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Wound biofilms: lessons learned from oral biofilms

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Abstract

Biofilms play an important role in the development and pathogenesis of many chronic infections. Oral biofilms, more commonly known as dental plaque,are a primary cause of oral diseases including caries, gingivitis and periodontitis. Oral biofilms are commonly studied as model biofilm systems as they are easily accessible, thus biofilm research in oral diseases is advanced with details of biofilm formation and bacterial interactions being well-elucidated. In contrast, wound research has relatively recently directed attentionto the role biofilms have in chronic wounds. This review discusses the biofilms in periodontal disease and chronic wounds with comparisons focusing on biofilm detection, biofilm formation, the immune response to biofilms, bacterial interaction and quorum sensing. Current treatment modalities used by both fields as well as future therapies are also discussed.

Keywords

Biofilm; chronic wound; periodontal disease

INTRODUCTION

Microorganisms colonize the human skin, mouth, digestive, and reproductive tracts, and it is estimated that the number of bacterial cells colonizing the human bodyexceeds the number of our own cells.[1]Bacteria have been studied for hundreds of years as scientist Anton van Leeuwenhock, credited as the father of microbiology, first observed bacteria with a microscope in 1683.[2]One of the first samples he studied was dental plaque, and from that time plaque has served a crucial role in furthering human understanding of microorganisms.

While bacteria have been extensively studied, it was relatively recently that a fundamental shift occurred in research regarding bacteria and various disease states. This shift was the knowledge that bacteria form biofilms. Biofilms are three dimensional structures consisting of densely packed aggregations of microbes that attach to each other and to a surface and are encased in a self-synthesized extracellular polymeric substance (EPS).[3] It is estimated thatbiofilms are composed of 75-95% EPS and only 5-25% bacteria.[2] In contrast to biofilms, planktonic bacteria, which have been the traditional focus of microbiology research, are free-floating microorganisms in an aqueous environment. The transition of planktonic microorganisms to a biofilm lifestyle can be damaging to a host as biofilms notoriously resist elimination by host defenses and antibiotics.[4]

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The details regarding biofilm formation are important as understanding these pathways is crucial to the development of future anti-biofilm therapy. The biofilm life cycle has distinct stages including attachment of planktonic cells to a surface, growth of the cells into a mature biofilm colony, and eventual dispersal of the cells from the colony into the surrounding environment.[5]All stages are complex, involving various environmental signals, signal transduction pathways, and effector molecules.

Attachment is initiated when planktonic bacteria form a reversible bond to a surface, mediated by various physical and chemical forces as well as bacterial and host cell adhesins, proteins that are key factorsin the attachment stage.[5] The bacteria then multiply on the surfaceand synthesize EPS whichdefines the second stage of biofilm development, also known as maturation. The extracellular polymeric matrix, which is composed of polysaccharides, proteins, DNA, RNA, and lipids,is important because it offers biofilms protection as well as mediates cell-to-cell and cell-surface interactions that allow forbiofilm stabilization.[6, 7]

The detachment of cells from the biofilm colony and their release into the environment comprises the final stage of biofilm development known as dispersion. This stage contributes to bacterial survival and disease transmission.[5]Mature biofilm is composed of microbial masses in species specific configurations with intermixed channels that allow for the exchange of nutrients and waste products, transfer of genetic material, transportation of signaling molecules used in quorum sensing, and other bacterial interactions.[5]

Biofilms occur commonly in nature, and they are known to form in water, waste pipes, on ship's hulls, and in food processing areas.[7] They are a topic of extensive research as they are implicated in the pathogenesis of many infections, with the US Centers for Disease Control and Prevention estimating that more than 65% of microbial infections are associated with biofilms.[8]Biofilms have a well-established role in oral diseases such as dental caries, gingivitis, and periodontitis, and more recently they have been implicated in the pathogenesis of chronic wounds.[8]

Biofilms create a therapeutic problem as they are often resistant to conventional antimicrobial treatment. Thus, the understanding of biofilms and ways to control them is imperative to the successful treatment of a multitude of diseases. This review aims to provide acomparison and discussion regarding biofilms in oral and wound disease states and current modalities of treatment with a goal of focusing on literature from oral biofilm investigation and how this can be applied to the understanding and future investigation of biofilms in chronic wounds.

BIOFILMS IN ORAL DISEASES AND CHRONIC WOUNDS

The common inflammatory diseases gingivitis and periodontitis are collectively referred to as periodontal disease. Gingivitis, estimated to affect 50-90% of adults worldwide, is a mild form of periodontal disease that is reversible with effective oral hygiene.[9, 10] Periodontitis, a more severe form of periodontal disease, occurs when inflammation extends deep into tissues and causes loss of gingival tissue, the periodontal ligament, and alveolar bone.[9] Mild periodontitis is estimated to be present in 21.6% of dentate US adults, while moderate to severe disease affects approximately 13%.[11]The economic impact of periodontal disease is striking, with treatment costs estimated to exceed $14 billion dollars per year in the United States.[12]

In addition to periodontal diseases, chronic skin wounds play a major role in healthcare. Chronic wounds typically affect patients with comorbid medical conditions and are those wounds that have failed to proceed through an orderly and timely reparative process.[13] The

major chronic wounds faced by clinicians are diabetic foot ulcers, venous leg ulcers, pressure ulcers, and ulcers resulting from peripheral vascular disease. It is estimated that1-2% of people will develop a chronic wound during their lifetime in developed countries, and in the United States chronic wounds affect approximately 6.5 million patients.[13] The economic impact of wounds is also staggering as upwards of $25 billion dollars is spent annually in the United States on the treatment of chronic wounds.[13]

The most widely accepted cause of periodontal diseases is the formation of tooth biofilm with pathogenic microorganisms, commonly known as dental plaque. In fact, research has demonstrated that within one day of discontinuation of oral hygiene procedures (e.g. brushing one's teeth), biofilms begin to develop andwithin 10-21 days gingivitis develops.[9]Due to the widespread presence and easy accessibility of dental biofilms, they are commonly studied as model systems for biofilm development and therapeutic techniques.[14]Listgarten et al. described the complex nature and architecture of oral biofilms decades ago through the use of light and electron microscopy on extracted teeth and epoxy resin crowns. Their work suggested that certain microorganisms are consistent with states of periodontal health and disease.[15, 16]Thoughat that time they were unable to determine the specific species present, their work laid the foundation for future oral biofilm research.

While biofilms have been widely accepted and studied in oral diseases for many years, recent research indicating that many chronic diseases result from bacterial biofilms initiated wound research's focus on biofilms as a possible cause of suboptimal wound healing.[17-19]One of the first associations of biofilms and wounds came from the electron microscopic examination of sutures and staples removed from healed surgical wounds.[20]Other studies then characterized in vitro and in vivo models for biofilm development in wounds. A study by Harrison-Balesta et al. demonstrated that wound-isolated *Pseudomonas aeruginosa* displays characteristics of a mature biofilm within 10 hours of in-vitro growth, thus suggesting that bacteria in wounds rapidly develop biofilms.[21]Charles et al. utilized a tissue-engineered skin equivalent, Graftskin, anddemonstrated time-dependent biofilm development on artificially created wounds inoculated with pathogenic *P. aeruginosa* and *Staphylococcusaureus.*[22]

Recently, a landmark study by James et al. investigated the presence of biofilms, evident by aggregated bacterial colonies surrounded by an extracellular matrix, in acute and chronic wounds using light and scanning electron microscopy (Figure 1).[17] They found that only 6% of acute wounds had biofilms, whereas 60% of chronic wounds exhibited biofilm formation, though it was not clear whether those chronic wounds with or without biofilms were refractory or healing.While biofilms are thought to be present in chronic wounds more so than acute wounds, both murine and porcine in vivo models have demonstrated acute wound biofilms.[23-27]Thus, although biofilms may bean important factor as to why chronic wounds do not follow the typical wound healing pattern, they may not be the only factor.

Interestingly, another study by Akiayama et al. demonstrated that *S. aureus* cultures from patients with impetigo, furuncle and atopic dermatitis grew biofilms on coverslips within 72 hours incubation at 37°C in the presence of plasma.[28] They inferred that biofilms also form in vivo. The implications of biofilms in not only wounds but also other dermatologic diseases including atopic dermatitis, acne, candidiasis, impetigo, and bullous diseases is now being recognized.[29, 30]

Detection of microorganisms in oral and wound biofilms

Research regarding oral biofilm composition has advanced considerably, mainly due to advanced technologies used for biofilm detection. While a study by Moore et al. demonstrated over 500 distinct bacterial species in dental plaque specimens via the use of

culture methods,[31] newer research indicates many more bacterial species are present that are uncultivable. Culture independent approaches, in particular the use of 16S rRNA gene sequencing, has revolutionized the understanding of bacterial species diversity in oral niches. Multiple studies utilizing this technique revealed previously unidentified species in the oral environment.[32-36]

Zijnge et al. investigated the location of periodontitis associated species in vivo using a panel of 16S and 18S rRNA targeted fluorescence in situ hybridization (FISH) probes.[14]Their researchrevealed not only the dominant bacterial species in plaque, but they also demonstrated that there are progressive differences in biofilm cell physiological activity depending on bacterial depth in the biofilm.[14] This finding was consistent with previous in vitro studies.[37]FISH overcomes traditional culture limits, allows for positional information regarding bacteria in intact biofilms and can relatively simply be extended to newly identified species and phylotypes.

Similarly to oral biofilm detection, culture has been the standard method used to detect bacterial infection in wounds.[38] Chronic wounds often have low bacterial burden measured by standard laboratory assays, however, and lack overt clinical signs of infection, making lack of clinical suspicion a confounding problem in wound biofilm identification.[18, 39]Culture techniqueslikelyfall shortin wounds as they do in oral biofilms atidentifying all microorganisms present as easily cultivable bacteria, such as *S. aureus*, are selected for and commonly used laboratory methods detect mostly planktonic bacteria.[38, 40]Multiplearticles highlight the limitations of culture methods in identifying skin and wound bacterial species and the increased microorganism diversity elucidated with molecular techniques such as 16S rRNA and FISH.[41-46] Other novel approaches to detect biofilm organisms in chronic wounds are based on detection of quorum sensing molecules found in debridement specimens.[47, 48]

Gjodsbol et al. utilized molecular and culture techniques to determine the microbial community of venous ulcers. They found that *S. aureus* was the most common wound bacteria, followed by *Enterococcus faecalis, P. aeruginosa*, coagulase-negative staphylococci, *Proteus*species, and anaerobic bacteria.[49]Dowd et al. investigated bacterial species present in chronic wounds of diabetic foot ulcers, venous leg ulcers, and pressure ulcers using molecular amplifications followed by pyrosequencing, shotgun Sanger sequencing, and denaturing gradient gel electrophoresis.[40] They found that across all wound types major populations of bacteria included Staphylococcus, Pseudomonas, Peptoniphilus, Enterobacter, Stenotrophomonas, Finegoldia, and *Serratia spp*. Other researchers identified additional genera such as*Streptococcus, Porphyromonas, Anaerococcus* and*Prevotella*in chronic wounds.[45, 46]Gardner et al. demonstrated an association between diabetic foot ulcer microorganisms and clinical factors including ulcer depth, ulcer duration, and glycemic control using 16S rRNA sequencing.[46]The previous studies demonstrate the identification of bacteria in wounds not traditionally thought of as wound pathogens and the diversity of microorganisms present in wounds.

As oral biofilm and now skin research have shown the identification of multiple additional species with the advent of molecular microbial identification techniques, further use of 16S rRNA probes along with FISH should be sought.However, whilepossible to detect biofilm organisms using these methods, molecular methods have historically been expensive and time intensive, and therefore generally not used in the everyday diagnosis of biofilm organisms.[38, 50]With continual advances in technology, hopefully 16S rRNA gene sequencing will become widely available to clinicians and routine clinical microbiology laboratories.

Biofilm formation and composition

In dental plaque, bacteria have been shown to form biofilms in an organized, concerted way with initial attachment of pioneer species subsequently followed by other colonizing species.[51] It is thought that each microorganism modifies the environment which allows for successful community development.In 1998, a landmark paper published by Socransky et al. described this sequential formation of dental plaquewith each successive colonization composed of increasingly periopathogenic bacteria.The end result was colonization by*Porphyromonasgingivalis, Treponemadenticola*, and *Tannerellaforsynthia,* a group of bacteria known to be present in periodontitis.[52]

As described earlier, attachment is the initial phase in biofilm formation.Within minutes of professional cleaning, a collection of host-derived molecules termed the acquired pellicle coats the enamel surface of teeth. The acquired pellicle serves as the source of receptors for the primary colonizers of dental plaque, with mucins, agglutinins, proline-rich proteins (PRPs), phosphate-rich proteins (statherin), and enzymes such as alpha-amylase being known receptors for various oral species.[53]Streptococci represent 60 – 90% of the early colonizerswith other bacteriaalso involved.[54] Crucial to the development of the initial biofilm are the interactions among these bacteria, many of which have been elucidated for dental biofilms.[53]

Secondary, or late, colonizers bind to the primary colonizers, and the sequential bacterial binding results in the formation of nascent surfaces that bridge with the next coaggregating bacterial cell. Late colonizers include many periodontal pathogens such as*P. gingivalis, T. denticola, Eubacterium* spp., Prevotellaintermedia, Aggregatibacteractinomycetemcomitans, T.forsythensis, and *Selenomonasflueggei*.[53]In dental biofilm formation, *F.nucleatum*, also considered a periodontal pathogen, is unique in that it acts as a bridge between primary and secondary colonizers.[53]It is the most numerous gram-negative species at healthy sites, but it also markedly increases in sites of periodontal disease.[31]An increase in plaque mass is observed at sites suffering from periodontal disease with increased species diversity. A rise in gram negative and obligate anaerobic species is also observed.[1]The proportion of certain bacterial species, such as *Streptococcusmutans* or *P. gingivalis*, is increased in patients with caries or periodontitis, respectively.[2]

Biofilms have only recently been shown to exist in chronic wounds, hence research regarding wound biofilm formation and composition is significantly behind the known biofilm intricacies in dental plaque. Chronic wound microbiology is shown to be complex with a wide variety of microorganisms implicated in delaying wound healing,[55] however little is actually known regarding how these organisms form a wound biofilm. *S. aureus* and *P.aeruginosa*are traditionally recognized as major wound pathogens, but they likely represent only a fraction of microbes involved in chronic wounds. The polymicrobial nature of wound infections and the role of anaerobic bacteria have been emphasized by Bowler et al. and James et al.[17, 56]

It is reasonable to suggest wound biofilms may form in a manner similar to oral biofilms with initial colonization of skin flora and secondary colonization of wound pathogens such as *S. aureus* and *P. aeruginosa*. The advent of molecular techniques with continual elucidation of species diversity in chronic wounds will serve as a foundation for future studies designed to determine if there is also sequential wound biofilm formation.

Bacterial interaction in biofilms

While little is known regarding bacterial interactions in wounds, oral research offers guidanceregarding processes that may also occur in wound biofilm formation.Coaggregation and coadherence are two mechanisms of interaction used by bacteria to establish specific

patterns of spatiotemporal development in the oral biofilm. Coaggregation describes the ability of genetically distinct cells to recognize one another in suspension with resultant clumping.[53, 57] It is thought to occur via physical interactions among bacteria that have been shown to display specific recognition patterns with partner cells.[58]Testing among 300 oral bacteria indicated that greater than 90% underwentcoaggregation, illustrating the importance of this interaction.[2]Coadhesion, the recognition of a specific bacterium in suspension to one already attached to a substratum,[53] and coaggregation bridges, a third bacterium bridging two bacteria together, are also important bacteria interactions.

While it is known that many species participate in the sequential colonization of the oral cavity to form biofilms, the exact mechanisms are still a subject of much research. Studies have shown that many aggregations are inhibited by lactose, with a study by McIntire reporting that *Streptococcus* and *Actinomyces* aggregation was inhibited 90% of the time by lactose and D-galactose.[59]Bacteria cooperate extensively via coaggregation and other mechanisms to facilitate survival and biofilm formation.For example, both *F. nucleatum* and *P. intermedia* help *P. gingivalis*, an acid-sensitive bacterium, survive in the oral environment by producing ammonia which results in a more neutral environment.Bacteria also compete with one another in the process of oral biofilm formation mainly via bacteriocin synthesis, quorum sensing, and hydrogen peroxide excretion. Bacteriocins are bacterial peptidescapable oflysing other bacteria, thus enhancing competitiveness of the producing species and consequently theirability to form biofilms.[60]Streptococci possess the strongest bacteriocin producing ability among oral bacteria.Further information regarding the detailed interactions of many pathogenic bacteria can be obtained in a comprehensive review by Huang et al.[2]

In regards to wounds, studies have found a correlation between the presence of multiple bacterial species and non-healing wounds rather than just one bacterial species. This suggests that similar to oral biofilms, cooperation and synergy exists in wounds among the various species present.[41, 55, 61]Dowd et al. also have introduced the concept of functional equivalent pathogroups (FEPs) as genetically distinct bacteria that symbiotically co-occur in appropriate mixtures to produce a pathogenic biofilm community.[40]Thisconcept is very similar to oral biofilm knowledge that certain bacteria are responsible for working together in various stages to form pathogenic biofilms.

Biofilm communication through quorum sensing

Microorganisms communicate via the secretion of one or more agents in response to changes in bacterial density and the surrounding environment. This communication process isintegral to bacterial cooperation and competition and is termed quorum sensing. Quorum sensing (QS) plays a critical role in biofilms as it regulates biofilm maturation and development through inducing differential protein expression.Numerous bacterial activities are thought to be controlled by QS systems including bacterial surface adhesion, extracellular matrix production, virulence factor expression, spore formation, biosurfactant synthesis, competency, and bioluminescence.[2, 62, 63]Various QS systems have been described withN-acyl-homoserine lactone (AHL) and 4-quinolone systems used by gram negative bacteria, oligopeptide(AgrD)systems by gram positives, and Autoinducer-2/LuxS systems by both gram positive and gram negative organisms.[2, 64]

Autoinducer-2 (AI-2) functions as an important signal molecule in multi-species biofilms as it promotes biofilm formation and maturation. In fact, the *luxS* gene encoding AI-2 is conserved among many species of oral pathogenic bacteria.[65, 66]Oral streptococci that lack the *luxS* gene don't express AI-2 which decreases bacterial communication and results in loose biofilm formation with significantly decreased bacterial density.[2]*S.mutans*QS system also involvesCompetence Stimulating Peptide (CSP), which interestingly has been shown to

cause this organisms' growth arrest and death when present in large concentrations in biofilms. Thus, QS systems may provide future therapeutic options in dental caries and chronic woundsas more information is learned about them.[67]

Similar to oral biofilms, wound biofilms use quorum sensing as a means of regulating the expression of biofilm specific genes. Gram positive bacteria communicate mainly via peptide signals such asautoinducing peptide 1 (AIP-1) produced by *S. aureus* and the delta toxin from *Staphylococcusepidermidis* and *S. aureus*. AIP-1 may play a role in biofilm dispersal through up-regulating the matrix degrading proteases aureolysin and serum protease 1.[5]Gram negative bacteria communicate mainly via N-acyl-homoserine lactones (AHLs), with wound pathogen *P. aeruginosa* biofilm dispersal having been shown to be regulated by multiple AHL signaling networks.[3, 5]

Immune response to biofilms

The innate immune response plays a role in the pathogenesis of both periodontal disease and chronic wounds. Important in the activation of the innate immune response is the presence of various cell pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and NOD-like receptors, that survey the extracellular and intracellular environments and detect microbe associated molecular patterns (MAMPS) present on or released from microbial cells.[1]Activation of PRRsstimulates the innate immune response and triggers the production of cytokines and other inflammatory mediators. Abnormal expression of PRRs that bind bacterial lipopolysaccharide have been linked to pre-disposition to periodontal disease, thus reflecting the importance of oral tissue host detection systems.[1]

The clinical entity gingivitis was demonstrated to be caused by the host's response to the accumulation of dental plaque in experimental gingivitis studies in the 1960s.[68] Likewise, chronic inflammation of the periodontium is initiated by periodontal pathogensin subgingival biofilms. The host response contributes to the developmentof periodontal disease through the recruitment of pro-inflammatory cells (neutrophils and macrophages), inflammatory mediator release (IL-1, PGE_2), and tissue destruction via matrix metalloproteinases (MMPs).[7, 51]The first-responders to inflammation, neutrophils, are shown to be hyper-responsive in periodontal diseases,and they contribute to the disease process through releasing destructive reactive oxygen species, proteinases, and other factors that damage the host gingiva and periodontal ligaments.[1, 69, 70] Neutrophils also increase pro-inflammatory cytokines such as leukotriene B4, IL-6, TNF-α, and IL-1β, and increased levels of these cytokines are consistently found in studies of gingival crevicular fluid (GCF) of patients with gingivitis and periodontitis.[71, 72]

Adaptive immunity also plays a role in the pathogenesis of periodontal diseases. Page and Schroeder observed that acute inflammation dominates early periodontal lesions, but later periodontal lesions have a predominance of T cells, B cells and plasma cells.[72, 73]The gingivitis lesion is primarily a T cell mediated response, while the periodontitis lesion is predominantly a B cell and plasma cell response, thus implying a switch in immune response in the development of periodontitis.[74]Control of this shift is mediated by a balance between Th1 and Th2 subsets of T cells, with T regulatory and Th17 cells also possibly involved in immunoregulation of periodontal disease.[74]

In wounds, normal healing in response to injury or trauma to the skin consists of three phases: inflammatory, proliferative, and remodeling. During the inflammatory phase the exposed extracellular matrix triggers the coagulation and plasminogen cascades, and these along with wound bacteria trigger the innate immune system.[39]Neutrophils and macrophages are abundant during this phase, and eventually macrophages down-regulate inflammatory signals to move the wound into the proliferative phase after wound debris and

bacteria are cleared.[75] The proliferative phase is composed mainly of angiogenesis as well as fibronectin and collagen deposition via fibroblasts with the formation of granulation tissue. The remodeling phase lasts up to 2 years and during this time collagen fibers reorganize and mature, returning the tissue to near original strength.[39]

Similar to periodontal disease, chronic wounds are thought to induce a sustained inflammatory response as the host attempts to eliminate the biofilm. The role biofilms play is not yet clear, however.Some research implies the biofilm releases planktonic bacteria and consequently maintains this response.[76]Other thoughts are that non-healing wounds are associated with compromised hosts that exhibit an inflammatory response incapable of managing the initial wound bacteria effectively. Bacteria, in particular *P. aeruginosa*, contribute to this as they combat the host's immune response via the production of various substances.[77-79] Either way, chronic wounds are thought to become fixed in the inflammatory wound healing stage, with the body unable to successfully progress to the proliferative stage.[39]

Numerous studies have supported the notion that a chronic inflammatory response occurs in chronic wounds. Pro-inflammatory cytokines such as IFN-α, IFN-γ, TNF-α, and interleukin 1, frequently produced in response to bacteria, are elevated in chronic wounds.[23] Trengove et al. observed increased cytokine and metalloprotease levels in chronic wounds compared to acute wounds.[80]The inflammatory response also results in numerous neutrophils and macrophages surrounding biofilms which secrete high levels of reactive oxygen species (ROS), proteases (matrix metalloproteinases), and elastase. Macrophage derived MMPs 2 and 9 and neutrophil-derived MMP-8 and elastase are commonly increased in chronic wounds.[38] While proteases aid in dislodging biofilms from tissues, they also damage normal and healing tissues. As mentioned previously, the inflammatory response is not always successful in removing the biofilm, and it has been hypothesized that the inflammatory response actually increases biofilm survival. An ineffective inflammatory response can result in increased vascular permeability and the production of wound exudate as fibrin slough builds up which serves as biofilm nutrition.[18]

While innate immune mechanisms are mostly observed in chronic wounds, *S. aureus* biofilms have been shown to elicit an adaptive immune response in ocular biofilm.[81] As research has shown the importance of adaptive immunity in the pathogenesis of periodontal diseases with differences between gingivitis and periodontitis, adaptive immunity may also play a role in chronic wound pathogenesis and the conversion of an acute to chronic wound. The role of adaptive immunity in response to biofilms opens the possibility for the development of biofilm-specific diagnostics and even vaccines, an interesting prospect to both oral and wound biofilm research.

Impact of the host environment on biofilm species and architecture

The host environment is selective and determines which organisms are able to colonize, grow, and become minor or major members of a microbial community, with the host-microflora interaction important in health and disease.Dental research has shown that the distribution of oral microflora is not random, but that most species prefer a specific oral site with microflora composition varying among locations such as the tongue, buccal mucosa, and teeth.[1, 5]Aas et al. investigatedoral microflora composition at nine sites in healthy subjects and found some species were common to all sites, while other species were site specific. They alsodetermined that most sites had 20 to 30 predominant species, and that periodontitis associated species were not detectedin the healthy gingiva samples.[82]For skin, bacteria colonize skin sites based on specific location and the underlying physiology of the skin, with certain bacteria associated with most, dry, and sebaceous environments.[83]In wounds, Dowd et al. showed that bacterial populations differed based on wound types, with

pressure ulcers having 62% obligate anaerobes.[40] Other studies have indicated that *P. aeruginosa* and *S. aureus* vary at different wound locations and appear non-randomly distributed in wounds.[44,84]

Composition and metabolic capability of an oral microbial community are impacted by the environment. Differences of over 2°C have been measured between periodontally healthy tissue and diseased states.[1, 85]Subgingival sites with increased temperatures likely resulting from the inflammatory response have been shown to have elevated proportions of periodontal pathogens.[1, 86]In addition to temperature, pH influences the growth and survival of microorganisms. After sugar consumption, the oral environmental pH falls due to acidic fermentation products, and this results in selection of acid-tolerant oral microogranisms, many of which are pathogenic.[1,87]Organisms such as *S.mutans* and lactobacilli become prevalent and shift the biofilm composition to one that predisposes to caries. This shift emphasizes the role that the environment and host characteristics, including diet, playin oral disease formation.

The environment also influences biofilm architecture.[88]Robinson et al. investigated the effects of mechanical manipulation, pH, ionic strength, and surfactant (0.25% sodium lauryl sulphate) on in vivo biofilm architecture.[88]They found that biofilms were robust, and only surfactant (the concentration used similar to that in toothpastes) removed or significantly reduced biofilm as evidenced by decreased biomass. The knowledge of biofilm architecture and the potential to manipulate it raises the possibility of future biofilm therapeutics and periodontal disease prevention. A summary of the literature presented in the previous chapters comparing oral and wound biofilms is shown in Table 1.

TREATMENT MODALITIES

Debridement

The mainstay of oral hygiene maintenance is the mechanical removal of bacterial biofilm from teeth via tooth brushing, flossing, or using other devices to remove plaque. Dentifrices and mouthwashes are adjuncts containing various biocides, surfactants, polymers, or other components aimed at reducing biofilm, andthese methods have been shown to reduce gingivitis.[9]Physical removal of biofilm bacteria is the standard means of managing biofilms in dentistry and in industry because it is the most effective method currently available.[39]

In wounds, debridement is also thought to be essential for effective biofilm control, with authors suggesting that sharp debridement is the best way to effectively remove and suppress biofilm.[89, 90]Interestingly, a study by Nusbaum et al. investigating a variety of debridement techniques found that plasma-mediated bipolar radiofrequency ablation (PBRA) treated biofilms had the lowest MRSA counts as compared to hydrosurgery, sharp debridement, and no debridement.[91]A decrease in MRSA counts was observed in all treatment groups relative to no debridement, though.[91]Studies have also shown that biofilms are able to rapidly recover from mechanical disruption to reform within 24 hours, thus implying that debridement may lead to only a brief window during which antimicrobial treatments are more effective in reducing both planktonic and biofilm microorganisms.[18]

Antibiotics and antiseptics

Biofilms evade the host's immune system and some studies estimate biofilms to be 1,000 to 1,500 times more resistant to antibiotics than planktonic cells.[92]Sedlacek and Walker studied subgingivaloral species in biofilm and planktonic states and found that almost all biofilm bacterial cells displayed a higher minimum inhibitory concentration than planktonic cells.[93] Multiple mechanisms forbiofilm antibiotic resistance were suggested including that biofilms contain a subpopulation of specialized survivor cells, the drug target may be

modified or unexpressed, the biofilm may contain less susceptible slow-growing bacteria, and the agent may not adequately penetrate the biofilm.[10]The latter is supported by Robinson et al. as they demonstrated increased biomass density from the saliva plaque interface inwards and a decreased frequency of channels and voids.[88]

In patients that lack signs of a local wound infection or systemic bacteremia, systemic antibiotic therapy has been shown to be minimally helpful with efficacy as low as 25 – 30%.[94] Topical antibiotics have also been widely used in chronic wounds with mixed results as wound biofilm bacteria are more resistant to topical antibiotics than planktonic bacteria.[94]Davis et al. investigated biofilm resistance to antibiotics by applying Mupirocin cream or triple antibiotic ointment after 15 minutes (representing planktonic bacteria) or 48 hours (representing biofilm bacteria) to a partial-thickness porcine wound that was inoculated with *S. aureus*.[27] They found that both treatments reduced planktonic bacteria but had decreased efficacy on *S. aureus* biofilms.

The use of antiseptics in the control of biofilm as examined by Kunisada et al. revealed that in vitro *P. aeruginosa*biofilms were reduced when exposed to 0.2% povidone-iodine, but no inhibition was detected on exposure to 0.2% solutions of chlorhexidinegluconate, benzalkoniumchloride or alkyldiaminoethylglycinehydrochloride.[95] Nakagawa et al. also found that povidone-iodine had rapid bactericidal activity against the causative bacteria of periodontal disease.[96]

Anti-biofilm agents

With increased knowledge of biofilm formation, anti-biofilm agents have been investigated in the role of biofilm control. Although disrupting community functions and defenses does not directly kill the bacteria, the perturbation allows other concomitant therapies and natural host mechanisms to work more effectively to promote healing.[39]Lactoferrin, a component of tears, mucus, and human milk, is thought to prevent biofilm formation via altering bacterial motility and decreasing bacterial surface attachment. Lactoferrin use in the disruption of *Pseudomonas* biofilms was described by Singh et al.[4]Lactoferrin has also been shown to act synergistically with xylitol which impairs matrix development.[94]

A study regarding biofilm-based wound care (BBWC) forwound treatment in patients with critical limb ischemia demonstrated a statistically significant increase in wound healing with BBWC as compared to a previous study by Fife.[97, 98] The study used standard wound care therapy as well as lactoferrin and xylitol compounded in a methylcellulose gel as the anti-biofilm agents. The authors concluded that managing wounds assuming that biofilms are present results in an improvement in healing outcomes.[97]

Further knowledge of biofilm molecular communication has led to the potential of a new therapeutic modality for biofilms: quorum sensing inhibitors. Preventing or confusing bacterial communication may decrease the expression of biofilm and virulence genes.[18] In fact, a recent study by Novak et al. demonstrated that knocking out QseBC, a two-component regulator system involved in controlling autoinducer-2 signaling for dental pathogen *A. actinomycetemocomitans*, resulted in reduced alveolar bone loss than a wild type murine model.[99] Thus, modulation of microbial signaling may represent a suitable therapeutic approach to oral and other biofilm diseases.[8]

Polysaccharides are an interesting potential therapeutic of both dental plaque and wound biofilms as studies have shown bacterial mutants deficient in polysaccharide production exhibit increased biofilm formation. Valle and colleagues identified the first anti-biofilm polysaccharideproduced by extra-intestinal *E. coli*.[100]Other anti-biofilm polysaccharides have since been discovered including PsI and PeI produced by *P. aeruginosa*, and crude

polysaccharide (released polysaccharide or r-EPS) from *Lactobacillus acidophilus*. While the details surrounding anti-biofilm polysaccharides need to be elucidated, they have potential to be applied in medical and industrial settings as most exhibit broadspecturm biofilm-inhibiting ability. Bacterial biofilms, therefore, constitute untapped sources of natural bioactive molecules capable of antagonizing adhesion or biofilm formation of other bacteria.[100, 101]

Biofilm extracellular enzymes

Integral to the success of microorganisms is the final stage of the biofilm life cycle, dispersion, which allows bacteria to release from the biofilm and colonize other areas. Dispersion, however, results in an increased number of easier to eradicate planktonic bacteria, and thus is a therapeutic topic of interest. One method of dispersion bacteria use is the production of extracellular enzymes such as glycosidases, proteases, and deoxyribonucleases which degrade the adhesive components in the biofilm matrix. The glycoside hydrolase dispersin B, produced by the periodontal pathogen *A. actinomycetemcomitans*, is known to degrade poly-N-Acetyglucosamine (PNAG), a matrix polysaccharide that functions in the attachment of *A. actinomycetemcomitans* to abiotic surfaces and other microbes.[5]

Non-oral bacteria, including two common wound pathogens *P. aeruginosa* and *S. aureus*, also have been shown to produce enzymes that degrade the biofilm matrix. *P. aeruginosa* produces alginate lyase, of which increased expression promotes biofilm cell detachment and antibiotic effectiveness.[102, 103] Regarding *S. aureus*, mutant strains that were no longer capable of producing extracellular proteases aureolysin and Sp1 resulted in increased biofilm formation and decreased planktonic cells.[104] In addition, deoxyribonucleases known as thermonuclease or micrococcal nucleases are also implicated in cell detachment in *S. aureus* biofilms.[105]

Vaccination and probiotics

As knowledge of the specific bacterial colonizers in periodontal diseases increased, this paved the way for the development of new treatment strategies, including the potential of vaccination against oral pathogens. Numerous studies have documented effective vaccination against oral pathogens.[8] One study by Liu et al. observed decreased bacterial co-agreggation, biofilm formation, and gum inflammation when a vaccine targeting *F.nucleatum*, a bacterium important in bridging primary and secondary colonizers during oral biofilm formation, was used.[106]

Other studies investigating the use of probiotics to replace periodontal pathogens with normal oral flora have been undertaken recently. A pilot human clinical trial assessing the probiotic mouthwash, ProBiora found that the mouthwash substantially decreased levels of periodontal pathogens, thus warranting future studies as probiotics may represent a useful aid in maintaining dental and periodontal health.[107] Similar therapeutic approaches regarding chronic wounds may be discovered as more knowledge is gained regarding chronic wounds and biofilms.

Laser and ultrasound

Another therapeutic modality currently being investigated is the use of lasertreatment in the control of biofilms and disease. In wounds, low-level laser therapy (LLLT) is thought to accelerate wound healing by increasing the proliferation of cells involved in wound healing and the synthesis of collagen, while also decreasing the inflammatory response.[108]Lasers also have been shown to cause disaggregation of microorganisms.[109] In periodontal disease, results from LLLT studies have shown significant bactericidal potential without damaging

oral tissues.[108] A study by Brasso et al. that evaluated LLLT on biofilms formed by *S.mutans* and *Candida albicans*found that LLLT reduced both cell viability and biofilm growth. Interestingly, when *S. mutans* was associated with *C. albicans* in a dual species biofilm, it was resistant to therapy with LLLT.This highlights the role and importance of understanding microorganism biofilm interactions.

In addition to lasers, ultrasonic therapy has been used to investigate oral biofilms as well as wound healing. Nishikawa et al. studied the efficacy of non-contact ultrasonic waves on *S. mutans*biofilms, and observed significant biofilm reduction in vitro as well as moderate biofilm removal in vivo.[110] Likewise, dental plaque biofilm was significantly reduced with the use of an ultrasound toothbrush without bristle contact in an in vitro assay of *S. mutans'* biofilm formed on hydroxyapatite disks.[111]Non-contact ultrasound therapy has also been investigated in wounds as a study by Escandon et al. found a significant reduction in wound area as well as decreased bacterial counts, inflammatory cytokines, and pain over a 4-week treatment period in 10 patients with chronic venous ulcers.[112] While this study did not directly involve biofilms, the results may have been related to decreased biofilm in chronic wounds as dental research has shown beneficial results of ultrasound on biofilms.

FUTURE CONSIDERATIONS

Both oral and wound biofilms are topics of extensive research. While oral biofilms are accepted as the cause of both gingivitis and periodontitis, wound biofilms are a relatively new concept and the role they play in chronic wounds is still being elucidated. This article highlights many similarities between oral diseases and chronic wounds, implying that biofilms likely play a significant role in chronic wound pathogenesis. Wound research canlearn a considerable amount regarding biofilms and the role they play in wounds through looking at what researchers have discovered regarding biofilms in oral diseases.

Wound research has started to utilize knowledge from oral biofilms regarding the use of advanced bacterial identification techniques, including 16S rRNA and FISH, as culture methods are inadequate in detecting the diversity of microorganisms present in both wounds and oral disease. The techniques used to identify bacterial species present in biofilms are crucial to furthering biofilm knowledge. While oral biofilm research has elucidated the sequential process of biofilm formation on teeth and many of the bacterial interactions that occur in oral biofilms, these are largely unknown for wound biofilms.Knowledge of both mechanisms will aid in determining future biofilm therapies.

The role of the innate and adaptive inflammatory immune response in the pathogenesis of both chronic wounds and periodontal disease serves as further evidence of the similarities between these fields of study. Wound research is beginning to investigate the role immunity may play in chronic wounds, and this research should be furthered as immunity is well known to have a role in periodontal disease.Thus, this article highlights the remarkable similarities between wound and oral biofilm associated diseases and suggests that despite of specific differences, future wound biofilm studies will benefit from following the path of oral biofilm research and discoveries.

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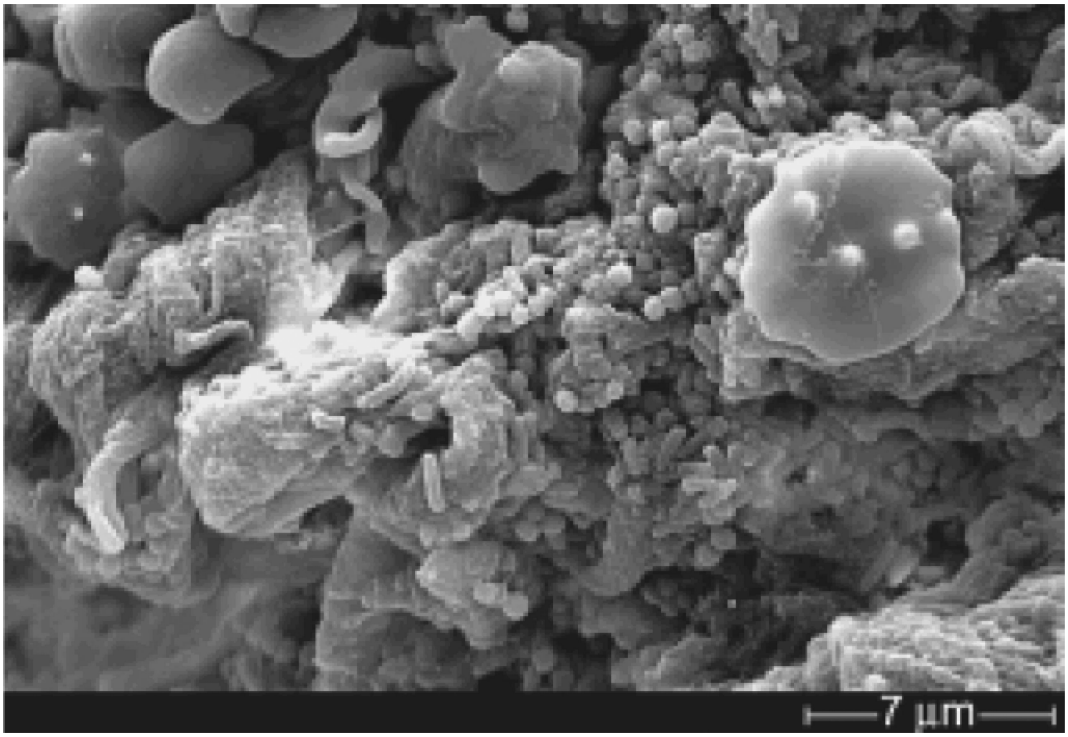

Figure 1.
Scanning electron micrograph of a chronic wound revealing the presence of a mixed species biofilm. Copyright 2008 Wiley. Used with permission from James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. *Wound Repair Regen* 2008;16(1):37-44.

Table 1

Comparison of oral and wound biofilms

	Oral Biofilms	Wound Biofilms
Species Identification	Culture techniques reveal numerous organisms but are thought to underestimate species diversity Molecular analysis utilizing 16SrRNA and FISH reveal over 700 species	**Culture techniques are widely used but likely detect mostly planktonic bacteria and underestimate the diversity of bacterial species present** 16S rRNA and FISH studies demonstrate increased bacterial diversity in wounds
Formation and Composition	Primary Colonizers (*Streptococci* and others) Crucial bridging bacteria (*Fusobacteriumnucleatum*) Late colonizers (periodontal pathogens)	***Staphylococcus aureus, Pseudomonas aeruginosa* and anaerobes play a role** **All bacterial species have not been identified yet**
Bacterial Interactions	Coaggregation, coadherence, coaggregation bridges all methods of bacterial biofilm interactions Various species assist in the survival of other species Complex interactions elucidated for many oral microorganisms	Functional Equivalent Pathogroups proposed as distinct bacterial groups that symbiotically exist in appropriate mixtures Correlation between non-healing wounds and the presence of multiple bacterial species vs. one bacterial species
Bacterial Communication	Autoinducer-2 functions as an important signal molecule with the luxS gene conserved among oral pathogens Gram positive species: peptides Gram negative species: homoserine lactones	Autoinducing peptide-1 and delta toxin serve as signal molecules for *S. aureus* *P. aeruginosa* thought to communicate via N-acyl-homoserine lactones
Immune Response	Innate: Abundant neutrophils and macrophages, tissue destruction by matrix metalloproteinases and ROS, inflammatory cytokines (IL-1, PGE_2, TNF-α, IL-1β) Adaptive: T cells - Gingivitis, B cells and plasma cells – Periodontitis	Innate: Abundant neutrophils and macrophages, tissue destruction by matrix metalloproteinases and ROS, inflammatory cytokines (IFN-α, IFN-γ, TNF-α, and IL- 1)
Environmental Influence	Supragingival and subgingival oral biofilms are composed of different species Bacterial species prefer certain sites in the oral cavity	*P. aeruginosa* and *S. aureus* vary at different wound locations and appear non-randomly distributed Variation in species has been observed with differing wound types

rRNA, ribosomal ribonucleic acid; FISH, fluorescence in-situ hybridization; IL-1, interleukin-1; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; INF-α, interferon-α; INF-γ, interferon-γ