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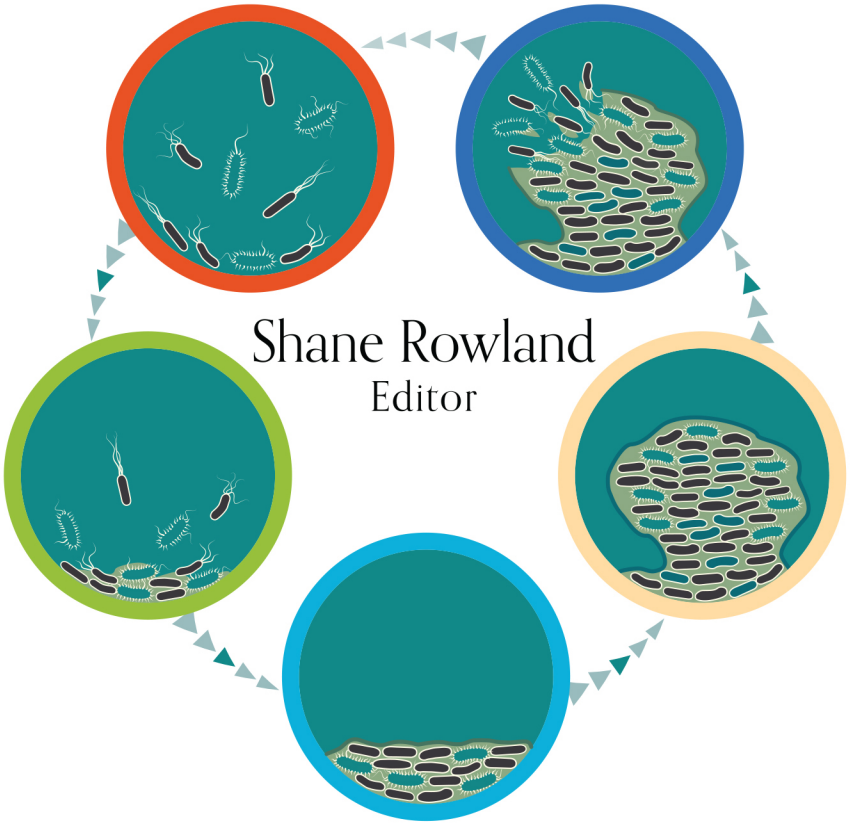

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SHANE ROWLAND
EDITOR

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Chapter 2

THE COMPLEXITY OF THE DYNAMIC ORAL MICROBIOME AND BIOFILMS IN ORAL CAVITY WITH THEIR MANAGEMENT

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ABSTRACT

The human oral cavity is a complex ecosystem and is an ideal environment for cell growth and development. Through molecular biology technology, approximately 1,000 different microbial species that live on the surface of teeth as dental biofilms were found. Diseases in the oral cavity as well as systemic diseases can be said to be closely related to the presence of biofilms in the oral cavity because these bacteria can spread to other parts of the body. The imbalance of interactions between microbes and the environment in the oral cavity can lead to disease development in the oral cavity because the oral microbiome plays an important role in maintaining the health of the oral cavity.

Biofilm is a component consisting of bacteria in a self-produced polymeric matrix, attached to an inert surface, alive, and can survive because of its ability to capture nutrients and withstand adverse environmental conditions. Biofilm is used for a term that describes complex communities of heterogeneous/multispecies microorganisms attached to the extracellular matrix. Bacterial interactions with each other in biofilms occur through nutritional interactions, genetic changes, and signal quorum sensing which are closely related to the development of dental biofilms and their relationship to clinical conditions can be examined using culture or genetic testing. However, biofilms also have unique self-protective properties and are resistant to the antimicrobials used, through their ability to generate gene expression and phenotype. Mature biofilms are complex consisting of approximately 100 species of bacteria that can cause diseases in the oral cavity such as caries, gingivitis, and periodontitis.

Recent research has focused on a deep understanding of the oral microbiome, including the nature of the microbes that play a role in oral disease, their interactions with the body, and how these communities affect health and disease progression in the oral cavity and can be used to determine the diagnosis of a disease, which will have important implications in determining management strategies for prevention and therapy related to biofilms in the future. In preventive efforts, chemical management of biofilms aims to prevent adhesion and inhibit the formation of biofilms due to the sensitivity of microorganisms to active biological components.

In recent years, much attention has been placed on the study of phytochemicals for their antibacterial activity and many studies have shown that phytochemicals exert their antibacterial activity through different mechanisms of action, such as damage to the bacterial membrane and suppression of virulence factors, including inhibition of the activity of enzymes and toxins, and bacterial biofilm formation. The uses of natural ingredients, especially medicinal plants, have increased along with the high price of medicines and the phenomenon of resistance from chemical drugs.

In this chapter, we will discuss how to manage oral microbial biofilm using chemical and herbal medicine.

Keywords: oral biofilms, preventive, management, chemical, phytochemicals

ORAL MICROBIOME

The oral cavity is a very complex ecosystem and is an ideal place for the growth of microorganisms because it has a temperature that is around ±37^{o}C and the ideal pH in the saliva is 6.5-7. Because of these conditions, in the oral cavity, there are various kinds of microbiota including bacteria, fungi, viruses, protozoa, archaea, and other microbes. This condition is known as the oral microbiome or oral microbiota. The oral microbiome can also be defined as a collection of microbial genomes found in the oral cavity. In humans, the oral microbiome is very unique and varies depending on their lifestyle, biological changes, and includes changes in the physiology of each individual that causes very rapid changes that occur both in activity and also in composition (Kilian et al. 2016; Willis and Gabaldón 2020; Deshmukh, 2019).

The development of the oral microbiome begins immediately after the baby is born, with the initial colonization of microbes in the baby's mouth due to contact with the uterus and vagina of the mother at birth or by food entering the baby's oral cavity. This is what causes the microbial acquisition process to begin, with the pioneer organism being *Streptococcus salivarius*. Entamoeba and Trichomonas are the most common and saprophytic protozoa, while the most common type of fungus in the oral cavity is Candida. Any changes in the oral environment will affect its development. The presence of this microbiota plays an essential role in maintaining oral health and the imbalance of these microbes will cause diseases in the oral cavity (Deshmukh, 2019; Oh et al. 2020; Nithya, Saxena, and Kharbanda 2020).

Microbes in the oral cavity can colonize on two kinds of surfaces, namely hard surfaces such as teeth and soft tissues of the oral mucosa such as the gingival sulcus, buccal mucosa, tongue, cheeks, tonsils, soft palate, hard palate, and floor of the mouth. When the tooth begins to erupt, various species of organisms will colonize the tooth surface and will develop in the gingival sulcus. Along with the aging process and when all the teeth fall out, the flora in the oral cavity will become similar to the condition when the teeth don't erupt (Deshmukh, 2019; Willis and Gabaldón 2020).

The microbiome in the oral cavity consists of the core microbiome which is the dominant species in healthy conditions and the variable microbiome which is the microbiome that has evolved in response to lifestyle changes. This oral microbiome plays an important role in biofilms formation, which will form an ecosystem. The balance between the host and microbes will maintain the oral cavity to remain in a healthy state so that if there is an imbalance it will cause disease in the oral cavity until systemic disease occurs. In a healthy state, microbes live in a homeostatic balance with the host, where these microbes will provide several advantages to the host. However, this balance can change and cause disease (Deshmukh, 2019; Kilian et al. 2016; Sterzenbach et al. 2020).

The oral microbiome can be identified by various methods, ranging from traditional methods such as culture to the development of “omics” methods, namely microbiomics and metagenomics. Through this technology, in the oral cavity can be found approximately 1,000 different species of microorganisms can live. These bacteria can colonize the tooth surface, such as on enamel and dentin, and can colonize the epithelial surface of the oral mucosa to form biofilms with various compositions. This can occur depending on the specific surface, location in the oral cavity, oral health status, and environmental conditions of the oral cavity, such as carbohydrate intake and gingival crevicular fluid flow (Deshmukh, 2019; Berger et al. 2018; Sterzenbach et al. 2020).

Biofilm

Biofilm is a dense community formed by approximately 95% of bacteria in the oral cavity, which then adheres to the surface to associate to form a multicellular community that is wrapped in the extracellular polysaccharide matrix (EPS) it produces and adheres to one another. EPS will create a different microenvironment and will affect changes in microbial behavior, including changes in redox, pH, and nutrients. In detailed biofilms, this EPS is produced by *Streptococcus mutans*. Biofilms also show changes in phenotypes related to growth rates and changes in gene expression patterns as a

form of adaptation to environmental changes, so that they will play a very important role as virulence factors in the formation of caries (Kilian et al. 2016; Rita Chandki, Priyank Banthia 2011; Berger et al. 2018; Jiao et al. 2019; Kuang, Chen, and Xu 2018; Nithya, Saxena, and Kharbanda 2020; Du and Bonsu 2015; Rajiv Saini and Sharma, 2011).

The growth of microorganisms in a specific environment is influenced by several factors, including environmental pH, nutrient availability, the presence of antimicrobial agents, and host defenses. In its development, these microorganisms will adapt physiologically and morphologically to various changes in the microenvironment that occur such as changes in oxygen, nutritional limitations, antimicrobial agents, and chemical changes to survive, resulting in the formation of a mature biofilm (Biradar et al. 2017; Rajiv Saini and Sharma, 2011; Yu et al. 2017).

Biofilm Formation

Biofilms are not new; their development is a dynamic process and can occur in both biotic and abiotic environments. The composition of the biofilm consists of bacterial cells and other materials bound in an extracellular polysaccharide matrix (±90% of the mass of the biofilm). In addition, biofilms are also composed of adhesive fibers, flagella, fili, proteins, and extracellular DNA (Nithya, Saxena, and Kharbanda 2020). The main characteristic of biofilms is microcolonies inside the biofilm which are attached to solid surfaces. Thus, an important first step in the development of biofilms is surface adhesion (Rajiv Saini and Sharma, 2011). When the bacteria are about 10-20 nm from the surface, the negative charge from the surface will repel the negative charge on the bacterial surface, which in turn will result in a mechanical bond between the bacteria and the surface through the surface, van der Waals forces, and the use of fimbriae and flagella (Du and Bonsu 2015).

The formation of biofilms occurs due to complex interactions between the host, food, and microorganisms in the biotic and abiotic environment. The development of biofilms occurs through several stages of the mechanism starting from the initial attachment consisting

of reversible attachment and irreversible attachment. After that, it was continued with biofilm maturation through passive transport of bacteria due to the weak long-distance attraction, and then a very strong bond occurred due to covalent bonds and hydrogen bonds. After the ripening stage, the biofilm will then spread. Pseudomonas, Vibrio, Escherichia, Salmonella, Listeria, Streptococcus, and Mycobacteria have been recognized as the cause of biofilm formation (Rita Chandki, Priyank Banthia 2011; Berger et al. 2018; Jiao et al. 2019; Kuang, Chen, and Xu 2018; Nithya, Saxena, and Kharbanda 2020; Du and Bonsu 2015).

Specifically, biofilm formation begins with the presence of an acquired pellicle which is an attachment for salivary proteins and peptides such as proline-rich proteins, lysozyme, alpha (α)-amylase, peroxidase, histatins, mucin, statherins, and also contains proteins and other macromolecules from the gingival crevicular fluid, mucosa, blood, bacteria, and food that are selectively absorbed on the tooth surface which is the site of attachment of bacteria and glucosyltransferases (Gtfs). The pellicle here has many benefits including as a lubricant, protecting the tooth surface, as a protective layer against erosion by preventing hard tissue decalcification. The pellicle also contains several antibacterial components such as lysozyme and peroxidase (Kalesinskas et al. 2014; Oh et al. 2020; Sterzenbach et al. 2020).

The first phase in biofilm formation is the attachment phase; this phase is the initial stage that lasts for a few seconds. The initial attachment of proteins or microorganisms is a diverse process through physical and chemical interactions. This stage is the transport of microorganisms on the surface which is followed by the occurrence of protein adsorption, the formation of a layer on the surface, and is reversible. This phase is modulated by the environment due to signals from several factors such as oxygen concentration, pH, temperature, nutrients, and so on. The occurrence of initial attachment is generally mediated by specific adhesins possessed by bacteria. The initial attachment of planktonic microbes occurs through fili, proteins, flagella, and polysaccharide adhesions on the surface. Currently, flagella and fili play an important role, where flagella play a role in the initial interaction between cells and the surface. Bacteria found in the early stages of biofilm formation include Streptococci, Neisseria, Rothia, Actinomyces,

or Veillonella (Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017; Du and Bonsu 2015; Sterzenbach et al. 2020).

The next phase is a transition phase, in this phase, the reversible attachment turns into an irreversible attachment. This phase occurs in a matter of minutes, at this time the attachment of planktonic bacteria and initial irreversible colonization occurs. Several hours later, auto aggregation (attraction between the same species) and coaggregation (attraction between different species) occurred which resulted in the functional organization of plaque bacteria and the formation of different morphological structures to form secondary colonies. Currently, the microenvironment is changing from aerobic/capnophilic to facultative anaerobic, decreasing pH, and releasing bacterial products, as well as communication between bacterial cells through chemical signals that are responsible for the formation of the extracellular matrix (EPS) which is the main matrix-forming biofilms by activating the mechanism genetic material so that a mature biofilm will be obtained (Rita Chandki, Priyank Banthia 2011; Berger et al. 2018; Jiao et al. 2019; Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017; Du and Bonsu 2015; Sterzenbach et al. 2020).

After 24 hours and the first biofilm layer was formed, then there was an organization and incorporation of new bacteria into the biofilm. The same or different bacterial species will be drawn into the biofilm. After that, the formation will reach its peak, which is then known as the maturation phase or maturation phase. In this phase, the plaque thickness will gradually increase over time and reach a thickness of 20 to 30 m within three days. The last phase is the dispersion phase or known as the dispersion phase, this phase is important for the biofilm life cycle. Biofilms can spread due to various factors, including lack of nutrients, large populations, and intense competition (Rita Chandki, Priyank Banthia 2011; Nithya, Saxena, and Kharbanda 2020; Du and Bonsu 2015).

The structure of the biofilm depends on several factors, including genotype factors, environmental physicochemical factors, nutritional sources, mechanical factors, and so on. This will also affect determining the important characteristics of bacteria in biofilms (Rita Chandki, Priyank Banthia 2011; Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017). The structure of a mature biofilm is usually

composed of several layers, starting from the presence of bacterial microcolonies, the formation of a matrix consisting of several extracellular polysaccharides, proteins, and deoxyribonucleic acid (DNA). In the biofilm, there are also channels filled with air, water, and nutrients for bacteria. Bacterial microcolonies in the biofilm structure contain various combinations of different bacterial species, for example, bacteria that live in the center of the microcolony will be anaerobic and other parts close to waterways will be aerobic (Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017; Rajiv Saini and Sharma, 2011; Du and Bonsu 2015).

The extracellular polysaccharide matrix (EPS) produced by bacteria is the major component of the biofilm mass, which is about 50-95% of the dry weight of the biofilm. This matrix is formed from water-insoluble glucan, which is the result of the interaction between Glucosyltransferase (Gtfs) with sucrose and starch hydrolysate. EPS will play an important role in maintaining the integrity and stabilization of the biofilm because this matrix acts as a structural builder of the biofilm. In addition, this matrix also plays a role in providing a place for bacteria to adhere and accumulate so that bacteria can be protected from changes in pH, act as a buffer, protect against ultraviolet radiation, osmotic changes, adhesion, as nutrients in the biofilm, provide mechanical stability, and increase the acidic nature of the biofilm. Because of this ability, this makes the biofilm difficult to remove from the surface (Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017; Rajiv Saini and Sharma, 2011; Kalesinskas et al. 2014; Oh et al. 2020).

Based on recent research, it is shown that EPS not only acts as a scaffold in the growth and maturation of biofilms but also shows the ability of biofilms as a surface attachment site and increases tolerance to antimicrobial agents. Therefore, it is necessary to investigate the most economical and effective way to control biofilms because of the increasing resistance to antibiotics and other antimicrobial agents (Kuang, Chen, and Xu 2018). Some proteins will adhere to the cell surface and polysaccharides to help the formation and stabilization of biofilms, as well as DNA which also has an important role in the formation of biofilms, especially when the biofilm attachment begins with a repulsion force on the initial attachment due to a negative charge which ultimately results in DNA interaction with receptors on the

substrate surface to facilitate adhesion This occurs when the distance between the cell and the surface becomes several nanometers (Du and Bonsu 2015).

The unique nature of the biofilm function depends on the signaling process associated with the regulation of specific genes that will mediate communication between bacterial cells and their colonies thus mediating gene expression in bacteria. Secreted peptides are signaling molecules in Gram-positive bacteria, whereas in Gram-negative bacteria there are two different quorum sensing systems, which use different types of automatic inducers. Changes in the physiological properties of bacteria can also lead to different properties for biofilms, by increasing the growth of beneficial bacteria and inhibiting the growth of competing bacteria. This can happen because of quorum-sensing (Rajiv Saini and Sharma, 2011; Lu et al. 2019).

Quorum-sensing or communication between cells also plays a role in regulating the expression of certain genes through the accumulation of signaling compounds between cells that mediate communication between cells. This quorum-sensing will result in the density of the bacterial cell population and produce different phenotypes in the development of biofilm formation (Rita Chandki, Priyank Banthia 2011; Nithya, Saxena, and Kharbanda 2020; Biradar et al. 2017). The density of bacterial cells in the biofilm will facilitate the horizontal exchange of genetic information between cells of one species or different species (Rajiv Saini and Sharma, 2011).

The presence of water channels in the biofilm located between the microbial microcolonies will facilitate the exchange of materials between the external and internal environment to provide sufficient nutrients through the diffusion process. This causes the bacteria in the biofilm to communicate both intraspecies communication (quorum-sensing) and inter-species communication (peptide molecule release), exchange genetic material, and also acquire new properties so that this channel can be called a circulating system in the biofilm (Biradar et al. 2017; Rajiv Saini and Sharma, 2011).

Biofilm Prevention and Management

Biofilms have a role in protecting microbial components from host defense mechanisms that can affect their environment by changing gene expression and the phenotype of the organism. In addition, biofilms can also play a role in increasing resistance to antimicrobial agents. A potential approach to controlling biofilm development is to interfere with the development of each stage of biofilm formation. This is a global challenge that can be done to prevent further infection. Various substances play an important role in interfering with the mechanism of biofilm formation (Jiao et al. 2019; Nithya, Saxena, and Kharbanda 2020; Kuang, Chen, and Xu 2018).

The latest developments in science and technology provide a new approach in the control and characterization of biofilms, so this is a new challenge in the field of dentistry. This view provides a general understanding of biofilm development, biofilm control by developing biofilm formation inhibitory agents aimed at preventing early biofilm formation or biofilm dispersion agents aimed at disrupting biofilm communities using chemical and biological methods, and an overview in analyzing biofilms. Compared to bacteria in the planktonic form, bacteria in biofilms require higher concentrations of antimicrobial agents to eliminate them, because these bacteria are more resistant (Deshmukh, 2019; Nithya, Saxena, and Kharbanda 2020; Jiao et al. 2019; Kuang, Chen, and Xu 2018; Yu et al. 2017).

In biofilms, extracellular polysaccharides produced by bacteria in the biofilm will act as a bacterial protective barrier in preventing the perfusion of antibacterial agents from hitting the target bacteria, protecting against environmental threats such as antibiotics, antibodies, surfactants, bacteriophages, and white blood cells. Based on the research that has been done, recently it is known that microorganisms in biofilms also show varied behavior and show new characters that produce more than one biofilm with functionally different properties. This can produce biofilms that are or are not resistant to environmental challenges. Microbial species that grow in the biofilm itself are more resistant, which is about 1000-1500 times greater to antibiotics than microbes that develop in planktonic form (Nithya, Saxena, and

Kharbanda 2020; Rajiv Saini and Sharma, 2011; Du and Bonsu 2015; Yu et al. 2017).

The management of biofilm-associated infections suffers a major setback due to the ineffectiveness of available antibiotics due to the protective layer built by cells within the biofilm. This results in limited antibiotic penetration so that the cell community persists (Du and Bonsu 2015). However, an important mechanism of resistance is the slower growth rate of bacteria within the biofilm, which makes them less susceptible to some antibiotics. Bacterial resistance to antibiotics is influenced by nutritional status, temperature, pH, growth rate, and previous exposure to less effective antimicrobial concentrations. Variations in any of these parameters can lead to varying responses to antibiotics. Slower growing bacteria often overexpress non-specific defense mechanisms including multidrug efflux pumps, heat-shock proteins, and an increase in exopolymer synthesis (Rajiv Saini and Sharma, 2011). The reason for the increase in antibiotic resistance in biofilms is still not fully understood, many factors play a role in protecting biofilms from antibiotic treatment. Several possible mechanisms of antibiotic resistance shown by biofilms are limited antibiotic penetration, horizontal gene transfer, reduced growth rate, persistent cells, efflux pumps, EPS matrix protection (Du and Bonsu 2015).

The biofilm structure itself has unique properties with its ability to influence changes in gene expression and phenotype that can protect microbes from disinfectant or antibiotic agents, causing an increase in antimicrobial tolerance which can also be caused by the presence of EPS in biofilms. In addition, EPS can also protect bacterial cells in deeper layers against antimicrobial agents by inhibiting the diffusion of these agents. Quorum-sensing is a communication tool from cell to cell that is closely related to the development of biofilm formation and inhibition. The resulting biofilm is a primary causative factor for caries, gingivitis, and periodontitis which in turn can cause serious public health problems due to the poor response of biofilms to traditional antimicrobial therapy. Therefore, various antimicrobial agents were developed to prevent the occurrence of initial bacterial attachment and biofilm formation through three strategies, namely: release of anti-microbial agents, contact killing, and multi-functionality. The destruction

of mature biofilms essentially requires disruption of the EPS matrix. The formation of new biofilms can be inhibited by preventing early processes such as cell attachment to the surface (Berger et al. 2018; Jiao et al. 2019; Kuang, Chen, and Xu 2018; Nithya, Saxena, and Kharbanda 2020; Du and Bonsu 2015; Takenaka, Ohsumi, and Noiri 2019; Sterzenbach et al. 2020).

There are several approaches that can be used to control biofilms in the oral cavity, including preventing the formation of biofilms by preventing initial attachment and destroying the biofilms that have already formed. Therefore, the biofilm control agent required is not only bacteriostatic but also has an anti-adhesive action to prevent the initial attachment of bacteria (Du and Bonsu 2015; Yu et al. 2017; Kouidhi, Al Qurashi, and Chaieb 2015).

Biofilm inhibition can be carried out in various steps, namely (Ghosh, Jayaraman, and Chatterji 2020):

Prevents Bacterial Surface Attachment or Initiation Step

The attachment of bacterial cells to the substrate or surface is the first step in the successful formation of biofilms. To prevent this from happening, it is necessary to modify the surface of the biomaterial. Modifications can be done with the help of metal ions, antibiotics, and other synthetic compounds. In the medical field, medical devices can be coated using bactericidal antibiotics that aim to kill pathogenic bacteria that come into contact with external surfaces, using zinc oxide nanoparticles on glass, and using iodine to coat titanium implants. In addition, the antimicrobial properties of covalent 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (QAS) can be exploited to prevent biofilm formation. In preventing biofilm formation, a similar approach aimed at preventing the initial attachment of bacteria to the device or host surface could use an anti-adhesion surface coating. This layer uses chemical compounds to change the surface texture, hydrophilicity and hydrophobicity, and surface roughness (Kuang, Chen, and Xu 2018; Ghosh, Jayaraman, and Chatterji 2020).

Disorders of the Quorum-Sensing (QS) System

Quorum-sensing (QS) refers to bacterial communication systems at the molecular level in which bacteria to controls the activity of many

mechanisms important for the microbial physiology, including production of biofilm. Quorum sensing responds to different environmental signals and translate messages by up-regulating or down-regulating a set of genes involved in stress virulence, tolerance, and biofilms (Ghosh, Jayaraman, and Chatterji 2020; Krzyżek 2019).

Modulation with Second Nucleotide Messenger Signaling Molecules

Cellular metabolic conditions can change due to the accumulation of second messenger nucleotide molecules under certain suboptimal growth which will translate into phenotypic changes. Guanosine pentaphosphate (pppGpp) and guanosine tetraphosphate (ppGpp) are the second most studied messenger molecules. Under nutrient-limited conditions, this molecule can play an important role in many biological processes to ensure its survival. Guanosine pentaphosphate (pppGpp) has also been shown to be associated with biofilm formation, biofilm dispersion, and quorum sensing, in many bacteria (Ghosh, Jayaraman, and Chatterji 2020; Fontaine, Duggal, and Weinert 2018).

Another important second messenger as a signaling molecule in bacteria is bis(3 bis,5′)-cyclic diguanylic acid (c-di-GMP) which is known to regulate various physiological processes in response to environmental stimuli. c-di-GMP has been shown to play a key role in biofilm formation and quorum-sensing.

Intracellular c-di-GMP levels also contribute to growth retardation, drug tolerance, and biofilm formation (Ghosh, Jayaraman, and Chatterji 2020; Fontaine, Duggal, and Weinert 2018).

The c-di-GMP and pppGpp signaling pathways directly contribute to the development of antimicrobial resistance and can therefore be used as promising therapeutic approaches to reduce biofilms (Ghosh, Jayaraman, and Chatterji 2020).

Chemical Inhibition of Biofilm Formation and Maturation

Recently, various methods have been developed to control the formation and maturation of biofilms, including the use of natural or synthetic antibiofilm agents aimed at disrupting the surface properties of Gram (+) and Gram (-) bacteria (Ghosh, Jayaraman, and Chatterji 2020).

Disrupts Mature Biofilm

A large number of bacterial infections are associated with the formation of biofilms that serve as drug-tolerant reservoirs. Cellular exopolysaccharides are important components of biofilms. Extracellular DNA (eDNA) is a target in the destruction of biofilms because it is one of the important components of biofilms (Ghosh, Jayaraman, and Chatterji 2020).

Several anti-biofilm strategies have been developed to inhibit every step of biofilm formation which can be done by adding agents to various oral health products such as toothpaste and mouthwash, which can inhibit the growth of bacteria in the oral cavity. Based on the research that has been done, mouthwash is more often used as a medium to carry antimicrobial agents that can assist in mechanical control and maintain oral health. The active components contained in mouthwash also come in different formulas, including delmopinol (Del), hexetidine, povidone-iodide, essential oil (EO), chlorhexidine gluconate (CHX), cetylpyridinium chloride (CPC), and hydrogen peroxide. The use of this agent can prevent the early stages of biofilm formation, namely preventing the initial attachment of bacteria or other microorganisms. Although the use of this agent is recommended as an antibacterial agent, some things must be considered, namely the presence of side effects in its use. For example, prolonged use of chlorhexidine gluconate can cause extrinsic staining of teeth, calculus formation, changes in taste sensation, burning sensation, hypersensitivity, anesthetic sensation, and other effects on soft tissues in the oral cavity, such as lesions on the oral mucosa (Kuang, Chen, and Xu 2018; Takenaka, Ohsumi, and Noiri 2019).

Prevention of biofilm formation can be done by inhibiting the initial attachment of bacteria to the surface and disrupting the quorum-sensing system by using small organic compounds. On the other hand, chemical inhibition of biofilm maturation, modulation with second nucleotide messenger molecules, and disruption of mature biofilms can be achieved by inhibition of specific pathways (Ghosh, Jayaraman, and Chatterji 2020). An ideal antibiofilm approach needs to be developed to eliminate pathogens and inhibit new biofilm formation while avoiding commensal deletion which can cause microecological dysbiosis. New treatment strategies to prevent biofilm formation aim to eliminate or

minimize drug resistance. This shows different results from the previous use of conventional antibiotics (Kuang, Chen, and Xu 2018).

In the following, we describe several new strategies to disrupt or inhibit biofilm formation in the oral cavity, including nanomaterials, small molecules, quaternary ammonium salts (QAS), arginine, and other natural products (Kuang, Chen, and Xu 2018).

Nanomaterials

Nanomaterials have been known and used in various fields since the 1980s and can be used to control the formation of biofilms, including silver, chitosan, copper oxide, titanium oxide, zinc oxide, quaternary ammonium polyethyleneimine, silica, and graphene. A "smart" drug delivery system is a drug delivery system by nanoparticles whose release is triggered by environmental stimuli such as pH, glucose, or bacterial products. The inhibition of the proliferation of microorganisms and the prevention of biofilm formation can also rely on the physicochemical properties of the nanoparticles (Kuang, Chen, and Xu 2018; Singh and Rengan 2019).

Various types of nanomaterials have excellent potential for microbial growth, whether they are based on lipids, metals, or polymers. Metal-based nanoparticles that are often used as antimicrobial agents are gold, silver, magnesium, iron, and copper. The most effective nanoparticles against oral pathogens are silver nanoparticles (AgNPs) and silver nitrate. The disadvantage of silver nitrate is that it can cause discoloration of the dentin, but its application as a dentin coating can protect dentin from tooth decay, plaque, and secondary caries. Chitosan is a natural cationic polysaccharide that has biocompatibility, biodegradability, non-toxicity, and has good antimicrobial activity under acidic conditions. This happens because chitosan has poor solubility when the pH is above 6.5. Chitosan nanoparticles (CNPs) have a smaller size than chitosan, but because they have a higher surface charge density, these nanoparticles still have antimicrobial activity. This causes CNPs to interact with the negatively charged surface of bacterial cells and cause bacterial cell death. Silica nanoparticles can inhibit bacterial attachment and have

the advantages of high drug molecule loading capacity, good dispersion, lower cost, relatively high biocompatibility, and are available for special designs (Kuang, Chen, and Xu 2018; Singh and Rengan 2019).

Small Molecules

Small molecules can be used to control biofilm formation because of their good stability, low toxicity, activity at low concentrations, antibiofilm activity, have good antibacterial activity, and prompt low drug resistance (Kuang, Chen, and Xu 2018; Hussain 2021).

Quaternary Ammonium Salts (QAS)

One material that has a broad spectrum of biological activity is quaternary ammonium salt (QAS). QAS has bacteriostatic, antiviral, fungistatic, tuberculostatic, sporostatic, and analgesic properties. When compared with other conventional antibacterial agents, quaternary ammonium salt (QAS) is chemically stable, has a high molecular weight, is non-volatile, has broad antimicrobial activity and low toxicity. Against *S. mutans*, *Prevotella nigrescens*, and *F. nucleatum* QAS showed strong antibacterial and antibiofilm effects. This effect can occur because of the bond between the positive charge and the negatively charged bacterial cell membrane, which then results in a QAS reaction with lipids and proteins in the cell membrane. This causes disorganization of the cell structure and leakage, which then causes low molecular weight components to exit the cell. Then, there is the degradation of proteins and nucleic acids in the cell which causes the lysis of the components of the bacterial cell membrane (Kuang, Chen, and Xu 2018; Hussain 2021; Kwaśniewska, Chen, and Wieczorek 2020).

Arginine

Arginine as a natural food supplement is used and is a basic generation substrate for oral bacteria that can play a role in preventing the formation of dental caries because it can act as an oral biofilm ecomodulator. Arginine has good activity against bacterial growth, coaggregation, virulence, and biofilm formation. This can occur by suppressing the production and composition of glucans on the extracellular membrane so that the attachment activity of *S. mutans* on the tooth surface is inhibited. Pathogenic bacteria in the oral cavity such as *P. gingivalis*, *E. faecalis*, *C. albicans*, *A. naeslundii*, *A. odontolyticus*, *F. nucleatum*, *Lactobacillus acidophilus*, and *A. actinomycetemcomitans* can be inhibited by the presence of polycationic proteins rich in arginine and protamine. L-arginine was able to reduce polymicrobial dental biofilm biomass, especially inhibiting *S. mutans* biofilm formation by reducing the production of water-insoluble EPS (Kuang, Chen, and Xu 2018; Hussain 2021).

Natural Products

Antibiotic therapy can lead to resistance to antimicrobials. This needs to be a concern because it can hinder the process of prevention and effective treatment of various infections. In recent years, great scientific and technological advances have occurred in the fields of chemistry and pharmacology aimed at obtaining new compounds having therapeutic properties. Some of these compounds may provide sustainable solutions to combat the drug resistance of microorganisms (Jose et al. 2017; Milho et al. 2021).

Medicinal plants are a rich source of biologically active/bioactive/bionutrient components, these components can be found in seeds, roots, leaves, flowers, or even all parts of the plant. Bioactive compounds contained in plants are secondary metabolites that can be classified based on their composition, a simple classification of bioactive compounds includes three main groups, consisting of phenolic compounds, terpenoids, and alkaloids, which represent about 90% of all secondary metabolite compounds. A small

group of secondary metabolites includes saponins, lipids, carbohydrates, ketones, isoflavonoids, tannins, coumarins, glycosides, and polyacetylenes, and others (Kuang, Chen, and Xu 2018; Nithya, Saxena, and Kharbanda 2020; Milho et al. 2021).

This biologically active ingredient has a promising biological activity to be used as an alternative therapy or additional therapy for oral biofilms due to its antimicrobial activity against planktonic and monospecies biofilm microorganisms. Many natural products of plant origin have antimicrobial and anti-biofilm functions in vitro. The anti-biofilm effect of natural products is on the inhibition of polymer matrix formation, suppression of cell adhesion and adhesion, interfering with ECM generation, and reducing the production of virulence factors, thereby blocking QS network and biofilm development. Based on the research that has been done, it is found that the use of medicinal plants is closely related to the occurrence of eradication of biofilms, by reducing the total number of bacteria from attached microorganisms. Polyphenols can be found as active compounds in various natural products which can be described as substances containing at least one aromatic ring with one or more hydroxyl groups and other substituents. polyphenols can be found in tea, wine, pine, coffee, manuka honey, propolis, pomegranate, cranberries, garlic, galla chinensis, and cocoa polyphenols (Kuang, Chen, and Xu 2018; Karygianni et al. 2016; Lu et al. 2019; Jose et al. 2017).

The use of herbal plants clearly shows how biologically active components have the potential to suppress pathogens and prevent disease development. Thus, the daily use of herbal extracts and their products is a promising and attractive alternative to synthetic compounds in the control of oral diseases. This plant-derived product can play a role in inhibiting the formation of biofilms in the oral cavity, disrupting and reducing the adhesion of pathogenic microbes to the oral surface, which is the first step in the formation of dental plaque. Plant extracts can act as bactericidal, inhibit one or all stages of plaque formation, by interfering with adhesion/aggregation/formation of biofilms, or inhibiting glycolytic acid production in cariogenic bacteria (Kuang, Chen, and Xu 2018; Nithya, Saxena, and Kharbanda 2020; Milho et al. 2021).

Plants undergo little differentiation and live long, rely on water and minerals from the earth, and can be produced as bioactive secondary metabolites. Physical and chemical defense is a condition for its survival. Plants can produce several defense chemicals and secondary metabolites that serve as a method of communication between plants and have significant anti-biofilm effects and modulate biofilm formation. Many natural products have been shown to interfere with biofilm formation, and it is hoped that large quantities of these compounds can be processed at a lower cost, quickly and efficiently. Phytochemicals, fractions, and extracts are grouped below according to the general class or active component of various natural products with potential anti-biofilm activity (Song et al. 2017).

Alkaloids

Alkaloids are a group of naturally occurring chemical compounds containing ring structures and nitrogen atoms, have great potential as compounds for drug discovery, and have significant biological activity for anti-cancer, anti-microbial, or anti-viral properties. These compounds are widely distributed and concentrated in higher plant species. Alkaloids isolated and identified from plants have been shown to have antimicrobial activity, including against certain oral pathogens. Alkaloids have antimicrobial mechanisms attributed to their ability to interact with DNA, resulting in impaired cell division and death. Antibacterial ability against *P. intermedia* (MIC at 3.8 mg/mL), F. nucleatum (MIC at 31.25 g/mL), and *E. faecalis* (MIC at 0.5 mg/mL), but antibiofilm activity is not good for multispecies culture (Song et al. 2017; Milho et al. 2021).

Polyphenol

Polyphenols are secondary metabolites in most plants that play an important role in resistance to pests and diseases. Polyphenols have antibacterial and anti-biofilm activity against microbes. This showed an inhibitory effect on the growth of *S. mutans* and other plaque-forming

streptococcal bacteria. Several studies have reported that polyphenols have an inhibitory effect on oral biofilm formation during the production and accumulation of dental biofilms (Lim et al. 2017; Milho et al. 2021).

Terpenes

Terpenes are a large and diverse group of hydrocarbons and are naturally synthesized in microorganisms, plants, and animals. This hydrocarbon has a high degree of structural diversity because it is derived from the incorporation of five-carbon isoprene units and has various anti-microbial activities against susceptible and resistant pathogens. Terpene derivatives have shown promising effects, with the ability to eliminate fungal and bacterial biofilm production (Song et al. 2017; Mahizan et al. 2019).

Flavonoids

Flavonoids, a family of polyphenolic compounds, widely distributed in the plant kingdom are consumed in significant amounts as part of the human diet. Flavonoids are effective against various microorganisms because they have an antimicrobial activity that occurs due to their ability to form complexes with extracellular proteins and bacterial cell membranes. This will increase cellular permeability and cause cell disruption. In addition, this compound can also act to inhibit the activity of DNA gyrase and -hydroxyacyl-acyl transport protein dehydratase (Milho et al. 2021; Gutiérrez-Venegas et al. 2019).

Essential Oils

The antibacterial properties of essential oils are well known, and their broad-spectrum activity against various pathogens has been widely reported. Numerous studies have shown that commercial mouthwashes containing essential oils are more beneficial for the maintenance of oral hygiene and are more popular for long-term daily

use than products with chlorhexidine. Essential oils can act as antimicrobial agents by fighting various biofilm-forming bacteria present in the oral cavity, such as *S. mutans*. Based on the research that has been done, it is known that essential oils can significantly inhibit the growth of bacteria, fungi and yeasts, thus inhibiting the production of biofilms. So it is reported to be an antimicrobial agent, insecticide, and antioxidant. Essential oils can affect biofilm formation by inhibiting peptidoglycan synthesis, damaging microbial membrane structures, and modulating quorum-sensing (Song et al. 2017; Milho et al. 2021; Kouidhi, Al Qurashi, and Chaieb 2015; Mahizan et al. 2019).

Sugar Alcohols

Sugar alcohols have been widely promoted as oral healthcare products and have a long history of being used in a wide variety of foods and beverages, from soft drinks to sugar-free products. This sugar alcohol is commonly found in fruits and vegetables. As for examples of sugar alcohols, namely: xylitol, polyalcohol or sugar alcohol which are generally used as anticariogenic agents which have been proven by several studies have been carried out and the results show anti-bacterial properties. Xylitol can inhibit the growth of S. mutans at a concentration of 1.56% (m/v), while at concentrations above 1.56% (m/v) it can inhibit other bacteria. Xylitol selectively inhibited the growth of S. mutans without affecting other strains such as S. salivarius, and S. sanguinis. Long-term use of xylitol decreased the glucosyltransferase B (gtfB) expression, which is responsible for synthesis of EPS of S. mutans (Song et al. 2017; Chan et al. 2020).

Plant Extracts

The results of phytochemical studies that tested its ability have been shown to inhibit the formation of dental biofilms or break down biofilms that have been formed previously. In the following section, the anti-biofilm activity of plant extracts will be discussed (Song et al. 2017).

Tea (*Camellia sinensis*)

Tea (*Camellia sinensis*) has many health benefits, therefore tea is one of the most popular beverages worldwide. These benefits include being an antioxidant, antibacterial, anti-inflammatory, antidiabetic, antimutagenic, hypocholesterolemic, and cancer prevention. In the oral cavity, tea has a role in disrupting the adhesion of microbes to various oral surfaces and also destroying bacterial cell membranes. The biologically active components in tea, among them are catechins. Catechins are the main antimicrobial components in tea that have a role in fighting oral pathogens and these catechins are polyphenols. The antimicrobial activity of these catechins is to cause permanent damage to the microbial cytoplasmic membrane, thereby suppressing the formation of cariogenic bacterial biofilms (Kuang, Chen, and Xu 2018; Karygianni et al. 2016).

Coffee

Caffeine and α-dicarbonyl compounds are found in coffee and show antibacterial activity against S. mutans. In preventing the formation of biofilms, coffee has an important role because of its ability to prevent the attachment of bacteria and as an anti-biofilm. Coffee itself contains substances with low molecular weight (chlorogenic acid), trigonelin, and high molecular weight substances (melanoidin) which can inhibit microbial adhesion to the surface to inhibit the initiation of the biofilm formation process, as well as interfere with substantial biofilm communication mechanisms such as quorum-sensing (Karygianni et al. 2016; Sharma et al. 2018; Almeida et al. 2012).

Propolis

Propolis can be derived from plants that can be used as a dietary supplement, it is non-toxic because it is a natural resin product. The condition of geographical origin will affect the chemical composition of proporlis which will also affect its pharmacological activity. Inhibitory

activity against glucosyltransferase (GTF) and mutans streptococci was shown by its ethanolic extract. The most effective antibacterial compound in propolis itself is tt-farnesol. The use of proporlis has been reported to play a role in reducing cell viability by disrupting the integrity of cell membranes. The tt-farnesol mechanism is associated with the interaction of lipophilic moieties with the bacterial cell membrane, which is consistent with reduced IPS accumulation in S. mutans biofilms treated with tt-farnesol (Kuang, Chen, and Xu 2018; Sharma et al. 2018).

Cranberries

Cranberry is a highly nutritious fruit that is rich in polyphenols and various other bioactive compounds including flavonols, tannins, anthocyanins, flavan-3-ols, and phenolic acid derivatives. Due to their high concentration of polyphenols, cranberries have been beneficial for fighting bacterial infections and recognized as an excellent antioxidant. It also exhibits antimicrobial activity against pathogens such as Helicobacter pylori, Staphylococcus aureus, Salmonella, and Escherichia coli. In addition, flavonol content is also the most active component of cranberries which can interfere with the formation of S. mutans biofilms. Polyphenols with high molecular weight will inhibit the formation of biofilms and prevent the attachment and colonization of pathogenic bacteria to host tissues (Kuang, Chen, and Xu 2018; Lu et al. 2019; Sharma et al. 2018).

Cacao

Cacao beans, which form the main constituent of chocolate, contain some polyphenols which exhibit anti-glucosyltransferase activity. Cocoa has significant anti-inflammatory, antioxidant, anticarcinogenic, anticariogenic benefits. The most important cocoa flavonoid compounds are catechins and epigallocatechin which have a role in inhibiting the activity of the dextran sukrase enzyme, resulting in a reduction in the production of extracellular polysaccharides derived

from sucrose and resulting in a decrease in biofilm formation (Karygianni et al. 2016; Sharma et al. 2018; Almeida et al. 2012).

Pomegranate (*Punica granatum*)

This fruit shows amazing antioxidant activity because it contains several compounds, namely tannins (punicalagins, punicalins), poliphenolic, anthocyanins, ellagic acid, and gallic acid. Tannins and polyphenols are the largest content of Punicagranatum L fruit extract which can play a role in inhibiting antibiotic-resistant and quorum-sensing bacterial strains among biofilm microorganisms, so that biofilm formation will be disrupted (Karygianni et al. 2016; Sharma et al. 2018).

Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is well known for its antimicrobial and antiviral activity due to its bioactive compound found in garlic, namely alliinase-derived allicin. Garlic also exhibits antibacterial action in dentistry, namely against several gram-positive and gram-negative planktonic bacteria through its inhibitory effect on QS by interfering with QS signaling through competitive inhibition of the transcriptional regulator LuxR and LasR by garlic extract (Karygianni et al. 2016; Lu et al. 2019).

Ginkgo biloba

Ginkgo biloba extract was reported to significantly inhibit biofilm formation by influencing biofilm formation and structure without affecting bacterial growth. The cinnamaldehyde content of ginkgo biloba plays a role in interfering with biofilm formation, by reducing the DNA binding ability of LuxR (Lu et al. 2019).

Citrus Limonoids

Citrus limonoids can interfere with cell-to-cell signaling and biofilm formation. This component is a secondary metabolite unique to a triterpenoid (Lu et al. 2019).

Quercetin

Quercetin, which is found in many fruits, vegetables, and whole grains, is a plant polyphenol. Quercetin and its derivatives, isorhamnetin and quercetin-3-glucuronide, may reduce the expression of some inflammatory genes. It was reported that quercetin can significantly inhibit biofilm formation and production of virulence factors including pyocyanins, proteases, and elastase at lower concentrations compared to most of the previously reported extracts and plant substances. Quercetin can interfere with biofilm formation through the suppression of sialic acid expression (Lu et al. 2019; Veloz, Alvear, and Salazar 2019).

Phloretin

Phloretin is an antioxidant that is abundant in apples, this content can play a role in reducing biofilm formation and fimbria production without affecting the growth of planktonic cells. Phloretin can act as an inhibitor of biofilm formation by inhibiting the production of fimbriae, as well as acting as an anti-inflammatory agent in inflammatory diseases (Lu et al. 2019; Du and Bonsu 2015).

Hordenine

Hordenine has potential as an anti-quorum sensing, so it can act as a competitive inhibitory agent for signaling molecules and a novel quorum sensing-based agent to defend against pathogens. Therefore, hordenine can effectively decrease virulence factors and gene

expression of *P. aeruginosa*. Hordenine was evaluated for its potential to block QS-controlled phenotypes and biofilm formation (Lu et al. 2019; Zhou et al. 2018).

In addition to those described above, the use of Antimicrobial Photodynamic Therapy (APDT) has also emerged as an alternative antimicrobial and biofilm removal. Its working principle involves the interaction between low-energy laser light in the presence of oxygen and a photosensitizer, to produce reactive oxygen species (ROS) so that microorganisms are unlikely to develop antimicrobial resistance. This technique exploits the bactericidal effect of APDT which is associated with oxidative damage to bacterial DNA and cell membrane systems. (Jiao et al. 2019).

Cold Atmospheric Plasma (CAP) is also a method that can be utilized because it can eliminate a broad spectrum of microorganisms, by utilizing a mixture of highly reactive ions and electrons, radical species, and molecules that can cause oxidative damage to cell membranes, DNA, and proteinaceous enzymes (Jiao et al. 2019).

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