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The Interactions Between Oral Biofilms, Antibiotic Resistance, and Dentistry

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Abstract

Antibiotic resistance is a pressing concern that demands further action to reduce and develop new countermeasures. Antibiotic resistance impacts all fields of health and medicine, including dentistry. Many of the preventive and restorative dental procedures are due to a bacterial cause. Knowledge of oral biofilm development mechanisms and the interactions and relationships between oral microbiota is necessary to combat the spread of antibiotic resistance and develop more effective dental treatments. Exploring how the oral microbiomes and their treatments impact the health of the rest of the body is another important consideration since dentistry is part of the general healthcare system. The purpose of this paper is to investigate the various research related to these topics and put together a detailed report on the current knowledge on the relationship between oral microbiota, dentistry, and antibiotic resistance. This research will hopefully provide valuable insight and will better prepare those entering and in the dental field.

General Characteristics of Biofilms

Introduction

The oral cavity consists of over 700 microbial species, a majority of which are nonpathogenic opportunistic commensals.[1,2] These species contribute to the oral microbiota and adhere to the different niches within the mouth, forming a structural community called a biofilm. The mouth is constantly exposed to airborne-, droplet-, food-, and water-borne microorganisms in the oral cavity. Oral microbiota act as a physical barrier against the attachment and infection of pathogenic bacteria the mouth is prone to encounter, pushing the new bacteria past the oral cavity and down into the microbicidal environment of the stomach.[1,2] Different conditions the oral cavity experiences also apply various pressures to the oral microbiota. These selective pressures can come from other bacteria, the host, and changes in the oral environment, resulting in the potential reshaping of the makeup of the microenvironments and the microbial community inside the dental biofilms.[1–3] Oral health is when the stability between the commensals and opportunistic pathogens is maintained. If there is a disruption into the dynamic between different types of bacteria, the mouth may shift towards dysbiosis, resulting in the plaque possibly becoming more pathogenic and increasing risk of oral disease development.[2,3] Environmental alterations can change the signaling molecules present. When this occurs, the microorganisms that can respond to these signals are more likely to survive in the new environment. The signaling molecules influence the genetic transcription of virulence genes, altering what virulence factors are expressed and the ability of the bacteria to adapt to the stressors. A better understanding on how biofilms develop and change in response to various environmental conditions will advance the knowledge on oral disease pathogenesis and develop better dental treatment options.[1–3]

Internal Environment of Oral Biofilms

The microbiome of the oral cavity establishes right after birth. The streptococci species are among the first and predominant bacteria of the oral microbiota, so they are typically the ones to initiate biofilm formation.[1] Biofilm development can be categorized as phases: early, middle, and late. Early phases of biofilm formation include reversible and irreversible adhesion stages.[4] Planktonic bacteria begin plaque formation by approaching and attaching to a surface. This attachment is weak, and the bacteria may release the attachment. It is easier to remove bacteria from the reversible stage because of the weak connection to the surface.[4] When there is a threshold number of attached bacteria, they begin to utilize certain fimbriae adhesins and secrete extracellular polymeric substances (EPS) to bind more permanently and start the biofilm structure.[4] This proceeds into the middle phase of biofilms where microcolonies and colonies start forming.[4]

A matrix provides adhesion and structural stability to the microbial population.[1–3] The EPS matrix supports the bacterial populations inside through protection from the external environmental pressures and traps nutrients within.[1] Due to the characteristics of the matrix, the distribution of nutrients and other substances creates distinct microenvironments within the dental plaque. Key environmental factors such as pH, nutrient availability, oxygenation, and redox reactions vary within the biofilm.[2,3] This variability enables both aerobic and anaerobic bacterial species to coexist within the same bacterial plaque. For example, the surface of the plaque consists of aerobes that consume the oxygen that diffuses into the biofilm, leaving smaller quantities of oxygen to diffuse into the lower layers where the anaerobes exist.[3] On top of the multiple microenvironments within biofilms, many possible niches within the oral cavity

produce varying compositions of oral microbiota, e.g., tooth surface, gum mucosa, tongue surfaces, etc. Each of these niches will contain populations of unique compositions.[1–3]

Biofilms enter the late phase once the plaque matures. A mature biofilm is composed of a compact biofilm structure that can house more coordinated functions.[4] This marks a point where interactions between microbial species start to function more like relationships. Biofilms provide greater instances of bacterial communication since the microorganisms are closer together. Signaling substances and metabolites involved in quorum sensing accumulate as the population grows, leading to more cell-to-cell communication.[1] What signals microorganisms express and how much they express can impact the characteristics of the signaling molecules of other species in response. Other microbes will sense the signals and may expand adhesion or other virulence factors, adding to the number of microorganisms creating the dental biofilm. Once the quantity of microorganisms reaches a certain point, synergistic and antagonistic relationships begin developing between species. Ecological factors can influence the ratios of species that engage in competitive behaviors with other species. For example, commensals have a significant advantage over cariogenic bacteria when the host's diet is low in carbohydrates, especially sucrose.[1,3] The changes to biofilm status, which determines if there is a shift towards dysbiosis, is caused by these ratio changes.

Biofilm vs. Planktonic Bacterial Cells

Plaques consist of bacterial cells that differ from their free-roaming counterparts and provide natural and acquired tolerance and resistance to antimicrobials. The signal to change from a planktonic to sessile phenotype is a regulated process depending on multiple environmental and genetic signals. Biofilm cells are physiologically heterogeneous, expressing different genes, metabolic activity, and phenotype to planktonic cells of the same species.[1,5] The

difference between phenotypes is in part due to the different conditions of the plaque environment versus the external environment.[1,5] Once the bacteria switch to the stationary lifestyle, their growth rate diminishes.[6] The bacteria remain in the stationary phase of growth for longer periods of time, which reduces the chances of antimicrobial killing since less bacteria are in the log phase, a stage where antimicrobials are most effective.[5,7] The diminished effect of antimicrobials is because there is less active replication while in stationary phase, so the bacteria are not as vulnerable and can divert the energy for growth towards other functions, leading to increased resistance. The thickness of the dental plaque can reduce antimicrobial penetration, so its diffusion does not reach satisfactory levels that can impact the bacterial growth or their survival. Diffusion happens more slowly, allowing the bacterial cells more time to generate an adaptable phenotype, increasing the tolerance of the populations in the biofilm.

The physical characteristics of plaque and the metabolic changes in the bacteria are factors that contribute to the natural resistance microorganisms exhibit in oral biofilms. On top of the natural resistance, the transfer of resistant molecules through transformation[5] or horizontal transfer[1,5] can increase the amount of resistance within the oral microbiota population.

Microorganism Interactions in Biofilm Development & Disease

Streptococcus sanguinis and Streptococcus gordonii are commonly found bacteria involved in the initial development of oral biofilms. The *S. sanguinis* fimbriae aid in the adhesion to the enamel on the tooth surface. *S. sanguinis* species SK36 also has three pili, PilA, PilB, and PilC, that help bind the bacteria to salivary components. When the pilus was mutated, *S. sanguinis* failed to accumulate on teeth and form plaques.[8] *S. sanguinis* is associated with oral health and a competitor of cariogenic species like *S. mutans*, a known acid-producer. *S. mutans* utilize glucosyltransferases (Gtfs) to synthesize adhesive glucans from environmental sucrose,

which enables *S. mutans* to attach to tooth surfaces. The glucans synthesized by GtfB and GtfC specifically are important for creating a matrix to adhere to the tooth surface and to attract other bacteria to join. *S. mutans* produce multiple glucan-binding proteins that help maintain the 3-dimensional structure of the dental plaque alongside other bacterial species. Surface protein antigen c (PAc) is one of the major surface proteins found on *S. mutans*, and PAc is often correlated with virulence in the formation of dental caries.[9]

Dental Caries and Streptococcus Antagonism

Children who have a larger ratio of *S. mutans* to *S. sanguinis* are more likely to have active caries compared to children with a larger ratio of *S. sanguinis* to *S. mutans*.[10] The competition between these two example species is more intense during the earlier stages of biofilms when the *S. sanguinis* colonizes and *S. mutans* tries to establish in the niches of the plaque. *S. sanguinis* (and *S. gordonii*[11]) produce hydrogen peroxide which inhibits the growth of *S. mutans*, while *S. mutans* secrete mutacins I and IV to decrease the survival of *S. sanguinis*.[1,8,10] When put into dual-species biofilms, *S. sanguinis* and *S. mutans* tend to reach an equilibrium where neither is able to outcompete the other. Each species releases virulence factors, but the other counters or has resistance to those virulence factors.[10] *S. sanguinis* is more sensitive to acidic environments than *S mutans.* If the host were to increase their sugar consumption in their diet, the oral biofilm environment can become more acidic due to aciduric bacteria like *S. mutans* producing acid from carbohydrate metabolism. As the acidity increases, *S. sanguinis* populations decrease, allowing greater growth of *S. mutans*, and a shift towards the development of dental caries due to the demineralization of the enamel from the acidic environment.[1,8,10] *S. sanguinis* can tolerate the initial rise in acidity, so they can help resist the shift towards dysbiosis to an extent. If the acidic direction is not reversed, the conditions will continue to put *S. sanguinis* at a

disadvantage, while the *S. mutans* will gain the advantage and proceed to increase in numbers.[10] This effect is one possible example of dental caries pathogenesis.

Periodontitis Pathogenesis

Periodontal disease occurs when there is a compositional shift in the biofilm flora from mostly Gram-positive facultative anaerobes to more Gram-negative obligate anaerobes. Gram-negative bacteria like *Porphyromonas gingivalis*, a periodontal-associated pathogen, use their short fimbriae and bind to several Gram-positive streptococcus species like *S. sanguinis* and *S. gordonii,*[12–14] infecting the oral biofilm sites. *S. sanguinus* inhibits the transcription of a *P. gingivalis* fimbriae *mfa1* gene by reducing the gene's promoter activity,[15] preventing the coadhesion of *P. gingivalis* to *S. gordonii.*[8] Additionally, some strains of *P. gingivalis* have a capsule. The capsule does not appear to help adhesion since the adhesion capacity of encapsulated *P. gingivalis* to epithelial cells is lower than unencapsulated *P. gingivalis*. This capsule does facilitate coaggregation with other periodontal-associated bacteria like *Fusobacterium nucleatum,*[16] but the binding is K-serotype-specific.[12] *F. nucleatum* can generate more tolerance in *P. gingivalis* towards aerobic conditions, expanding *P. gingivalis* growth in oxygen environments that would hinder the bacteria without the presence of *F. nucleatum.*[16] This association may be a mechanism for the progression and spread of periodontal disease. *F. nucleatum* can also coaggreate with other bacteria species like *S. sanguinis* and *S. mutans*, and *F. nucleatum* can enhance the coaggregation between *S. sanguinis* and *P. gingivalis.*[8] *P. gingivalis* can enable a range of motility to nonmotile bacteria like *F. nucleatum* and *S. sanguinis*. These bacteria can bind to the surface of *P. gingivalis* and travel. This can impact the characteristics of the oral microbial community by transporting health-associated or -promoting bacteria into and out of the biofilm.[17]

There are many interactions between various bacterial species, making the generation of oral diseases not as simple as one disease-causing bacteria. One species may be associated with healthier dental plaques but lead to the establishment of pathogenic species. For example, *S. sanguinis* may promote oral health or survive better in healthier environments, but the species is also a target for the adhesion of *P. gingivalis* and *F. nucleatum* pathogens.[8] A better understanding of the various species interactions within a biofilm will enhance the knowledge of the pathogenesis of oral diseases and create more effective treatment options that manage dental biofilms before they can cause oral diseases.

Fungal-bacterial Interactions

Bacterial interactions are not the only exchanges that occur within oral biofilms. Fungal species are another occupant of the oral microbiota. Although detected amounts of fungi are smaller than bacteria, fungal species like *Candida albicans*, the most abundant, can cooperate with different bacteria species and develop and modify plaque environments.[14] *C. albicans* have the adaptive ability to survive in various niches, which allows them to interact with strictly anaerobic bacteria under aerobic conditions, enabling the bacterial survival in an unfavorable oxygen condition. This interaction promotes the possibility of a pathogenic phenotype. Patients with periodontal disease and/or dental caries have higher amounts of *C. albicans* present in their oral microflora.[14] During hypha development, *C. albicans*, produce compounds on the surface of the hypha that allow co-adhesion with various bacteria. Some of these surface molecules include glucans and mannans. For example, *C. albicans*, through the protein Als3, interacts synergistically with surface protein SspB on *S. gordonii* to promote adhesion and biofilm formation.[14,17] The hyphae establish a hypoxic environment in mature dental plaques, leading to increased *P. gingivalis* adherence and cell viability. Since *C. albicans* are found in periodontal

pockets under the gums, they can create protective environments for anaerobic bacteria, potentially increasing the concentration of periodontal-associated bacteria and shift towards periodontal pathogenesis.[14]

C. albicans is also in caries-associated dental plaques alongside *S. mutans*. Glucosyltransferases (Gtfs) aid the coexistence between *C. albicans* and *S. mutans* in sucrose-rich environments. The GtfB of *S. mutans* binds strongly to purified mannans produced by *C. albicans* hypha. If either *S. mutans* or *C. albicans* were deficient in their respective components, no biofilm development occurs between the two species. Additionally, the binding of *S. mutans* to *C. albicans* surface is low when sucrose is limited or absent. As sucrose levels increase, a synergistic relationship between the fungus and bacteria develops further, exacerbating the damage done by *S. mutans* virulence factors.[18,19]

Antibiotic Resistance

Background

The breakthrough of antibiotics significantly propelled the science community to greater heights, but in the past decades, the overuse and misuse of antibiotics has become a serious concern. With the increasing levels of antibiotic resistance seen globally, standards for effective and proper antibiotic prescription and treatment are continuously altered. There are around 700,000 deaths globally per year caused by antibiotic-resistant bacteria, and the number is predicted to reach 10 million by 2050.[20] Alongside the health complications, the healthcare expense will also increase.

The physical structure of the oral biofilm inherently provides resistance to selective pressures like antibiotics. More time is required for antibiotics to diffuse across the biofilm matrix and act upon its target. Many microbes within the plaque are in a stationary stage of

growth, so the effectiveness of the antibiotic is further reduced. There are external factors that can contribute to antibiotic resistance like overuse of antibiotics, unnecessary prescribing, and inappropriate dosing and duration of antibiotic treatments.[21] Antibiotics create a selective pressure on the microbiome when taken. The amount of antibiotic consumption determines the extent of the selective pressure, contributing to the frequency of resistance being developed.[21] The longer the exposure and how frequent the microorganisms are exposed to antibiotics, the greater chance of developing resistance or acquiring resistance. Broad-spectrum antibiotics are more likely to promote this resistance than narrow-spectrum antibiotics because of the wider targeting range, more bacteria are impacted, so more bacteria may become resistant.[21]

When the antibiotic leaves the system, the population of resistant bacteria decreases, and there is a shift back towards sensitive-type bacterial populations.[21] The expended energy used in the resistance processes and expression is no longer beneficial to survival, so the sensitive bacteria begin repopulating the biome since their use of energy is more effective, increasing their survival. The reduction in antibiotic consumption also diminishes the portion of resistance in microbiomes.[21,22] With the global increase in antibiotic consumption, the forces shifting towards resistant-type bacteria are becoming more frequent.[21] Gram-negative bacteria especially are becoming more resistant to multiple antibiotics,[22] which can have negative implications for treating periodontitis.[21]

Dentists make up about 10%[20,23] of the antibiotic prescriptions, and around 30-85%[24] of these prescriptions are for managing dental pain and to avoid complications after surgeries.[20,24] The percentage may seem small but oral diseases are prevalent in every geographical region, regardless of age or race. Dental caries and periodontal disease are the more frequent oral health problems.[25] There are currently no specific guidelines for antibiotic use established by

organizations like the American Dental Association in the United States, so dentists typically rely more on personal experience when deciding antibiotic use.[20,24] With the rise in health risks and cost-related concerns related to antibiotic resistance, appropriate use of antibiotics is a critical issue not just in dentistry but in all of healthcare. Establishing guidelines on antibiotic use in dentistry will help hinder the progression of antibiotic resistance.

In dentistry, antibiotics are prescribed without knowledge of the microflora makeup of the dental plaque present.[20] A wide range of broad systemic antibiotics, especially penicillin, are authorized by dentists for their outpatient care without considering the impact on the oral microbiota, or the microbiota in other areas of the body. For instance, with *Candida albicans* and oral streptococci, overusing broad antibiotics can sometimes increase the growth of *C. albicans* since the antibiotics do not kill the fungus.[26] The increase of *C. albicans* can lead to more adherence of bacteria like *S. mutans,*[27] which may create conditions favoring the shift towards oral disease. Better knowledge on the interactions between biofilm microorganisms and how antibiotics interact with them should be considered for knowing when to prescribe an antibiotic and prescribing the optimal antibiotic for treatments.

Systemic Antibiotic Therapies for Chronic or Refractory Periodontitis

The primary treatment for periodontitis is the removal of the dental plaque and cleaning of periodontal pockets in the gums, eliminating the periodontopathic bacteria present and restoring tissue health. Non-surgical treatments like scaling and root planing (SRP) are effective in treating periodontitis and initial chronic periodontitis.[28] The accessibility of mechanical debridement is limited, so there are some cases where completely cleaning a periodontal pocket is not possible. The use of local or systemic antibiotic therapies are beneficial in managing chronic- or refractory-types of periodontal disease alongside non-surgical treatment.[28–30] There

are risks and limitations to local and systemic antibiotic therapies. Both local and systemic are unable to achieve sufficient concentrations in the gingival crevicular fluid.[28,29] The issue of retention is a limitation for local antibiotics.[28] Systemic antibiotics carry a greater risk of generating antibiotic resistant microbes than local antibiotics[29]. Therefore, local antibiotic therapies are preferred despite their limitations because the overall impact and risk associated with systemic antibiotics is greater.[28] There are some cases like with refractory periodontitis where systemic antibiotic therapy is necessary, the use of antibiotic combinations may be a beneficial treatment since a variety of periodontopathic bacteria with different levels of susceptibility can be targeted, and the antibiotics can overcome the protective effects of the oral biofilm.[29] Combinational systemic antibiotic therapy should be used alongside mechanical periodontal therapy to increase the effectiveness of the treatment and limit the need for frequent or prolonged antibiotic therapy, reducing the development of antibiotic resistance.

Prophylactic Use in Dental Procedures

Recent studies have begun to evaluate the effectiveness and need for an antibiotic prescription for several invasive dental procedures. The results of these studies can be used to develop clearer guidelines for dentists and reduce antibiotic misuse and overuse.

In standard periodontics surgeries, antibiotics are usually prescribed to reduce the risk of infection following the dental surgery. Previous experiments have been done to observe if antibiotics had an influence on the healing process after regenerative periodontal surgery in immunocompetent patients. A year after surgery, the antibiotic showed no additional postoperative benefits.[31] For wisdom teeth extractions, antibiotics are used to prevent postoperative complications. Studies on antibiotic necessity for these surgeries have shown no significant evidence to support the need for antibiotic prophylaxis for wisdom tooth extraction(s)

in immunocompetent young adults[23] and adults.[32] There have not been signs of significant increases in resistant genes after the antibiotic courses either.[32] The length of the extraction procedure is an important factor to consider since longer procedures correlate with higher incidences of infection. Preoperative antibiotics may be more beneficial for complex extractions requiring ostectomy, so providers should consider the necessity of antibiotics for longer, complex surgeries until further research expands on this knowledge.[23]

Similar results in some implant studies for antibiotic use during implant procedures determined no statistically significant evidence to support the routine use of antibiotics to reduce the risk of implant failure and postoperative complications.[23] However, there have been other studies that obtained results that conflict on the outcomes of antibiotic benefits for implants. Some studies showed that antibiotics were beneficial for reducing implant failure but not significant in preventing postoperative complications or differences in the level of adverse effects.[23] Others found no evidence that antibiotics provided improvements to postoperative healing and symptoms, nor evidence demonstrating antibiotics reduced postoperative complications either for pre-, peri-, or postoperative prophylactic antibiotics.[33] The body of evidence may suggest that antibiotic use may have some benefits in certain situations but not others, but more research regarding prophylactic antibiotics and which antibiotics would be more potent are necessary.[23]

There are similar overlapping limitations to the studies on prophylactic antibiotic use and various dental operations. Many of these studies are underpowered, lacking in substantial population size, so broader application of the results is interpreted with caution. An experiment outcome for a small sample may display no increase in resistance genes, but if applied to a larger population size, the chance for mutations and gene transfer from the selective pressure

significantly increases. Some experiments did not contain a control for comparison, but for more serious clinical procedures, it may not be ethical to withhold antibiotic treatment, especially when current research conclusions remain unclear whether antibiotics play an important role during and after treatments. To combat these limitations and gain a clearer idea on the effectiveness and benefits of the prophylactic use of antibiotics, further research is required with larger sampling sizes and clearer distinctions on what outcomes can be contributed to the antibiotic or the processes of the patient. As the demand for these dental procedures continues to grow globally, the need for a better understanding on the usefulness of prophylactic antibiotics becomes more essential, especially with the continued expansion of antibiotic resistance in healthcare.

Microbiome Impact Beyond Oral Cavity

The health of the oral cavity can be an indicator for the overall health of the body. Chronic oral diseases like periodontitis can foster numerous life-threatening systemic diseases in the body from diseases in the digestive system to diseases in the nervous system.[25,34] What occurs in the mouth can impact other body systems in various ways. Two major pathways the oral microbiota influence overall health is 1) biofilm bacteria can restart their planktonic lifestyle and circulate to other parts of the body, and 2) the interaction between microbes and the host immune system may impact host susceptibility to certain disease development and/or the byproducts from the immune response can negatively affect other parts of the body when prolonged.[35,36]

The impact of antibiotic use has a similar connection. Antibiotics taken to treat an oral disease can impact other microbiomes in the body alongside the oral microbiome,[37,38] promoting the risk of opportunistic pathogens elsewhere in the body.[37] In comparing the impact of a single

treatment with various broad-spectrum antibiotics on different microbiomes, one study found the impact on the salivary microbiome was benign and short-lived, while there was a severe and prolonged effect on the gut microbiome from most of the antibiotics tested.[38] Clindamycin and ciprofloxacin especially had the most severe effect on the gut microbiome.[38] Clindamycin is a commonly prescribed antibiotic in dentistry for patients with penicillin allergies. The oral microbiota may be able to bounce back quickly after a broad-spectrum antibiotic course, while the gut microbiota may take longer to return to initial levels, so the influence of antibiotics on other microbiomes should be considered when prescribing a broad-spectrum antibiotic for healthy patients. The study did not collect the baseline microbiome compositions or the last oral hygiene practice like teeth brushing from the subjects beforehand.[38] These limitations should be considered since the acquisition of microbial populations and their amounts of resistant or sensitive bacteria can affect the outcomes of antibiotic use.

Concluding Thoughts and Going Forward

Dysbiosis Prevention

Development of species-specific agents and treatments would be the most effective options for preventing and remedying oral diseases. The optimal choice would be one that reduces or eliminates pathogenic species while preserving health-associated bacteria. Dysbiosis is not only caused by a shift in conditions favoring pathogenic bacteria but the depletion of beneficial bacteria also. Investigation into these possible agents and treatments is in early stages. There needs to be more research on multiple topics associated with understanding and treating oral health, but there are numerous possibilities currently being explored.

Target disease-causing bacteria

One mode of preventing the development or progression of some oral diseases is manipulating the oral environment. Since conditions like diet can influence the signal expression of bacteria, affecting or possibly changing the status of the oral microbiome, countermeasures against disease-prone diets may help maintain or reestablish health in the oral cavity. For example, high sugar diets are known to influence the pathogenesis of dental caries since bacteria like *S. mutans* benefit from the acidic environment and expand, while the number of health-associated bacteria like *S. sanguinis* decrease. Some of these bacteria, including *S. sanguinis*, produce ammonia through the arginine deiminase system (ADS). The ammonia neutralizes the acids produced by other bacteria like *S.* mutans.[39] If the environment shifts unfavorably for the ADS bacteria, their numbers will be impacted, changing the proportions between the oral bacteria, which can lead to the acidic byproducts outweighing the basic products. As a result, the biofilm becomes acidic and demineralizes the protective enamel of the teeth.

Increasing the level of arginine to the free-floating amount in the oral cavity could help shift and maintain an advantageous condition for alkali producing health-related bacteria. In experiments, arginine can reduce plaque biomass in polymicrobial biofilms by inhibiting EPS formation and limiting *S. mutans* adherence, while also contributing to pH homeostasis.[39] With reduced EPS, the thickness of the plaque is thinner, which makes it easier to remove[39] and more susceptible to antimicrobial treatments when necessary. Arginine does not kill *S. mutans* biofilm cells,[39] so there may not be a dramatic shift from a healthy ratio of bacteria. Arginine as more than a preventative additive requires more investigation. As a preventative measure, 1.5% arginine and fluoride toothpaste show promising results on the de- and remineralization balance of the tooth enamel, increasing the efficacy for cavity prevention than fluoride toothpaste

alone.[39,40] This could mean that using arginine with fluoride may be a useful force against dysbiosis shifts. Beyond dental caries, arginine can inhibit co-aggregation between *P. gingivalis* and other bacterial biofilm species.[39] For either dental caries or periodontal treatment, the long-term efficacy and impact of arginine on the microbiota should be better understood to determine any negative effects or limitations to arginine agents.

Other molecules that affect *S. mutans*' glucosyltransferases (gtfBCD) can diminish the adhesion of planktonic bacteria, reducing biofilm formation or weakening its architecture.[41] This will have similar results of easier removal and increased susceptibility to antimicrobials.

Chlorhexidine is an antiseptic used in hand soaps, wipes, surface cleaning solutions, and mouthwash. There is growing concern regarding the possible selection for resistant microbes with widespread and consistent application of chlorhexidine in hand hygiene practices.[42,43] If applicable, there should be consideration on how the resistance to chlorhexidine mouthwashes influences gingivitis treatments, where these mouthwashes are mostly used. The cross-resistance connection to antibiotics is controversial.[43] For skin applications, if there is an immense exposure to chlorhexidine, there may be an increased risk of resistance to antibiotics like ampicillin, gentamicin, and tetracycline[44] through certain genes shared in the response mechanisms between the two microbicidal agents.[43] The measurement of chlorhexidine resistance in the oral cavity has very little testing. Studies that have been done on these mouthwashes determined that a high chlorhexidine concentration was insufficient at eliminating all bacterial cells in a biofilm,[42] so there is the existence of tolerant microbes which may or may not contribute to the risk of resistance development. If oral or skin resistance is possible, the broader implications of skin and oral resistance transfer should be determined.

Blocking Biofilm Formation

There is research on developing antimicrobial surfaces that limit various adherence mechanisms and quorum sensing.[4,40,45] These methods may be excellent in application to artificial surfaces like implants and restorative materials to prevent secondary caries, specifically on the surfaces within the filled cavity. Places that would benefit from biofilm inhibition should be the target of biofilm blocking agents. The natural oral niches like teeth, gums, tongue, etc. are unlikely to benefit from biofilm prevention since oral health is tied to oral microbiota similar to the relationship between gut health and gut microbiota. Also, like the gut microbiome, the health of the oral cavity carries over to the health of various systems in the body. Preventing biofilm formation can also block commensal bacteria from adhering, thus leaving the body more susceptible to infection and disease.

Probiotics/prebiotics

There is ongoing research into the benefits and effectiveness in probiotics and prebiotics on gut microbiota and controlling gastrointestinal diseases. This research has led to the question if the results from the gut can be translated to the mouth.[26] Probiotics could be used as part of the prevention of pathogenic bacteria adherence or colonization by competing for binding sites and/or nutrients just as commensal microorganisms do.[26] The effectiveness of around a million cultured bacteria versus over a billion bacteria in the microbiomes is a question of interest. Under normal, healthy conditions, is it possible for probiotic numbers to be significant enough to influence the microbiomes already present? Perhaps under depleted circumstances like after taking a broad-spectrum antibiotic, will a probiotic have an effect on the microbiota state. During those conditions, if there is an effect, then the question of controlling the shifts in microbes and their interactions should be evaluated. A beneficial species of bacteria or a certain ratio of

beneficial bacteria may not remain beneficial once established in the microbiome and runs the risk of possibly becoming pathogenic or causing harm. There is also the question of administration. If the target is the oral cavity, then determining how the probiotic should be delivered without causing harmful side effects is important.

The oral cavity shares a passageway with the gastrointestinal microbiomes. Probiotics or prebiotics taken to influence the oral microbiota will also influence the microbiomes along the rest of the digestive system and possibly the other microbiomes of the body. This body-wide connection makes studying broad applications of probiotics and prebiotics (and also antimicrobial agents) difficult to design and execute. Narrower, local applications may prove easier to analyze the effectiveness of probiotics and prebiotics on the oral microbiota.

Areas Needing More Research

More research on the development of oral biofilms, biofilm microbial interactions, conditions that favor dysbiosis, and other factors that shift the microbiota to become more pathogenic is necessary to better understand and advance dental and oral treatment and therapies. The health of the oral cavity is determined by the bacteria present, their numbers, and their interactions with other microorganisms. Additionally, there is less research on the influences of other microorganisms like fungi, viruses, and protozoa in the oral microbiome, and their role in oral health and disease pathogenesis. A clearer picture on the biofilm interactions between oral microbes and how they may respond to various environmental shifts will allow better efficacy in the necessary antibiotics. More effective antibiotic treatment will help reduce the rate of resistance development and spread in healthcare.

An individual’s immune function, diet, and hygiene abilities change as their lives progress; likewise, the oral microbiota change throughout life also. Children, especially, are

currently developing their microbiomes, so they are more vulnerable to changes to the biofilms due to external pressures. Since children are more likely to be treated with antibiotics compared to adults, the outcomes of antibiotic use and potential risk of shifts towards future oral diseases should be considered in future antibiotic research. The relationship between antibiotics and oral microbiota in the elderly should also be examined, particularly because immune competency and physical abilities diminish with age. The greater chance of antibiotic prescriptions and less proper oral hygiene practices in the elderly may pose a greater risk of antibiotic resistance and the status of their oral health.

Procedures in dentistry that use long-term antibiotic treatment and its impact on resistance development in the mouth, and its spread to other microbiomes in the body should be examined. The evaluation on the advantages and efficacy of prophylactic antibiotics for various dental and oral procedures can help create a general antibiotic guideline for dentists in the United States.

Dental practices and other healthcare providers may want to consider the inclusion of an overall history of antibiotic prescriptions from the patients similar to general medical history. This would include information on what antibiotics they have taken, how frequently they are prescribed an antibiotic, the dosage and duration of the antibiotic course, and when and why they had to take an antibiotic. This knowledge will help when determining if an antibiotic is beneficial versus too risky, or when determining which antibiotic would be more optimal, treating the patient efficiently and quickly to reduce any risk of developing resistance.

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