Life on the Edge:
The Clinical Implications of Gastrointestinal Biofilm

Stephen Olmstead, MD, Dennis Meiss, PhD, and Janet Ralston, BS

ABSTRACT: The perceived knowledge of microorganisms is largely a laboratory artifact. In nature, microbes are usually not free-living, but prefer life within biofilm, a collection of phenotypically distinct, sessile organisms inside a hydrated, self-produced matrix. The matrix is principally composed of exopolysaccharides cross-linked by calcium and magnesium. Biofilm existence confers an number of survival and reproductive advantages. The microorganisms are protected from dislodgement, predation, and host immune responses. They are 10-1000-times more resistant to antimicrobials than their planktonic counterparts. Biofilm formation is triggered by adhesion to an edge or surface and is mediated by a mechanism termed quorum sensing. Quorum sensing molecules also facilitate microbial perception of host metabolic and stress status. Biofilms have attracted increased medical research interest because of their important roles in a variety of acute and chronic diseases. The healthful gastrointestinal microbiota reside within biofilm, which provides protective barrier function and colonization resistance against pathogens. Pathogenic biofilms occur in a variety of dysbiotic disorders from Barrett's esophagus and Helicobacter pylori-associated disorders to inflammatory bowel disease and chronic fatigue syndrome. Pathogenic biofilm disruption is the focus of intense pharmaceutical research. An antibiofilm strategy involving nutritional supplements is available. This strategy involves the coadministration of hydrolytic enzymes, chelating agents, and antimicrobials in the fasting state to disrupt pathogenic biofilm and kill pathogens. Pre- and probiotics are given at a separate time of day to support the healthful endogenous microflora, displace pathogens, and further disrupt pathogenic biofilm through probiotic secretion of surfactants and other molecules. More clinical research is needed to develop optimal antibiofilm regimens based on patient clinical status, the specific pathogen involved, and the gastrointestinal site affected.

INTRODUCTION TO BIOFILM

In the microbiology world, a quiet revolution in the way microorganisms are perceived has been occurring that has only begun to catch the attention of medical practitioners. Much of what was and is commonly accepted about microbial structure, function, and ecology consists of laboratory artifacts.[1] Microbes do not normally live as free-floating, planktonic organisms in enriched media on agar plates or in flasks. Microorganisms naturally prefer life on the edge, in protected communities nestled within biofilm.[2] Biofilm is a gathering of sessile microorganisms encased by a self-generated hydrated exopolysaccharide matrix, strongly attached to a surface.[2,3] Familiar biofilms are dental plaque and the film covering the oral cavity on awakening each morning.[4,5] Bacteria and fungi living within biofilm differ substantially from free-living, planktonic members of the same species.[2] Gene expression in sessile, biofilm-associated microbes significantly varies from that of planktonic organisms.[6] Genes mediating adhesion, growth, and motility are downregulated while those mediating expression of exopolysaccharide and exoprotein synthesis as well as antibiotic resistance are upregulated.[7,8] Biofilm populations are quite heterogeneous.[9] Biofilms may be multispecies and sometimes multikingdom communities. Even when populated by a single species, heterogeneity occurs due to diverse gene expression and metabolism related to the dissimilar microenvironments inhabited by organisms within the matrix. Biofilms are complex, interactive communities that have been analogized to multicellular organisms.[10] Microbes within biofilms communicate with each other, display metabolic specialization, and some even appear to undergo programmed cell death similar to apoptosis so as to benefit the greater biofilm community. Biofilms are ubiquitous in nature including the human gastrointestinal tract, oropharynx, skin, and genitalia.

BIOFILMS AND MICROBIAL SURVIVAL

Microbes appear to prefer the biofilm mode of existence because it increases their survival.[2,9] Biofilms confer significant protection to their inhabitants. They are highly resistant to physical stresses such as shear force that occurs in the gastrointestinal tract due to peristalsis. This resistance allows biofilms to develop and persist in otherwise favorable environments without dislodgement. Shear stress is, in fact, a powerful stimulus to biofilm development: the higher the shear stress, the stronger the biofilm.[2] Within biofilms, microorganisms are protected from predation by amoebas, bacteriophages, and other agents.[11] Biofilm shields microbes from the body's humoral and cell-mediated immune responses.[9] The body's phagocytic assault on biofilm generally results in collateral damage to surrounding tissue with no impact on the pathogens. Resistance to antimicrobial agents such as antibiotics and disinfectants is intrinsic to the biofilm mode of life.[2] Depending on the species and antibiotic, biofilm phenotypes are between 10- to over 1000-times more resistant to antibiotics than their planktonic comrades. The mechanisms of antibiotic resistance are not well understood. Reduced antimicrobial diffusion into biofilms is one means applicable to agents such as aminoglycosides, fluoroquinolones, and glycopeptides.[12-15] In some cases, the exopolymer matrix presents an effective barrier to antimicrobial diffusion. In other instances, antibiotics may react with exopolymers rendering them ineffective. However, many antimicrobials, such as rifampin, clindamycin, and macrolides, readily diffuse within biofilms, but remain relatively ineffective.

Biofilm shields microbes from the body's humoral and cell-mediated immune responses. The body's phagocytic assault on biofilm generally results in collateral damage to surrounding tissue with no impact on the pathogens.

A variety of mechanisms have been proposed to explain microbial resistance in the face of what should be effective concentrations.[16-18] These include reduced growth rates, nutrient limitations, toxic metabolite accumulations, adaptive stress responses, and persister

cell development. Finally, in some organisms such as *Candida albicans*, surface adhesion and biofilm formation activates expression of genes mediating classical antimicrobial resistance. All of these mechanisms combine to provide biofilm communities with a multifaceted defense system.

BIOFILMS AND REPRODUCTIVE FITNESS

While biofilms clearly increase survival, evolutionarily, individual survival within a species means little unless reproductive fitness is enhanced. Although it may seem counterintuitive that reduced growth rates within biofilms coexist with improved reproductive fitness, this appears to be a key reason why microbes prefer life within biofilms.[10]

Biofilms are fortified niches that give rise to new generations of adaptive, more highly resistant microorganisms. As parts of the biofilm detach or matrix lysis occurs, sessile biofilm organisms transform into free-living, often virulent, planktonic phenotypes.

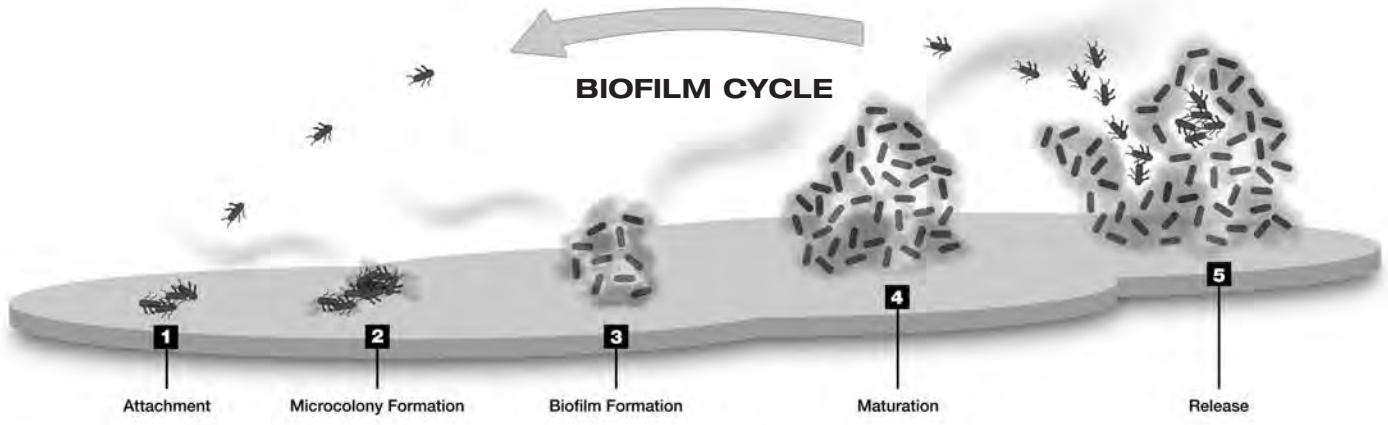
Biofilms are excellent environments for horizontal gene transfer.[9] Among streptococcal species, competence factors mediating biofilm formation have been shown to promote assimilation of external DNA.[20] Biofilms promote conjugation, genetic exchanges, including plasmids, and the spread of genes such as those coding for antibiotic resistance, all of which increase the ability of microbes to survive and reproduce.[2] Biofilms are fortified niches that give rise to new generations of adaptive, more highly resistant microorganisms.[2] These new generations spread as parts of the biofilm detach or matrix lysis occurs and sessile biofilm organisms transform into free-living, often virulent, planktonic phenotypes.[11]

BIOFILM FORMATION

Microbes must adhere to an edge or interface in order for biofilm genesis to occur. Nonmotile organisms reach surfaces by random movement through the suspending medium while motile microbes may actually seek out an interface through some form of tactic response.[3] Adhesion directly to an edge generally does not happen. In the environment and in biological systems, adhesive interactions occur with an overlying stratum of molecules called a conditioning film. This film is predominately composed of glycoproteins and, in multicellular organisms, proteins such as albumin, collagen, fibrinogen, and fibrinonectin. Adhesion triggers a host of changes in gene transcription as microorganisms transform from planktonic to sessile phenotypes. In one study of biofilm formation by *Escherichia coli*, adhesion and biofilm genesis affected the transcription of 38% of the organisms' genes.[21] These genetic alterations lead to changes in electron-transport activity, exopolymer synthesis, metabolic substrate uptake and catabolism, aerobic oxygen uptake, diminished heat generation, and reduced growth rates. Adhesion is quickly followed by biofilm colonization characterized by exopolymer matrix synthesis, proliferation of adherent microbes, and attachment of other organisms of the same or disparate species in a process called co-adhesion. As cell density increases, a cell-to-cell communication phenomenon known as quorum sensing takes place.[22] Quorum sensing is carried out using small hormone-like molecules referred to as autoinducers.[23] Among Gram-negative bacteria quorum sensing is mediated by *N*-acyl-L-homoserine lactones, 4-quinolones, fatty acids, and fatty acid methyl esters while Gram-positive bacteria utilize oligopeptides.[24] Both Gram-negative and Gram-positive microbes employ a family of interconvertible furanone molecules called autoinducer-2. A fourth highly conserved quorum sensing molecule is autoinducer-3 (AI-3).[25] AI-3 is produced by the normal gastrointestinal microbiota as well as by an array of pathogens. It appears to be central to microbial interspecies communication as well as to mediate interkingdom messaging. AI-3 crosstalks with the eukaryotic hormones epinephrine and norepinephrine providing a means for both healthful microbes and pathogens to sense host metabolic status and stress levels. AI-3 may allow the highly pathogenic enterohemorrhagic *Escherichia coli* (EHEC) to switch on virulence factors when host stress is detected.[26] Quorum sensing is essential for maturation of biofilm architecture and sessile phenotype. Mature biofilms are heterogeneous compositions of microorganisms, exopolysaccharides and other exopolymers, water, metals, and debris. Sophisticated microscopy reveals mature biofilms are composed of colonies of cells in matrix-encased structures resembling tiny mushroom shaped turrets or towers crisscrossed by microcanals.[2]

BIOFILM MATRIX EXOPOLYMERS

Biofilm matrix is predominately comprised of exopolysaccharides.[3] Different species synthesize differing biofilm polysaccharides. For example, biofilm made by *E. coli* is characterized by a high colanic acid content, biofilm produced by *Pseudomonas aeruginosus* is rich in alginate, and *Streptococcus mutans* biofilms are mostly mutan and frutan. Most biofilm exopolysaccharides contain the sugars fructose, galactose, glucose, mannose, rhamnose, and *N*-acetylglucosamine. The exopolysaccharides of Gram-negative bacteria are typically anionic and bind cations such as calcium and magnesium leading to polymer cross-linking and increased strength.[27] In contrast, biofilm produced by Gram-positives such as coagulase negative staphylococci may be positively charged.[28] While the dominant matrix exopolysaccharides vary from species to species, common components have been



described.[29] Two widespread polysaccharide biofilm constituents are poly-β-1,6-*N*-acetylglucosamine (PNAG) and cellulose. Poly-β-1,6-*N*-acetylglucosamine is also known as polysaccharide intercellular adhesin (PIA). Although PNAG was first described in staphylococcal species, its production as a biofilm matrix constituent is highly conserved among eubacteria. Cellulose, a polymer of β-(1→4) linked D-glucose sugars associated with plants, is a particularly ubiquitous component of biofilms made by microorganisms in the *Enterobacteriaceae* family although it is also produced by some Gram-positive organisms and cyanobacteria.

HEALTH IMPLICATIONS OF BIOFILMS

Biofilm is increasingly recognized as a major factor in chronic, persistent diseases.[2,11,30] (See Table 1) Disorders acknowledged to be caused by biofilm include dental caries, periodontal disease, endocarditis, osteomyelitis, and otitis media. Infections related to medical devices invariably involve biofilm. The presence of biofilm explains why many common infections are difficult to treat with antimicrobial agents and are characterized by recurrent relapses. Antimicrobial agents may transiently improve symptoms as planktonic pathogens are killed, but the underlying source of the infection, sessile pathogens ensconced within their protective biofilm, cannot be eradicated. Biofilm also explains why infected medical devices do not respond well to antibiotic treatment. The resistance intrinsic to biofilm means that infected devices usually need to be removed for successful treatment. Persistent biofilm may cause chronic symptoms as the body's immune responses are deflected and inflammation damages tissue. Biofilm serves as a nidus for recurring bouts of acute infection.

Table 1 – Examples of Human Infections Associated with Biofilm

Native Tissue Infections	Medical Device Infections
Biliary tract infections	Arteriovenous shunts
Chronic bacterial prostatitis	Artificial heart valves
Chronic Candida infections	Biliary stents
Chronic tonsillitis	Cerebral spinal fluid shunts
Cystic fibrosis pneumonia	Contact lens
Dental caries	Endotracheal tubes
Endocarditis	Endovascular catheters
Kidney stone infections	Intrauterine devices
Osteomyelitis	Orthopedic prostheses
Periodonditis	Penile prostheses

HEALTHY GASTROINTESTINAL BIOFILMS

Hundreds of microbial species call the human gastrointestinal tract home.[31] They outnumber human cells by a factor of 10 and possess more than 100 times the amount of genetic information. The gut microbiota may be considered a distinct, essential organ displaying a metabolic activity on a par with the liver.[31,32] As with virtually all other prokaryocytes, the gastrointestinal microflora prefers life within biofilm.[33,34] Gut microorganisms inhabit a myriad of microdomains and metabolic alcoves. Biofilm is found on the gastrointestinal mucosa, within the overlying mucus layer, and associated with food and other particles within the luminal contents.[33] The only areas of the intestinal mucosa not normally associated with biofilm are colonic crypts.[35] Studies have been at odds as to whether the normal microflora biofilm is mostly associated with mucosal epithelial cells[36] or primarily nestled within the mucus layer.[37] Current evidence based on sophisticated electronic microscopic techniques shows that biofilm spans from the epithelial surface though the mucus layer. Resuspension and culture of human gut biofilm samples are beginning to shed light on its complex taxonomy and microecology. Gastrointestinal biofilm communities are invariably multispecies.[33] Predominate healthy gut biofilm organisms include *Bacteroides*, *Bifidobacterium*, and *Clostridium* species as well as various Gram-positive cocci.[34] *Lactobacillus* species unequivocally form biofilms,[38] but their presence in gastrointestinal biofilm has not been sufficiently studied. Certain organisms, such as *Fusobacterium* species, appear to promote biofilm formation and perform a bridging function bringing together microbes to aggregate on and adhere to epithelial surfaces and expand into the mucus layer.[39] Significant metabolic differences have been described between microbes inhabiting mucosal and food particle biofilm and their planktonic kindred.[40] Biofilm bacteria are more efficient at metabolizing mucin and polysaccharides producing acetate as the primary fermentation end product while free-living organisms ferment oligosaccharides and primarily produce butyrate. The healthful mucosal biofilm embodies gastrointestinal barrier function.[41] It is directly responsible for colonization resistance to pathogenic organisms, an interface for gastrointestinal and systemic immune function modulation, a site for gut detoxification, and a source of calories and nutrients. Disruption of the healthy gastrointestinal biofilm facilitates an inflammatory response to normal commensal microflora and compromises host defenses against a variety of pathogens.

PATHOGENIC GASTROINTESTINAL BIOFILMS

Although research into pathogenic gastrointestinal biofilms is relatively scant, interest is rapidly expanding. Pathogenic biofilms are now implicated in disorders ranging from Barrett's esophagus and *Helicobacter pylori*-associated disorders to inflammatory bowel disease and chronic fatigue syndrome.[33,35,42-44]

Barrett's Esophagus

Barrett's esophagus is caused by gastroesophageal reflux. The normal esophageal squamous mucosa is transformed by chronic acid exposure. People with Barrett's esophagus are at increased risk of developing cancer of the esophagus. Pharmacological and surgical therapies do not prevent Barrett's esophagus or the development of adenocarcinoma of the esophagus. Extensive epithelial biofilm microcolony formation characterizes Barrett's esophagus.[45] This biofilm contains a much higher number of bacteria and greater species diversity than found in the healthy esophagus. Far greater numbers of nitrate-reducing microbes such as *Campylobacter* and *Veillonella* are found. These organisms may cause tissue damage and induce cancer by producing carcinogenic *N*-nitroso substances and nitric oxide, which is mutagenic in high concentrations. Interventions aimed at disrupting pathogenic biofilm in Barrett's esophagus may offer a therapeutic means to reduce the risk of cancer in these patients.

Helicobacter pylori

H. pylori is a helical Gram-negative, microaerophilic bacterium harbored by approximately 80% of people in developing countries and by about 40% of the population in industrialized countries.[46] It is much more prevalent in adults than in children and adolescents. *H. pylori* infection of the gastric epithelium results in chronic gastritis. While most people with *H. pylori* remain asymptomatic, about 10 to 15% will develop ulcer disease. Infection is associated with an increased risk of both duodenal and gastric ulcers. Chronic inflammation induced by *H. pylori* infection leads to the destruction

of gastric glands, fibrosis, atrophic gastritis, and intestinal metaplasia. These atrophic and metaplastic changes may give rise to stomach cancer. *H. pylori* infection increases the risk of gastric carcinoma by 5- to 90-fold depending on the distribution and magnitude of the metaplastic response. *H. pylori* has been long known to form biofilms.[47] *H. pylori* biofilms have been described in people suffering from peptic ulcer disease and in one endoscopic study covered an average of 97.3% of the gastric mucosa in urease positive patients.[42] Despite aggressive antibiotic treatment, *H. pylori* persists in 10-20% of infected patients.[48] Biofilm is hypothesized to play a major role in *H. pylori*'s resistance to treatment and persistence in the gastric mucosa.[49] Recognition that *H. pylori* resides within biofilm on the gastric mucosa may lead to innovative approaches to eradicating this class I carcinogen from the gastrointestinal tract.

Inflammatory Bowel Disease

Inflammatory bowel disease refers to two chronic, relapsing or remitting diseases: ulcerative colitis and Crohn's disease. Ulcerative colitis is an inflammatory disease of the colon and rectum. The colon mucosa becomes inflamed and develops ulcers. Crohn's disease most commonly affects the terminal ileum and parts of the large intestine. However, it can attack any part of the digestive tract. The inflammation of Crohn's disease generally involves the entire bowel wall. Although the etiology of inflammatory bowel disease is unknown, several lines of evidence have implicated an abnormal response to certain commensal gut bacteria in its pathogenesis.[50] Animals used to model inflammatory bowel disease are healthy when raised in a germ-free environment and only develop intestinal inflammation on exposure to normal gut microbiota. In inflammatory bowel disease, higher numbers of biofilm-residing *Bacterioides*, *Enterobacteriaceae*, and *Peptostreptococcus* and lower numbers of *Bifidobacterium* are found in regions of inflamed mucosa than are encountered in healthy controls.[35,51] In both Crohn's disease and pouchitis, a complication of surgical therapy for ulcerative colitis in which an ileal pouch connected to the anus becomes inflamed, inflammation is subdued when fecal flow is diverted and flares when fecal flow is restored suggesting that exposure to luminal contents causes inflammation. Conversely, certain commensal biofilm bacteria, such as *Bifidobacterium*, protect the intestinal mucosa from inflammation by competing with proinflammatory species, reducing intestinal permeability, improving epithelial defense mechanisms, and modulating innate and acquired immune responses. Strategies for modification of gut commensal biofilm communities such as the use of probiotics and prebiotics have been shown to reduce mucosal concentrations of proinflammatory cytokines and resolve inflammation and eliminate abscess formation in patients with ulcerative colitis.[52]

Chronic Fatigue Syndrome

Chronic fatigue syndrome consists of debilitating fatigue often associated with arthralgias, myalgias, chills, feverishness, and lymphadenopathy.[53] Symptoms are usually exacerbated by exercise. There is significant clinical overlap between chronic fatigue syndrome and other illnesses such as irritable bowel syndrome and fibromyalgia. Studies show that the normal gastrointestinal microbiota is frequently disrupted in people with chronic fatigue syndrome, fibromyalgia, and irritable bowel syndrome. Chronic fatigue syndrome and fibromyalgia are associated with a gut microflora low in *Bifidobacterium* and high in *Enterococcus* species.[55] Higher enterococcal counts correlate with more severe neurological and cognitive symptoms. In people with diarrhea-predominate irritable bowel syndrome, *Lactobacillus* numbers are reduced while in patients with constipation, greater numbers of *Veillonella* species are present compared to healthy controls.[56] While its etiology is unknown, one longstanding, albeit controversial, hypothesis is that chronic fatigue syndrome is caused by an immune response to intestinal colonization by *C. albicans*.[57,58] Increased fecal counts of *C. albicans* have been described during the early phase of the syndrome.[59] Treatment with antifungal agents together with a special diet has been described as improving the symptomatology of patients with chronic fatigue symptomatology.[60]

Candida species ubiquitously form biofilm communities and most manifestations of candidiasis are associated with biofilm formation.

Improvements are often transient and the role of *C. albicans* in chronic fatigue syndrome and related disorders remains contentious in part because there is no definitive test and yeast overgrowth as documented by fungal cultures is uncommon in these patients. A refinement of the *C. albicans* hypothesis is that chronic fatigue symptoms are caused by an immune response to *Candida* species residing within biofilm in the gastrointestinal tract. *Candida* species ubiquitously form biofilm communities and most manifestations of candidiasis are associated with biofilm formation.[44,61] Transition to the biofilm phenotype is associated with up regulation of genes coding for multidrug efflux pumps which would contribute to treatment resistance of biofilm *Candida* species.[44] The biofilm mode characteristics of slow growth and presence of persister microorganisms would explain the tendency for patients to relapse following a beneficial symptomatic response to one or more courses of therapy. The combination of antifungals with agents designed to disrupt biofilm along with pro- and prebiotics to reestablish a balanced, healthy gastrointestinal microbiota may offer an innovative approach to treating patients with chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, and other chronic disorders associated with gastrointestinal dysbiosis and *Candida* biofilms.

APPROACHES TO PATHOGENIC GASTROINTESTINAL BIOFILM

The pivotal importance of pathogenic microbial biofilms in human disease has generated intense research efforts to find pharmacological agents that prevent and disrupt biofilm formation. A major focus has been on agents that disrupt quorum sensing.

A nonpharmaceutical approach to pathogenic biofilm eradication involves the use of digestive enzymes and chelating agents available as nutritional supplements together with naturally occurring antimicrobial agents.

Other interventional targets include cell-wall biosynthetic enzymes, cell-wall cross-linking enzymes, cell-wall proteins including adhesins, enzymes involved in biofilm polysaccharide production, polysaccharides and proteins involved in biofilm formation, and chelation of metals essential to biofilm formation and maintenance. A nonpharmaceutical approach to pathogenic biofilm eradication involves the use of digestive enzymes and chelating agents available as nutritional supplements together with naturally occurring antimicrobial agents.

Polysaccharidases

Enzymes that disrupt the polysaccharides involved in biofilm formation offer great potential as antibiofilm agents. Cellulose is a major component of most biofilms.[29] Cellulase is a group of

glycoside hydrolase enzymes that hydrolyze the β-(1→4) glycosidic bonds in cellulose. Cellulase has been shown to significantly reduce biofilm formation by the important pathogen *P. aeruginosa* as measured by biomass and colony forming unit counts.[63] The antibiofilm effect of cellulase alone was only partial underscoring the need to combine cellulase with other enzymes and agents that break down biofilm matrix and impair cell wall function. Beta-glucanase is one such enzyme. β-(1→3)- and β-(1→6)-glucans compose up to 60% of the cell wall of fungi like *C. albicans* and are the major component of candidal biofilms. The inclusion of a beta-glucanase with β-(1→3) and β-(1→6) lytic activity would provide powerful anti-candidal biofilm activity.

Peptidases/Proteases

Peptidases and proteases have been shown to prevent and disrupt biofilm formed by a variety of microbes especially *Staphylococcus* species.[65] *In vitro*, proteases inhibit biofilm formation and cause rapid detachment of *S. aureus* biofilms increasing the organism's susceptibility to antibiotic treatment.[66] One of the more important antibiofilm proteases is *Serratia* peptidase, a zinc metalloprotease derived from non-pathogenic strains of enterobacteria genus *Serratia*.[67] *Serratia* peptidase degrades biofilm and supports against both infection and inflammation. In one *in vitro* study, *Serratia* peptidase was more effective than clostridiopeptidase, fibrinolysin, and streptokinase at disrupting biofilm and augmenting the sensitivity of biofilm-embedded *P. aeruginosa* and *S. epidermidis* to the antibiotic ofloxacin.[68] Research also shows that *Serratia* peptidase significantly decreases the ability of *Listeria monocytogenes* to produce biofilms and invade intestinal-like Caco-2 cells.[69] Animal studies confirm that *Serratia* peptidase increases the efficacy of antibiotics to eradicate biofilm-forming pathogens.[70] Proteolytic biofilm degradation is believed to be the mechanism whereby *Serratia* peptidase enhances antibiotic activity. Combining *Serratia* peptidase with broad spectrum proteases and select polysaccharides can deliver potent biofilm disruptive effects.

Metal Chelating Agents

Metals such as calcium, magnesium, and iron are critical to biofilm formation and maintenance.[27] The disodium salt of ethylenediaminetetraacetic acid (EDTA) is a powerful chelator of bi- and trivalent cations.[71] Disodium EDTA has well established antibiofilm activity mediated by complexing with metals required for cross-linking biofilm matrices. Disodium EDTA also causes structural damage to bacterial cell walls making them more permeable to antimicrobial agents.[72,73] EDTA has been shown to inhibit filamentation and biofilm formation by *C. albicans*. Disodium EDTA effectively inhibits the growth of microorganisms residing within biofilms. In one *in vitro* study, disodium EDTA alone dramatically decreased the number of biofilm *P. aeruginosa* cells by up to 99% in a dose-dependent fashion.[74] The combination of EDTA and gentamicin completely eliminated the pathogens. In another study, EDTA effectively reduced or eliminated Gram-negative, Gram-positive, and mixed populations of microorganisms within intravascular catheters removed from hemodialysis patients. Another effective antibiofilm chelator is a component of the innate immune system.[76] Lactoferrin, a copious constituent of human external secretions, binds iron. It dramatically reduces biofilm formation by *P. aeruginosa* by preventing pathogen aggregation. These studies indicate that EDTA and lactoferrin may potently augment the antibiofilm activity of hydrolytic enzymes in a comprehensive antibiofilm protocol.

Antimicrobial Agents

A major survival and reproductive advantage conferred by life within biofilm is resistance to antimicrobial agents and immune responses.[2,11] Any successful approach to pathogenic biofilm must combine agents that disrupt biofilm, such as hydrolytic enzymes and chelators, with antimicrobial agents. Disruption of biofilm alone is unlikely to have a major impact unless measures are taken to inhibit and destroy the microbes residing within the biofilm. Antimicrobial agents should be taken contemporaneously with hydrolytic enzymes and chelators for maximal effect and should be taken away from meals to minimize any interference by ingested foods. Antimicrobials employed span the spectrum from natural agents such as berberine and undecylenic acid to prescription antibiotics and antifungals based on the type of biofilm and the anticipated sensitivities of the pathogen to be treated.

Prebiotics & Probiotics for Upper Gastrointestinal Biofilm

Depending on the location of the pathogen gastrointestinal biofilm, pre- and probiotics may have a significant role in the treatment of pathogenic biofilm. The role of prebiotics in the management of disease-associated upper gastrointestinal biofilm may be limited because normal populations of commensal flora in these areas are quite low. A number of probiotics have been shown to have significant antagonistic effects against *H. pylori*. In studies using solid media and an *in vitro* model simulating gastric conditions, *Lactobacillus rhamnosus*, *L. paracasei*, and *L. plantarum* antagonized *H. pylori*. In a human study, *L. casei* together with quadruple therapy augmented successful eradication of *H. pylori* after a failed first round of triple antimicrobial therapy. In children, intake of a dairy product containing *L. johnsonii* was shown to reduce *H. pylori* gastric colonization in children. A recent review of probiotics as treatment for *H. pylori* concluded that select probiotic strains which in addition to the above, included *L. acidophilus*, *L. salivarius*, *L. reuteri*, and *L. lactis*, antagonize *H. pylori* and attenuate *H. pylori*-associated gastritis in animal models.[80] Seven of 9 human studies have shown that probiotics improve *H. pylori* gastritis and decrease *H. pylori* populations. The addition of one or more probiotics to an antibiotic regimen significantly improves eradication rates and reduces side effects by 50%. When selecting a probiotic as an adjunctive to *H. pylori* therapy, it is very important to choose a formulation without any enteric coating or acid protective technology so that the organisms are free to interact within the stomach. It is also essential to consume the probiotics 1 hour before or at least 2 hours after an antibiotic dose so as to minimize the bacteriocidal effects of the antibiotic(s) on the probiotic organisms.

Prebiotics & Probiotics for Lower Gastrointestinal Biofilm

Prebiotics show greater benefit in the management of pathogenic biofilms in the colon. Prebiotics uniformly stimulate the growth of endogenous *Bifidobacterium* species and to a lesser extent augment populations of *Eubacterium* species.[81,82] By increasing healthful populations of normal commensal flora, pathogens residing within biofilm may be displaced from their microecological niches. In the setting of ulcerative colitis, the combination of prebiotics with probiotics (symbiotic) has shown greater benefit in the modulation of colonic biofilm communities than either a prebiotic or probiotic alone.[52] In addition to beneficially modifying mucosal biofilms, the prebiotic inulin has been found to stimulate proliferation in crypts, augment mucin release, and favorably alter mucin composition which may enhance gut barrier function. Probiotics show great efficacy in displacing pathogenic biofilm by combating pathogens through a number of mechanisms including secretion of organic acids, hydrogen peroxide, and bacteriocins.[84] Less well appreciated is the ability of probiotic organisms to directly disrupt pathogenic

biofilm. Surfactants produced by various strains of *L. acidophilus* have been shown to significantly reduce biofilm formation by both *Staphylococcus aureus* and *S. epidermidis*.[85] *Streptococcocus thermophilus* has been found to secrete a surfactant that impairs adhesion of *C. albicans* to surfaces.[86] As research progresses, it is highly likely that much of the ability of probiotics to interfere with pathogen adhesion and displacement will be found to be due to specific antibiofilm activities.[87,88] In the management of pathogenic lower gastrointestinal biofilms, a combination of pre- and probiotics represents the most comprehensive strategy for the displacement of pathogens and their replacement by healthful microbes. Pre- and probiotics should be taken with meals and not contemporaneously with hydrolytic enzymes to minimize their effect on healthful biofilm formation. The selection of a probiotic formulation with high numbers and enteric coating or a acid stabilization technology represents a prudent approach to ensuring high numbers of viable microorganisms arrive in the distal small bowel and colon.

CONCLUSION

Bacteria and fungi prefer life within biofilm communities where they are protected from predation, antimicrobials, and host immune responses. Biofilm is increasingly implicated in a variety of acute and chronic diseases. The normal gastrointestinal microbiota resides within biofilm and a number of gastrointestinal diseases associated with dysbiosis are related to the formation of pathogenic biofilm and the toxic effects of the pathogen or the body's immune responses to persistent, resistant microbes. A combination of hydrolytic enzymes available as nutritional supplements represents a viable approach to degrading pathogenic biofilms by lysing biofilm exopolymers. Chelating agents degrade biofilm by binding metals required for pathogen aggregation and exopolymer cross-linkage. Hydrolytic enzymes may be combined with one or more chelating agents for a synergistic antibiofilm effect. In treating pathogenic biofilm, it is essential to administer enzymes and chelators together with antimicrobial agents, but apart from food to ensure pathogen destruction as biofilm is degraded. Pre- and probiotics are optimally used in an antibiofilm regimen to support the healthy intestinal microflora, displace pathogens, and disrupt pathogenic biofilm by the secretion of surfactants and other molecules. There exists a critical need for clinical research to establish effective combinations of hydrolytic enzymes, chelators, pre- and probiotics and to work out optimal doses and regimens for various pathogens.

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