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Biofilm: Its Relevance In Kidney Disease

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Biofilm/bioslime is a complex, dynamically interactive multicellular community protected within a heterogeneous exopolysaccharide matrix. Its formation results in the genesis or perpetuation of infection, enhancement of inflammation, and tissue damage or death. Industrial financial losses result from biofilm/bioslime formation; however, the consequences in the medical realm are equally devastating. The relation of biofilm to patients with chronic kidney disease is often covert and extends beyond the colonization of hemodialysis circuits and vascular accesses. Urinary tract device– and vascular access–related biofilms may also increase the burden of cardiovascular risk borne by chronic kidney disease patients, synergizing with the chronic inflammatory state already incurred by these individuals. Current anti-infective strategies are aimed at rapidly killing planktonic forms of microorganisms without specifically targeting the sessile forms that perpetuate their planktonic brethren. Future treatments of infections must ultimately target these reservoirs of infection aiming for their complete eradication. Presently, included among these novel weapons of microdestruction are molecular blockading techniques, electrical enhancement of anti-infectives, and bacterial interference. Nonetheless, the best approach against biofilm formation remains the prevention of microbial colonization, which can be largely by sterile handling of patient-related devices, the most well-established biofilm reservoirs.

Index Words: Biofilm; Chronic kidney disease; End-stage renal disease; Autoinducer; Quorum sensing; Acylhomoserine lactones

Pathogenic organisms have withstood the onslaught of anti-infective therapies since their inception. The major reason for their persistence and ability to evolve is their commensal property of biofilm formation. Although the incidence of biofilm or bioslime formation remains unknown, these polymeric, genetically controlled communities of microorganisms adherent to surfaces, are estimated to cause at least 60% of bacterial infections in the developed world.[1] Accordingly, biofilm constitutes an alarmingly high proportion of infection-related medical costs, which in 2002, have been estimated at $6.7 billion by the Centers for Disease Control and Prevention.[2] Patients with chronic kidney disease incur a disproportionate share of infections and, therefore, are similarly at higher risk for biofilm formation. For example, direct charges for *Staphylococcus aureus* bacteremia in hemodialysis patients has been estimated at $24, 033 per episode.[3] Moreover, biofilm represents a pathogenic force in the realm of peritoneal dialysis, kidney stone formers and any patient housing an indwelling medical device, particularly within the confines of an intensive care unit in which resident biofilms may become abundant. In addition, hemodialysis-dependent individuals are periodically exposed to a circuitry of tubes and sensors that may be contaminated by hardy biofilms. Presently, the accrual of the molecular underpinnings of biofilm formation is now bearing fruit. The conception and development of novel molecular and pharmacological treatment modalities that target not the intact pathogen per se, but the regulation and control of its biofilm-making machinery enhances the conventional approach of antimicrobial/antibiotic therapy and paves the way for improved therapy of all patients with infections.

Epidemiology of Biofilm

Biofilm is a sessile multicellular community comprised of an exopolysaccharide matrix embedded with living microorganisms that evolves to overcome local microenvironmental physical and chemical stressors. Bacterial adherence on inert and living surfaces plays an important role in the industrial, agricul-

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tural, and medical fields, often with highly damaging financial outcomes. Biofilm-mediated corrosion of water- and oil-carrying pipes, tubes, tanks, and reservoirs is a common industrial concern. The presence of biofilms within oil pipelines leads to corrosion of the internal pipe wall, and within industrial water systems they cause biofouling, the phenomenon whereby surfaces in contact with water are colonized by microorganisms, often with secondary accretion of local minerals, making the bioslime even less penetrable.[4] In water-distribution systems, biofilms degrade the safety and quality of drinking water.

However, industry-ruinous organisms must first gain a foothold on inert surfaces before they unleash their uniquely damaging capabilities, one of which is altering the native galvanic currents on metallic surfaces to optimize their adherence. Several bacterial species can absorb nitrogen and sulfur from the atmosphere and/or their host metallic surfaces to produce structurally damaging nitric and sulfuric acids. The mining industry's leaching processes (mixing the ore with chemical in order to separate materials) also inadvertently support the growth of massive biofilm formation. Other industries similarly afflicted include those involving airplanes, papermaking, and oil distribution. In agriculture, the health of crops and livestock is compromised by biofilm. As an example, the xylem-clogging organism, *Xylella fastidiosa*, wreaks destruction on the grape industry without ever directly physically damaging the plant.[5] "Ring rot" by *Clavibacter* or *Rhizobia* species, on the other hand, directly infects its potato hosts, thereby killing them.[6]

In medicine, biofilm is hypothesized to cause more than 60% of all infections.[1] Biofilm formations have a major impact on temporary and permanent implants or devices placed in the human body. Electron microscopic studies of infected medical devices have revealed heavy colonization and biofilm formation on such devices.[7] Endotracheal tubes, urinary catheters, pacemaker wires, and orthopedic joint replacements have all been inhabited by biofilms, with consequent infection. In dentistry, biofilms play an important role in the formation of dental plaque, which contributes to infection, tooth decay, and chronic gum disease such as inflammatory periodontal disease. Dental implants, braces, and bridges also represent ideal places for biofilms, bathed in an environment replete with commensal bacteria that possess the appropriate molecular machinery to stick to such prosthetics.

Aside from these sources of biofilm, one must never forget that biofilms live within our own residences, on cutting boards, kitchen counters, and in toilets. Failure to cleanse such surfaces properly may contribute to infection, particularly in immunocompromised individuals or those with long-term indwelling urinary and vascular catheters for home-infusion therapies.

Biofilm Formation and Regulation

Bacteria moor themselves to inert and tissue surfaces, particularly those that have been previously scored or roughened, via their fimbriae and pili (see Fig 1 of article by Lok in this issue). After adherence, these sessile forms reinforce their anchorage through their production of a "sticky" exopolymer. This polymer is composed primarily of exopolysaccharide and water, and its mass may be 100-fold larger than the microorganisms that it shelters. Production of this matrix precedes an initial growth phase that culminates in microcolony formation. Expansion of microcolonies and coalescence ultimately leads to a much larger, mature, heterogeneous, and multilayered biofilm, housing a biomass that can sustain itself with nutritive flow through multiple channels that also permits biological communication among the sessile form of microorganisms throughout the biofilm. From these sessile organisms, free-living or planktonic forms are derived that can multiply rapidly and disperse after programmed detachment from the host biofilm to produce acute infectivity. A more greatly detailed expository of the orchestration of the multiple steps in the biofilm life cycle follows using prototypical examples of several pathogenic species.

Bacteria produce toxic exomolecules only when in higher densities (postexponential phase of growth). In early exponential growth, when at lower densities, the bacteria express surface molecules, such as fibronectin-binding proteins and fibrinogen-binding protein, facilitating their attachment per se to

a central venous catheter with a fibrin sheath. These allow the organism to adhere to and colonize host cells.[8] For example, the ability of *S aureus* to differentially express surface adhesion molecules and toxin exomolecules is regulated primarily by RNAIII, encoded by the *agr* locus. Synthesis of RNAIII is regulated by quorum sensing, a process by which bacterial signaling molecules (autoinducers) are produced and secreted until a critical threshold concentration is reached and RNAIII is synthesized.[9]

On surface contact, bacteria such as *Pseudomonas aeruginosa* transcribe specific genes (*algC*, *algD*, and *algU*) to synthesize extracellular polysaccharides that increase there surfacial attachment.[10] Again via a quorum sensing regulatory process, when the resulting microcolony aggregates achieve critical density, autoinducer acylhomoserine lactones (AHL) encoded at the *LasR-Lasl* loci, are produced.[10] AHLs subsequently facilitate thickening of biofilm, establishment of intercellular signaling regarding cell-density relationships and production of virulence factors, with downstream cytokine regulation. Although the exact mechanisms of quorum sensing differ between gram-negative and gram-positive bacterial species, a commonality exists: quorum sensing regulates bacterial symbiosis, virulence, antimicrobial production, and biofilm formation and is a fertile field for innovative pharmacotherapeutics.[10]

Biofilm in Kidney Disease

By their attachment to inert (eg, stone matrix) or biological surfaces (eg, mucosal scars), biofilms gain importance in kidney disease because of their promulgation of infections of the urinary tract, vascular accesses, central venous catheters, arteriovenous grafts, peritoneal catheters, dialysis circuits, and dedicated water systems.[1,7] Moreover, the proinflammatory mediators emanating from biofilms may promulgate cardiovascular risk, enhance the chronic inflammatory state of chronic kidney disease, and worsen anemia management.[11]

Urinary Tract Infections

Bacteria and/or fungi can initiate biofilm formation on urinary catheters in 48 hours. When present on the surface of such a device, the escape of planktonic forms renders the patient susceptible to urosepsis. The subsequent incorporation of endogenously produced proteins such as coagulation proteins can enhance the growth of the biofilm and cosset the organisms from anti-infective therapy and eradication. Therefore, surveillance for urinary tract infections is paramount in individuals who require long-term catheterization. Chronic kidney disease may result from staghorn calculus formation, and struvite formation from urease producing bacteria is the principal pathogenetic event. However, the initiating event is the adherence of the inciting organism to a stone nidus, disrupted urothelium, or a foreign body, including urethral catheters, stents, and nephrostomy tubes in the renal parenchyma and/or bladder. Adherence is followed by colony formation and biofilm expansion, which now permits the organism to live on the stone that it has produced and continually reinfect the urine. Furthermore, the biofilm protects the organism from antibiotic/antimicrobial penetration[12] and urine-acidifying therapy, as with *Proteus mirabilis*.[13] Lastly, crystals entrapped in the biofilm matrix resist environmental pH changes and exhibit rapid structural growth aggravating the stone burden.[13] Chronic urinary tract infections may also result from chronic prostatitis, and this too is a biofilm-related disorder. Evidence of the persistence of bacterial microcolonies or biofilms within the prostatic ducts or incorporated into corpora amylacea or calculi within the prostate permits long-term infection that becomes increasingly difficult to eradicate.[14]

Vascular Access–Related Infections

In general, infections are the second leading cause of mortality among patients with end-stage renal disease. Many of these infections are caused by bacteremias that occur with a frequency of 1 per 100 patient-care months. The source of the infections is most often attributed to external skin-resident microbes gaining access to the blood space by migration along the external environs of a vascular access device, the consequence of which is an associated 3-month mortality of 19% to 34%.[3,15,16] Hence, it is not surprising that

staphyloccocal species account for 60% to 100% of episodes of catheter-related bloodstream infection in hemodialysis patients.[3,15] Obviously, efforts should be made to create and maintain native arteriovenous fistulas that carry the lowest risk of infection among all vascular access modalities.[15,16] On the other hand, bacteremias associated with indwelling vascular catheters predispose to the development of infective endocarditis in 2% to 6% percent of hemodialysis patients.[17,18] Direct analysis of tissue vegetations reveals the existence of matrix-embedded microcolonies of bacteria.[1] Indeed, vegetations represent macroscopic bacterial biofilms.[19]

Bacteria can potentially initiate biofilm formation on the walls of urinary and vascular catheters within 48 hours of their placement, predisposing such patients to the development of sepsis from catheter-related infections.[20] However, simple colonization is necessary but not sufficient to produce catheter-related bloodstream infection. Planktonic forms of the pathogen must be present and "break" free from the biofilm to induce bacteremia and/or sepsis.[21] This observation correlates directly with the finding that just 11% of organisms can be cultured from indwelling central venous catheters that harbor biofilms.[21] Furthermore, flow stagnation in venous catheters or a biosynthetic arteriovenous graft, from whichever cause, offers an ideal environment in which immobilized bacteria can proliferate and expand their biomass.[22] Therefore, hemodialysis catheter dysfunction manifested by diminishing forward flows facilitates biofilm formation.

Peritonitis

Peritoneal catheter-related infections (exit-site infections, tunnel infections, and peritonitis) are mostly associated with technique failures with continuous ambulatory peritoneal dialysis.[23] As with all long-term medical devices, peritoneal catheters frequently acquire bacterial biofilm, thereby inducing the aforementioned infectious complications and their recurrence. This proposal has been shown in an analysis of 32 Tenckhoff peritoneal dialysis catheters from patients with peritonitis. Here, viable mixed microbial biofilms were present on 81% of catheters analyzed.[24] Moreover, the influence of deposition of extracellular matrix and coagulation protein agglomeration was shown in a case in which disruption of biofilm by tissue plasminogen activator led to resolution of a recurrent catheter-related peritonitis[25]; the authors expanded on a prior observation wherein heparin had yielded a similarly efficacious outcome.

Dialysis Circuits and Water Systems

Hemodialysis water systems have been fouled by biofilm. Affected sites have included, paradoxically enough, the water treatment system itself, hydraulic monitors, and water distribution pipelines. Part of the problem has been attributed to the favorable environment sought by contaminating water-borne bacteria (ie, the organic nutrient content and high pH of commonly used bicarbonate-buffered solutions). In addition, physical factors, such as dead ends, low fluxes, and "stop flow" intervals, may favor biofilm formation.[26,27] However, the highest risk area for bacterial contamination likely derives from the water tubing that connects to the reverse osmosis-water distribution loop with the individual hemodialysis monitors.[28] Unfortunately, bacteria- and endotoxin-free dialysate does not exclude the risks and hazards of bacteria and endotoxin discharge from preexistent biofilm in fluid pathway tubing, serving as a continuous contaminatorium from which both bacteria and algae have been isolated.[26]

A multimodal plan of attack that thwarts biofilm formation within the silicone tubing of dialysis machines ought to include the following: direct microscopic observation for biofilm formation, biofilm removal with a mechanical scraping device, quantitative analysis with culturable and total bacteria counts, and endotoxin level measurement.[29] In summary, biofilm formation represents the starting point for biofouling and resistance to disinfection and bacterial regrowth.[30] Its residence within the hydraulic dialysis circuit consequently modifies the efficacy of differential disinfection modalities against bacteria and endotoxin concentrations.[31]

Cardiovascular Risk and Chronic Inflammation

Recurrent bacteremias that originate from biofilm bacterial colonization has been associated with an approximately 2-fold increase in the risk of myocardial infarctions, stroke, heart failure, and peripheral vascular disease.[11] Therefore, bioslime represents an ongoing source of chronic, subclinical inflammation resulting from repetitive cycles of the release of noxious proinflammatory cytokines derived from monocytes/macrophage stimulation. This pathophysiological sequence potentially explains some the isolated febrile responses that occur during hemodialysis sessions in patients who have no obvious cause for infection.[20,32] In patients with catheters, flow turbulence and shear forces within and around the catheter blood ingress and egress sites may convert sessile organisms into planktonic ones. The cytokine release from biofilms during dialytic and interdialytic intervals may also favor coagulation pathway activation, thereby enhancing the probability for vascular access thrombosis and erythropoietin resistance.[20] In clinical studies regarding the anemia of end-stage renal disease, vascular access infections have been shown to increase the dose requirement of erythropoietin.[33]

Biofilm Detection and Eradication

No method presently exists that detects biofilm in vivo with sufficient sensitivity to confirm its eradication (ie, direct bright field microscopy). More highly sensitive techniques (eg, confocal microscopy) remain relegated to the research laboratory and cannot be used practically on a widespread scale in the clinical realm. In vascular access catheters that have been removed, surface ultrastructural analysis alone or in combination with specific staining techniques that detect bacterial nuclear DNA with membrane-permeable or -impermeable fluorochromes has disclosed the presence of microorganisms thriving within their glycocalyx cocoon.[20] However, an indirect assay that detects viable organisms via endotoxin level measurement with chromogenic kinetic assays may provide a more efficient and practically-based assay for uncovering microorganisms resident within biofilm.[34]

Adaptive Resistance

Bacterial biofilms show adaptive resistance in response to antimicrobial stress more effectively than corresponding purely planktonic populations.[30] The mechanisms of resistance of biofilms differ from the well-described methods by which bacteria develop innate resistance (ie, plasmid-mediated gene transfer, transposons and mutations that provide survival advantages[30]). Biofilm-mediated antimicrobial resistance is partially attributable to the relatively slow growth of biofilms, whereas planktonic organisms are highly susceptible to antimicrobials that operate optimally against rapidly dividing cells. Another possible mechanism for antimicrobial resistance is the poor nonfacilitated diffusion of antimicrobials or antiseptic solutions into and through biofilm. This anti-infective process is further diluted by active degradation of these agents by biofilm. Furthermore, anaerobiosis with deeper and dense biofilm layers and local accrual of waste products wherefrom may produce downstream antagonism of antimicrobials.[30] Worse yet is that certain small bacterial populations become truly invulnerable "persister" cells, which neither grow nor die in the presence of bactericidal agents, hence exhibiting multidrug tolerance.

Persisters are protected from host defenses by their contiguous matrices and after declines in antimicrobial concentration, these survivors propagate with recrudescent infection.[35] Lastly, plasmid transfer occurs at a greater rate between cells in biofilms than between planktonic cells, likely the consequence of the closer apposition of cells matrixed within the organic polymer.[36,37] Because plasmids encode for resistance to multiple antimicrobial agents, biofilm facilitates the spread of bacterial resistance to antimicrobial agents.[38]

The genetic and biochemical details of the biofilm defenses are being elucidated because each gene and gene product contributing to this resistance may be a target for the development of newer agents. Disabling biofilm resistance may enhance the ability of currently used antimicrobials to discharge infections

Table 1. Treatment Modalities in Biofilm Eradication

Mechanism	*Treatment Modality*	*Examples*
Enzymes and detergents	Industrial products	Citric and peracetic acid
Fibrinolysis	Thrombolytics	Recombinant tissue plasminogen activator, Streptokinase
Transcriptional inhibition of production of virulence factors and autoinducers	Antimicrobials	
	Systemic	Systemic: "subinhibitory" erythromycin, clindamycin concentration
	Impregnanted catheters	Impregnated: minocycline and rifampin
	Antimicrobial catheter lock	Lock: vancomycin, linezolid, gentamycin
	Tetrasodium EDTA	
Quorum sensing inhibitors	Targeted blockade	
	Analogs	Furanone derivatives
	Natural inhibitors	RNA III inhibitory peptide (RIP)
Bacterial Interference	Catheters coated with nonpathogenic bacteria avoid colonization by uropathogens	Catheters coated with nonpathogenic *E coli*
Electrical enhancement of antimicrobials	Intermittent electrical field enhances anti-infective therapy	Direct current plus antimicrobial

Abbreviations: EDTA, ethylenediaminetetraacetatic acid; tPT, tissue plasminogen activator.

that have been refractory to therapy because of biofilm development. In this regard, the distinct and possibly complementary modalities of biofilm eradication are enumerated in Table 1.

Hygiene

The genotypes of community-associated methicillin-resistant *S aureus* are different from nosocomial strains that express the distinctive type IV methicillin-resistance chromosomal cassette and Panton-Valentine leukocidin virulence factor, which primarily promotes skin and soft-tissue infection.[39] Currently, outbreaks of MRSA infection, frequently found among young individuals, are thought to be caused by complex interactions that accrue from an environment contaminated by MRSA, indiscriminate use of antimicrobial drugs and personal hygienic factors. Nonetheless, the most important approach to biofilm formation is prevention. Consequently, excellent personal hygiene is and always has been essential to the prevention and elimination of community-associated methicillin-resistant *S aureus* infections, including catheter-related infections.[40] In hemodialysis centers, it must be reemphasized that the careful and sterile handling by health care personnel of vascular accesses during treatment delivery remains primary as the most important step in avoiding microbial colonization of the skin and the catheter. Although specific trials addressing this issue are lacking, it is highly probable that prevention of bacterial colonization and contamination would reduce the frequency or magnitude of biofilm formation.

Dialysis Circuits

Other preventive strategies for biofilm formation in dialysis water treatment systems involve the delivery of highly purified water, which is also treated by regular disinfection procedures. The generation of such ultrapure water leads to a significant reduction in biofilm formation, bacterial growth, and endotoxin levels in water-treatment systems.[41] Regular disinfection of the entire blood and dialysate paths are requisite to biofilm prophylaxis. The bicarbonate concentrate must be handled with extra care because it constitutes an excellent growth medium of microbes.[26] Ideally, one should use a dialysis fluid that has fewer than 100 colony-forming units (CFUs) per milliliter and less than 0.25 IU/mL of endotoxin. Ultrafiltration just before the dialyzer can produce ultrapure dialysis fluid, namely less than 10^{-1} CFU/mL with endotoxin less than 0.03 IU/mL. Lastly, an additional controlled ultrafiltration step will afford sterile and pyrogen-free fluids ($<10^{-6}$ CFU/mL and endotoxin <0.03 IU/mL).[42]

Lastly, it is of paramount importance to prevent the penetration of bacteria into the bloodstream at the time of connection (or disconnection) of the patient to the hemodialysis circuit. This inherent risk underscores the critical role of nursing care and clinical practice in managing the vascular access and in preventing bacterial contamination.[43,44]

All current disinfection protocols have been validated in relation to microbial killing but not for biofilm prevention and eradication.[20] Because biofilms markedly reduces the efficiency of disinfection modalities in industrial applications, it is recommended that hemodialysis monitors should periodically undergo decontamination by a combination of citric acid descaling and physical/chemical agents. In addition, circuit monitors should be immersed in dilute peracetic acid when not in use.[31] One alternative approach is to combine enzymatic and detergent cleansings to detach less adherent cells, which could contribute to reestablishment and extension of its biofilm.[45,46]

Catheters

New techniques to eliminate biofilms from vascular access catheters have been introduced. However, catheter exchanges over a guidewire neither eliminate remnant bacteria in subcutaneous tunnels nor prevent bacterial inoculation of the "new" catheter with organisms upon its insertion over a clean guidewire.[19] Nonetheless, guidewire exchange has been shown to result in a lower frequency of catheter-related bacteremias than had it not been done. Antimicrobial-impregnated noncuffed catheters can decrease the risk of catheter-related bloodstream infection and its associated hospital costs.[47,48] In peritoneal dialysis catheters, the attempt to use silver-ion implanted catheters to reduce peritoneal infections has not proven beneficial.[49] The effect of antimicrobial lock therapy on vascular access catheters has been studied in relation to biofilm formation. Linezolid and vancomycin have been shown to eradicate *Staphylococcus epidermidis* in vitro models of biofilm.[50] Similarly, tetrasodium EDTA in vitro has produced biofilm reduction or eradication from central venous catheters.[51,52]

Antimicrobial Inhibition

In animal models, systemic erythromycin at low-growth inhibitory concentrations reduces the production of adhesion factors (lectins) and quorum-sensing signaling molecules (eg, homoserine lactone autoinducers[53]). In *S aureus*, subinhibitory clindamycin concentrations block the production of several virulence factors at the level of transcription. However, the expression of *agr* and *sar*, global regulators of exoprotein synthesis and virulence response regulated by quorum sensing mechanisms, are minimally affected.[54]

Molecular Biofilm Blockade

One staphylococcal-specific quorum-sensing inhibitor that has been developed to abrogate toxin production and block biofilm formation locks the organism into its planktonic phenotype. This RNAIII-inhibiting peptide (RIP) analog of the native RNAIII-activating peptide signal reduces in vitro adherence to polystyrene-, polyurethane- and silicon-based catheters. Most predominantly, RIP activity is synergistic with antimicrobials and may prevent the materialization of drug-resistant *S aureus*. In addition, RIP is bactericidal in the presence of biomatrix,[55] and, in a rat model, dacron vascular catheters coated with RNAIII-inhibiting peptide had reduced graft infections.[56]

Innovative Biofilm-Eradication Approaches

As a preventive method, bacterial interference uses nonpathogenic "benign" organisms to form a catheter-associated biofilm. In vitro, incubating urinary catheters with nonpathogenic *Escherichia coli* before exposing the catheters to uropathogens effectively impedes catheter colonization.[57,58] Such a strategy, involving a multicenter, prospective, placebo-controlled trial of direct bladder instillation of *E coli* to prevent urinary tract infection in persons with spinal cord injury,[59] has recently applied.

Novel treatment that takes advantage of the naturally occurring pea seedling exudate has also been examined. Extracts of the pea, *Pisum sativum*, contain several separable activities that mimic AHL signals, thereby inhibiting the communication systems of certain

gram negative bacterial strains.[60] Along this line, natural furanone derivatives have been found to attenuate the virulence of *Pseudomonas aeruginosa* by inhibiting quorum sensing and virulence factor expression, with a consequent augmentation of bacterial susceptibility to tobramycin.[61,62]

Among the innovative strategies that propose to render biofilms more susceptible to conventional antimicrobial treatment include the application of direct current fields and precisely directed ultrasonic wave forms.[63] There is evidence that the application of a weak intermittent electrical field enhances anti-infective therapy.[64,65] The combination of direct current of just 1 mA plus antimicrobial exerts an 8-log increase in killing activity versus controls during in vitro experiments in which biofilm is observed in an isolation chamber. However, direct current alone does not affect biofilm formation. The optimal current and antimicrobial concentration must be determined for each experiment, rendering this modality as a therapeutic challenge for future clinical applications.

Combined approaches to control biofilm infections have been studied in the orthopedic realm, and such approaches are currently being engineered using the bactericidal effects of direct and indirect electrical fields and antimicrobials, in conjunction with quorum sensing inhibitors that reside within strategically localized implant-associated reservoirs.[66] Other novel approaches include the use of surfactant application to prevent pulmonary infection. This methodology is enhances the negativity of charge of bacterial cell walls, thereby reducing the adherence capabilities of flagellated organisms and inhibiting surface attachment. Successful modeling of this approach has been achieved in vitro and reduces membrane-attached biofilms.[67] Mucoid, *mucA*-mutant *P aeruginosa species*, a scourge of cystic fibrosis (CF) patients, are hardy and resistant to phagocytosis and antibiotics. These organisms, however, can be killed within anaerobic biofilms by the exposure nitrous acid (HNO_2) via a presumed NO-dependent mechanism. This bactericidal mechanism has also been successful in vitro from ultrasupernatants of airway secretions from cystic fibrosis lung explants explanted CF patient lungs and in mouse lungs in vivo in a pH-dependent fashion, with total microorganism eradication after 16 days' exposure to HNO_2, without discernible tissue damage, showing a promising mechanism for pseudomonal reduction in CF patients.[68]

Conclusions

The medical and financial impact of biofilm formation, regulation, and persistence is obvious. The prevalent use and misuse of antimicrobial care as well as the proliferation of indwelling medical devices will only enlarge the biofilm mass and result in the enhancement of new and relapsing infections. It must be remembered that, technically, the device itself is not infected but provides the environment for infection through its colonization. Biofilm that is not immediately fatal and that is permitted to persist invokes an escalating inflammatory response and may foster cardiovascular illness and adverse outcomes. In patients with CKD, biofilm formation may synergize with the inherent intrinsic inflammatory processes and cardiovascular risks (ie, biofilm is an underappreciated risk multiplier in CKD). Novel detection schemes that recognize signaling molecules during quorum sensing and autoinduction are required, particularly on device surfaces intimately associated with hemodialysis circuits. Emerging therapies to abrogate biofilm formation must become the standard of care in CKD-related infections and in medicine, in general. Failure is not an option.

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