The Oral Microbiome: A New View of Plaque Biofilm

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Conflict of Interest Disclosure Statement

- Dr. Salme Lavigne is a member of the dentalcare.com advisory board. She has no relevant financial relationships to disclose.
- Dr. Pamela Overman has done consulting work for P&G. She has no relevant financial relationships to disclose.

Short Description

This course provides a guide to assist both clinicians and students to navigate through the recently introduced Classification of Periodontal and Peri-implant diseases by the American Academy of Periodontology and the European Federation of Periodontology in 2018. Key dynamics that played a role in the creation of this classification are discussed including both new discoveries resulting from the human microbiome project as well as the concept of Precision Medicine. This classification system is a major paradigm shift from the previous 1999 classification. Thus, an easy four-step approach for determining a periodontal diagnosis is presented along with clinical photos and radiographs for each case type.

Course Contents

Overview

The primary learning objective for this course is to increase your general knowledge of the evolution of various plaque hypotheses throughout the years, highlighting the current view of plaque biofilm as an integral part of the oral microbiome; its role in health and disease; and the ramifications for periodontal therapy.

Learning Objectives

Upon completion of this course, the dental professional should be able to:

- Compare and contrast the differing plaque hypotheses from the 19th century to current times.
- Define plaque as a biofilm.
- Compare and contrast the behavior of bacteria as grown on culture plates with their behavior in biofilms.
- Discuss the positive and negative aspects of biofilm formation in nature.
- Provide examples of how biofilm provides benefits and harms.
- Discuss microbial biofilms as part of the oral microbiome.
- Identify the key microbial species associated with the conversion of a symbiotic biofilm to one of dysbiosis.
- Discuss the various bacterial color complexes involved in the sequential colonization of plaque microorganisms.
- Describe the role that commensal microorganisms play in plaque biofilm.
- Summarize how plaque microorganisms enter a state of dysbiosis.
- Discuss current thought on the role that inflammation plays in conversion of gingivitis to periodontitis using the IMPEDE model.
- Discuss the ramifications of total microbial elimination.
- Describe both traditional and novel strategies for the control of oral biofilm targeted to maintain the oral cavity in a state of symbiosis.

Background

The primary learning objective for this course is to increase your general knowledge of the various ways that dental professionals have viewed plaque throughout the years, highlighting its evolution from a sticky mass of microorganisms to a plaque biofilm that is now recognized as an important part of the oral microbiome. Learnings from the 10-year Human Microbiome Project funded by the National Institutes of Health (NIH) from 2008-2017,[1] have created a major paradigm shift in terms of the way we now view dental plaque, plaque biofilm, and its relationship to the oral microbiome. The oral microbiome is second only to the gut microbiome in the number of microbial inhabitants with over 700 different species listed in the *Human Oral Microbiome Database*.[2]

The oral microbiome has been identified as an integral part of our well-being when considered to be in a state of symbiosis where both commensal microbial species co-exist with those who have the potential to be disease producing. These commensal microorganisms are responsible for the smooth running of our initial food digestion, play a critical role in maintaining oral homeostasis, and protecting the oral cavity from disease.[3] However, if this symbiotic balance is disrupted and more disease-producing microbes overpower the beneficial species, an immune response is triggered, dysbiosis of the oral microbiome occurs, and periodontal disease ensues. Understanding the mechanisms that cause this state of dysbiosis is integral to how we as dental professionals can help our patients in both maintaining and/or returning the periodontium into a symbiotic state.

Dental researchers have attempted to understand the microbial nature of oral diseases over the past 130 years. Their view of plaque and its constituent microorganisms has shifted numerous times from a non-specific plaque hypothesis, to a specific plaque hypothesis and then back again to a non-specific theory that led to first the ecological plaque hypothesis, the keystone pathogen hypothesis and finally the currently introduced IMPEDE model (Inflammation-Mediated Polymicrobial-Emergence and Dysbiotic-Exacerbation).[4] At this time, no one microbe has yet been shown to be responsible for causing periodontal disease, rather host-related factors such as genetic variations, inflammatory-immune response to pathogens, environmental factors all contribute to the initiation and progression of periodontal disease.[5] More specifically, local environmental conditions have been shown to impact the microbial composition, and the impact of inflammation on the microbiome has been shown to be modifiable. Echoed by the new classification of periodontal disease in 2017, created by the American Academy of Periodontology in partnership with the European Federation of Periodontology, periodontitis was redefined as: *"a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus"*.[6] This shifting view of plaque has important implications for future efforts in research, treatment and prevention.

Glossary

Biofilm: In general, a biofilm is a well-organized, cooperative community of microorganisms that is found on surfaces anywhere in nature that typically form under fluid conditions.[7]

Plaque Biofilm: The biofilm unique to the oral cavity occurring on numerous surfaces such as the cheeks, tongue and teeth comprised of a sticky mass of proteins, lipids, glycoproteins, and glycolipids housing oral microbial communities with special chemical and nutritional gradients.[3,8,9]

Microbiome: The human microbiota consists of the 10-100 trillion symbiotic microbial cells harbored by each person; the human microbiome consists of the genes these cells harbor.[10]

Symbionts: Symbiont is the term used to refer to an organism living in symbiosis.[11]

Pathobionts: Pathobionts are opportunistic microbes that emerge as a result of perturbations in the healthy microbiome due to complex interactions of various genetic, exposomal, microbial, and host factors that lead to their selection and expansion.[12]

Commensal organisms: Beneficial bacterial species that protect against pathogens, perform multiple immunological functions and maintain health.[13]

Symbiosis: A condition that occurs in health dominated by commensal gram-positive organisms that are in homeostasis with the host.[4]

Dysbiosis: The transition of the polymicrobial community from largely gram-positive commensal microbes to a gram-negative enriched inflammogenic community.[4]

Metagenomics: Is a set of techniques which detects bacteria that cannot be cultured and identifies the genomic diversity of microbes by genomic analysis of the DNA of the entire community of microbes that involves the whole-genome shotgun sequencing (WGS).[3]

16S rRNA sequencing: Involves sequencing of the conserved 16S rRNA gene.[3]

Metabolomics: the study of molecules in biological samples which may be produced by the host or its microbiome.[14]

Meta-transcriptomics: Sequencing of mRNA in a sample providing a snapshot of transcriptionally active microbes.[14]

Keystone Pathogens: specific microbes that are present in low numbers such as Porphyromonas gingivalis (P. gingivalis) and Filifactor alocis (F. alocis) that are capable of triggering inflammation by interfering with the innate immune system causing a shift in the host response.[15]

Introduction

Despite the best efforts of dental health professionals, oral infections are still widespread. Nearly 90% of U.S. adults between 20 and 64 have had dental caries, and 1 in 4 adults in that age group have untreated dental caries.[16] The most recent National Health and Nutrition Examination Survey (NHANES) gathered probing depth data to measure the prevalence of periodontitis between 2009-2014. Their findings estimated 42% of dentate US adults 30 years or older had periodontitis, with 7.8% having severe periodontitis.[17]

There is now universal recognition these oral infections are multifactorial in nature, with a large variety of microbial species residing in intraoral plaque biofilms, with some species being beneficial (commensal) while others being

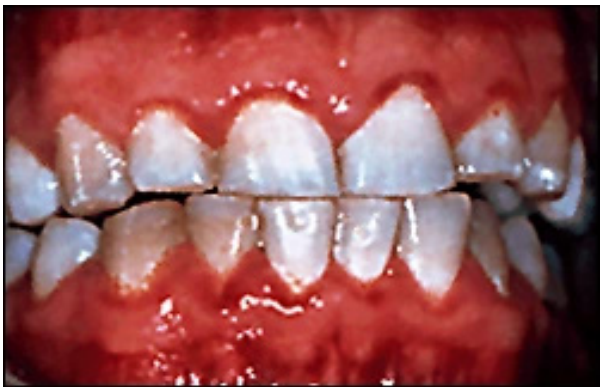

Figure 1. Gingivitis.

capable of producing disease (pathobionts). The plaque biofilm, unique to the oral cavity, occurs on numerous surfaces such as the cheeks, tongue and teeth and is comprised of a sticky mass of proteins, lipids, glycoproteins, and glycolipids housing oral microbial communities with special chemical and nutritional gradients.[3,9]

It is also now recognized that these microbes live in a symbiotic or balanced relationship in health and do not cause disease. However, when this homeostatic state is disrupted, oral disease ensues. (Figure 1) Exactly how this symbiotic state is turned into a dysbiotic one is still not clearly understood despite over a century of research.

How dental plaque and its resident microorganisms are viewed has been dictated by the analytical tools available to study it. Historically, dental plaque microorganisms were first identified by the Dutch microbiologist Anton Von Leeuwenhoek and referenced in his letter to the British Royal Society in 1677 as little animalcules.[18] Since that time, numerous hypotheses have followed regarding the exact nature of plaque and its role in oral diseases such as caries, gingivitis and periodontitis. Research has been evolving rapidly over the past several decades with the availability of newer scientific methods and technologies enabling better identification of specific microbial species. Results from the more recent Human Microbiome Project,[1] funded by the NIH, have provided more specific evidence about these microorganisms and their genomes which are now referred to collectively as the oral microbiome that form the oral ecosystem and will guide newer treatment approaches for plaque control and prevention strategies.

Figure 2. Plaque-dwelling microorganisms.

Oral microorganisms in dental plaque showing typical "corn-cob" structure of bacterium.

Plaque as a Biofilm

Plaque was first recognized as a biofilm in the early 1990's and began to be referred to as plaque biofilm rather than plaque alone. This discovery consequently, had an influence on some of the more current strategies used to control and prevent dental diseases.[7]

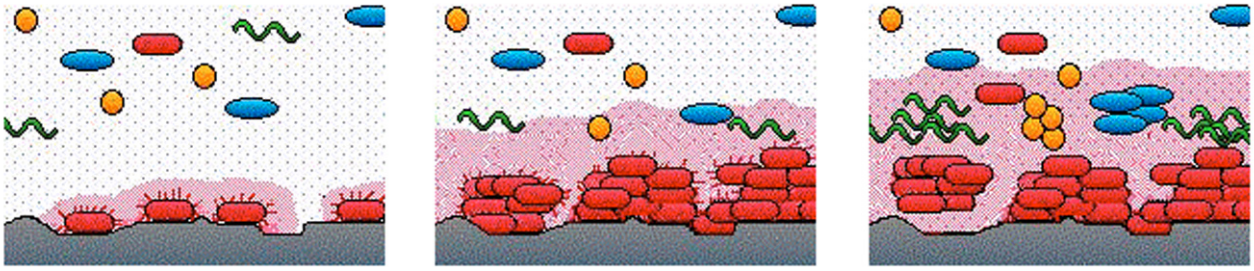

Figure 3. Artistic Depiction of Plaque Biofilm.

Previously, bacteria were studied as colonies growing on culture plates in the laboratory. Newer and more sophisticated technologies, such as confocal scanning laser and two-photon excitation technology, as well as molecular analysis methods, such as DNA-DNA hybridization and gene expressions and metabolomics, permitted examination and understanding of oral biofilms in their natural states.[7,8,9]

Microorganisms in biofilm behave differently than planktonic (free-floating) bacteria or those in a culture medium (Table 1).

In general a biofilm is a well-organized, cooperative community of microorganisms.[7,8] The slime layer that forms on rocks in streams is a classic example of a biofilm (Figure 3). Biofilms are everywhere in nature and typically form under fluid conditions. It is estimated over 95 percent of microorganisms existing in nature live in biofilms.[9] Sometimes biofilms in nature are seen as positive, such as their use for detoxification of waste water and sewage.

One familiar example of a biofilm for dental professionals is the slime layer that forms in dental unit water lines that requires daily flushing. (Figure 4) Biofilms can also be found lining oil pipelines, fish tanks, indwelling catheters, internal implants, contact lenses, and prosthetic devices. Occasionally biofilms are deadly. Legionnaire's disease that killed 29 persons in Philadelphia in 1976 was ultimately traced to bacteria in the biofilm of the air conditioning system. Millions of dollars are spent each year working to control these biofilms.[19,20]

Figure 4. Biofilm found on dental equipment.

The microorganisms living with humans also live in biofilms. However, unlike environmental biofilms, humans have a symbiotic relationship with their microbiome in health as it plays a critical role in physiologic, metabolic and immunological functions such as food digestion, energy generation and balances pro-inflammatory and anti-inflammatory processes. Thus, it controls homeostasis and prevents us from disease.19 However, if a shift in this symbiotic state occurs, the microbiome changes to a dysbiotic state ultimately resulting in disease.

Microorganisms in biofilm behave differently than planktonic (free-floating) bacteria or those in a culture medium (Table 1).

Seen through a microscope, bacteria in a biofilm are not distributed evenly. They are grouped in microcolonies surrounded by an enveloping intermicrobial matrix (Figures 5 & 6).

Table 1. Basic Biofilm Properties[20,21]

- ✓ Cooperating community of various types of microorganisms
- ✓ Microorganisms are arranged in microcolonies with channels between the microcolonies
- ✓ Microcolonies are surrounded by protective matrix
- ✓ Differing environments within the microcolonies in the biofilm
- ✓ Microbial gene expression differes when microorganisms are in a biofilm
- ✓ Microorganisms have primitive communication system
- ✓ Microorganisms in biofilm are resistant to antibiotics, antimicrobials, and host response

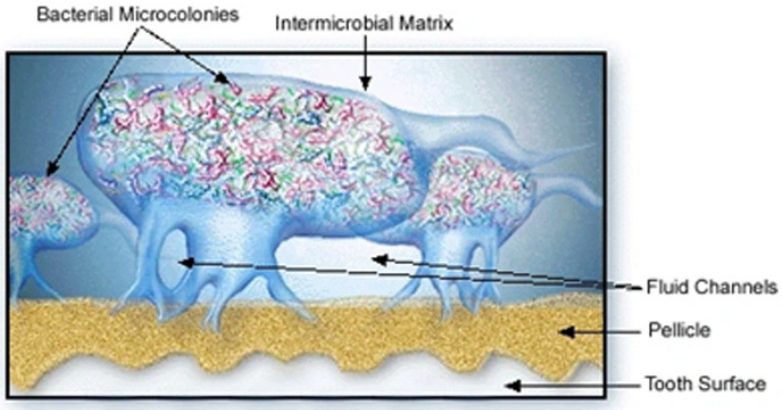

Figure 5. Artistic Depiction of Plaque Biofilm.

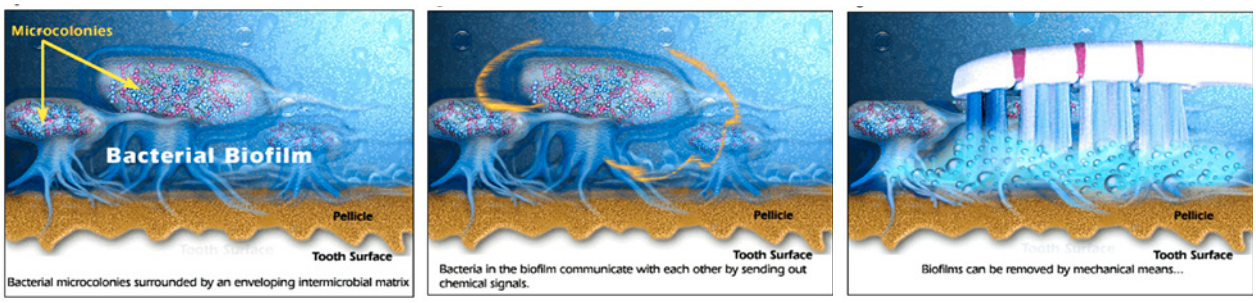

Figure 6. Animation of Biofilm

The biofilm matrix is penetrated by fluid channels that conduct the flow of nutrients, waste products, enzymes, metabolites, and oxygen. The microcolonies within the biofilm have micro-environments with differing pH's, nutrient availability, and oxygen concentrations (Figure 7). The bacteria in a biofilm use a communication system termed quorum sensing that involves sending out chemical signals (Figure 8). These chemical signals trigger the bacteria to produce potentially harmful proteins and enzymes, virulence factors that help the intraoral biofilm bypass host defense systems.[9,23]

Our previous attempts to predict and control periodontal diseases have been based on the performance of bacteria cultured under laboratory conditions.[9,16] Increased

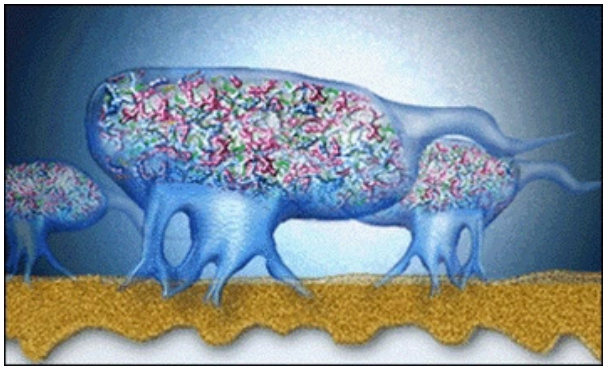

Figure 7. Biofilm Fluid Channels.

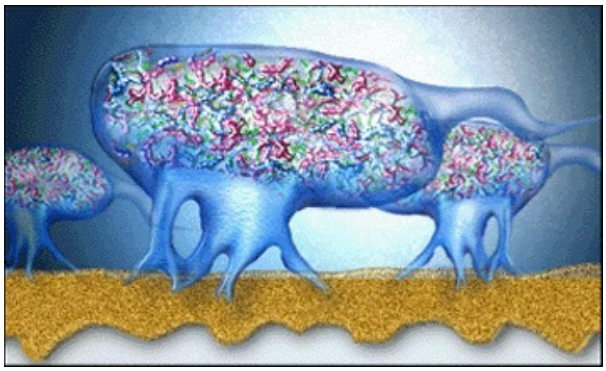

Figure 8. Communication Signals
Biofilm bacteria communicate by sending out chemical signals.

understanding of biofilms have demonstrated great differences between bacterial behavior in laboratory culture and in their natural ecosystems. For example, bacteria in biofilm produce compounds in biofilm that they do not produce when in culture. Also, the biofilm matrix surrounding the microcolonies serves as a protective barrier. This helps explain why systemic and locally delivered antimicrobials have not always proven successful, even when they were targeted at specific microorganisms. Researchers have estimated that it can take 1,000 times the drug to kill a microorganism in a biofilm as it does to kill the same organism in a free floating or planktonic environment.[21,22] The protective matrix of biofilm also helps explain why mechanical plaque control and personal oral hygiene have continued to be an integral part of periodontal therapy in an attempt to penetrate through the matrix to disrupt the colonies of bacteria inside the biofilm. Biofilms can be removed by mechanical means, however, they immediately begin to reform and are difficult to penetrate, so the search continues for ways to lyse and remove pathogenic biofilms.

The Sequential Theory of Colonization

It has been well established that prior to formation of the plaque biofilm, the enamel surfaces first become coated with a sticky film comprised of proteins, lipids, glycoproteins and glycolipids enabling the adherence of the primary microbial colonizers. This sticky coating is referred to as the acquired pellicle.[14] Interestingly, findings from the human microbiome project revealed that most, although not all oral microbes demonstrate site specificity.[14,24] An example of this site specificity is from the Streptococcus species, where *S. salivarius* and *S. parasanguinis* colonize on the dorsum of the tongue while S. sanguis and S. gordonii reside in dental plaque.[24] As illustrated in the following video, primary colonizers are gram-positive rods and cocci followed by *Actinomyces*, *Gemella, Veillonella, Rothia* and *Neisseria* species that help facilitate attachment of *F. nucleatum* that is thought to play a bridging role between early and late colonizers.[14]

Between 18 hours to 4 days, primary colonizers predominate, however there is a slow increase in anaerobic species such as *Porphyromonas*, *Fusobacterium*, *Prevotella* and *Capnocytophaga*. These microbes situated at the dentogingival border stimulate the host immune response and both inflammation and further dysbiosis ensues with the introduction of keystone pathogens such as *P. gingivalis* and *Filifactor alocis*. It has been difficult to determine which comes first, the dysbiosis or the inflammation. There is now some speculation that this initial inflammation may be the driver of the dysbiosis.[4]

The Relationship Between Plaque Biofilm and Disease

Despite all the recent discoveries, particularly over the past 10 years, it still remains unclear as to how the microbes within plaque biofilm become dysbiotic and cause disease. There has been a lot of speculation about the temporality of events leading to

Click on image to view video online.

Figure 9. Sequential Colonization of Plaque

periodontal disease and more specifically, which comes first, inflammation or dysbiosis of the polymicrobial biofilm? A new model has recently been proposed by Van Dyke and colleagues[4] suggesting that initial inflammation caused by the host innate immune response attempting to maintain and/or restore homeostasis during health and gingivitis, is the first stage of the process. If attempts made by the host response are not successful in maintaining balance in the microbiome, then growth of pathobionts overcome the balance causing dysbiosis that ultimately leads to periodontitis.[4] This new Model, now referred to as the "Inflammation-Mediated-Polymicrobial-Emergence and Dysbiotic-Exacerbation" (IMPEDE) model was designed by its authors as a subsequent follow-up to the 2017 World Workshop Classification of Periodontitis.[4]

The Evolution of Plaque Hypotheses in Periodontal Disease Progression

When Anton Von Leeuwenhoek first discovered the existence of microbes in the 17th Century, there was no way of identifying specific bacterial species. In the late 19th Century, with advances in science, several specific pathogens were identified to be associated with a variety of systemic diseases, however none were found for oral diseases despite ongoing searches. Subsequently, dental scientists believed that periodontal disease was linked with some constitutional defect in the individual.[25] That time period is referred to as the '**Golden Age of Microbiology**'.

The search for specific periodontal bacteria continued beyond the mid-part of the 20th century. However, since no specific bacteria could be identified, all plaque was viewed as bad plaque. Mechanical irritants such as calculus and overhanging restorations were also thought to play a major role in the pathogenesis of periodontal disease.[26] Stringent plaque control thus became the focus of periodontal therapy. This period of time was referred to as the **Non-specific Plaque Hypothesis** since no specific microorganisms were identified.

Discoveries in the late 1960's and early 1970's marked a return to the idea of a **Specific Plaque Hypothesis** when researchers successfully demonstrated that periodontal

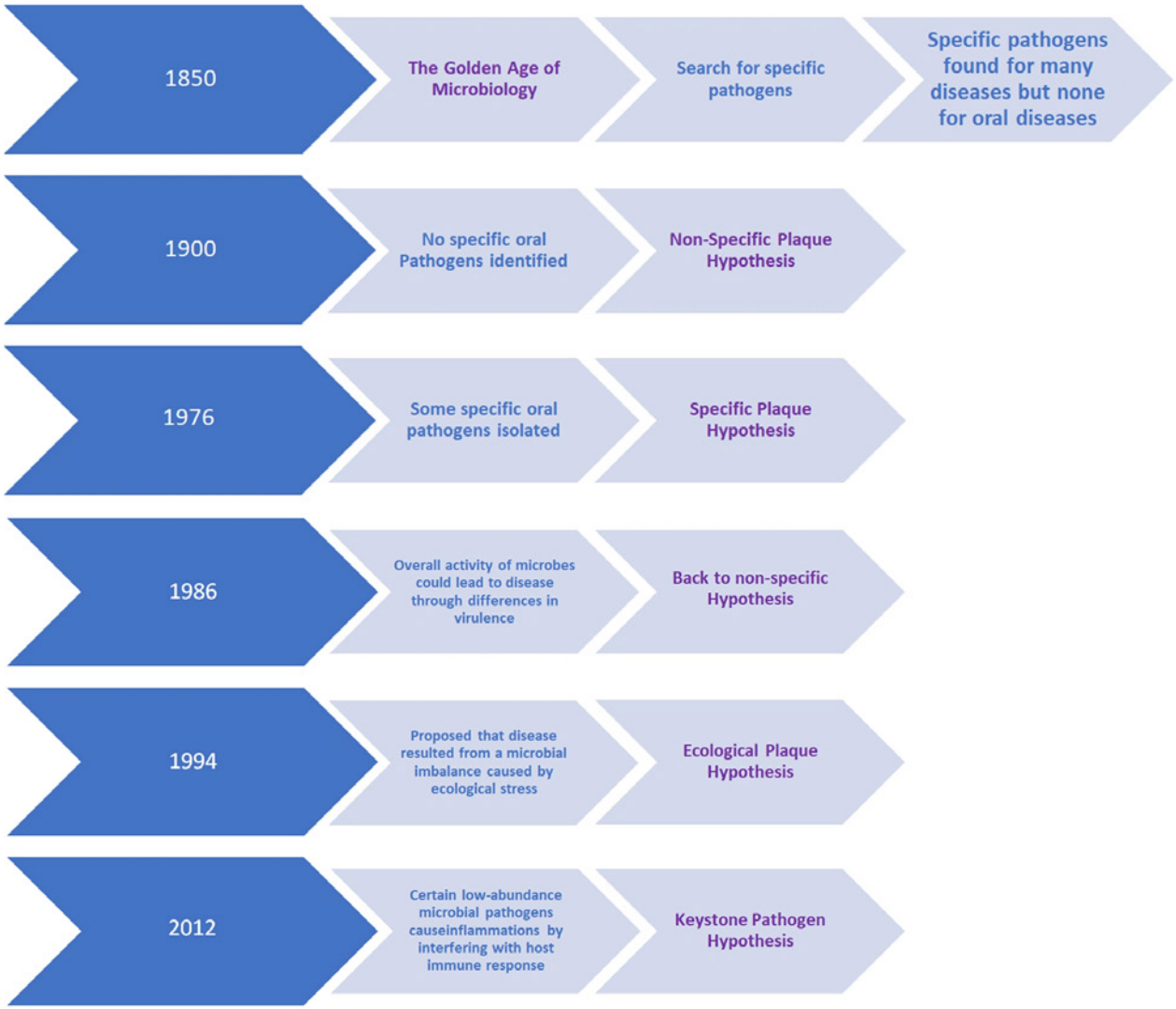

Figure 10. The Evolution of Plaque Hypothesis

disease could be transmitted between hamsters.[27] The specific plaque hypothesis identified a shift from predominantly gram positive aerobes to gram negative anaerobes in oral communities. Research efforts also identified specific groups of bacteria that were significantly associated with periodontitis. Socransky used DNA-DNA hybridization to identify complexes, or groups of bacteria that were thought to be major etiologic contributors to periodontal diseases. Yellow, green, blue and purple complexes were thought to be compatible with gingival health, while orange and red were associated with disease. Once it was identified that some of these bacteria could also be present in the absence of disease, additional refinement was indicated to support the specific plaque and Socransky's Microbial Complexes concepts.[28] In 1976 scientists proposed that only a few species from the total microflora were actively involved in disease and thus once again the search for a specific microbial periodontal pathogen began and treatment was aimed at the causative agent.[28] That period however did not last long. In 1986 there was a return to the **Non-specific Plaque Hypothesis** because scientists began to suspect that the overall activity of the microflora could lead to disease by taking into account differences in virulence among the various species of bacteria.[28]

In 1994, researchers combined the key concepts of the earlier two hypotheses proposing that the disease was the result of an imbalance in the microflora that could be caused by ecological stress resulting in an enrichment of certain disease-related microorganisms.[28] This became known as the **Ecological Plaque Hypothesis** and was the beginning of the concept of dysbiosis that is now in the current literature.[29]

More advanced bacterial profiling techniques available in the early 2000's such as 16SrRNA-based bacterial profiling using next generation sequencing, reverse transcription-polymerase chain reaction, microarray and pyrosequencing technology, enabled the launching of the Human Microbiome project by the NIH in 2008. This project resulted in the identification of over 700 species of distinct oral microbial species with suggestions of numbers as high as 1,200-1,500.[1] Of course not all 700+ species have been found to be associated with periodontal disease.

Following these discoveries made during the Human Microbiome project, the ecological plaque hypothesis was taken one step further in 2012 by Hajishengalis and colleagues[30] who proposed that certain low-abundance microbes could integrate with the host immune system and remodel the microbiota thereby causing inflammatory disease. In line with earlier findings, gram negative anaerobic bacteria were most commonly found to be associated with periodontitis such as *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* (Red complex bacteria); *Prevotella intermedia, Fusobacterium nucleatum, Parvimonas micros,* Campylobacter recta, multiple species of Eubacterium, and multiple species of Bacteroides (Orange complex); *Aggregatibacter actinomycetemcomitans* (Green complex); and *F. alocis* (gram positive rod more recently identified).[15,35] Of this group of bacteria, only two are considered as "keystone" microbes, *P. gingivalis* and *F. alocis*. Keystone microbes are classified as those appearing in lower numbers but who have inherent virulence factors that allow them to interact with the host innate immune system and alter a symbiotic microbiota into one that is dysbiotic.[15] In addition to being keystone microbes, both *P. gingivalis* and *F. alocis* are highly virulent microbes that have a possible commensal relationship and also are able to by-pass the host immune response.[15,37]

This became known as the **Keystone Pathogen Hypothesis**.[30] This hypothesis was in direct contrast to earlier beliefs that dominant species when abundant were what influenced inflammation. This new hypothesis suggested that keystone pathogens such as *Porphyromonas gingivalis (P. gingivalis)* triggered inflammation when they were present in "low" numbers by interfering with the innate immune system causing a shift in the host response triggering inflammation. Research began to demonstrate that commensal bacteria must be present to trigger other bacteria to cause disease.[30]

Although this hypothesis is the most recent and offers a plausible explanation of the significance of the microbial community when compared with patients who have periodontal disease and those who are healthy, there are still some unknowns. The problem is that keystone pathogens can be any species and some that are not necessarily pathogenic.[29] Newer research has surfaced exploring what actually triggers commensal microbes to alter the symbiotic state and suspect that this alteration ultimately leads to localized inflammation, and if not controlled by the host innate immune system, is what leads to the ultimate state of dysbiosis found in periodontitis.[4] This new model, proposed by Van Dyke et al. the "Inflammation-Mediated-Polymicrobial-Emergence and Dysbiotic-Exacerbation" (IMPEDE) model was designed by its authors as a subsequent follow-up to the 2017 World Workshop Classification of Periodontitis.[4] It hypothesizes that initial inflammation initiated by the innate immune system in its attempt to restore symbiosis, is what ultimately leads to the dysbiosis that causes periodontitis. It also suggests that there are multiple factors that are at play such as one's genetics, environmental factors, and host response to pathogens.[5]

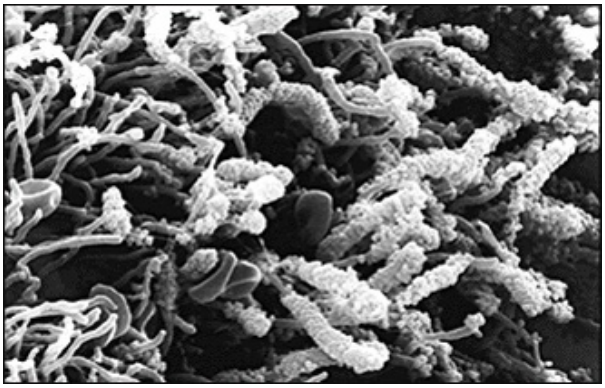

Figure 11. SEM of mature human dental plaque demonstrating corn cob formation. Bar = 10 microns at an original magnification of 2,020.

Image courtesy of Dr. Charles Cobb. University of Missouri-Kansas City

The Polymicrobial Oral Biofilm

The lengthy search for the identification of specific oral microbes contributing to periodontal disease has progressed over the past 130 years with increasingly newer methods of microbial analysis ranging from darkfield microscopy, transmission electron microscopy, scanning electron microscopy, DNA probes, DNA hybridization, BANA hydrolysis, and immunoassay aiding the search.[31] Even newer techniques are now available such as metagenomics and meta-transcriptomics, thus enabling researchers to propose that even more diverse periodontitis-associated microbiota may be involved in periodontal disease.[34] In addition, the recent discovery of commensal microbial species and their role in maintaining symbiosis, has created a major paradigm shift in the way we now look at oral biofilm. Restoration of microbial symbiosis has now become the focus rather than destruction of all microbes.

Fanas and colleagues in 2021 conducted a study ranking the most prevalent bacterial species present during Stage 2 periodontitis classified according to Socransky's color complexes and also included a number of health-related species.[34] (Table 2) Using newer more advanced techniques and Socransky and Haffajee color complexes, these specific microbe color groups were categorized based on their pathogenicity and prevalence in Stage 2 periodontitis.[34]

Future Treatment Implications

Researchers have sought to develop diagnostic tests such as chairside microbial samplings as well as salivary testing, for detection and treatments designed to target periodontal pathogens. Treatments targeting biofilms include systemic and topical agents, including host modulators. Systemic antibiotics such as metronidazole, clindamycin, doxycycline, ciprofloxacin, azithromycin alone or in combination have been used.[32] Local delivery of antimicrobials (tetracycline fibers, metronidazole and minocycline gels, chlorhexidine chips, and doxycycline polymer) have also been introduced.[36] Host modulation focuses on changing how the body responds to the bacterial challenge rather than solely reducing the bacterial challenge of the plaque biofilm. While these approaches have enhanced our ability to manage periodontal diseases to some extent, they have still failed to provide uniform success.

Viewing plaque as a polymicrobial biofilm that includes both commensal or beneficial species that are essential to health, along with other species that have the potential to become pathological (pathobionts), may be an explanation as to why these older approaches have not been as successful as hoped. If all microbes, including the commensal ones, are totally wiped out by antibiotics for example, that could seriously have an impact on the innate immune system that may no longer be able to control disease progression as well as having a negative effect on other necessary physiological functions.

With the new knowledge about the critical role of these commensal microorganisms, found in biofilm, it is becoming clear that *maintenance of symbiosis* must be the goal of plaque control, rather than total destruction of all species. This presents a major paradigm shift to older beliefs where all plaque was thought to be bad, and treatment approaches were targeted to eliminate all plaque microorganisms. We can no longer condone treatments that propose to eliminate or kill 99% of all microbes.[3,14]

Table 2. Microbial Color Complexes Prevalent in Stage 2 Periodontitis[34]

Symbiosis

Color Complex[33]	Order of Disease Prevalence[34]
Red Complex	*T. denticola* *P. gingivalis* *T. forsythia*
Orange Complex	*F. nucleatum* *P. intermedia* *C. rectus* *P. micros*
Yellow Complex	*S. Mitis* *S. gordinii* *S. intermedius* *S. sanguis*
Green Complex	*Capnocytophaga spp.* *E. corrodens*
Purple Complex	*Veillonella spp.* *Actinomyces spp.*
Health Related Species[34]	*Neisseria spp.* *Gemella spp.* *Rotia spp.* *Kingella spp.*

Traditional Treatment Approaches

Mechanical

Traditional mechanical methods of controlling plaque accumulation with twice daily toothbrushing and interdental plaque control methods such as dental floss, interdental brushes, water flossers etc. must continue as they have an impact on "disrupting" the biofilm rather than totally annihilating all of its resident microbes. Use of electric toothbrushes has been shown through a plethora of studies to have superior results to manual toothbrushes and should be encouraged. However brushing alone has only been shown to be partially effective, thus chemical means of plaque control should be used to complement these mechanical approaches.

Chemical

Antimicrobial agents incorporated into oral care products such as toothpastes and mouthrinses, complement mechanical plaque control devices. Continuing research explores new antimicrobial agents that target only the pathogenic microbes without destroying the beneficial species. With the focus being on maintaining symbiosis rather than killing all microbes, these products must be at concentrations that inhibit the growth of pathobionts without elimination of beneficial species.

Recent studies of the effects of chlorhexidine on the oral microbiome, have resulted in evidence of reductions in numerous beneficial species.

Along with these reductions in commensal species, this alteration in oral homeostasis resulted in significant reductions in salivary PH, reductions in the buffering capacity of saliva, and increases in salivary lactate. These alterations have all been associated with tooth decay and periodontal disease. Another detrimental effect of chlorhexidine found in these studies was decreased concentrations of oral nitrite, which plays a significant role in stabilizing blood pressure.[14] Further studies however are required to explore the actual extent of the impact chlorhexidine has on altering blood pressure. Use of antimicrobial mouthrinses such as chlorhexidine digluconate, along with others having high enough concentrations of alcohol to kill oral microbes, should be reconsidered.

Other Novel Approaches

With what we now know about the polymicrobial nature of the oral microbiome and the importance of maintaining a symbiotic relationship, controlling disease must include not only maintenance of good oral hygiene, but must focus on environmental and inflammatory/immune factors that drive dysbiosis. These other approaches involve interfering with environmental factors that may drive dysbiosis such as inhibiting growth of pathobionts such as *P. gingivalis*, reducing nutrient supply of GCF, increasing alkalinity of plaque, inhibiting destructive host pathways, and decreasing inflammatory markers.

Varying the oxygen concentration, pH, and nutrient availability in plaque have all been shown to modulate biofilm microflora and may prove useful. For example, periodontal pathogens require a low redox potential for growth. Addition of a redox agent, such as methylene blue, to periodontal pockets has been shown to inhibit the growth of *P. Gingivalis*.[37] Since increased gingival crevicular flow (GCF) increases the nutrient supply for subgingival biofilm, control of GCF may be used in the future to control subgingival biofilm. Use of novel anti-inflammatory agents such as lipoxins, resolvins, and protectins[38] may not only help inhibit destructive host pathways, these anti-inflammatory agents may also reduce the nutrient supply of GCF for the biofilm community. Other popular strategies being introduced are the use of oral probiotics designed to increase the alkalinity of the plaque, while others are targeted towards specific pathogenic organisms eg. *S. mutans*.[14] A major source of alkalinity occurs during the breakdown of arginine in the oral cavity. Some studies have shown that addition of arginine supplements have been able to inhibit the occurrence of dental caries. *Lactobacillus* brevis is one probiotic species that has been shown to have superior ability to produce arginine deaminase and has shown substantial decreases in inflammatory markers for periodontal pathogens.[14,39]

Table 3. Possible Strategies to Maintain Symbiosis

Strategy	Outcome
Redox Agents like Methylene Blue into Periodontal Pockets	Inhibits growth of *P. gingivalis*
Novel Anti-inflammatory Agents: Resolvins, Lipoxins, Protectins	• Reduces nutrient supply of GCF for biofilm community • Inhibits destructive host pathways
Oral Probiotics • Introduce species targeted towards specific pathogens • Addition of Arginine Supplements such as Lactobacillus brevis	• Increase alkalinity of plaque • Substantial decreases in inflammatory markers

Conclusion

Dental researchers have attempted to understand the microbial nature of oral diseases over the past 130 years. A lot of progress has been made particularly in the last decade with findings from the human microbiome project, enabled by major technological advancements, to identify over 700 microbial species present in the mouth alone. Building on the past knowledge of biofilms and how these microbes interact with each other, research has now evolved from numerous previous hypotheses to several new ones such as the keystone plaque hypothesis suggesting that certain keystone pathogens are responsible for the initial tip towards a dysbiotic plaque biofilm. Additionally, the new IMPEDE model takes the keystone concept one step further by suggesting that inflammation is the driver of the ultimate dysbiosis that leads to periodontitis rather than the pathogenic microbes themselves, providing a better understanding of how disease occurs. These new discoveries have created a paradigm shift in how we manage plaque biofilms with the ultimate goal being restoration of a state of symbiosis which means not destroying the beneficial species.

Course Test Preview

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1. The plaque biofilm unique to the oral cavity that is located on numerous oral surfaces can BEST be described as a sticky mass of:

A. proteins and disorganized bacteria.
B. proteins, lipids, glycoproteins, and glycolipids housing organized microbial communities with special chemical and nutrient gradients.
C. food debris, materia alba and a variety of pathogenic microorganisms.
D. proteins, carbohydrates, fats, bacteria, viruses and protozoa.

2. In general, a biofilm occurring anywhere in the environment including the oral cavity is a(n):

A. loose collection of free-floating bacteria
B. calcified collection of bacteria that cannot be easily removed
C. acellular translucent, homogeneous film covering moist surfaces
D. well-organized, cooperative community of microorganisms

3. One positive use of biofilms found in nature is:

A. detoxification of human waste products
B. lining of indwelling catheters
C. cleansing of fish tanks
D. decontaminating dental unit water lines

4. The difference between microorganisms living in biofilms found in the environment compared to those living in humans, is that in humans, they:

A. have a dysbiotic relationship in health.
B. cause disease when found in larger numbers.
C. disrupt physiologic, metabolic and immunologic functions.
D. have a symbiotic relationship in health.

5. The plaque biofilm matrix is penetrated by fluid channels that conduct the flow of:

A. nutrients and waste products
B. enzymes and metabolites
C. oxygen
D. all of the above

6. The best explanation of why systemic and locally delivered antimicrobial agents have not always been successful in the elimination of plaque microbes is that the biofilm:

A. prevents the antimicrobial agent from entering the periodontal pocket.
B. matrix surrounding the microcolonies serves as a protective barrier.
C. fluid channels direct the antimicrobial agent out of the pocket.
D. changes the pH of the antimicrobial agent and inactivates it.

7. The period in time when all plaque was viewed as bad plaque and mechanical irritants such as calculus and overhanging restorations were thought to play a major role in the pathogenesis of periodontal disease was referred to as the era of the:

A. non-specific plaque hypothesis
B. specific plaque hypothesis
C. Golden Age of Microbiology
D. ecological plaque hypothesis

8. The hypothesis that was the beginning of the concept of dysbiosis that is now more aligned with current thought, is the:

A. specific plaque hypothesis
B. keystone plaque hypothesis
C. ecological plaque hypothesis
D. IMPEDE model

9. Findings from the Human Microbiome project led to the development of another plaque hypothesis that suggested that low-abundance microbes could trigger inflammation by interfering with the innate host response. That hypothesis is referred to as the:

A. specific plaque hypothesis
B. keystone pathogen hypothesis
C. ecological plaque hypothesis
D. IMPEDE model

10. Using newer DNA hybridization techniques, Socransky and Haffajee grouped specific microorganisms into various color complexes according to their pathogenicity. The complex most notable in periodontal disease is the:

A. orange complex
B. red complex
C. green complex
D. purple complex

11. Using more advanced bacterial profiling techniques such as 16SrRNA sequencing, findings from the Human Microbiome Project identified over ____microbial species comprising the oral microbiome.

A. 300
B. 500
C. 700
D. 1,000

12. The two microbes that have been shown to be able to by-pass the host immune response and are identified as keystone pathogens are:

A. P. gingivalis and A.actinomycetemcomitans
B. T. forsythia and T.denticola
C. P. gingivalis and F. alocis
D. F. nucleatum and F. alocis

13. The new Inflammation-Mediated-Polymicrobial-Emergence and Dysbiotic-Exacerbation (IMPEDE) model is based on the premise that:

A. inflammation precedes the dysbiosis leading to periodontitis
B. initial dysbiosis causes the inflammation leading to periodontitis
C. the commensal bacteria must outnumber the pathobionts to cause dysbiosis
D. the innate immune system initially is disabled

14. Given what we now know about the critical role of commensal microorganisms found in plaque biofilm, the new goal of plaque control must now be:

A. total elimination of all plaque microorganisms
B. thorough oral disinfection
C. maintenance of oral symbiosis
D. specifically targeting the keystone pathogens

15. In addition to traditional oral hygiene practices, other novel strategies to oral biofilm control include administration of:

A. redox agents such as methylene blue
B. resolvins, protectins and lipoxins
C. oral probiotics
D. All of the above

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Additional Resources

- No Additional Resources Available.

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