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Oral Biofilms in Health and Disease

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Springer Series on Biofilms presents carefully refereed volumes on selected topics on this field of research. All volumes reflect the latest findings and developments. Once anchored to a surface, biofilm microorganisms carry out a variety of detrimental (like the plaque on teeth) or beneficial (bioremediating hazardous waste sites) reactions. International experts will write, or edit, volumes on specific aspects of biofilms, like research methods, or the roles of biofilms in diseases. While based in microbiology, biofilms are of intense interest to many other scientists, because they affect whole ecosystems, and lie at the root of chronic bacterial infections.

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Preface

Biofilms consist of microorganisms associated with an interface and protected within an extracellular matrix of microbially-produced polymers and host-derived biomolecules. Biofilm growth enables microbes to persist on surfaces and to work co-operatively for the extraction of nutrients from complex microenvironments. Importantly, biofilms also protect the resident microorganisms from exogenous physical or chemical attack. In fact, the ability of biofilm microbes to tolerate antimicrobials is so fundamental to their function that it has become an integral part of the definition of medically relevant biofilms (Parsek and Singh 2003). Although the term "biofilm" was not introduced until the 1970s, the concept of biofilms providing protection for microbes has been known since the earliest days of microbiology. In a letter to the Royal Society in London written in 1683, Antonie van Leeuwenhoek described experiments conducted on his own dental biofilms ("scurf") by swilling with an antimicrobial mouth rinse, "very strong wine-Vinegar" (Leewenhoeck 1684). Observing the loss of motility as microbial cells ("Animals") were inactivated, van Leeuwenhoek commented that "From hence I conclude, that the Vinegar with which I washt my Teeth, kill'd only those Animals which were on the outside of the scurf, but did not pass through the whole substance of it." Over 300 years later and with the assistance of the latest cutting-edge techniques, a study identified the motile bacterium *Selenomonas sputigena* as a potential caries pathogen and showed it to be protected within biofilm microcolonies by polysaccharides derived from *Streptococcus mutans* (Cho et al. 2022). Although studies like this are improving our understanding of oral biofilms, the challenges of eradicating microbes that hide in the depths of the structure remain.

Oral microbiology has since contributed with invaluable advances in the understanding of basic biofilm biology. However, in contrast to many clinically relevant biofilms (e.g., implant-related biofilms, wound biofilms), oral biofilms are archetypes of polymicrobial communities, consisting of prokaryotic and eukaryotic microorganisms in close and direct contact with hard a-biotic, hard biotic, and soft biotic surfaces. Research on oral biofilms has been invaluable for studying polymicrobial diseases. Periodontal disease and dental caries have served as excellent models for studying and refining the role of the microbiome on mucosal and hard

surfaces, which helped advance the field of (oral) microbial ecology and established the importance of polymicrobial interactions in health and disease.

The concept of microbial dysbiosis, defined as an imbalance in microbial diversity leading to an increase in abundance of disease-associated species, is now unequivocally accepted for many biofilm-associated diseases that have both local and systemic implications. For example, the oral-gut-brain axis connecting anatomically the oral cavity with the gut and establishing communication with the brain through specific nerve interactions has been implicated in neurodegenerative diseases. Moreover, the connection of periodontal disease with systemic diseases such as cardiovascular disease, diabetes, and systemic inflammation is undeniable. The various examples of oral bacterial species that are found in extraoral locations demonstrate the importance of the oral microbiome beyond the mouth. Conversely, oral microbiology also highlighted the importance of the oral microbiota in maintaining oral and systemic health. Efforts to survey the oral environment have early on focused on identifying species associated with oral health. This particular knowledge served greatly in developing concepts and applications for oral health maintenance and, potentially, reversal of dysbiosis. Therapeutic approaches that include nanotechnology to deliver effective antimicrobials targeting dysbiosis-associated species and novel probiotics to restore microbial homeostasis are being currently developed and will provide much needed alternatives in the treatment of polymicrobial diseases.

Oral microbiology has a history of pioneering studies that advanced our understanding of fundamental microbial processes and evolved into a field that studies complex microbial interactions. Early (and ongoing studies) at the molecular level demonstrated the facets of virulence regulation in the most extensively studied oral bacteria, *Streptococcus mutans* and *Porphyromonas gingivalis*, often revealing novel regulatory mechanisms. Moreover, bacterial interaction studies of coaggregation patterns, dividing plaque development into early and late colonizers, have guided oral microbial research for over two decades. Oral biofilms are ideal models to study the biology of biofilm formation. They are unique in that they are involved in health maintenance (commensal biofilms) as well as associated with the most common microbial diseases on Earth, dental caries, and gum diseases such as gingivitis and periodontitis. In addition, the easily accessible oral cavity allows for sample collection, in vitro-in vivo comparisons, and evaluation of antibiofilm strategies. Building on early evidence that individual oral microbes associate with other species during biofilm development, research has evolved toward the concept of a functional microbiome – one in which microbial species (including different kingdoms) communicate, interact, compete, adapt, and actively shape their microenvironment. Furthermore, the studies in oral biogeography revealed stunning variations in the biofilm's spatial organization at a macro, micro, and sub-micrometer scale. For example, oral polymicrobial communities can develop specific spatiotemporal patterns depending on the local environmental variations, nutrient availability, and interspecies interactions (Fig. 1). Oral microbiology also contributed greatly to the understanding of the biofilm matrix, the “functional extracellular matter.” For example, the detailed composition and structure of extracellular polymeric matrix (matrixome) and how it ultimately drives the spatial organization of specific species inside the biofilm have been directly linked to community scaffolding, function, and

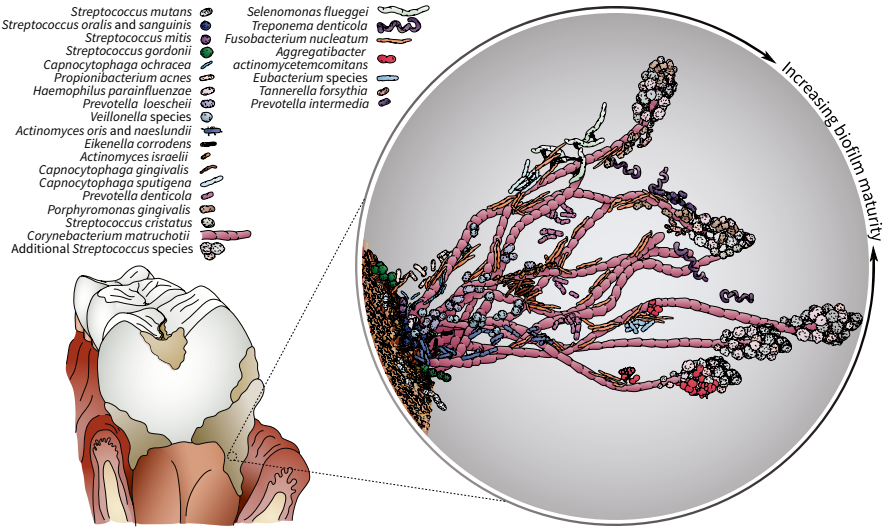

Fig. 1 Structure of the dental biofilm microbial community. The field of oral microbiology has led the way in understanding the spatial structure of complex microbial biofilms. Studies on coaggregation between bacteria have revealed many specific interactions between cell surface adhesins and receptors. Similar adhesin–receptor interactions mediate the initial adhesion of pioneer colonizers to the acquired enamel pellicle. These interactions are thought to drive the spatiotemporal formation of biofilms on tooth surfaces. For further information, see chapter "Molecular, Cellular and Ecological Aspects of Dental Biofilm Development"

disease development. Now as the technology advances with real-time microscopy-spectroscopy and spatial transcriptomics, exciting opportunities are at hand to assess the function of structured oral communities.

Oral microbiology as a research field has tremendous potential to advance the understanding of the functional microbiome. Oral microbiome sequencing has given us a detailed inventory of its members. Unlike the gut microbiome, the oral microbiome is easily accessible and an unprecedented number of species are actually cultivable, thus can be experimentally investigated using both laboratory and *in vivo* mechanistic models. The genetic tools for microbial manipulation are ever expanding and include more and more amenable species. Community dynamics can now be deciphered on the molecular level and will further advance microbial ecology. Questions about why certain species can only be found at a specific intraoral location – site-specialists –, while others reside across distinct niches – generalists –, are currently being answered by oral microbiologists. This will greatly benefit not only our field, but (medical) microbiology in general, since concepts developed in oral microbiology will be directly applicable to other polymicrobial communities living across different niches in the human body or on implanted surfaces. We envision a bold future in which oral microbiology informs novel strategies for treating human polymicrobial diseases and developing preventive measures or precision therapeutics to intercept disease before clinical onset.

In this book we have attempted to bring the oral research field together and present an overview of past and current developments. The book is organized in four

sections: (1) fundamentals of oral biofilms, (2) biofilm-related oral diseases, (3) experimental models to study oral biofilms, and (4) clinical studies on oral biofilms. An understanding of oral microbiome communities in their homeostatic or dysbiotic state and the manner in which they participate in oral disease processes requires cross-disciplinary approaches encompassing the fields of genetics and genomics, ecology, biochemistry, biophysics and bioengineering, and bioinformatics, among others. In this book we present 24 chapters.

Outline of the Book

Part I describes the fundamental principles governing the formation of biofilms, and oral biofilms in particular. All biofilms share common principles including cycles of biofilm formation, maturation and detachment, extracellular matrix production, and heterogeneity throughout the structure, and these processes are discussed in chapter "The Biofilm Microenvironment: Structure and Function". The concept of heterogeneity is elaborated further in chapter "Coping with Stress in Bacterial Communities: Heterogeneity of Stress Responses and Methods for Mapping Them" in the context of nutrient limitation, osmotic stress, and acid stress. Chapter "The Oral Biofilm Microenvironment" presents an overview of the chemical gradients in oral biofilms and the role of the matrix in modulating the stability and robustness of the structure. Attachment strength of dental biofilms is determined to a great extent by the initial adhesion of microorganisms to the tooth surface. Microbes do not directly attach to teeth, but instead bind components of the acquired enamel pellicle, described in detail in chapter "Pellicle, Adhesion and Early Colonization". Subsequently, chapter "The Extracellular Matrix: A Scaffold for Microbial Community Assembly and Function" returns to the topic of the biofilm matrix, a critical structural and functional component of biofilms as they mature. This chapter describes the many different macromolecules produced by bacteria and fungal microorganisms as matrix material and the contribution of the matrix to protection from antimicrobials, modulating the fitness and virulence potential of oral microbes and controlling the arrangement of microbial cells. The structural arrangement of cells is also driven by interactions between cell surface adhesin proteins and receptor polysaccharides that bind to the pellicle, matrix and directly to other cells, and these are the focus of chapter "Molecular, Cellular and Ecological Aspects of Dental Biofilm Development". Most of our understanding of adhesion in oral biofilms comes from studies on bacteria in dental biofilms, but bacteria are not the only microbes present and biofilms on hard tissues are not the only relevant microbial communities in the human oral cavity. Therefore, chapter "The Oral Mucosa-Fungi Interactome" extends the discussion of oral biofilms to consider fungal biofilms on mucosal surfaces and the cross-talk between microbe and host. This chapter also describes fungal biofilms in diseases such as candidiasis and denture stomatitis.

Diseases become the focus of Part II, which starts with an overview of biofilms in the pathogenesis of dental caries (Chapter "Cariogenic Biofilms"). Dental caries is the main source of endodontic infections, which can lead to periapical abscesses and acute pain, necessitating urgent dental treatment. Chapter "Endodontic Biofilms" discusses

the microbiology of endodontic infections, including intraradicular and extraradicular infections, and potential therapeutic approaches. Different perspectives on biofilms associated with gingivitis and periodontitis are provided in chapters "Microbial Biofilms and Pathobiology of Gingivitis and Periodontitis" and "Subgingival Ecology of the Periodontal Pocket". Chapter "Microbial Biofilms and Pathobiology of Gingivitis and Periodontitis" provides an in-depth review of periodontitis including risk factors, associations with systemic diseases, models to study periodontal biofilms, biogeography of biofilms, and interactions with the immune system, all of which are key topics for understanding the etiology of periodontitis. Chapter "Subgingival Ecology of the Periodontal Pocket" then zooms in on the ecological aspects of subgingival dental plaque and how these modulate the disease process. Subgingival dental plaque is also a key driver of peri-implant infections. The microbiology of peri-implant infections and potential preventative or treatment approaches for managing infections are the subject of chapter "Implant Related Biofilms".

Dramatic advances have been made in our understanding of oral biofilms through the development of a wide variety of tools and techniques for biofilm modeling and analyses, which are presented in detail within Part III. This section starts with a description of novel molecular tools for studying the roles of specific genes and proteins in oral microbial biofilms, from mutagenesis of individual gene targets to forward genetic screens (Chapter "Molecular Tools for Oral Biofilm Analyses"). Examples are given of how these tools have been used to decipher different aspects of oral biofilm biology. Modeling oral biofilms *in vitro* has been a cornerstone of oral biofilm research. Chapter "Modelling Holistic Oral Biofilm Communities, Including Candidate Phyla Radiation Group and Bacteriophage" discusses some of the single-, dual-, and multi-species synthetic community models that have been developed. This includes studies on newly identified ultrasmall bacteria belonging to the Candidate Phyla Radiation group. Multi-species models formed from the natural population of microbes in saliva (microcosm models) are becoming increasingly common as tools for community analysis improve and chapter "Multispecies Oral Biofilm Models" describes some of the methods that have been used in different studies. In the mouth, biofilms grow in the presence of saliva and this is a critical fluid for biofilm modeling. Chapter "Role of Saliva in Oral Biofilm Models" presents an overview of saliva, including its roles in biofilms on hard and soft tissues, and emphasizes the importance of including saliva in biofilm models as a source of the conditioning layer (pellicle) and microorganisms, as well as a culture medium. Biofilms grown *in situ* provide the most natural model of dental plaque, although there are limitations on how these can be used. Chapter "Preclinical Models and Methodologies to Study Biofilms Associated with Dental Caries" describes some of the *in situ* models as well as *ex vivo* models to study the structure and biochemistry of biofilms associated with dental caries. Different models are required to study the interactions of biofilms with soft tissues and chapter "Models to Study Interactions Between Host and Oral Biofilms" describes some of the animal models and 3D tissue culture systems that have been employed for this purpose.

Part IV focuses on clinical studies that have been done to understand the role of biofilms in human health and disease. In chapter "Cohort, Longitudinal and Cross-Sectional Studies: Dental Caries", clinical studies of dental caries are discussed

including the measurement of biomass and other characteristics of dental plaque and the microbiome associated with dental caries. Chapter "Cohort, Longitudinal and Cross-Sectional Studies: Periodontal Diseases" gives a detailed description of studies that have addressed the microbiology of periodontal disease from the experimental gingivitis model introduced in the 1960s through to modern metagenomics approaches. Factors that modulate the microbiome, including smoking, diabetes, pregnancy, genetics, and autoimmune disorders are considered and longitudinal studies of the impact of periodontitis progression and treatment on the microbiome are described. Chapter "Fundamentals of Omics Applications in the Study of Common Oral Diseases" provides a primer on conducting-omics studies for oral microbial ecology including reporting guidelines, study designs that support inferences, and the importance of sharing code and data appropriately to ensure reusability of data. Despite the wealth of information on oral biofilms, oral diseases remain the most prevalent condition in human populations worldwide according to repeated global burden of diseases studies (GBD 2017 Oral Disorders Collaborators, 2020). New treatment options are urgently needed. Chapter "Ecological Modulation of Oral Biofilms: Novel Approaches to Treat and Prevent Oral Diseases" presents treatment options that hold promise for oral biofilm control including pre- and probiotics, phage therapy, antimicrobial peptides, and quorum sensing inhibitors. Chapter "Innovative, Non-antibiotic-Based Approaches to Oral Biofilm-Related Diseases" gives a different perspective on controlling biofilms using prebiotics and synbiotics while extending to new nano/biotechnology-based approaches, including catalytic nanoparticles and plant-engineered enzymes. Finally, Part V presents our thoughts on the future for the field in chapter "A Perspective on Oral Biofilms Research". Thanks to all outstanding scientists and researchers for contributing to this book. Hope you enjoy it!

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The Biofilm Microenvironment: Structure and Function

Logan L. Bulock and Kenneth W. Bayles

Abstract Biofilm formation is a preferred mode of growth for bacteria and an intricately complex developmental process. Biofilms on hard and soft tissues in the oral cavity are critical for determining the balance between oral health and disease. Many studies have demonstrated the presence of heterogeneous niches formed during biofilm development, leading to significant variation in gene expression across the biofilm architecture. Although much of this heterogeneity can be attributed to microenvironmental differences formed within the biofilm, recent studies have revealed that some aspects of biofilm heterogeneity arise as a result of the stochastic expression of genes and/or cellular processes. This previously unrecognized diversification is predicted to benefit the population through the division of labor and as a "bet hedging" strategy that better prepares for unpredictable changes in environmental conditions. In this chapter, we explore how biofilm heterogeneity is due to both environmental influences and genetic factors and explore the predicted benefits it may serve for the biofilm community.

1 Introduction

The discovery of the biofilm mode of growth has led to a much greater appreciation for the complexity of bacterial survival within often rapidly changing environmental conditions. Not unlike eukaryotic organisms, many bacteria communicate with each other, form aggregated structures, and behave cooperatively (Rutherford and Bassler 2012; Nadell et al. 2016; Xavier and Foster 2007). In fact, these structured and adherent communities are the most common form of bacterial growth found in nature (Geesey et al. 1977; Costerton et al. 1978; Hall-Stoodley et al. 2004). In contrast to their planktonic counterparts, biofilms are surface attached, structured

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communities that are encased in an extracellular matrix (ECM) comprised of a combination of exopolysaccharides, proteins, and nucleic acids. Most microorganisms can form a biofilm, and many use the unique biofilm lifestyle to resist antibiotics and/or the host immune defenses during infection. Biofilms form in the oral cavity on hard and soft tissues, and those on tooth surfaces (dental plaque) are particularly important for oral health. Due to the clinical relevance of the biofilm mode of growth, much work has been done to characterize biofilm formation and the complex and heterogeneous nature of the biofilm lifestyle. However, despite the tremendous amount of progress made in understanding bacterial biofilms, there remains much to learn about how they form and survive in the face of rapidly changing and often stressful environmental conditions.

Perhaps the best place to start our discussion of biofilm resilience would be deep in the ocean, near hydrothermal vents, where the evolution of Earth's earliest lifeforms has been proposed to occur. There is a unique chemistry at these vents, where mixing of reduced hydrothermal fluids with seawater provides an environment with the energy and substrates required for metabolic reactions (Klein et al. 2015). The oldest known fossils are of ancient microorganisms from at least 3770 million years ago, that dwelled in submarine-hydrothermal environments (Dodd et al. 2017). At current hydrothermal vents, microbial life can be found in abundance compared to the otherwise desolate deep-sea floor (Lutz and Kennish 1993). Here, chemolithotrophs use ferric and sulfuric minerals for oxidation and form an essential part of the sulfur cycle (Ruby et al. 1981; Gugliandolo and Maugeria 1993; Emerson and Moyer 2002). The discovery of microbial mats near hydrothermal vents, along with nearly all microbial life in nature being in the biofilm state, suggests that natural selection in the hostile environment found on primordial planet Earth favored lifeforms that could adhere to a surface and persist near a nutrient source, like hydrothermal vents, where there is stiff competition from competitors and rapidly changing environmental conditions (Dodd et al. 2017; Baross and Hoffmann 1985; Rasmussen 2000; Stoodley et al. 2002). Given the challenging and inhospitable origins of biofilms, it is not surprising that "modern" biofilms are resistant to starvation, immune defenses, and antimicrobial agents (Costerton 1995; Yamada and Kielian 2019; Le et al. 2018; Gonzalez et al. 2018; Stewart 2014).

In addition to resistance to stressful conditions, the hydrothermal vents of our primordial Earth likely helped drive biofilm evolution in another way: toward the heterogeneity and division of labor that are essential for maximizing fitness of the species. As will be discussed at length, bacteria grown in their natural biofilm form display marked heterogeneity, from task allocation and specialized matrix-producers to subpopulations of antibiotic-tolerant cells. Historically, studies of bacteria were conducted only in the context of planktonic cultures measuring averages within the population, completely overlooking the primary mode of bacterial growth (biofilm) and overlooking differences among the individuals in the population. In other words, we were trying to make sense of these complex organisms by averaging the characteristics of cells growing in planktonic culture, rather than examining the interactions and diversity of individuals living within a community. Imagine trying to understand organ systems by studying cultured cells. We would likely learn a

great deal about the growth characteristics of these cells, but completely miss the intricate details of the communal behavior that is essential to their ultimate function, and to the survival of the whole organism. Yet despite taking cells out of their natural form of growth, planktonic cells still exhibit marked heterogeneity suggesting the processes eliciting heterogenic behavior are deeply engrained in the functionality of the cell (Avery 2006).

The key to understanding bacterial communities may be to gain a better understanding of the developmental processes driving their formation. Once thought of as merely a collection of cells, more or less randomly organized within a matrix, biofilm development is now known to be an intricate process culminating in the formation of complex multicellular structures with common features shared among most microorganisms, such as differential gene expression, physiological heterogeneity, and division of labor. The complexity of these structures manifest in multiple ways, including the formation of fruiting bodies, microcolonies, and floating aggregates, depending on the environment and characteristics of the organism. For example, motile bacteria like *Bacillus subtilis* and *Myxococcus xanthus* form stalk-like structures called fruiting bodies, which can become hot spots for spore formation (Branda et al. 2001; Kaiser and Welch 2004). Other bacteria simply form mounds or "towers" that are often referred to as "microcolonies" (Moormeier and Bayles 2017).

In this review, we will summarize what is known about the development of some well-characterized biofilm-forming bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *B. subtilis*, and *Staphylococcus aureus*. These bacteria belong to several different branches of the Bacteriae domain of life, yet these organisms evolved to form biofilms with the same developmental steps, underscoring the apparent necessity and importance of biofilm growth to their survival (Hall-Stoodley et al. 2004; Moormeier and Bayles 2017; Sharma et al. 2016). We then discuss how physiological heterogeneity is a part of the developmental process, as well as describe the potential role(s) biofilm heterogeneity plays in long-term biofilm survival. The fundamental principles of biofilm growth are relevant to all biofilms, including those that form on oral tissues.

2 Biofilm Development

Bacterial biofilm formation was documented as early as the 1930s with the observation of bacterial growth on submerged surfaces (Henrici 1933). In the 1980s, significant interest in biofilm formation was renewed with the recognition that most bacterial growth in streams was in the form of biofilms (Geesey et al. 1977). Early into this renewed interest in biofilms, it was postulated that biofilm growth was the result of a microbial developmental process (O'Toole et al. 2000). Indeed, much evidence has been found to support the notion that microbes follow a largely conserved developmental process that progresses in multiple stages. Here, we present a general overview of biofilm development. Specific mechanisms of attachment,

proliferation, maturation and dispersal of oral biofilms are considered elsewhere in this Part I of this book.

Attachment The first step is to attach to a surface, which can provide several benefits to an organism. First, all surfaces adsorb proteins and polysaccharides through molecular interactions, forming a conditioning film upon which bacteria can adhere and consume nutrients (Gubner and Beech 2000; Lorite et al. 2011; Mittelman 1996; Palmer et al. 2007; Ren et al. 2018). The conditioning film has been shown to change the properties of surfaces, such as the hydrophobicity and surface charge (Sheng et al. 2008; Li and Logan 2004). These changes affect the ability of bacteria to adhere to the surface, with different bacteria attaching better to different materials and conditioning films (Hwang et al. 2013; Talluri et al. 2020). Importantly, this surface film can be a source of proteins and/or polysaccharides while also driving away competing organisms that may not favor the physicochemical properties of a surface/condition film.

Second, attached bacteria can remain in a favorable environment, such as near hydrothermal vents, rather than drifting away to a less suitable environment (Klein et al. 2015). As mentioned above, this may be why biofilm formation is conserved among ancient bacterial and archaeal species (Jahnke et al. 2001; Reysenbach et al. 2000), as natural selection may have favored communally driven bacteria (Hall-Stoodley et al. 2004).

Finally, compared to planktonic bacteria, adherent bacteria can better withstand nutrient deprivation, pH changes, immune defenses, and antimicrobial agents (Yamada and Kielian 2019; Le et al. 2018; Gonzalez et al. 2018; Stewart 2014; Costerton et al. 1995). Adherence to a surface allows the bacteria to resist these stressors, through a variety of mechanisms. In one model, the matrix serves as a diffusion barrier to limit the penetration of molecules into the inner section of the biofilm, thus protecting the interior cells from antimicrobial agents and immune defenses (Tseng et al. 2013). In another model, a subpopulation of biofilm-associated cells broadly resistant to stress (persisters) are already present prior to exposure to stress (the so-called, "bet-hedging" strategy) (Lewis 2008). Thus, the presence of an external stress (such as an antibiotic) results in the elimination of a large portion of the population leaving behind the persister cells, which can go on to form a new biofilm. Although other models of stress resistance exist, it is clear that the ability of bacterial cells to adhere to a surface and form a biofilm are likely to be essential for the initiation of one or more of these processes.

Adhesion requires a net attraction between the surface/conditioning film and the bacterium. Together, the van der Waals, electrostatic, and hydrophobic interactions must favor interaction for bacteria to adhere to a surface (Berne et al. 2018). Motile bacteria, like *E. coli* and *P. aeruginosa*, have flagella that are used to stay near the surface as molecular interactions form with the surface (Friedlander et al. 2013; Belas 2014). These interactions are part of the initial stage of biofilm development for motile bacteria, called reversible attachment (Belas 2014). As the name implies, bacteria may freely attach and detach from the surface. These initial interactions are weak, perhaps providing the bacteria time to explore the surface by detaching and

attaching to find the optimal location to adhere (Berne et al. 2018). Also, for rod-shaped bacteria, these interactions are polar, meaning contact between the surface and flagella is at one pole or the other of the bacteria. This triggers a cellular response that results in repositioning of the cell body longitudinally to the surface, downregulating flagellar biosynthesis, and upregulating production of adhesins, fimbriae, and pili (Berne et al. 2018). These culminate in stronger interactions that irreversibly adhere the bacteria to the surface in the next stage of biofilm development, irreversible attachment.

As a non-motile bacterium, *S. aureus* (and other Gram-positive cocci) utilizes an array of proteinaceous and non-proteinaceous adhesins to strongly adhere to biotic and abiotic surfaces (Heilmann 2011). The proteinaceous adhesins can be grouped into cell wall-anchored (CWA) proteins and non-covalently linked surface-associated proteins, whereas the non-proteinaceous adhesins consist of polysaccharide intracellular adhesin (PIA), wall teichoic acids (WTA), or lipoteichoic acid (LTA). Of the CWA proteins, the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) are the most prevalent, and consist of biofilm-associated protein (Bap) (Cucarella et al. 2001), clumping factor B (ClfB) (Abraham and Jefferson 2012), fibronection-binding protein A (FnBPA) and FnBPB (O'Neill et al. 2008; Geoghegan et al. 2013), *S. aureus* surface protein C (SasC) and SasG (Geoghegan et al. 2010; Corrigan et al. 2007; Schroeder et al. 2009), protein A (Merino et al. 2009), and serine-aspartate repeat protein SdrC (Feuillie et al. 2017). These proteins contain an LPXTG motif that is recognized by sortase, which translocates these proteins across the membrane and covalently links them to peptidoglycan (Mazmanian et al. 1999). These proteins contain ligand-binding domains for interaction with components of the host ECM, such as fibrinogen, fibronectin, and collagen (Patti et al. 1994; Patti and Hook 1994; Deivanayagam et al. 2002; Foster et al. 2014). Non-covalently linked proteins involved in surface interactions are autolysin (Atl) (Biswas et al. 2006; Houston et al. 2011; Kohler et al. 2014) and secretable expanded repertoire adhesive molecules (SERAM) proteins (Chavakis et al. 2005). Utilizing this array of adhesins, *S. aureus* is quite successful at forming biofilms on a number of surfaces, including skin, heart valves, and catheters.

Proliferation and Maturation After attachment, biofilm cells start to proliferate and produce an ECM that is composed of polysaccharides, extracellular DNA (eDNA), and/or proteins (Flemming and Wingender 2010; Kostakioti et al. 2013). The exact composition of the ECM depends on both the organism forming the biofilm and the specific signals present in the environment. In addition to its role as a structural scaffold, the ECM can retain nutrients through electrostatic interactions with anionic fermentation products (such as formate, lactate, or acetate) and positively charged matrix components (Foulston et al. 2014; Graf et al. 2019; Stewart and Franklin 2008).

Like most bacteria, *E. coli* matrix composition is comprised of a variety of different molecules. There are three major polysaccharides produced by *E. coli* during biofilm maturation: polyglucosamine (PGA), colanic acid, and cellulose (Matthysse et al. 2008; Danese et al. 2000; Serra and Hengge 2017; Vogeleer et al. 2014).