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What are We Learning and What Can We Learn from the Human Oral Microbiome Project?

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Abstract

Extraordinary technological advances have greatly accelerated our ability to identify bacteria, at the species level, present in clinical samples taken from the human mouth. In addition, technologies are evolving such that the oral samples can be analyzed for their protein and metabolic products.

As a result, pictures are the advent of personalized dental medicine is becoming closer to reality.

Keywords

microbiomes; oral health; oral disease; caries; periodontal disease; oral cancer

1. Introductory comments: The advent of the microbiome-era in human oral biology

During his 2015 State of the Union address, President Obama announced an allocation of \$215 million dollars to fund a Precision Medicine Initiative, to be driven by the National Institutes of Health, with the goal of developing the technology to prevent, diagnose, and treat diseases at the level of individual patients [1]. The elucidation and publication of the human genome in 2001 had initiated a new era in biology, one in which ‘precision-medicine’ became the concept on which the future of healthcare is being built. The foundation of precision medicine is the human genome and the genomes of the bacteria, viruses, fungi, and protists that are carried along on, and in, the human body, and how those organisms relate to their hosts.

A series of Nobel Prize-winning discoveries were essential for establishing DNA sequences in the human genome, and indeed the genome of all living things. The elucidation of DNA structure [2]; its organization into groups, or ‘codons’, of deoxyribonucleic bases that act as the translating chemicals between the structure of DNA and the amino acids that comprise

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Compliance with Ethics Guidelines

Conflict of Interest

Benjamin Cross, Roberta C. Faustoferri and Robert G. Quivey, Jr. declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

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proteins [3]; the necessity of three codons to encode an amino acid [4]; and finally, deciphering the genetic code itself, showing that individual codons were specific for particular amino acids, thereby providing a basis for what is now thought of as biotechnology and genetic engineering. [5-7]. Following the Nobel Prize-winning development, by Frederick Sanger in the 1970's, of a method to chemically determine the order of bases in a strand of DNA, referred to as "DNA sequencing" [8], it became a certainty that the genetic content – or genome – of all living things would be established.

The first sequence of the human genome, referred to as a draft sequence, was published in 2001 and constituted approx. 90% of the genome [9], at an estimated cost of \$3 billion dollars (U.S.). Thus, the first blueprint for human genomes was established, providing a known sequence on which additional human genomes, such as individual patients, could be mapped and compared, at the level of individual bases.

As the logical extension of the Human Genome project, the Human Microbiome Project (HMP) was launched in 2007, with the initial goal of establishing 3000 microbial genomes to serve as reference sequences for deep sequencing of samples from microbial communities, isolated from five regions of the human body: skin, mouth, esophagus, stomach, colon, and vagina [10]. The magnitude of the project was immediately clear, as it was already understood that bacterial cells alone outnumbered cells in the human body by approx. 10-fold. Further, it was also understood, from the genetic content of known bacterial genomes, that the number of individual bacterial genes likely out-numbered human genes by an order of magnitude, at least.

2. Microbiomics of dental caries and periodontal disease – what have we learned?

Completion of the human genome project was accompanied by the parallel, and continuing, development of commercially available high-throughput DNA sequencing instruments, which have in turn, facilitated the acquisition of genomic information from human oral samples. The majority of initial studies focused on identifying oral bacteria associated with health and disease. These types of studies relied on the use of 16S rRNA-encoding DNA as a target gene that can be used to identify and enumerate the number and kinds of bacteria in a given sample, including dental plaque samples and samples taken from periodontal pockets.

The results of a myriad "16S" studies culminated in the first publication of the Human Oral Microbiome Database (HOMD), which identified bacteria in samples taken from specific locations in the oral cavity: teeth, the gingival sulcus, tongue, cheek, hard and soft palates, and tonsils, all of which are colonized by bacteria [11]. HOMD represents, at least, 700 taxa, or bacteria, at the species level, as well as identified subsets of bacteria residing at different habitats. HOMD was the first curated database of a human microbiome, providing an essential, and continuously accumulating, database for comparison of oral bacterial genomic DNA sequences that continues to grow from ongoing studies.

Genome-sequencing protocols and instrumentation have evolved rapidly over the past decade, resulting in improvements in the amount of sequencing information that could be

obtained from the relatively small, or limited, samples from the oral cavity. Moreover, the ability to obtain complete genome sequences from all bacteria in oral samples, have made possible the field of meta-genomics: the ability to identify bacteria at the level of individual genes in complex samples.

What is now known from classical microbiological experiments, and from dramatically deeper information arising from metagenomic sequencing of oral plaque samples, is that specific bacteria are clearly associated with oral health and oral disease. Bacteria that have been associated with periodontal disease using advanced DNA sequencing approaches [12], fully support results from classical microbiology data accumulated over several, earlier, decades [13, 14], including the following species *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*. The bacterial genera that appeared at elevated levels in periodontal disease have included *Campylobacter*, *Selenomonas*, *Deferribacteres*, *Dialister*, *Catonella*, *Tannerella*, *Streptococcus*, *Atopobium*, *Eubacterium* and *Treponema* [15]. Additional organisms, not well studied at the time, but of strong recent interest included *Peptostreptococcus* and *Filifactor*. *Streptococcus* and *Veillonella* were also found in high numbers in subjects with healthy periodontium, along with lower abundance organisms. What is also now fully realized, is that human immune components, or lack thereof, and lifestyle attributes such as diet and smoking, influence the composition and abundance of periodontal bacteria and the extent of periodontal disease, see for example [16, 17, 15, 18, 19, 12].

The bacterial composition of supragingival plaque, associated with dental caries has also been the object of sequencing studies [20-23]. Generally, streptococci associated with the well-known mutans group have been shown to dominate the bacterial composition of dental plaque associated with caries, though approx. 10% of samples reveal absence or low abundance of *S. mutans* [21, 22]. *Veillonella* species are also often found in substantial numbers associated with samples from carious lesions, suggesting that these bacteria facilitate dental caries. It is appreciated that the metabolism of *Veillonella* relies on the metabolism of lactic acid produced by streptococci, not just aciduric streptococci, such that its role in caries formation and progression is not entirely clear. However, it is clear that the *Veillonella* is generally in high abundance in supragingival plaque samples containing streptococci.

In addition to identifying associations of bacteria with health and disease, the composite take-home message from the metagenomic studies reported to date can be simply stated: that periodontal disease and dental caries are complex diseases, reflecting changes in bacterial composition in disease sites, diet, and interface with the human immune system [24]. The available data strongly support the ecological hypothesis of Marsh, who, early-on, opined that oral disease of bacterial etiology would reflect the environment, the bacteria, and the condition of the host [25, 26]. Changes in one or more of these components of a microbial niche may cause a potential pathobiont to become dominant, creating a dysbiotic, or disease-favoring environment. Perhaps most interestingly, the microbial communities associated with dental caries show reduced diversity in the extent of microbial species [21, 22]; whereas, the periodontal disease pockets exhibit greater diversity [27, 12, 28, 29], likely indicating that availability of human cell constituents, crevicular fluid, and blood provide

abundant substrates for growth of many bacterial species. Whereas, on the tooth surfaces, erosion of enamel by bacteria is caused by prolonged exposure to organic acids, leading to localized pH values well below the limits of survival by many competing bacteria. *S. mutans*, as an example, can grow at pH values of 5 and continue to protect itself from acidification, via proton-pumping at pH values of 3.5 [30, 31]. That the acidogenic/aciduric species in the oral cavity can cause dental caries and survive the inimical influences of acidification, there is, in this era no doubt.

3. The rise of Meta-omics – the connection of microbiomes, proteomes, and metabolomes as tools to establish health and disease

The principal remaining questions in clinical oral microbiology revolve around how dysbiosis begins in the microbial niches of the oral cavity. Moreover, oral microbiology in the laboratory has consisted of primarily one species culture over the last century, with mixed species experiments being utilized in a small number of laboratories. It is fully understood that the meta-genomes resident in the oral cavity represent health and the risk of disease. How that balance turns to disease is now being explored by the combination of multiple, technology-driven, sciences: including the concepts of individual genomes, that is, determining the DNA sequence of all patients; along with a determination of all proteins present in disease sites, which represents the collective output of the bacteria at those sites; and, most recently, the science of metabolomics, which is the metabolic output of all bacteria in a given site. This would include, particularly in periodontal pockets, considerable amounts of biological material derived from the patient themselves. Proteomics is driven by ever more sensitive mass spectrometers to separate biological materials by mass, and to identify compounds by comparison to existing libraries of compounds; and, to do so, in a quantitative manner. Likewise, metabolomics is also driven by mass spectrometric identification of bacterial, viral, fungal, and human products in disease sites. As the reader will rightly imagine, the application of these technologies to problems in oral biology would seem extremely complex. Nevertheless, the technologies continue to develop greater speed, precision, and depth at great rate, such that reports are now emerging that detail studies of mixes of bacteria, primarily model systems that have been constructed to report on known bacteria. For example, the microbiota in salivary samples [32, 33] have been reported, underscoring the proximity of scientists and clinicians are to realizing the possibility of using these technologies in a practical fashion to predict oral microbial disease. Specific interactions of *P. gingivalis* and *Streptococcus*, highly related to the onset of periodontal disease, are also being explored [34-36]. The combination of meta-genomics and metabolomics has recently been reported in studies with multiple oral bacterial species, in work designed to follow the development of bacterial products over time, as would be expected in healthy and disease sites [37]. As impressive as the science has been, it is clear that the models, and technologies are rapidly advancing, with their potential usefulness in personalized dental medicine now in the realm of the conceivable [29, 28, 38, 39].

4. What can we still learn from Microbiomic Information?

The metagenome of the oral cavity is comprised of hundreds of individual genomes. The most influential of these is arguably the human genome. Human genetic variation can influence phenotypes with obvious impacts on microbiome composition such as salivary flow rate, the composition of saliva, immune system function, and possibly taste preference [40-42]. Twin studies also suggested a genetic component to caries long before the human genome project was complete [43, 44].

A thorough analysis of 93 individuals by Bleckhman *et al.* [45] analyzed the correlation of host genetic profiles with specific species and microbiome alpha diversity at 15 body sites. Principal component analysis revealed a correlation between host genetic variation and alpha diversity in the supragingival plaque, the throat, and the tongue dorsum, suggesting genetics plays an especially important role in the diversity of the oral microbiome, compared to other body sites.

A pathway-based analysis was used to test associations between specific taxa and single-nucleotide polymorphisms (SNPs). The leptin signaling pathway had the most significant effect on microbiome composition. SNPs in leptin signaling were correlated with the presence of *Lachnospiraceae* and *Haemophilus* on the attached keratinized gingiva, and *Propionibacterium* in subgingival plaque [45]. Leptin is a hormone that influences many diverse metabolic functions including insulin secretion, appetite, inflammation, activation of leukocytes, and proliferation of oral keratinocytes [46, 47]. [45] correlated with specific taxa in the oral cavity include G Protein Signaling Mediated by Tubby, P2Y Purigenic Receptor Signaling, and Melatonin Signaling.

Finally, Blekhman *et al.* examined the variability of the taxa-associated SNPs between different human populations. They found that the allele frequency differentiation (measured by F_{ST}) of SNPs linked to specific taxa was significantly higher than the genome average [45]. This increased variability suggests two possible scenarios: humans evolve adaptations in response to local, population-specific microbiomes, or past defensive adaptations to specific pathogens are having a lasting effect on the present commensal microbiome. These two possibilities are not mutually exclusive. It is likely that examples of each can be found in our evolutionary history.

Many questions about humans' coevolution with their oral microbiome could be addressed by the emerging field of paleomicrobiology. The dental calculus can preserve bacterial DNA, mitochondrial DNA, and dietary fossils for thousands of years, giving us snapshots of ancient oral microbiomes [48-50]. There is currently not enough data in this field to address the subtleties of coevolution, but there is enough data to draw conclusions about the effects of major changes in the human diet. Consumption of cariogenic wild plants coincided with changes in the composition of the oral microbiota, including expansion of *S. mutans* in populations of Pleistocene hunter-gathers [51]. European archaeological data sets show a major increase in the prevalence of dental caries and periodontitis coinciding with the introduction of farming in the Neolithic period [52]. Another marked increase in the prevalence of dental caries occurred in the mid-19th century, during the industrial revolution

[53]. DNA evidence suggests that during both of these time periods overall diversity in the oral microbiome decreased, and the prevalence of *S. mutans* increased [54]. Neolithic farming is also associated with a significant increase in the prevalence of *P. gingivalis* [54]. Warinner *et. al.* [50] sampled dental calculus from human skeletons with evidence of periodontal disease buried between 950-1200 CE in Dalheim, Germany. Sequencing 16S rRNA revealed an abundance of *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola*, three species commonly associated with modern periodontitis [55]. These findings provide strong evidence in favor of the ecological theory of caries, which suggests that changes in carbohydrate intake affect the ecosystem of the mouth. Increases in carbohydrate intake in the modern era, resulting from the consumption of processed grains, favor pathogenic ecologies [25, 56].

One of the main goals of the Human Microbiome Project is to find differences between microbiome compositions in healthy and diseased states [57]. Because of the unique nature of every person's microbiome, it may be many years before we can clearly define what it means to have a "healthy" oral bacterial community. As our knowledge in this area becomes more complete, what clinical benefits will we gain? Knowledge of microbiomes, in general, is not enough. For any patient-oriented applications, we must develop technology to make regular monitoring of a patient's oral microbiome practical. Based on the progress of DNA sequencing technologies in the past twenty years, there is no reason to doubt that this technology will eventually exist.

Testing for the simple presence or absence of bacterial species is probably not a practical way to screen for the risk of caries or periodontitis. These are multifactorial diseases that do not depend on any one pathogenic species [12]. There are some opportunistic pathogens, however, that maintain a reservoir in the oral microbiome and cause infections outside of the oral cavity [58]. Infective endocarditis is often the result of oral bacteria entering the bloodstream [59, 58]. There is an ongoing debate about the efficacy of providing prophylactic antibiotics to patients undergoing dental procedures that are at heightened risk for endocarditis [60-62]. Perhaps a more accurate risk assessment could be made by testing each patient for the presence of species (or serotypes) that have been known to cause infective endocarditis [63, 64]. Similarly, nosocomial pneumonia is correlated with periodontitis and poor oral health [65, 66]. Screening the oral microbiome for respiratory pathogens may help assess a patient's risk of developing pneumonia while in the hospital.

Disease prevention is always preferable to disease treatment, so how could information about a patient's microbiome be used to predict the risk of caries or periodontitis? If there are distinct healthy and diseased microbiomes for the teeth and gums, then there must also be transitional states. Some research has been done regarding transitional states in the gut and lungs, but the concept is mostly speculation [67, 68]. Are transitional states somewhere on a spectrum between health and illness, or are there species that play an important role in the transition without being prevalent in either health or disease? How long do transitional states last? As these questions are answered, we may gain a tool for predicting the onset of caries or periodontitis long before any visible signs of demineralization or inflammation occur.

If a disease is already severe, understanding the patient's microbiome could still be beneficial. The number of species combinations in the microbiome that could be considered unhealthy is potentially infinite. The best treatment for a case of periodontitis dominated by *P. gingivalis* and *T. denticola* may be different from the best treatment for periodontitis dominated by *P. gingivalis* and *T. forsythia*. The complex interactions between multiple species are known to influence the effectiveness of antibiotics [69]. Future research will provide more definite insight into increasing the specificity of treatments.

One significant problem with finding the best treatments for specific microbiome maladies is our current lack of narrow-spectrum antibiotics. Broad spectrum antibiotics have saved millions of lives, but side effects from their disruption to the normal microbiome can be devastating, especially in the gut [70, 71]. The knowledge of healthy versus unhealthy microbiomes will not be very useful to patients if we lack the ability to manipulate their microbiome composition. This problem could possibly be solved using narrow-spectrum antibiotics; however, the antibiotics currently available are most likely not specific enough to produce the desired effects. This is especially true for dental caries, where different species from the same genus, *Streptococcus*, have been shown to contribute to caries and also prevent them [24, 12]. Fecal transplantation has been shown to effectively alter the microbiome of the gut [72, 73], but this method would be very difficult to implement in the oral cavity. The salivary flow and normal use of the mouth complicate the delivery of the new microbiome, and pre-established biofilms on enamel surfaces inhibit colonization by new species and are difficult to remove [69, 74]. Bacteriocins, antibiotic peptides produced by bacteria, are a potential new class of narrow-spectrum antibiotics [75, 76]. They can have very specific targets, but may be difficult to produce and administer [77].

The best way to deliver bacteriocins may be via whole, living bacteria. Probiotics as a method for improving oral health have been heavily researched [78-80], there is even a method for using metagenomics to screen for potential probiotics [24], but no definite conclusions have been made regarding future treatments. Biofilms may prevent a complete change in microbiome, but research has found several specific species that are able to infiltrate established dental biofilms [78]. If these species could be engineered to produce bacteriocins, the target species could be eliminated and prevented from returning, the process being dependent on the continued presence of the probiotic species. There are many other helpful products besides bacteriocins that could be produced by engineered probiotics. Overproduction of natural metabolic products like ammonia-containing compounds (to increase pH) or peroxide (for oxidative stress) could alter the environment, and therefore the microbiome, in a positive way [81].

It has been argued that observing the prevalent metabolic pathways in the oral microbiome could be more important than observing the species [82]. This requires sequencing all of the DNA or RNA present, as opposed to only small sections of 16S rRNA, but it may be more useful as a diagnostic tool. For many bacterial species in the oral cavity, the 16S sequence is the only thing known about them, so the effect of their presence is currently unknown. If the major metabolic pathways present in a microbiome are known, it should be easier to predict how that microbiome will react to different environmental perturbations. Potentially informative pathways include antibiotic resistance mechanisms, lactic acid fermentation,

short-chain fatty acid production [83], and many more. Perhaps future microbiologists will be able to discuss microbiomes in terms of their ability to lower pH or induce inflammation without much knowledge of the species involved.

What can we learn from the human oral microbiome project? Like any other large research undertaking, every answer provides us with new questions. Knowledge of the oral microbiome is driving research in new directions, many of which don't rely on large-scale DNA sequencing. Experiments using live cells are especially important for conducting experiments that depend on specific spatial or temporal arrangements of bacteria, such as adherence to enamel. He *et. al.* [74] identified a specific "social structure" of three species from the mouse oral microbiome that synergistically cooperate to inhibit the colonization by *Escherichia coli*. *Staphylococcus saprophyticus* senses the *E. coli* LPS and produces a diffusible signal that directly increases peroxide production by *Streptococcus sanguinis*. *S. saprophyticus* also disrupts the ability of *Streptococcus infantis* to inhibit peroxide production by *S. sanguinis*.

There are hundreds, maybe thousands, of polymicrobial interactions like the one described by He simultaneously occurring in the microbiome, and they are subtly different for every individual. Here, the main examples focused on bacteria, but fungi and viruses are important components of the microbiome, each with their own unique metabolic contributions. Organizing this vast amount of information has become a science in it's own right [84, 85]. Bioinformatics is already indispensable, and will grow in importance as more data is gathered. Public databases such as the National Center for Biotechnology Information (NCBI) and HOMD represent the principal, major, resources for gaining an understanding of the oral microbiome [86]. Microbiomes are as unique as a fingerprint, but common patterns will have to be found, and studied, using large data sets gathered from many individuals.

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References

• Of importance

1. Steele M. Obama to Announce Major Personalized Medicine Initiative. US News & World Report HealthDay. Jan.2015 2015 30
2. Watson JD, Crick FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature. 1953; 171(4356):737–8. [PubMed: 13054692]
3. Gamow G, Ycas M. Statistical correlation of protein and ribonucleic acid composition. Proc Natl Acad Sci USA. 1955; 41(12):1011–9. [PubMed: 16589789]
4. Crick FH, Barnett L, Brenner S, Watts-Tobin RJ. General nature of the genetic code for proteins. Nature. 1961; 192:1227–32. [PubMed: 13882203]
5. Nirenberg MW, Matthaei JH. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. Proc Natl Acad Sci USA. 1961; 47:1588–602. [PubMed: 14479932]
6. Matthaei JH, Jones OW, Martin RG, Nirenberg MW. Characteristics and composition of RNA coding units. Proc Natl Acad Sci USA. 1962; 48:666–77. [PubMed: 14471390]

7. Last JA, Stanley WM Jr, Salas M, Hille MB, Wahba AJ, Ochoa S. Translation of the genetic message, IV. UAA as a chain termination codon. *Proc Natl Acad Sci USA*. 1967; 57(4):1062–7. [PubMed: 5231346]
8. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA*. 1977; 74(12):5463–7. [PubMed: 271968]
9. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001; 409(6822):860–921. doi:10.1038/35057062. [PubMed: 11237011]
10. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature*. 2007; 449(7164):804–10. doi:10.1038/nature06244. doi:10.1038/nature06244. [PubMed: 17943116]
11. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. *J Bacteriol*. 2010; 192(19):5002–17. doi:10.1128/JB.00542-10. [PubMed: 20656903]
12. Wade WG. The oral microbiome in health and disease. *Pharmacol Res*. 2013; 69:137–43. doi: 10.1016/j.phrs.2012.11.006. [PubMed: 23201354]
13. Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. *J Clin Perio*. 2000; 27(10):722–32.
14. Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *J Clin Perio*. 2000; 27(9):648–57.
15. Kumar PS, Griffen AL, Moeschberger ML, Leys EJ. Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. *J Clin Microbiol*. 2005; 43(8):3944–55. doi:10.1128/JCM.43.8.3944-3955.2005. [PubMed: 16081935]
16. Schlafer S, Riep B, Griffen AL, Petrich A, Hubner J, Berning M, et al. *Filifactor alocis*–involvement in periodontal biofilms. *BMC microbiol*. 2010; 10:66. doi:10.1186/1471-2180-10-66. [PubMed: 20193074]
17. Kumar PS, Leys EJ, Bryk JM, Martinez FJ, Moeschberger ML, Griffen AL. Changes in periodontal health status are associated with bacterial community shifts as assessed by quantitative 16S cloning and sequencing. *J Clin Microbiol*. 2006; 44(10):3665–73. doi:10.1128/JCM.00317-06. [PubMed: 17021095]
18. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of *Porphyromonas gingivalis* and periodontal health status. *J Clin Microbiol*. 1998; 36(11):3239–42. [PubMed: 9774572]
19. Teles R, Sakellari D, Teles F, Konstantinidis A, Kent R, Socransky S, et al. Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontal disease, and the subgingival microbiota. *J Perio*. 2010; 81(1):89–98. doi:10.1902/jop.2009.090397.
20. Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, et al. Molecular analysis of bacterial species associated with childhood caries. *J Clin Microbiol*. 2002; 40(3):1001–9. [PubMed: 11880430]
21. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond *Streptococcus mutans*: dental caries onset linked to multiple species by 16S rRNA community analysis. *PloS one*. 2012; 7(10):e47722. • . doi:10.1371/journal.pone.0047722. Gross et al provide a compelling argument that multiple species participate in the human disease known as dental caries and show that over time, mutans streptococci can become dominant members of the supragingival flora. [PubMed: 23091642]
22. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, et al. Bacterial 16S sequence analysis of severe caries in young permanent teeth. *J Clin Microbiol*. 2010; 48(11): 4121–8. doi:10.1128/JCM.01232-10. [PubMed: 20826648]
23. Griffen AL, Beall CJ, Firestone ND, Gross EL, Difrancia JM, Hardman JH, et al. CORE: a phylogenetically-curated 16S rDNA database of the core oral microbiome. *PloS One*. 2011; 6(4):e19051. doi:10.1371/journal.pone.0019051. [PubMed: 21544197]
24. Belda-Ferre P, Alcaraz LD, Cabrera-Rubio R, Romero H, Simón-Soro A, Pignatelli M, et al. The oral metagenome in health and disease. *The ISME journal*. 2012; 6:46–56. doi:10.1038/ismej.2011.85. [PubMed: 21716308]

25. Marsh PD. Dental plaque as a microbial biofilm. *Caries Res.* 2004; 38(3):204–11. doi: 10.1159/000077756. [PubMed: 15153690]
26. Marsh PD, Head DA, Devine DA. Ecological approaches to oral biofilms: Control without killing. *Caries Res.* 2015; 49:46–54. doi:10.1159/000377732. [PubMed: 25871418]
27. Curtis MA, Zenobia C, Darveau RP. The relationship of the oral microbiota to periodontal health and disease. *Cell Host & Microbe.* 2011; 10(4):302–6. doi:10.1016/j.chom.2011.09.008. [PubMed: 22018230]
28. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiol.* 2009; 9:259. doi:10.1186/1471-2180-9-259. [PubMed: 20003481]
29. Zaura E, ten Cate JM. Towards understanding oral health. *Caries Res.* 2015; 49(Suppl 1):55–61. doi:10.1159/000377733. [PubMed: 25871419]
30. Sutton SV, Marquis RE. Membrane-associated and solubilized ATPases of *Streptococcus mutans* and *Streptococcus sanguis*. *J Dent Res.* 1987; 66(6):1095–8. [PubMed: 2887601]
31. Bender GR, Sutton SV, Marquis RE. Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. *Inf Immun.* 1986; 53(2):331–8.
32. Jagtap PD, Blakely A, Murray K, Stewart S, Kooren J, Johnson JE, et al. Metaproteomic analysis using the Galaxy framework. *Proteomics.* 2015; 15(20):3553–65. doi:10.1002/pmic.201500074. [PubMed: 26058579]
33. Rudney JD, Xie H, Rhodus NL, Ondrey FG, Griffin TJ. A metaproteomic analysis of the human salivary microbiota by three-dimensional peptide fractionation and tandem mass spectrometry. *Molec Oral Microbiol.* 2010; 25(1):38–49. doi:10.1111/j.2041-1014.2009.00558.x. [PubMed: 20331792]
34. Lamont RJ, Meila M, Xia Q, Hackett M. Mass spectrometry-based proteomics and its application to studies of *Porphyromonas gingivalis* invasion and pathogenicity. *Infectious Dis Drug Targets.* 2006; 6(3):311–25.
35. Xia Q, Hendrickson EL, Wang T, Lamont RJ, Leigh JA, Hackett M. Protein abundance ratios for global studies of prokaryotes. *Proteomics.* 2007; 7(16):2904–19. doi:10.1002/pmic.200700267. [PubMed: 17639608]
36. Xia Q, Wang T, Taub F, Park Y, Capestany CA, Lamont RJ, et al. Quantitative proteomics of intracellular *Porphyromonas gingivalis*. *Proteomics.* 2007; 7(23):4323–37. doi:10.1002/pmic.200700543. [PubMed: 17979175]
37. Edlund A, Yang Y, Yooseph S, Hall AP, Nguyen DD, Dorrestein PC, et al. Meta-omics uncover temporal regulation of pathways across oral microbiome genera during *in vitro* sugar metabolism. *The ISME Journal.* 2015 • . doi:10.1038/ismej.2015.72. The wave of microbiome sequencing provided the evidence that the genomes of microbiota must also be considered in the context of the products produced by a given microbial population, which in turn influences the transcriptomes and, eventually, the microbiota present in a given microbial community. The term “omics” is fully developed in this tour de force of technology meeting the oral microbiome.
38. Zaura E. Next-generation sequencing approaches to understanding the oral microbiome. *Adv Dent Res.* 2012; 24(2):81–5. doi:10.1177/0022034512449466. [PubMed: 22899686]
39. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Dis.* 2012; 18(2):109–20. doi:10.1111/j.1601-0825.2011.01851.x. [PubMed: 21902769]
40. Hart TC, Kornman KS. Genetic factors in the pathogenesis of periodontitis. *Periodontology* 2000. 1997; 14:202–15. doi:10.1111/j.1600-0757.1997.tb00198.x. [PubMed: 9567972]
41. Hjorth JP. Genetic variation in mouse salivary amylase rate of synthesis. *Biochem Genetics.* 1979; 17:665–82. doi:10.1007/BF00502125. [PubMed: 94264]
42. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome-wide scan finds suggestive caries loci. *J Dent Res.* 2008; 87:435–9. doi:10.1177/154405910808700506. [PubMed: 18434572]
43. Boraas JC, Messer LB, Till MJ. A genetic contribution to dental caries, occlusion, and morphology as demonstrated by twins reared apart. *J Dent Res.* 2008; 87:1150–5. [PubMed: 3165997]

44. Conry JP, Messer LB, Boraas JC, Aepli DP, Bouchard Jr TJ. Dental caries and treatment characteristics in human twins reared apart. *Arch Oral Biol.* 1993; 38:937–43. doi: 10.1016/0003-9969(93)90106-V. [PubMed: 8297257]
45. Blekman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, et al. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol.* 2015; 16:191. •. doi: 10.1186/s13059-015-0759-1. Blekman et al show clearly that the microbiome of the human host is in fact affected by the genome of that host, indicating a fluidity to what must be considered the human “microbiome” across all humanity. [PubMed: 26374288]
46. Gröschl M, Topf H-G, Kratzsch J, Dötsch J, Rascher W, Rauh M. Salivary leptin induces increased expression of growth factors in oral keratinocytes. *J Mol Endocrinol.* 2005; 34:353–66. doi: 10.1677/jme.1.01658. [PubMed: 15821102]
47. La Cava A, Matarese G. The weight of leptin in immunity. *Nat Rev Immun.* 2004; 4:371–9. doi: 10.1038/nri1350.
48. Fox CL, Pérez-Pérez A, Juan J. Dietary information through the examination of plant phytoliths on the enamel surface of human dentition. *J Archaeol Sci.* 1994; 21:29–34. doi:10.1006/jasc.1994.1005.
49. Preus HR, Marvik OJ, Selvig KA, Bennike P. Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. *J Archaeol Sci.* 2011; 38:1827–31. doi:10.1016/j.jas.2011.03.020.
50. Warinner C, Speller C, Collins MJ. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Phil Trans Royal Society of London B: Biol Sci.* 2015; 370:20130376. doi:10.1098/rstb.2013.0376.
51. Humphrey LT, De Groote I, Morales J, Barton N, Collcutt S, Bronk Ramsey C, et al. Earliest evidence for caries and exploitation of starchy plant foods in Pleistocene hunter-gatherers from Morocco. *Proc Natl Acad Sci USA.* 2014; 111(3):954–9. doi:10.1073/pnas.1318176111. [PubMed: 24395774]
52. Roberts C. The Cambridge encyclopedia of human paleopathology. *Med History.* 2000; 44:121–3.
53. Burt BA. Influences for Change in the Dental Health Status of Populations: An Historical Perspective. *J Publ Health Dent.* 1978; 38:272–88. doi:10.1111/j.1752-7325.1978.tb03753.x.
54. Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nature Genetics.* 2013; 45:450–5. doi:10.1038/ng.2536. [PubMed: 23416520]
55. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontology 2000.* 2005; 38:135–87. doi:10.1111/j.1600-0757.2005.00107.x. [PubMed: 15853940]
56. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. *J Dent Res.* 2011; 90(3):294–303. doi:10.1177/0022034510379602. [PubMed: 20924061]
57. Group TNHW. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, et al. The NIH Human Microbiome Project. *Genome Res.* 2009; 19:2317–23. doi:10.1101/gr.096651.109. [PubMed: 19819907]
58. Gendron R, Grenier D, Maheu-Robert L-F. The oral cavity as a reservoir of bacterial pathogens for focal infections. *Microb Infect.* 2000; 2:897–906. doi:10.1016/S1286-4579(00)00391-9.
59. Fiehn NE, Gutschik E, Larsen T, Bangsbo JM. Identity of streptococcal blood isolates and oral isolates from two patients with infective endocarditis. *J Clin Microbiol.* 1995; 33:1399–401. [PubMed: 7615767]
60. Dajani AS, Taubert KA, Wilson W, Bolger AF, Bayer A, Ferrieri P, et al. Prevention of bacterial endocarditis recommendations by the American Heart Association. *Circulation.* 1997; 96:358–66. doi:10.1161/01.CIR.96.1.358. [PubMed: 9236458]
61. Duval X, Delahaye F, Alla F, Tattévin P, Obadia J-F, Le Moing V, et al. Temporal trends in infective endocarditis in the context of prophylaxis guideline modifications: three successive population-based surveys. *J American Coll Cardio.* 2012; 59:1968–76. doi:10.1016/j.jacc.2012.02.029.
62. Gouriet F, Habib G, Raoult D. Infective endocarditis and antibiotic prophylaxis. *The Lancet.* 2015; 386:528. doi:10.1016/S0140-6736(15)61466-0.

63. Avilés-Reyes A, Miller JH, Simpson-Haidaris PJ, Hagen FK, Abranches J, Lemos JA. Modification of *Streptococcus mutans* Cnm by PgfS contributes to adhesion, endothelial cell invasion, and virulence. *J Bact.* 2014; 196:2789–97. doi:10.1128/JB.01783-14. [PubMed: 24837294]
64. Nakano K, Nemoto H, Nomura R, Inaba H, Yoshioka H, Taniguchi K, et al. Detection of oral bacteria in cardiovascular specimens. *Oral Microbiol Immunol.* 2009; 24:64–8. doi:10.1111/j.1399-302X.2008.00479.x. [PubMed: 19121072]
65. Paju S, Scannapieco FA. Oral biofilms, periodontitis, and pulmonary infections. *Oral Dis.* 2007; 13:508–12. doi:10.1111/j.1601-0825.2007.01410a.x. [PubMed: 17944664]
66. Russell SL, Boylan RJ, Kaslick RS, Scannapieco FA, Katz RV. Respiratory pathogen colonization of the dental plaque of institutionalized elders. *Spec Care Dent.* 1999; 19:128–34. doi:10.1111/j.1754-4505.1999.tb01413.x.
67. Han MK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. *The Lancet Resp Med.* 2014; 2:548–56. doi:10.1016/S2213-2600(14)70069-4.
68. Kostic Aleksandar D, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen A-M, et al. The dynamics of the human infant gut microbiome in development and in progression toward Type 1 diabetes. *Cell Host & Microbe.* 2015; 17:260–73. doi:10.1016/j.chom.2015.01.001. [PubMed: 25662751]
69. Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Appl Environ Microbiol.* 2006; 72:3916–23. doi:10.1128/AEM.03022-05. [PubMed: 16751497]
70. Rea MC, Dobson A, O'Sullivan O, Crispie F, Fouhy F, Cotter PD, et al. Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *Proc Natl Acad Sci USA.* 2011; 108:4639–44. doi:10.1073/pnas.1001224107. [PubMed: 20616009]
71. Thomas C, Stevenson M, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile*-associated diarrhoea: a systematic review. *J Antimicrob Chemo.* 2003; 51:1339–50. doi: 10.1093/jac/dkg254.
72. Jorup-Rönström C, Håkanson A, Sandell S, Edvinsson O, Midtvedt T, Persson A-K, et al. Fecal transplant against relapsing *Clostridium difficile*-associated diarrhea in 32 patients. *Scand J Gastroenterol.* 2012; 47:548–52. doi:10.3109/00365521.2012.672587. [PubMed: 22468996]
73. Mattila E, Uusitalo-Seppälä R, Wuorela M, Lehtola L, Nurmi H, Ristikankare M, et al. Fecal transplantation, through colonoscopy, is effective therapy for recurrent *Clostridium difficile* infection. *Gastroenterol.* 2012; 142:490–6. doi:10.1053/j.gastro.2011.11.037.
74. He X, McLean JS, Guo L, Lux R, Shi W. The social structure of microbial community involved in colonization resistance. *The ISME journal.* 2014; 8:564–74. doi:10.1038/ismej.2013.172. [PubMed: 24088624]
75. Cavera VL, Arthur TD, Kashtanov D, Chikindas ML. Bacteriocins and their position in the next wave of conventional antibiotics. *Int J Antimicrob Agents.* doi:10.1016/j.ijantimicag.2015.07.011.
76. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci USA.* 2007; 104:7617–21. doi:10.1073/pnas.0700440104. [PubMed: 17456596]
77. Agyei D, Danquah MK. Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. *Biotech Adv.* 2011; 29:272–7. doi:10.1016/j.biotechadv.2011.01.001.
78. Comelli EM, Guggenheim B, Stingle F, Neeser J-R. Selection of dairy bacterial strains as probiotics for oral health. *Eur J of Oral Sci.* 2002; 110:218–24. doi:10.1034/j.1600-0447.2002.21216.x. [PubMed: 12120707]
79. Meurman J, Stamatova I. Probiotics: contributions to oral health. *Oral Dis.* 2007; 13:443–51. doi: 10.1111/j.1601-0825.2007.01386.x. [PubMed: 17714346]
80. Wescombe PA, Heng NCK, Burton JP, Chilcott CN, Tagg JR. Streptococcal bacteriocins and the case for *Streptococcus salivarius* as model oral probiotics. *Future Microbiol.* 2009; 4:819–35. doi: 10.2217/fmb.09.61. [PubMed: 19722837]

81. Lemos JA, Burne RA. A model of efficiency: stress tolerance by *Streptococcus mutans*. Microbiology. 2008; 154:3247–55. Pt 11. doi:10.1099/mic.0.2008/023770-0. [PubMed: 18957579]
82. Jorth P, Turner KH, Gumus P, Nizam N, Buduneli N, Whiteley M. Metatranscriptomics of the human oral microbiome during health and disease. mBio. 2014; 5:e01012–14. doi:10.1128/mBio.01012-14. [PubMed: 24692635]
83. Niederman R, Buyle-Bodin Y, Lu B-Y, Robinson P, Naleway C. Short-chain carboxylic acid concentration in human gingival crevicular fluid. J Dent Res. 1997; 76:575–9. doi: 10.1177/00220345970760010801. [PubMed: 9042080]
84. Gupta S, Misra G, Khurana SMP. Bioinformatics: promises and progress. Internat J Bioinformatics Res Appl. 2015; 11:462–7. doi:10.1504/IJBRA.2015.071945.
85. Scholz MB, Lo C-C, Chain PS. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. Curr Opin Biotech. 2012; 23:9–15. doi:10.1016/j.copbio.2011.11.013. [PubMed: 22154470]
86. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database. 2010; 2010:baq013. doi:10.1093/database/baq013. [PubMed: 20624719]

Figure.

Possible analysis and treatment options for future dentists and clinicians. This flow chart represents a potential new paradigm for observing and treating oral diseases with a microbiological component. The specific technique that will be most helpful for assessing disease risk and treatment options is not yet known (blue box). All of these techniques will require significant advances in technology and years of clinical study before becoming practical.