microbiology
Baylor College of Medicine
Dr. Hoffman is an assistant professor in the Department of Molecular Virology and Microbiology at Baylor College of Medicine in Houston, Texas. She obtained her Ph.D. from Baylor in 2012, studying the role of estrogen receptors in bladder cancer. During her graduate studies she became interested in the role of diet in bladder cancer risk and progression and went on to earn a Master's in Public Health with a focus in Food, Nutrition, and Health at the Johns Hopkins Bloomberg School of Public Health. Through her coursework she came to appreciate the potential of the microbiome in modulating the body's response to diet and its role in cancer etiology and progression. She explored these relationships further as a postdoctoral fellow in the Cancer Prevention Research Training Program at M.D. Anderson Cancer Center, where she developed a particular fondness for the oral microbiome as a biomarker of disease risk. In 2018 she returned to Baylor College of Medicine as a project manager with the Center for Metagenomics and Microbiome Research. She currently leads the Center's project management team and continues to explore the potential of extraenteric microbiota to predict inflammatory disease states. She loves all things microbiome, teaching, and helping others plan and execute their metagenomic studies.

Mark Willcox, Ph.D., D.Sc.

Professor
School of Optometry and Vision Science
Faculty of Medicine and Health
University of New South Wales
Professor Willcox is a medical microbiologist who specializes in ocular surface microbiology and how it changes during contact lens wear and during ocular surface antibiotic therapy. For the past five years his team has been studying the ocular surface microbiome, including composition, stability, and geography.

Michael A. Steinmetz, Ph.D.
Director
Division of Extramural Science Programs
National Eye Institute, NIH
Dr. Steinmetz is a graduate of the University of Michigan and Michigan State University and did postdoctoral training in the Laboratory of Vernon Mountcastle in the Department of Physiology at The Johns Hopkins Medical School. He remained at Johns Hopkins for more than twenty years as a faculty member in the Department of Neuroscience and the Zanvyl Krieger Mind-Brain Institute. His research program studied the neurophysiological mechanisms of selective attention and spatial perception by combining behavioral studies with single-unit electrophysiology in awake monkeys and fMRI experiments in humans and non-human primates. Dr. Steinmetz joined the National Eye Institute after serving five years as the Scientific Review Administrator for the Central Visual Processing and Cognitive Neuroscience study sections, and as a Referral Officer for the Health of the Population and Risk Prevention and Health Behavior review groups at NIH's Center for Scientific Review. He served as the Director of the Strabismus, Amblyopia, and Visual Processing Program at NEI from 2007 to 2014, and as the Director of the Division of Extramural Science Programs since October of 2014. He serves on the coordinating committees of numerous trans-NIH and trans-agency initiatives including the NIH BRAIN and Neuroscience Blueprint programs, and the Programmatic Panel of the DOD's Vision Research Program.

Michael E. Zegans, M.D.
Professor
Department of Surgery (Ophthalmology) and Department of Microbiology and Immunology
Dartmouth-Hitchcock Medical Center
Geisel School of Medicine at Dartmouth
Dr. Zegans has been on the faculty at Geisel School of Medicine at Dartmouth in Hanover, New Hampshire, since 1998. He is a professor of surgery and of microbiology and immunology and the section chief of ophthalmology at Dartmouth-Hitchcock Medical Center as well. He has served as a co-investigator for the Steroids for Corneal Ulcers Trial and Mycotic Ulcer Treatment Trial. Additionally, he is the Dartmouth site director for the National Eye Institute-funded Standardization of Uveitis Nomenclature (SUN) Study and on the steering committee for the Zoster Eye Disease Study. Dr. Zegans' current laboratory research focuses on fungal corneal infection.

Michael F. Chiang, M.D.

Director
Office of the Director
National Eye Institute, NIH
Dr. Chiang is director of the National Eye Institute, at the National Institutes of Health in Bethesda, Maryland. His clinical practice focuses on pediatric ophthalmology and strabismus, and he is board-certified in clinical informatics. His research develops and applies biomedical informatics methods to clinical ophthalmology in areas such as retinopathy of prematurity, telehealth, artificial intelligence, clinical information systems, data science, and genotype-phenotype correlation. His group has published over 200 peer-reviewed papers and has developed an assistive artificial intelligence system for ROP that received breakthrough status from the U.S. Food and Drug Administration

Russell N. Van Gelder, M.D., Ph.D.
Professor and Chair
Department of Ophthalmology
University of Washington
Dr. Van Gelder is a clinician-scientist. His clinical expertise is in ocular inflammatory disease. Dr. Van Gelder's laboratory has been pursuing nucleic acid-based diagnostics of ocular infectious disease for the past 20 years.

Sinem Beyhan, Ph.D.
Assistant Professor
University of California at San Diego
J. Craig Venter Institute
Dr. Beyhan is interested in host-microbe interactions in general, and has been studying fungal pathogens, *Histoplasma* and *Coccidioides* species. Lately, she has been developing *in vitro* models to study host-microbe interactions to provide a reproducible platform to study host and microbial response to infection.

Suzanne M. J. Fleiszig, O.D., Ph.D., FAAO
Professor
Optometry and Vision Science, Infectious Disease and Immunity, and Microbiology
University of California, Berkeley
Dr. Fleiszig is an optometrist with a Ph.D. in microbiology, trained at the University of Melbourne. Since completing a postdoctoral fellowship in the Department of Medicine, Division of Infectious Disease at Harvard Medical School, she has been a professor at the University of California Berkeley with appointments in multiple programs. She also holds an adjunct appointment at the Proctor Foundation at the University of California, San Francisco. As both an ocular biologist and microbiologist, Dr. Fleiszig has spent more than three decades focused on understanding how the cornea resists bacterial colonization, mechanisms by which that resistance becomes compromised, and strategies bacteria use to colonize susceptible corneas. She is the current chair of the council on Microbial Science for the American Society for Microbiology. She is also a standing member of the NIH Bacterial Pathogenesis Study Section.

Thuy Doan, M.D., Ph.D.

Associate Professor
Proctor Foundation
University of California at San Francisco
Dr. Doan is a clinician scientist. His laboratory at the University of California San Francisco is a metagenomic epidemiology laboratory that takes innovative approaches to understand how the various human microbiomes (ocular, gut, and upper respiratory tract) respond to clinically relevant perturbations in randomized controlled trials. Specifically, the laboratory seeks to identify mechanisms by which mass drug distribution to preschool children in Sub-Saharan countries leads to improvement in childhood mortality. Concurrently, antibiotic resistance is carefully tracked in these communities to inform public health policies. In addition to the molecular epidemiology work, Dr. Doan's group focuses on using genomic technologies to efficiently identify causes of ocular inflammation.

Timothy A. Blenkinsop, Ph.D.
Associate Professor
Department of Ophthalmology
Icahn School of Medicine at Mount Sinai
Dr. Blenkinsop's laboratory studies the plasticity of the adult human eye in normal and pathological states. They currently focus on the role of the retinal pigment epithelium (RPE), a layer of cells supporting the photoreceptors of the retina. The RPE exhibits regenerative capacity in some conditions and Dr. Blenkinsop's objective is to better understand the epigenetic state and molecular architecture of this plasticity to find ways to activate this potential endogenously. The approach is to develop in vitro models of eye diseases using cells isolated from human donor tissues, in the effort to more closely mimic human eye disorders and examine their cellular, molecular, and physiological foundation. In parallel, Dr. Blenkinsop's team is developing cell transplantation therapies for patients with retinal degeneration due to diseases such as age-related macular degeneration and eye injury. During quarantine, to learn more about SARS-CoV-2 and COVID-19, the group has been testing whether direct exposure to the eye can lead to systemic infection by SARS-CoV-2.

About NEI

NEI leads the federal government's research on the visual system and eye diseases. NEI supports basic and clinical science programs to develop sight-saving treatments and address special needs of people with vision loss. For more information, visit https://www.nei.nih.gov/.

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