
Joellen Coates
University of Wyoming, j.coates80@outlook.com

Follow this and additional works at: http://repository.uwyo.edu/honors_theses_16-17
Part of the Dentistry Commons

Recommended Citation
http://repository.uwyo.edu/honors_theses_16-17/86

This Honors Thesis is brought to you for free and open access by the Undergraduate Honors Theses at Wyoming Scholars Repository. It has been accepted for inclusion in Honors Theses AY 16/17 by an authorized administrator of Wyoming Scholars Repository. For more information, please contact scholcom@uwyo.edu.

Senior Honors Capstone Project
Spring 2017

Joellen Coates
Mentor: Dr. John Willford
Department of Microbiology
University of Wyoming
Acknowledgements

I would like to extend my thanks and gratitude to several people who have helped me throughout this project and at UW. I would like to thank Dr. John Willford for his support, guidance, and mentorship, not only with my Honors project, but as an advisor during my time at UW. I cannot express how much he has helped me over these past four years, whether it was giving life advice, becoming an advisor for one of my clubs, and dealing with me during registration. I also would like to thank him for supporting me at Undergraduate Research day and reading my draft and final paper. Thank you, John! Thank you to Rachel Watson for making Microbiology so fun and engaging, and reminding me why Microbiology is so great! Thank you to the Honors Program for providing a variety of enjoyable classes with interesting topics and engaging professors. Thank you to all of my supportive friends who have helped, and dealt, with me for the last 4 years; I couldn’t have done it without you all! Finally, thank you to my mom and family who has supported me throughout this whole process, especially when I was stressed out and irritated at the world, and pushing me to challenge myself during my time at UW. Thank you!
Abstract
Within the last decade, the human microbiome, especially the gut and oral microbiomes, has been heavily researched to understand its relationship to our health and its diversity. The oral microbiome has become of increasing interest in the last few years to determine how the microbiome affects oral health and the presence of dental diseases, such as dental caries (cavities) and periodontitis. It has been suggested that the oral microbiome is in a state of imbalance, or dysbiosis, during oral diseases and can be affected by several factors, such as genetics, oral hygiene habits, and more importantly diet. Ancient dental remains, specifically dental calculus, have been analyzed to provide a better understanding of ancient civilizations and emergence of modern diseases in relation to diet and oral microbiome changes. To better understand the relationship between the oral microbiome, diet, and teeth, a literature review of current research articles on the oral microbiome and diet, and the impact of those on oral health were examined. Current research suggests that it will become more important to incorporate the knowledge of the benefits of the oral microbiome and its relation to oral diseases when treating a patient. A patient’s unique individual oral microbiome is expected to be utilized to prevent oral diseases from occurring and as an indication of health. The extensive research on the oral microbiome, and the impact of diet on oral and overall health, could shape the future of dentistry.
Introduction

Oral diseases, such as dental caries (cavities) and periodontitis, are still prevalent worldwide despite advancements in dental treatments and oral hygiene products. Dental caries is one of the most common diseases worldwide, and in the United States (US), and affects individuals throughout the entirety of their life (1, 2). In the US, it was estimated, from 2005-2008 and 2007-2010 data, that 23.7% of adults and 15.7% of children had untreated dental caries (2). In addition, periodontitis affects approximately 743 million people worldwide and often is present in later stages of life (3). Although there has been a decline in the prevalence of these diseases due to access to dental care and fluoridation of water, dental caries and periodontitis are still prevalent in populations where public waters are not fluoridated (4). To better understand the relationship between our oral microbiome, health, diet, and disease, analyzing ancient remains can provide important data regarding shifts in the prevalence of the oral diseases and predictions for the future.

The oral cavity is composed of several hundred taxa that compose the oral microbiome (5, 6). The term “microbiome” was introduced by Joshua Lederberg, in 2001, to acknowledge the community of all microorganisms, including pathogenic, commensal, and symbiotic microorganisms, that share the human body and have been largely ignored as determinants of health and disease (6–8). In 2008, the National Institutes of Health (NIH) launched the Human Microbiome Project (HMP) with the purpose of characterizing and understanding more about the human microbiome and its role in disease (6). Since then, there has been abundant research on the relationship between the oral microbiome and oral diseases, as well as systemic diseases (5). The oral microbiome consists of balanced and complex interactions, which when disturbed, or in a state of dysbiosis, can lead to formation of disease (6). Diet is one of many factors that can upset the balance of the oral microbiome, and is an important factor in the susceptibility of dental
caries and periodontitis (6, 7). Modern diets have significantly changed from the diets of ancient civilizations and analysis of ancient microbiomes can provide patterns and shifts in human disease, as well as in microbiomes.

Analysis from both ancient and modern samples strongly suggests diet affects the prevalence of dental caries and periodontitis, as well as the oral microbiome balance. Paleomicrobiology is providing more data on human microbiomes allowing for reconstruction of ancient human history and evolutionary patterns of diseases (9). Ancient microbiomes can provide insight into the emergence of specific diseases, and patterns with changing environments (7). Corporlites and dental calculus, or mineralized dental plaque on the surface of the tooth, are two reliable and accurate sources to analyze ancient microbiomes, with dental calculus being the most important for analyzing ancient oral microbiomes (7, 10). Dental calculus is present in all ancient human samples and has the potential to reconstruct a timeline of oral microbiome and diet shifts (10). Major shifts in diets in ancient civilizations have been associated with increases in the frequency of dental caries, and periodontitis (10).

With emerging research on the importance of the oral microbiome and its benefits to maintaining oral health, the future of dentistry is suggested to change to incorporate a patient’s unique oral microbiome to prevent oral diseases (6). A visit to the dentist could become even more personal, and individualized, within the next few years.

Results
Ancient Microbiomes

Paleomicrobiology is an evolving archaeology field that analyzes and studies ancient samples and microbiomes to better understand human evolution, emergence of specific diseases, and any patterns that may exist (9). In addition, paleomicrobiology can provide more insight into the relationship between changes in diet, lifestyles, the oral microbiome, and how the oral
microbiome has co-evolved with humans (9). Advancement and developments in sequencing technology has provided researchers powerful tools to analyze ancient samples and better understand the role of the microbiome in health (7). Understanding changes in oral microbiomes, lifestyles in ancient civilizations, and the emergence of oral diseases can help shift the focus towards promoting a healthier oral microbiome and oral health (10).

**Dental Calculus**

There are some challenges in analyzing certain ancient remains. The microbiome changes with soft tissue decomposition, which makes it a less reliable source to accurately analyze ancient microbiomes (7). There are two ancient human samples that remain stable after death, which are used to provide insight into the composition of ancient human microbiomes: desiccated or mineralized fecal material, known as corporlites, or dental calculus, which is mineralized dental plaque on the surface of the tooth (7). Corporlites are not individual to a person, as they are commonly found in communal sites, and commonly contain over one billion microorganisms (7). In contrast to corporlites, dental calculus is abundant in all human populations, both ancient and present, and in those with poor oral hygiene habits (7, 10). Dental calculus provides highly preserved DNA, proteins, and cellular components, which are used to analyze the oral microbiome, health, and diets of individuals (10).

Dental calculus is formed through a periodical mineralization process in which calcium phosphate ions within the saliva and gingival crevicular fluid deposit onto dental plaque present on the surface of the tooth and below the gingiva (10). This process destroys the microbiota present on the surface and essentially fixes, and preserves, the cells and other components in that specific time period (10). Dental calculus provides a unique benefit to understanding ancient oral
microbiomes since it highly preserves key biomolecules for analysis and provides a layered history unique to an individual (7).

Utilizing dental calculus as a reliable sample source allows researchers to observe precise changes in diets and environment, and the impact on the oral microbiome and health (10). Developments and advancements in sequencing technologies allows for comparison of modern microbiomes to ancient ones, as well as providing records of dietary and health changes in ancient civilizations (10, 11). With the recent data from dental calculus, it is clear diet significantly impacts oral health, the microbiome, and prevalence of dental caries and periodontitis (7, 10, 11).

**Dietary Changes**

![Figure 1](image_url). An idealized timeline of European history from major dental calculus findings, including major time periods and analysis techniques to support findings (10).

Dental calculus analysis provides information to create a timeline of distinct periods in human evolution in relation to diet and dental diseases (10). A major dietary shift towards an
increase in carbohydrate consumption in ancient civilizations is associated with an increase in the frequency of dental caries (10–12). The Neolithic, or farming, period, from 4500-2500 BCE, is identified as the first significant change in the diet to an increase in consumption of cereals (Figure 1, (11)). The Neolithic period is also characterized by the emergence and presence of dental caries and periodontitis (11). In addition to the Neolithic period, the frequency of dental caries increased in the 18th century due to the accessibility of refined flour and sugars (10). In many studies, the increase in frequency of dental caries is most likely associated with the introduction of agriculture, with increasing prevalence of dental caries and periodontitis due to the availability of refined sugar and carbohydrates (10, 11). To further support the relationship of diet and prevalence of dental caries and periodontitis to the introduction of agriculture, dental caries and periodontitis were rare in pre-Neolithic populations (11). With more data supporting the impact of dietary shifts on the presence of dental diseases, it is clear diet negatively impacts the oral microbiome and oral health.

**Ancient Microbiomes and Diet**

The reduced instances of dental caries and periodontitis observed prior to the introduction of agriculture are coupled with a decreased abundance in the microorganisms with which they are commonly associated (11, 13). The oral ecosystem drastically shifted due to the increase in carbohydrate and refined sugar consumption during the Industrial revolution, and was accompanied by a shift in increased prevalence of dental caries, periodontitis, and disease associated bacteria (11, 13). Alder et al. (2013) analyzed thirty-four DNA sequences from ancient European dental calculus to identify bacterial species composing the oral microbiome and to determine, if any, compositional shifts were associated with diet. The results from the study show the modern oral microbiome is less diverse compared to ancient samples, and could
be composed of more opportunistic cariogenic bacteria, such as *Streptococcus mutans* (11). The prevalence of *S. mutans* was much higher in modern, post-industrialized samples than in pre-agriculture samples, suggesting the presence of dental caries associated bacteria have recently become dominant due shifts in diet (7, 11). In addition to *S. mutans*, periodontitis associated bacteria, such as *Porphyromonas gingivalis*, *Treponema* species and *Tannerella* species, were much more abundant in farming populations than in hunter-gatherer populations (11). These findings support the association between a significant dietary shift due to the onset of agriculture and increase in refine sugar consumption, and the prevalence of dental diseases (7, 11). It is suggested with a less biodiverse oral microbiome adapting to changes in the oral environment, whether it is an imbalance in microbial composition or other factors, is less efficient and resilient (11, 12).

Dietary changes in ancient hunter-gatherer human populations affected the evolution and adaptation of the oral cavity (13). A significant adaption of the oral cavity was the evolution of the teeth to become one of the hardest biological surfaces and more resistant to mechanical wear (13). Additionally, production of saliva with bicarbonate ions to buffer against low pH dietary foods was a result of evolutionary selective pressures (13). These evolutionary adaptations in response to diet demonstrate the significant impact of diet on the evolution of the oral cavity and oral microbiome. The oral cavity and microbiome may be slowly adapting to the increased consumption of carbohydrates in modern diets.

The research and data from studying ancient dental calculus provides a better understanding on the importance of diet and oral health, especially with the emergence of dental caries and periodontitis. Since dental calculus accurately preserves microbiomes, there is great potential to reconstruct past civilizations and track states of health, disease, and populations, and
to track the evolution of the oral microbiome with changes experienced by ancient populations (10). A better understanding of past microbiomes and civilizations could help in predicting future directions of the oral microbiome if modern diets are unchanged.

**The Oral Microbiome**

The oral cavity is heavily colonized and consists of several distinct microbial habits, such as teeth, gingiva, or gums, cheek, tongue, and soft palate (6, 8). There are different populations of microorganisms, resident and transient, within the oral cavity and comparison between human and environmental studies allows for identification/characterization of transient and resident bacteria (8, 14). There have been approximately 700 taxa detected within the oral cavity, with isolation of only half (280 species) by traditional microbiological methods (5, 6, 8). The microorganisms within the oral microbiome often form complex and specific interactions with each other in specific environmental conditions, which often cannot be recreated in the lab. To identify and characterize more microbial species within the oral microbiome, non-culturable techniques have been developed and utilized, such as 16S rRNA sequencing in combination with PCR (4, 6–8). Dewhirst et al. (2010) analyzed 16S rRNA sequences to determine whether any additional bacterial taxa needed to be added to the Human Oral Microbiome Database (HOMD) and to develop a scheme for naming unnamed isolated oral bacteria. Six major bacterial phyla were isolated and included: *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria*, as well as additional phyla, genera and species within an isolated taxa (8). Within the specific phyla, the most abundant genera of bacteria were also determined, such as *Streptococcus* within the most *Fusobacteria* phylum, and *Prevotella*, within the *Bacteriodetes* phylum (8).
Bik et al. (2010) characterized the genera of bacteria from healthy oral cavities of participants to determine if there was a common, core oral microbiome associated with health (5). The data from this study, summarized in figure 2, suggested that there was a “core” genera of bacteria associated with healthy state in all of the participants, as well as bacteria more unique to an individual (5). It is important to acknowledge that in addition to bacteria, viruses, fungi, archaea, and protozoa also comprise the oral microbiome (14). Although there are bacterial species not yet characterized and isolated, it is clear there is great diversity within the community of the microbiome.

Figure 2. Results from a study done by Bik et al. (2010) characterizing bacterial species found in the oral cavity of 10 individuals (5).

**Acquiring the Oral Microbiome**

Since the oral microbiome is important for health, how does one acquire their oral microbiome? HMP research suggests the transfer of bacteria from the mother during pregnancy, and after birth, to the infant is an essential process, and a significant factor, in acquiring a “normal” microbiome (15). Although most transfer of microbiota occur postnatally, there is research that supports that this transfer occurs before birth with the placenta and entry of oral
bacteria from pregnancy gingivitis, umbilical cord, and amniotic fluid (15). During birth the mother’s microbiota is transferred to the infant, but the type of microorganisms the infant is initially exposed to depends on the mode of delivery (6, 15). Infants vaginally born have a higher taxonomic diversity oral microbiome three months after birth and may delay the colonization of S. mutans compared to those born via Caesarian section with early colonizers (6, 15). Breast feeding affects the infant oral microbiome, as oral lactobacilli possessing antimicrobial properties are present in breast-fed infants compared to those formula-fed (6, 15). In addition to vertical transmission, there is some evidence of horizontal transmission of the oral microbiome, which occurs with siblings or others who share the same environment (15).

Maintenance of the oral microbiome is important once it is established. Both microbial and host derived factors contribute to maintaining the oral microbiome (6, 15). Since the oral microbiome is exposed, and disrupted by, external factors, innate and adaptive immunity determine the composition of the microbiome (15). Additionally, microbe derived factors prevent colonization of foreign microorganisms through a variety of mechanisms, such as competing for nutritional resources, neutralizing virulence factors, and antagonism (15). The oral microbiome is constantly exposed to the external environment and the balanced interactions within the communities are important in maintaining a “healthy” microbiome.

The Oral Microbiome and Oral Health Diseases
The oral microbiome is an integral part of maintaining oral health. The oral microbiome is distinctly different from other microbiomes within the human body due to continual exposure with the external environment and variety of environmental niches within the oral cavity, but is also unique to an individual (5). The oral microbiome has co-evolved with humans and established a symbiotic and mutualistic relationship, providing benefits and functions for humans
(15). The oral microbiome maintains a healthy state by providing enzymes that aid in digestion and metabolism, vitamin production, immune system regulation and stimulation, and preventing colonization of pathogens through competition for nutritional resources (4, 7, 12, 14, 15). A beneficial oral bacterium, *Streptococcus salivarius* strain K12, produces bacteriocins, which inhibits the growth of Gram-negative species and maintains oral health by preventing halitosis (bad breath) (14).

A “healthy” oral microbiome is balanced, stable, and in a symbiotic state, however, the presence of certain factors, such as poor diet, illness, and stress, disrupts the balance and shifts the oral microbiome to a state of dysbiosis and results in shifted diversity and proportions of microorganisms found in the oral cavity (5–7). Figure 3 depicts a model suggested by Marsh (2003) and adapted by Kilian, et al. (2016), which accounts for the interaction of these factors in promoting dysbiosis (6). Oral biofilms, or dental plaque, are complex communities of microorganisms that colonize surfaces in the oral cavity, which traps and sequesters microorganisms from the immune system and removal from saliva (5, 16). During an “unhealthy” state, the biofilm detaches and the oral microbiome is in a state of dybiosis, which results in a shifted diversity and proportions of microorganisms in the oral cavity (5, 6). Oral “pathogens”, such as *S. mutans*, *P. gingivalis*, *Treponema* species, and *Tannerella* species, are found in low numbers in healthy individuals, but due to the disruption in the balance the “pathogens” proliferate and grow to high proportions and cause dental caries or periodontitis (6).
Non-microbial Factors of Oral Disease

Saliva is important in maintaining a balanced biofilm and oral microbiome. Saliva provides the optimum environment for biofilms to grow, which includes proteins, enzymes, and minerals, and removes layers of plaque that are present on the tooth (5, 6, 17). Additionally, saliva protects the enamel layer of the tooth and the host from other non-oral infections (5). Research suggests saliva composition and flow, like the oral microbiome, are unique to an individual and may determine the composition of dental plaque (5, 15). Another benefit of saliva is the protective secretory immunoglobulin A (S-IgA), which regulates microbial adhesion and colonization (15). Although S-IgA regulates microbial adhesion, some commensal streptococci, such as *Streptococcus mitis*, produce IgA proteases that neutralize S-IgA and allows colonization (15).

In addition, oral hygiene contributes to the composition of the oral microbiome (5). With poor oral hygiene, dental plaque accumulates on the surface of the tooth and may promote the
growth of bacteria associated with dental caries and periodontitis (5). Also, in some studies determining the composition of biofilms in association with oral hygiene, those with great oral hygiene mostly had Gram-positive cocci and rods, such as streptococci, but with poor oral hygiene, anaerobic Gram-negative bacteria dominate and shift the composition of the biofilm (4, 5).

The consequence of having a dysbiotic oral microbiome on oral health is clear and no longer ignored. Maintaining the intricate and complex balance of a “healthy” oral microbiome is important in preventing certain dental diseases, such as dental caries and periodontitis, and for continuing to provide beneficiary functions for the host.

**Oral Microbiome and Diet**

Modern diet affects the oral microbiome and prevalence of oral disease, which is consistent with the data on the oral microbiome and dietary shifts seen in ancient microbiomes. Research shows that diets high in carbohydrates and sucrose lead to accumulation of plaque and gingivitis (18). To test the effect of modern diets on the oral microbiome, Baumgartner et al. (2009) presented participants a Stone Age diet to determine the effect of a lack of modern oral hygiene on the oral microbiome and presence of gingivitis (18). The results showed that there was a change in the oral microbiome and presence of pathogenic bacteria due to Stone Age diet, which lacked refined sugars and carbohydrates, and decreased clinical symptoms of gingivitis, such as bleeding on probation (18). This study supports that changing the diet to reduce the consumption of carbohydrates and refined sugars positively benefits the oral microbiome composition, as well as oral health. Although this simulation of a Stone Age diet focused primarily on individuals eating a Swiss diet, the results of this study, as well as others, support
the suggestion of restricting carbohydrate intake to prevent the formation of dental caries, and maintain balance of the oral microbiome (3, 18).

It is important to maintain a balanced and “healthy” oral microbiome to prevent the presence of certain dental diseases, such as dental caries, and periodontitis. Although some of the factors that affect the composition of the oral microbiome and presence of disease cannot be controlled, there are some factors within the control of the individual to maintain a “healthy” oral microbiome. As a future dentist, I would expect factors such as diet, lifestyle and habits, and genetic composition, to directly affect the oral microbiome.

**Dental Caries**

Dental caries is one of the most common preventable chronic noncommunicable disease worldwide and in the US and affects all age groups causing pain and tooth loss, and even anxiety, in those affected (1, 2). Not only do dental caries impact the person affected, but also the economy of the health care industry. It is estimated to be the fourth most expensive disease to treat and costs approximately 10% of the health care budget in industrialized countries (2). As observed with ancient dental calculus samples, the presence of caries has emerged with the introduction of agriculture, and most importantly, the consumption of refined sugars and fermentable carbohydrates. Frequent intake of dietary carbohydrates and acidification of oral biofilms can affect the microbial composition and the presence of caries (19). While the prevalence of dental caries is declining in some countries, such as the US, Japan, England, and Sweden, there is still risk of dental caries later in life, and questions still exist about how to further reduce this oral disease (2). The solution to reducing the prevalence of dental caries may be a simple change in our diets, as modern diets are drastically different than ancient diets and the effects of diet on oral health is clear.
Dental Caries and the Oral Microbiome

Dental caries is considered a polymicrobial disease, affected by many factors (4). In a balanced biofilm, the pH is close to neutral (pH 7), which inhibits the growth, and damage, from acidogenic and aciduric bacteria, which produce acid and tolerate low acid environments, respectively, such as *S. mutans* (13). There are two biological molecules that are effective at maintaining a balanced biofilm by buffering low pH: arginine, which is metabolized by oral biofilms to ornithine and two molecules of ammonia, and urea, which increases the pH and inhibits the growth of cariogenic bacteria (19). The production of ammonia from arginine can determine the prevalence of dental caries, as individuals who had no clinical history of dental caries had significantly higher levels of arginine deiminase enzyme, which converts arginine to ammonia, than those who had caries (19).

To understand the process of the formation of biofilms and development of oral diseases, such as dental caries and periodontitis, several plaque hypotheses have been presented and revised with more knowledge on the disease associated bacteria and communities. These hypotheses include: the Traditional and Updated Non-Specific Plaque Hypothesis (T-NSPH, U-NSPH), Specific Plaque Hypothesis (SPH), Ecological Plaque Hypothesis (EPH), and the Keystone Pathogen Hypothesis (KPH) (16). These hypotheses provide important background and understanding on the development of biofilms and oral diseases, especially in dental caries and periodontitis.

The earliest hypothesis, the T-NSPH, proposed dental diseases/infections were caused by overgrowth of non-specific bacteria in the dental plaque (6, 16). It was thought the amount of plaque determined the pathogenicity and dental disease would develop when the specific threshold of combating virulence factors was exceeded (16). The non-specific removal plaque
was thought to be the best way to prevent disease, however, improvement in technologies to identify bacteria, rejected this hypothesis (16).

A new hypothesis was formulated to include more information known about disease associated bacteria and to improve the previous hypothesis on the development of dental caries. The discovery of the effectiveness of kanamycin, an antibiotic, against streptococci species associated with caries, led to the formation of the SPH and the idea of using antibiotics to remove certain bacterial species as prevention of dental caries (16). The SPH proposed that there were specific bacteria in the dental plaque, the most important being “mutans streptococci”, such as *S. mutans* and *Streptococcus sobrinus*, that caused dental caries (16). Although it was thought kanamycin was effective to treat and prevent dental caries, it was later discovered that dental caries associated bacteria increased in certain oral sites (16). In addition, long-term use of kanamycin resulted in antibiotic resistance and the increase of certain bacterial species suggested there were native species to the oral cavity (16). Later work on identifying different microorganisms within the oral cavity and those associated with periodontitis suggested oral diseases could be caused by specific species (16). From the SPH, the NSPH was updated to the U-NSPH hypothesis, which accounted for the differences in virulence of some bacteria found within plaque and that plaque formation changes with health and disease (16).

One of the more updated hypotheses was the EPH, which combined the principles and knowledge of the earlier hypotheses. The EPH proposed that disease was due to the imbalance from ecological stress, which can promote the growth of specific disease-related microbes due to the presence of certain ecological factors, such as nutrients and cofactors and pH (16). The presence of ecological factors on the microbial composition is supported by the selection of acid-tolerant species in an acidic environment (16). Although the EPH supports that microbial
composition of dental plaque does depend on the environment, it does not take into account genetic factors, such as encoding for a permanent immune system disorder affecting the microbiome, absence of genes encoding for protective proteins within saliva, and promotion of pathogenic bacteria rather than beneficial bacteria, that can significantly impact the microbial composition and susceptibility to oral diseases (16).

Finally, the KPH addressed the disproportions of certain microbes, which disrupt the microbial community and increase the relative abundance of some bacterial species (16). Low abundance pathogenic species can trigger inflammation and when isolated or detected in high numbers, the disease progresses to advanced stages (16). The KPH was formed by observing the ability of *P. gingivalis*, a “red complex” bacterium associated with periodontitis, to change the composition of dental plaque but not cause disease solely by itself (16).

These hypotheses, especially the most updated, provide a better understanding of how plaque forms and contributes to the formation of disease. Although these hypotheses acknowledge the different bacterial species involved in diseases, there is more emerging research on the formation of plaque, which will lead to updates to these hypotheses to create a more cohesive model of how plaque forms and its association with disease.

The surface of the tooth can be colonized by several different bacterial species. Early colonizers of a cleaned tooth surface include many non-mutans streptococci, such as *Streptococcus sanguinis, Streptococcus oralis, and S. mitis* 1, which are disincticly different than the mutans streptococci, and the genera *Actinomyces* (20). Mutans streptococci, including *S. mutans, S. sobrinus,* and *Streptococcus cricetus,* are abundant in dental caries and in the plaque on the surface of the tooth (4, 20). Mutans streptococci are often associated with dental caries, induce dental caries formation in animals fed a sucrose-rich diet, are highly acidogenic and
aciduric, and promote bacterial adhesion to the surface of the tooth and other bacteria (4, 20). Although mutans streptococci are commonly isolated from dental caries, the group only comprises approximately 2% of the initial streptococci population, and are only in high abundance during disease (13, 20). *S. mutans* flourishes in dental caries plaque, since it tolerates, grows, and metabolizes carbohydrates in an acidic environment (4, 19). The proportions of the mutans streptococci and non-mutans streptococci are suggested to be associated with different stages in dental caries, as the relative abundance of mutans streptococci is higher when dental caries are rampant (20). It is suggested the changing microbial compositions and other bacterial species, such as lactobacilli, *Actinomyces* spp., and *Bifidobacterium* spp., may be important in the development of dental caries (20).

Changes in microbial composition of biofilms, due to a variety of factors, results in an increased risk of dental caries and in some cases, leads to the presence of dental caries. Although *S. mutans* is commonly associated with dental caries, there are many other bacteria important in the process of maintaining a homeostasis in the biofilm, keeping pathogenic organisms in low numbers, and promoting a balanced microbiome.

**Dental Caries, Diet, and the Oral Microbiome**

Dental caries is significantly impacted by the diet, especially one with high amount of fermentable carbohydrates, selects for bacterial species that have the ability to ferment the carbohydrates and produce acids, which supports the EPH (16). Dental caries requires both the presence of cariogenic bacteria in plaque and fermentable dietary carbohydrates, which can be converted to lactic acid (4). The production of acids is key in the development of dental caries, which leads to lower pH in the biofilm, optimum environment for cariogenic bacteria, and risk of dental caries (6, 16, 19)). Although the production of acid is crucial in the formation of dental
caries, the overall process is quite complex and intricate. Figure 4 illustrates some of the complex interactions within the biofilm that are involved in the development of dental caries.

![Figure 4. Modern model of host-microbe interactions in the development of dental caries (6).](image)

Although there are many components to a diet, sugar has consistently been identified as a major driver of the presence of dental caries. An increase in consumption of carbohydrates was observed with a significant increase in the frequency of dental caries in Woodland populations in North America, around 500 CE (10). Since then, modern diets have been filled with refined sugars, carbohydrates and sugary drinks, significantly different from hunter-gatherer populations. This increase in consumption of sugars, or a decrease in saliva flow, exposes the biofilm to a low pH for an extended period of time, which selects for cariogenic bacteria, especially *S. mutans* (6). In an optimum acidic environment, *S. mutans* up-regulates proteins to increase survival in the environment (6, 16). The biofilm progresses through various acidic stages due to the presence of carbohydrates, including acidic and aciduric stages, which shift the microbial composition and select for bacteria able to survive within the environment (19).
Sugary drinks, which are especially popular in Western diets, pose a risk to the integrity of the tooth. Determining the precise effects of a variety of different drinks on enamel erosion is difficult, since there are many ways to measure the erosivity of drinks (21). A study done by professors in the Department of Operative and Preventative Dentistry in Germany, used gravimetric analysis to weigh *in vitro* tooth samples before and after treatment of different sugary drinks with varying aciditity, such as Coca Cola, Sprite, and Orange juice, as well as water, and to identify the impact of drinks on erosion of the enamel (21). The erosive potential of Coca Cola, which has a lower pH than orange juice, was higher within the first few minutes of exposure than orange juice, but after the first few minutes, orange juice showed higher erosive potential than Coca Cola (21). Although the results from this study demonstrate the variability in the erosive potential of sugary drinks, it does present findings that could help dental providers recommending alternative drinks that have lower erosive potential (21).

*Other Factors Associated with Dental Caries*

In addition to the composition of the oral microbiome, saliva has been found to be an important factor for preventing certain oral diseases. Saliva coats the oral cavity and provides essential nutrients to the bacteria in the oral biofilm (1). Although saliva promotes the growth of microorganisms within the mouth, it also inhibits the growth of microorganisms through mechanical removal and antimicrobial enzymes (1, 17). Some antimicrobial enzymes include: lysozymes, which lyse bacterial cells, lactoferrin, which inhibit growth of bacteria, and oral mucin, which prevents the colonization of bacteria on the surface of the tooth and promotes their removal when swallowing (1, 17). Neutralization properties of saliva maintains a pH that prevents the demineralization of the enamel and decreases the length of exposure to acidic environments due to diet (1).
The flow of saliva is an important factor in the prevention of dental caries, since in combination with oral hygiene habits, it removes colonized bacteria in dental plaque (1). Saliva flow can be affected by many different factors, including medication, and genetic disorders, and can lead to an optimum environment for aciduric microorganisms and disrupt the microbial balance (20). A reduction in saliva flow and production demonstrated that, sugars, acids, and dietary carbohydrates, are not removed from the surface of the tooth and increases the risk of dental caries, especially if oral hygiene habits are poor (20).

It is clear the Western diet rich in carbohydrates is detrimental to not only the oral microbiome, but oral health and cavity. Diet is a major factor that is controllable and could help reduce the prevalence of, and prevent, dental caries and maintain a balanced oral microbiome.

**Periodontitis**

![Figure 5](image.png)

Figure 5. The stages of periodontal disease and destruction, including the appearance of gingiva and the tooth in each of the stages (4).
Periodontitis is a chronic inflammatory dental disease that affects many adults within the United States, and 743 million people globally (3, 22, 23). Specifically, periodontitis affects the supporting tissues of the tooth, such as the periodontal ligament, alveolar bone, which supports the tooth, and gingiva, which are the gums, and can lead to tooth loss (Figure 5, (4, 23)). In addition to tooth loss, periodontitis impacts system health and is associated with systemic diseases, such as rheumatoid arthritis, cardiovascular disease, and atherosclerosis (3, 23). The presence of gingivitis is essential for the progression to periodontitis and is characterized by a change in appearance of the gums from pink to red, as well as sensitivity and tenderness (3, 4). Often bleeding upon probation may occur with gingivitis and some individuals may not experience pain (4). There are several factors that affect the presence of periodontitis, as well as the oral microbiome, and include diet, smoking, and obesity (3). Severe inflammation of the surrounding tissues, due to the immune system, is associated with periodontitis, which allows for growth of periodontitis associated bacteria to proliferate and cause damage (23). Since periodontitis is associated with systemic diseases, it is important to understand the relationship between risk factors, especially diet, the oral microbiome, and periodontitis to effectively treat and prevent periodontitis.

**The Oral Microbiome and Periodontitis**

The HMP research is providing a better understanding of the types of bacterial species commonly associated with periodontitis and the dynamics of the oral microbiome in association with periodontitis. The KSH, previously mentioned, was formed by observing *P. gingivalis*, one bacterium in the “red complex”, with *Treponema denticola*, and *Tannerella forsythia*, being the two other bacteria (16). The keystone “red complex” bacteria were thought to be the main cause of periodontitis, since they were commonly associated with sites of periodontal disease in the
oral cavity (4, 17, 23). Now, with more research and sequencing of DNA from the oral cavity, there are more bacteria than originally thought to be associated with periodontitis and that instead, the oral microbiome shifts from a balanced to a dysbiotic state (Figure 6, (23)). The “red complex” bacteria have been found in healthy individuals at low levels and it is thought there is a strong association between the “red complex” and periodontitis due to pathogenicity factors aid in inhibition or evasion of host immune responses (17). It is important to note that the presence of *P. gingivalis* does not cause periodontitis, since in healthy individuals, it is present in low numbers, which is similar to dental caries and the abundance of *S. mutans* (23).

![Figure 6. Oral microbiome communities and interactions leading to periodontitis. Top diagram: The different stages and interactions within the biofilm leading to periodontitis. Bottom diagram: Physical differences between a healthy tooth and a diseased tooth, including the progression from gingivitis to periodontitis. Additionally, the interactions between the biofilm and tooth are shown (23).](image)

Ancient microbiomes of pre-agriculture populations show that *P. gingivalis*, and other periodontal associated bacteria, were not present which supports the detrimental impact of diet on the oral microbiome (10, 23). In a symbiotic and synergistic state, there are mostly bacterial
species from *Actinomyces* and *Streptococcus* genera, but in a dysbiotic state, anaerobic bacteria from *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and other phyla, dominate (23). In this dysbiotic state, the microbiota beneath the gums produce virulence factors that aid survival, and proliferation, of keystone bacteria in an inflammatory environment, which shift the microbial composition (4, 6, 16, 23). Subgingival microbiota interact with the host immune system, and the virulence factors allow for host immune dysregulation and subversion, which leads overgrowth of bacteria (23). The host immune system plays an important role in regulating the growth of periodontal pathogens to prevent disease.

The biofilm is also important in periodontitis. Accumulation of bacteria on the surface of the tooth leads to gingivitis, and is strongly associated with periodontitis (Figure 5, (16, 17)). The biofilm is formed in sequential sequences of colonization, starting with early then intermediate and late colonizers (17). One bacterium, *Streptococcus gordonii*, is an early colonizer in the biofilm and acts as a binding platform for *P. gingivalis* and other colonizers, demonstrating the ability of periodontal associated bacteria to colonize the biofilm early (17). In addition, antimicrobial enzymes and mucin in the saliva are important in maintaining homeostasis in the biofilm and preventing the colonization, and build up, of bacteria on the surface of the tooth (17).

Although there are certain bacteria commonly associated with periodontitis, there are other bacteria that contribute to the dysbiosis of the oral microbiome and development of periodontitis. The complex interactions between the microbial community and microbe-host interactions are still being characterized, which will allow for a better understanding of the specific mechanisms that lead to periodontitis.

*Diet, Periodontitis, and the Oral Microbiome*
The frequency of periodontitis, like dental caries, increased after the introduction of agriculture and was not present in hunter-gatherer populations (10, 23). Ancient microbiomes also shifted to higher abundance of \textit{P. gingivalis} after significant dietary changes (10, 23). Modern diets are high in carbohydrates and fermentable sugars, as well as fatty acids. A sugary diet promotes plaque formation and leads to dental decay in combination with poor oral hygiene (24). Consumption of certain fatty acid ratios, such as omega-6/omega-3 poly unsaturated fatty acids, in overweight people has been shown to increase inflammation and risk of periodontitis, as well as the potential to predict periodontitis (25). The ratio of omega-6 to omega-3 increased to a 15 to 1 ratio in Western diets, compared to hunter-gatherer diets (3). One study characterizing the oral microbiome in relation to the types of diet and foods eaten, found that saturated fatty acids were associated with a diverse and complex microbial community, including pathogens (25). In addition, deficiencies in vitamins have been found to affect periodontitis (24). Specifically, deficiencies in Vitamins C and D affect inflammation associated with gingivitis and periodontitis (3, 24). Periodontal bones loss, irritated and inflamed gums, as well as scurvy result from a deficiency in Vitamin C (3, 24). A deficiency in Vitamin D is thought to lead to periodontal inflammation, however, the relationship between the two is not well defined (3, 24).

\textbf{Other Factors Associated with Periodontitis}

There are other factors strongly associated with periodontitis. Obesity and smoking, as well as diet, are major risk factors for periodontitis (22). Although there are confounding variables, such as oral hygiene and plaque levels, smoking is one of the major factors of periodontitis, with mild smoking increasing the risk of tooth loss by at least 4 fold (22). Obesity is associated with many systemic health problems, such as diabetes and heart disease, and a
proinflammatory response (22). The oral microbiome is suggested to be affected by the proinflammatory response and have higher levels of *T. forsythia* compared to non-obese individuals (22). In addition to life style factors, periodontitis is affected by the same factors as dental caries, including saliva flow and oral hygiene habits. The host inflammatory response against the relevant associated microbes is a key factor in periodontitis, and if the immune system is not tightly controlled, dysbiosis and inflammation amplify each other (4, 23).

Periodontitis, like dental caries, depends on many factors, including diet and the oral microbiome. The same patterns in shifts in the ancient oral microbiome due to diet were observed in both periodontitis and dental caries. Since periodontitis is prevalent within the United States and is associated with other detrimental systemic diseases, understanding the interactions between all the risk factors and oral microbiome, will help to make any necessary changes to reduce the prevalence of periodontitis.

**Future of Dentistry**

The future of dentistry is suggested to change to incorporate the data and key findings on the impact of the diet on the oral microbiome and its effect on oral health. The impact of the oral microbiome on oral and systemic health when in dysbiosis will be too important to ignore when treating a patient.

**Dietary Recommendations for Dental Caries and Periodontitis**

To prevent dental caries and periodontitis, it is necessary to suggest dietary recommendations and updates, especially since the impact of a Western diet on oral health is clear. Restriction of refined sugars and carbohydrates in the diet can help reduce the prevalence of caries (2). It has been suggested that the intake of carbohydrates promotes dysbiosis of the oral microbiome and chronic inflammation, through promotion of apoptosis (3). Current research
supports that a diet low in carbohydrates, high non-vegetable fats, high in micronutrients, and high in protein can prevent dental caries and periodontitis, and improve overall oral health (26). Although there are many similar dietary recommendations for improving oral health and preventing both dental caries and periodontitis, there are some that are specific to each dental disease.

**Dietary Recommendations for Preventing Dental Caries**

Diet remains one of the main factors to preventing dental caries. In 2002, the World Health Organization (WHO) provided guidelines on restricting the consumption of sugar to less than 10% of energy intake (2). In 2015, WHO presented new guidelines to reduce the intake of free sugars throughout life (2). The impacts of sugar consumption on the oral microbiome and oral health are clearly observed, which makes reducing sugar consumption a logical recommendation.

There are certain foods that are nutritious and benefit oral health, while also being effective against cariogenic bacteria. These foods are categorized as functional foods and counteract microbial products (27). Some functional foods include: tea, cranberry, cocoa, coffee, apples, grapes and wine, and dairy products with bioactive peptides (27). Otake et al. (1991), cited by Van Loveren et al., utilized specific pathogen free rats who were fed a cariogenic diet with unsweetened green tea and infected with *S. mutans* (27). The rats fed the diet with green tea had a lower frequency of caries and has been observed in humans who drank unsweetened green tea (27). Food additives and food preservatives have also been considered for their potential to decrease dental caries (27). Milk and dairy products have been suggested as benefiting the oral microbiome due to beneficial properties and components (27–29). Milk contains calcium, phosphate, as well as lipids and the milk protein, casein, which have been shown to inhibit the
growth of *S. mutans* (27). Ravishankar et al. (2012) looked at the effects of ions found in milk on the levels in dental plaque and found that high levels of calcium and phosphate ions promote remineralization (29). Also, consumption of probiotic products, such as yogurt and unsweetened milk, can be beneficial for oral health (28, 29).

Another solution to preventing dental caries is fluoride. Fluoridation of public water has greatly reduced the prevalence of caries, which can be seen in populations with no exposure to fluoride and lack of access to dental care (4). Duggal et al. (2001) analyzed the effect of using fluoride toothpaste, after consumption of acidogenic foods, on enamel demineralization (30). The results show that even with frequently drinking high carbohydrate containing drinks, brushing twice a day with fluoride toothpaste promoted remineralization rather than demineralization of the enamel (30). By promoting remineralization and inhibiting demineralization, the use of fluoride can prevent dental caries (30).

**Periodontitis and Dietary Recommendations**

Dietary recommendations for periodontitis are similar to those for dental caries, including reducing carbohydrate intake, since accumulation of plaque triggers dysbiosis of the oral microbiome and gingivitis (6). Woelber et al. (2016) demonstrated the effects of recommending an oral health optimized diet resulting in lower periodontitis and gingivitis inflammation (3). The diet set for participants comprised of reducing the intake of carbohydrates below 130 grams per day, daily intake of omega-3 fatty acids, including fish oil capsules, decrease in omega-6 fatty acids, daily intake of Vitamin C and Vitamin D, and daily intake of antioxidants (3). The results suggested that the “oral optimized health diet” proposed could significantly reduce gingival and periodontal inflammation within the clinically important range, even without a change in oral hygiene habits (3). Although these results are promising and demonstrate the potential for a
change in diet, it is difficult to distinguish which specific food impacted inflammation the most (3). A low fat diet rich in fiber has also been shown to improve periodontal health (25). The results from the “oral optimized health diet” demonstrates that the Western lifestyle, which includes large amounts of refined carbohydrates and trans-fatty acids, is detrimental to oral health (3).

Vitamins have been found to be beneficial to periodontal health (24). These vitamins include: Vitamin A, Vitamin B Complex, Vitamin C, Vitamin D, Vitamin E, and Vitamin K (24). Both Vitamin C and D have shown to positively impact periodontal and gingival inflammation, and should be included in the diet to benefit overall and oral health (3).

**Focus on individual oral microbiome**

The oral microbiome will soon become a key tool to better treat a patient. The oral microbiome is unique to an individual and deeper understanding of the impact of microorganisms on the body will provide more personalized dentistry (5). By understanding an individual’s oral microbiome, monitoring of the health status of the oral cavity can help dental professionals in treating, and diagnosing, an unhealthy oral cavity before onset of periodontitis, and caries (5). Treating an unhealthy oral cavity is suggested to focus on restoring the oral microbiome to the original, balanced state, through modifying oral hygiene habits, diet, and lifestyle changes (6). Therapeutic treatment plans and therapeutics could be “personalized” to an individual to direct the oral microbiome back to a healthy state from dysbiosis (31). Dentists and dental hygienists can work to help educate patients on the importance of maintaining a healthy oral microbiome and the impacts on oral health of an imbalanced microbiome (6).

**Future of Dental Related Products**
New dental related products are being developed in response to the emerging and expanding knowledge on the oral microbiome. CS Bioscience, developed a toothpaste that contains prebiotics, which are key nutritional components, minerals and other components to help promote the growth of beneficial bacteria (32). Adams et al. (2016) developed toothpastes with fluoride and other enzymes and proteins with antimicrobial effects to test the impact of the toothpastes on the oral microbiome after brushing (33). The results from the study indicated there was a change in the composition of the oral microbiome to a higher abundance of health associated bacteria species and a decrease in disease associated bacteria species (33). This study is promising for the potential for development of more oral hygiene products incorporating knowledge on the oral microbiome.

Utilizing the Oral Microbiome
There has been increasing interest in probiotics to help promote a healthier microbiome. Probiotics are being researched to determine the potential benefits of use on the oral health and microbiome (27). The use of probiotics would shift the emphasis of treating the disease to utilizing the oral microbiome maintaining a healthy oral microbiome (27). Oral lactobacilli have been tested for inhibitory effects against strains associated with caries and periodontitis, such as *S. mutans*, *P. gingivalis*, and *Prevotella intermedia* (27). A mouthwash composed of three natural oral streptococci species, was found to have a significant effect on the bacterial levels of cariogenic and periodontal associated bacteria (27). More research is needed to determine the effects of probiotics, as well as developing probiotics to utilize the oral microbiome to prevent disease, however, the possibilities of using probiotics to prevent dental caries and periodontitis is exciting (27).

Concluding Remarks
The research from the HMP and understanding of the relationship between the oral microbiome and disease will change the focus of dentistry and medicine. There has been a strong association between diet, specifically the consumption of fermentable carbohydrates and sugars, and the presence of dental diseases, such as dental caries and periodontitis. Dental caries and periodontitis were rare in ancient civilizations and their emergence can be traced to specific time periods with the introduction of agriculture, which significantly changed the diet of hunter gatherers. The importance of a balanced and healthy microbiome is becoming even clearer with more research on the impacts and effects on our oral and overall health. By taking care of the oral microbiome and changing lifestyles and diets to promote a balanced oral microbiome, the prevalence of caries and periodontitis could be reduced even further. The unique oral microbiome of an individual could be utilized more to help prevent dental caries and periodontitis. Dentists and dental hygienists can play an integral part in the ongoing discussion about the oral microbiome and changes to diet to shift the focus to preventing oral diseases, rather than treatment once a disease is present. It is an exciting time within dentistry to incorporate the knowledge of the importance and benefits of the oral microbiome to provide more personalized treatment to a patient.
References: