DERMATOLOGY PEARLS

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BIOFILMS IN VETERINARY DERMATOLOGY

What are biofilms?

Biofilms are accumulations of bacteria (usually comprising several different microbial species) enmeshed in an extracellular matrix (ECM) collectively secreted by constituent members and composed of extracellular DNA, polysaccharides, protein, amyloid and bacteriophages. This matrix functions to sequester nutrients, acts to diffuse waste products, oxygen and chemical signals such as nitric oxide throughout the biofilm, and also as a shield to protect the bacteria from damage due to antibiotics and disinfectants. These structured, functionally coordinated communities form on a vast array of living and non-living surfaces and probably represent the most common form of existence for microbes in natural environments.

Biofilms begin when motile (planktonic) bacteria attach to a surface (such as teeth, a wound, or a medical device such as a catheter). Adherence is always the first step towards biofilm development. Once the bacteria attach, they transform into three dimensional communities with altered phenotypes and growth characteristics, and begin communicating with each other via quorum sensing (QS) molecules (typically peptides and fatty acid derivatives) to regulate growth and development of the biofilm. The QS molecules exert their effect by regulating expression of genes involved in the production of virulence factors, sporulation, DNA uptake and biofilm formation. The final step in biofilm formation is detachment and dispersal of bacteria from the biofilm; dispersion is influenced by environmental cues (e.g. nutrients, oxygen depletion, c-di-GMP and QS). Detachment can be initiated by several factors including mechanical perturbations (e.g. changes in shear forces or abrasion), enzymatic degradation of the biofilm matrix (e.g. dispersin B and alginate lyase), enzymatic degradation of the biofilm substrate (e.g. hyaluronidase), induction of motility, production of surfactants (e.g. rhamnolipids), release of EPS and surface-binding proteins or cell death and cell lysis. The released bacteria can then colonize new surfaces.

Bacteria in biofilms are 50-500 times more resistant to antibiotics then their planktonic counterparts, due to several reasons: the physical barrier of the ECM prevents effective antibiotic penetration into the biofilm, altered bacterial growth and metabolism reduces antibiotic effects, increased bacterial mutation frequency and gene transfer allows for rapid development of multidrug resistance, the variety of genotypes (ie. bacterial species) and phenotypes (cells of the same bacterial species expressing different proteins and operating at varied levels of metabolic activity), and spore like bacterial "persister cells" which produce proteins that shut down antibiotic targets. In non-healing wounds, biofilms impair healing due to induction of local immune dysfunction, keratinocyte death, alter production of enzymes and growth factors by endothelial cells, and inhibit fibroblast migration and proliferation. Biofilms of *Staph. aureus* have also been documented in human atopic dermatitis skin lesions and may play a role in canine atopic dermatitis.

How are biofilms treated?

Since topical and systemic antibiotics cannot eradicate a biofilm, effective treatment of a biofilm must use a combination of specific anti-biofilm strategies with traditional topical and systemic therapies to weaken the biofilm to a point where the host's immunity can fight infection. Antibiotics should be used judiciously as some can enhance biofilm formation if used at sub MIC concentrations. Additionally, antibiotics eliminate both pathogenic and commensal organisms, and thus allow "persister cells" to proliferate in the absence of competition. Therapies which do not rely on antibiotics are therefore important, and include topical therapies to prevent initial bacterial attachment and so prevent biofilm formation, as well as to disrupt formation of established biofilms. Research is currently actively investigating molecules that interfere with biofilm cell-to-cell communication (QS) such as RNA III-inhibiting peptide (RIP), as well as molecules which stimulate biofilm dispersion enzyme (dispersin B, DNAase I, and nitric oxide via regulation of the intracellular concentrations of cyclic di-GMP), and amphipathic molecules (ie. rhamnolipids) to reduce surface tension to facilitate detachment and dispersal.

Topical therapies - Human studies:
1. Four antiseptics were tested in vitro against biofilms of *P. aeruginosa* and *Burkholderia cepacia* on Teflon chips. Results showed that 0.2% povidone-iodine effected a 6-log reduction in 10 minutes, whereas inhibition was not detected after 60 minutes exposure to 0.2% solutions of chlorhexidine gluconate, benzalkonium chloride or alkyldiaminoethylglycine hydrochloride.

2. Four disinfectants used for preparation of intact skin for catheter insertion (70% isopropyl alcohol (IPA), 0.5% chlorhexidene in 70% IPA, 2% chlorhexidene in 70% IPA, and 10% povidone iodine) all reduced bacterial population after 30 sec contact time in an in vitro biofilm model of *Staph. epidermidis*.
3. Central venous catheters impregnated with chlorhexidene and silver sulfadiazine reduce bacterial adherence and biofilm formation.
4. Silver impregnated dressings are popular for treatment of chronic wounds. When tested against in vitro biofilms of *Pseudomonas aeruginosa*, silver sulfadiazine at a concentration of 5-10ug/ml eradicated the biofilm, while a lower concentration of 1ug/ml had no effect. In another in vitro study of the effect of silver sulfadiazine and silver nitrate on *S. aureus* biofilms, bacterial cell counts were effectively reduced at a silver concentration of 0.302%.
5. Sucrose in high concentration (70%) has induced adverse effects on immature *S. aureus* biofilms, especially in combination with other agents including levofloxacin and 10% povidone iodine.
6. In the food service industry, enzymes such as polysaccharidases and proteases, when combined with a buffer containing surfactants and dispersing and chelating agents, were found to be effective in removal of biofilms of *Bacillus* and *P. fluorescens*. Enzymes have been used to remove bacterial biofilms from surfaces, such as stainless steel, polypropylene, and soft contact lenses, and may also be helpful for wound treatment. Specifically, glucose oxidase combined with lactoperoxidase was bactericidal against biofilm bacteria but did not remove the biofilm from the substrata. A complex mixture of polysaccharide-hydrolyzing enzymes was able to remove bacterial biofilm from steel and polypropylene substrata but did not have a significant bactericidal activity. Combining oxidoreductases with polysaccharide-hydrolyzing enzymes resulted in bactericidal activity as well as removal of the biofilm.
7. Lactoferrin is a constituent of human secretions that is found in tears, mucus and human milk. It can prevent biofilm formation by sequestering iron. In the presence of bovine lactoferrin, *Pseudomonas* is unable to attach and form biofilms.

Veterinary Products or studies:
There are very few veterinary products with a substantiated claim of anti-biofilm affects, though as listed above, we often use products with potential anti-biofilm affects such as chlorhexidene, alcohol, povidone iodine, silver sulfadiazine, silver impregnated wound dressings and sometimes sugar bandages.

1. Epi-Otic Advanced formula (Virbac Animal Health) contains a monosaccharide complex (l-rhamnose, d-galactose, d-mannose) which in an in-vitro study caused decreased adhesion of Pseudomonas to canine corneocytes. Additionally an in vivo study using the cleaner BID for 2 weeks in dogs with bacterial otitis demonstrated clinical and microbiological improvement.
2. Advanced Formula Zymox Plus (PKB Inc.) claims to reduce biofilm formation and contains lysozyme, lacroferrin, lactoperoxidase, beta-glucanase, cellulose, pectinase, protease and glucose oxidase, which have a rational basis for the claim based on in vitro studies of the components on inanimate surfaces listed above. However product information available for review online included only in vitro studies of bacterial inhibition and no studies documenting decreased bacterial adherence or biofilm efficacy, nor in vivo studies of clinical efficacy.
3. Synoplex (Syndegen Inc.) is a recently FDA approved wound flush product for the treatment of chronic and infected wounds in elephants. According to company information, it contains a "novel non-toxic biopolymer, poly (acetyl, arginyl) glucosamine (PAAG) which prevents and removes biofilms, reduces inflammation, and facilitates a more rapid resolution of wound infections." According to company information, MRSA biofilms were reduced by 99.9% within 1 hour of treatment with 0.5% PAAG rinse or 1% PAAG gel in vitro compared to untreated biofilms, and MRSA biofilms in porcine wounds that were rinsed with 0.1% PAAG were reduced by 95.8% compared to untreated wounds. However, in company information available online this research was classified as "preliminary" or "pilot," and published papers were unavailable for review. The same company has also used the same technology to develop oral rinses, wound irrigation rinses/gels and mucolytics for human use, which are listed as "near-term development."
4. In one in vivo study using porcine skin, wounds were created and then inoculated with *S. aureus*. Wounds were then treated with either one of two topical antimicrobial agents (mupirocin cream or triple antibiotic ointment) within 15 minutes to represent planktonic bacteria, or 48 hours after initial inoculation to represent biofilm-associated wound infection. Using light microscopy, scanning electron microscopy and epifluorescence microscopy, biofilms were observed in wounds after 48 hours of inoculation and occlusion. Study results demonstrated that both mupirocin cream and the triple antibiotic ointment were effective in reducing planktonic *S. aureus* but had reduced efficacy against biofilm-embedded *S. aureus*.