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Oral microbiome findings challenge dentistry dogma

Complex microbial communities in the mouth clarify the causes of, and provide new treatments for, dental disease.

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The surface of the tongue hosts a complex microbial biofilm made up of distinct clusters of bacterial species (coloured dots). Credit: S. A. Wilbert *et al. Cell Rep.* **30**, 4003–4015 (2020)

The bacterium *Streptococcus mutans* was first implicated in causing dental caries in 1924, when an English dentist named J. Kilian Clarke found this microorganism at the scene of a cavity and declared it the culprit. Because the bacterium was easy to culture and study outside the mouth, scientists in subsequent decades were able to gather more and more evidence to support the guilty verdict: *S. mutans* was adept at attaching itself to hard tooth surfaces; it loved dietary sugars; and it churned out acid — in fact, it thrived in the kind of acidic milieu that carved holes in tooth enamel. By 1960, many dentists considered *S. mutans* the cause of dental caries (tooth decay) and by the mid-1970s, scientists were developing a caries vaccine from whole bacterial cells.

Little did these researchers know that the vaccine was misdirected. It turned out that *S. mutans* was not acting alone to cause dental damage. It was receiving assistance from other microbial cells. Scientists have been aware of microbes in the mouth for centuries, since Antonie van Leeuwenhoek famously reported scraping live bacteria from the inside of his mouth and observing them "very prettily a-moving" under an early microscope in the late 1600s. Subsequently, they cultured what they could from sites in the mouth and studied the characteristics of specific bacteria, leading them to pin oral diseases on certain microbes. But with next-generation gene-sequencing techniques becoming widespread over the past two decades, along with innovative imaging tools, scientists are now equipped with other ways to interrogate the microbes of the oral cavity. Researchers can determine which ones are present, how they're structured and what their functions are in human health. Oral microbiome research is changing long-held views about how microbes contribute to dental health — and to overall health, too. Fresh ways to leverage the mouth's microbial community for better oral and general health might be just around the corner.

Structure and community

The members of the microbial population remain constant, despite a regular influx of microbes that enter the mouth during eating, breathing or nail biting. Not all microbes survive in the oral environment, but the average person has around 250 species[1] from a pool of around 700 documented oral residents[2]. These species can evade the anti-microbial

defences of saliva and are also adapted to living in a warm, wet environment that is regularly bathed in oxygen.

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A closer look, however, reveals that the oral microbiome is not a homogeneous collection of life forms, but rather an assortment of mini microbiomes at different sites in the mouth. The microbes seem to be specialists for the niche that they occupy, whether that is the most commonly studied sites of saliva or dental plaque, or other areas of the oral cavity: tongue, cheek, palate, throat or tonsils.

"Different places in the mouth that are only millimetres apart from each other are inhabited by completely different microbial communities," says Jessica Mark Welch, a microbial ecologist at the Marine Biological Laboratory in Woods Hole, Massachusetts. She and several of her colleagues started to wonder how bacteria ended up in each niche, and how they managed to thrive. "We wanted to understand how the bacteria work together and how they interact with each other, and to understand that, we had to investigate spatial structure," she says.

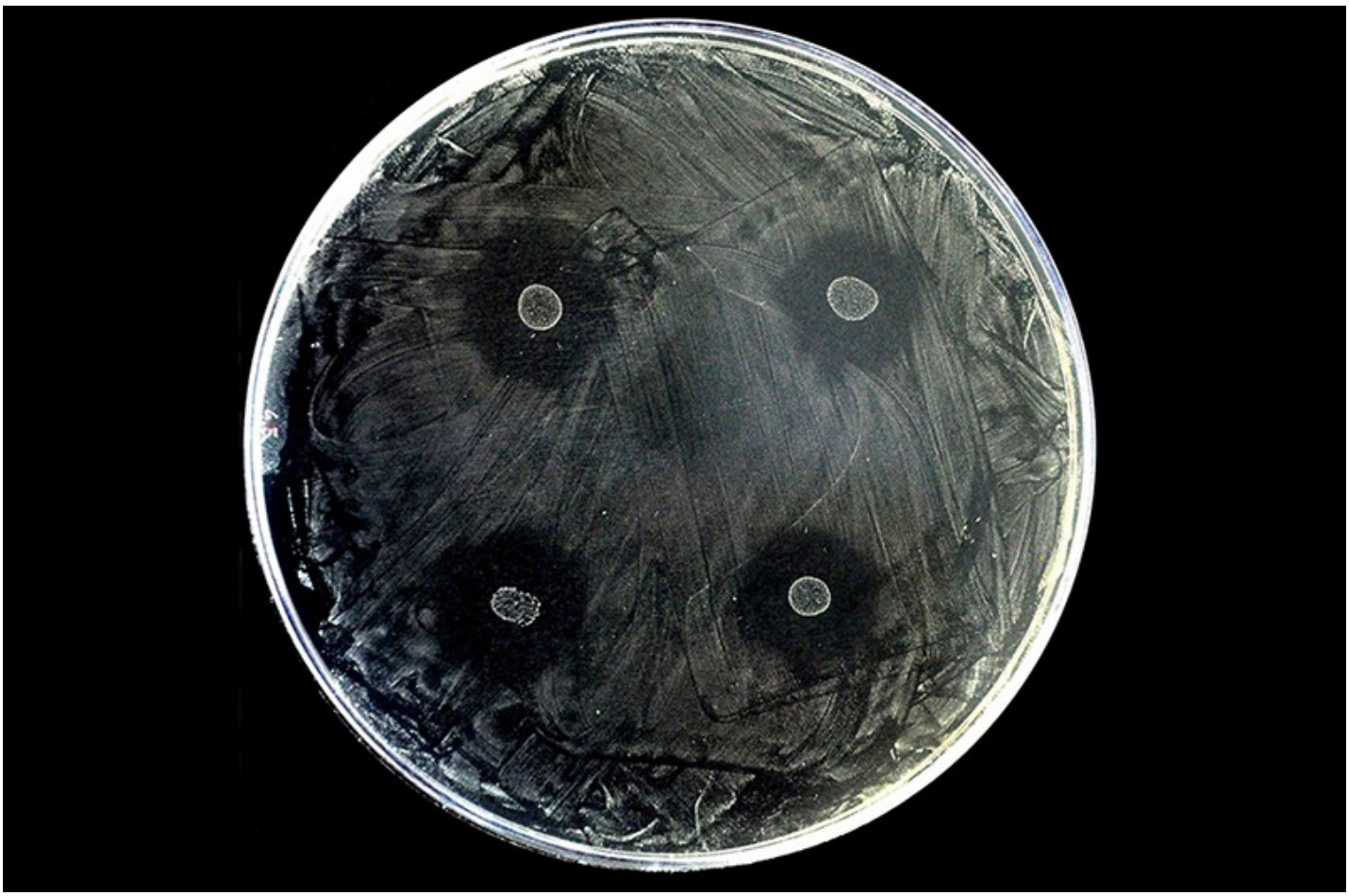
Mark Welch's colleague Gary Borisy, a cell biologist and imaging specialist now at The Forsyth Institute in Cambridge, Massachusetts, helped to develop a microscopy method that uses spectral fluorescence to image 15 or more bacterial species at the same time[3]. He and Mark Welch used this technique to look at dental plaque, a biofilm composed of self-assembling microorganisms. The microbes had organized themselves into an arrangement resembling a hedgehog: filamentous bacteria had stacked on top of each other to build a main spiny structure, with other organisms nestled in between the spikes. This happened in a healthy mouth, but other work has shown that the microbial species composition, as well as the biofilm's physical structure, change in the transition from health to disease.

Egija Zaura, an oral microbial ecologist at the Free University of Amsterdam and the University of Amsterdam, says that during her dental training in the early 1990s, she was taught that oral biofilms were always bad. Now that view has changed — it seems the effect depends on which microbes the biofilm contains. Zaura says that given the clear benefits of

brushing on oral health, wiping out the biofilms through regular brushing (for people who consume lots of starches and sugars, at least) must have positive overall effects on the oral microbiome. The long-term stability of the community supports the likelihood that any health-promoting microbes present in a biofilm will grow back after brushing and actually protect the teeth from their next acid bath. One study showed that people with periodontitis — also known as gum disease — gain a different biofilm with an increased richness of microbial species after a professional dental cleaning[4].

How oral microbes promote health

It is now clear that the entire microbial community in a healthy mouth works together to provide services for the human host. Some microbial residents, such as *Streptococcus salivarius*, have the ability to inhibit inflammation[5]. The bacterial community members also help to regulate the acidity of the mouth: people without caries have species that are able to convert arginine or urea in the diet to pH-balancing ammonia. Furthermore, products of metabolism from the bacterial community help to kill oral pathogens.



Streptococcus dentisani (in the four circular colonies) produces antimicrobial molecules that kill *Streptococcus mutans*, a contributor to tooth decay. Killing is indicated by the empty rings around each colony. Credit: P. Belda-Ferre *et al. ISME J.* **6**, 46–56 (2012)

However, microbes in the mouth support more than just oral health. They also transform the nitrate that we ingest in fruit and vegetables into nitrite, which is then turned into nitric oxide, helping to regulate blood pressure. Alex Mira, a bacterial geneticist at the FISABIO research institute in Valencia, Spain, says that as humans evolved, they (like many other mammals) apparently outsourced the job of making nitrite from dietary nitrate to their resident oral microbes. "Our body doesn't have the enzymes to do that," he explains. "We entirely rely on our oral organisms."

In fact, over-the-counter antiseptic mouthwashes can wipe out beneficial oral microbes and interfere with this crucial process. Unlike toothpaste, many brands of mouthwash have an active ingredient called chlorhexidine that destroys the microbes themselves. Mira says that even ten years ago the predominant thinking in dentistry was that people needed to keep the oral cavity 'clean' by getting rid of as many oral bacteria as possible. But this is now seen as folly — a 2020 study showed that healthy people who rinsed with chlorhexidine mouthwash experienced a major shift in their salivary microbiomes, leading to more acidity in the mouth and lower nitrite availability, with a trend toward higher blood pressure[6].

Dental culprits re-examined

Until recently, dentists pinned different dental health disorders on different microbes: *S. mutans* caused dental caries, *Porphyromonas gingivalis* led to periodontitis and *Candida albicans* caused oral candidiasis (commonly known as oral thrush).

"For dental conditions, we were very much influenced by the idea that every disease should have a single causal microbial agent," says Mira. Researchers started to modify their views on the bacterial causes of dental disease in the late 1990s, when scientists at The Forsyth Institute proposed how complexes of organisms in plaque below the gum line could be responsible for dental disease. Floyd Dewhirst, a dentist, pharmacologist and microbiologist at Forsyth, was one of the scientists working on periodontal disease who realized the

importance of microbial communities in the mouth well before inexpensive DNA-sequencing techniques became available.

In the early 2000s, as next-generation sequencing techniques became more widely available, Dewhirst identified a barrier to progress in the field: even though researchers could distinguish between different human oral bacteria by their unique genetic sequences — looking at 16S ribosomal RNA genes — they could not assign names to these bacteria or understand their relationships with other microbes. His work in developing a comprehensive database and provisional naming system yielded an important resource for the field, the Human Oral Microbiome Database.

Work on this database by Dewhirst and others made it possible to catalogue the entire known array of microbes at different oral microbiome sites as part of the Human Microbiome Project, one of the world's first large-scale surveys of the microbiomes of healthy individuals. The project included specimens from nine places in the oral cavity. Microbiome composition varied widely from person to person, which unfortunately precluded the identification of characteristics that signalled a healthy microbiome. Dominant microbial groups at each site tended to be shared, however, whereas less-dominant ones were highly personalized.

The Human Microbiome Project data confirmed that oral pathogens were present in people with and without oral disease. When oral disease occurred, other acid-producing bacteria — such as bifidobacteria or lactobacilli — were sometimes implicated; later research confirmed that different microbial profiles were associated with caries in different individuals.

The latest theories posit that a shift in the oral microbial community permits bacteria that are normally kept in check by other microbes to become virulent, causing oral disease. "Those bacteria are kept under control in a healthy ecosystem," Mira says. "They increase in proportion, they change the profile of gene expression, then they become pathogens." He and others have written about oral diseases as having a polymicrobial origin. He thinks the reason that efforts to produce a caries vaccine have not proved successful in clinical trials so far is that they each target a single microbe that is irregularly implicated.

Another conundrum that could be addressed with knowledge about oral microbial communities is why only a fraction of people with gingivitis (mild gum inflammation) progress to full-blown gum disease. It might be that the presence of a dental biofilm is less important than the exact microbes in the biofilm, because those individuals who develop gum disease have oral microbes that interact differently with the immune system and trigger more tissue-destroying inflammation as the person advances to periodontitis. Microbes that benefit from the ecological niche created by inflammation will thrive, creating a vicious cycle of ecosystem disturbance and inflammation.

Thinking of oral bacteria and their host as part of the same overall ecosystem allows a more comprehensive picture of what determines dental health for individuals. In this new framework for dental disease, lifestyle factors such as the consumption of sweets or how often someone's teeth come in contact with fluoride through drinking water or toothpaste might exert pressure on the entire microbial community and shift it in the direction of health or disease. In other words, lifestyle is important but not deterministic.

Association studies have found altered oral microbiome compositions in a growing list of diseases and conditions, including colorectal cancer, rheumatoid arthritis and Alzheimer's disease[7].

Any existing causal links between these diseases and the oral microbiome might take time to unravel, but in the meantime scientists are investigating whether oral microbial shifts could reliably signal some aspect of disease aetiology or progression. The oral microbiome is of particular interest in the search for biomarkers because of its accessibility and convenience. Furthermore, the influence of dietary factors on the oral microbiome seem minimal compared with on the gut microbiome, so the stability of the oral microbial community gives it strong biomarker potential.

Using one of the world's largest supercomputers, a project at the Oak Ridge National Laboratory (ORNL) in Tennessee is processing large quantities of data on the microbiome of various body sites, including the oral microbiome, to search for patterns that could lead to medically relevant biomarkers for neurological disorders such as Parkinson's disease, Alzheimer's disease and motor neuron disease — also known as amyotrophic lateral sclerosis

(ALS). “There are associations of microbes in periodontal disease with Alzheimer’s. Could we perhaps find an association in Parkinson’s and ALS?” says J. Chris Ellis, a computational microbiologist at ORNL. “One of the goals is to find early indicators that someone may be at risk for a disease. Perhaps then we’ll be able to create new medicines that are able to treat or prevent the onset of these disease states.”

Towards better dental care

The current mainstays of dental care are surgical and antibiotic treatments. But oral microbiome research could usher in an era in which the microbes of the mouth can be precisely manipulated — perhaps even purposely adding bacteria to maintain dental health.

The goal, says Mark Welch, would be to screen for aggressive strains in an individual’s mouth, and look to replace them with other strains, fine-tuning the oral community. Currently available probiotics are probably not up to the task, however, because the oral microbial community is so resistant to change. “To get a random external bacterium to incorporate into the oral microbiome is not easy,” says Dewhirst, “because it does not have the adhesion networks of organisms recruited over a lifetime for their ability to interact.”

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Mira’s group has identified a candidate oral probiotic, a bacterium called *Streptococcus dentisani*. This microbe, which the researchers isolated from people without caries, contributes to killing oral pathogens — the name ‘*dentisani*’ translates from Latin as ‘healthy teeth’. The team is now tackling the technical challenges of delivering these finicky bacteria while looking to address regulatory hurdles in bringing the resulting probiotic to market.

Another approach could be to shape the chemistry of the oral environment in a way that discourages cavities and other disorders. Toothpaste with ammonia-producing, pH-lowering arginine is already available on the market, but Mira thinks the next closest application might be the use of prebiotics: nitrate in toothpaste, for example, either as a salt or vegetable extract, could be used to selectively feed certain bacteria to better support health.

Zaura points out that regular preventive visits to the dentist could be used to pursue more focused prevention strategies. She suggests that dentists might examine the activity of someone's microbial community just as they examine the physical condition of the person's teeth. That would enable dentists to identify people's vulnerabilities and implement strategies to change the oral microbial ecology to steer away from oral, and possibly other, diseases. "By focusing on oral health", Zaura says, "you can do a lot to support general health."

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