Biofilms in Oral Cavity of Dogs and Implication in Zoonotic Infections

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Abstract
Biofilm forming bacteria in oral cavity of dogs have an important impact on human health because they can transmit zoonotic infection after dog bites. Knowing the microbial composition of dental plaque biofilm is crucial for understanding oral and systemic diseases in dogs but also those transmitted to humans. Recently it was found that biofilm is a key factor in delaying wound healing process of the skin. The skin is an essential component of nonspecific immune system, protecting the host of potential pathogens from the environment. Thus, breaches that appear in this protective barrier are a form of compromised immunity, which predisposes the patient to infection. Bites and scratches caused by animals and humans can inoculate microorganisms over the protective barrier of the skin. The objective of this study is to reveal that microbiology of infected wounds produced by dog bites is very similar to that of oral cavity in dogs. Delaying wound healing process is achieved by the fact that biofilms stimulate an ineffective inflammatory response, microorganisms in the biofilm become protected and an increase of the production of exudate provides nutritional source for biofilm growth.

Keywords: biofilm; dog bites; wound healing; zoonotic infection

1. Introduction
Dog bites are the most common form of skin injury encountered. Dogs have the ability to produce enough force (150-450 lbs./sq. in.) to crush tissues. Such wounds can become susceptible to infection because of the direct inoculation of bacteria into this affected area. Therefore, basic wound care is of great importance. There are a lot of studies that have been performed in order to evaluate the microorganisms that cause infection following dog bites [1].
A wide variety of organisms have been cultured from the oral cavity of dogs and have the ability to form oral biofilms that can confer resistance and cause zoonotic infections following dog bites. Microorganisms from infected wounds generally originate from the oropharyngeal flora of the biting dog.

Interest on the study of biofilms in the pathology of chronic wounds has been increased by the recent publications. Evidence from these studies suggests that biofilms play an important role in wound chronicity. There are different microscopic techniques used for evaluation of microorganisms from chronic wounds that indicate the presence of biofilm [2].

Biofilm forming bacteria in the oral cavity of biting dogs have an important impact on human health and are a major factor in delaying wound healing process of the skin by the capacity of inoculation over the protective barrier of the skin. It is known that over 80% of bacteria that participate in biofilm formation are able to detach and migrate to other places and form new groups [3, 4]. Therefore, regeneration of the damaged tissue of the skin becomes a long process and includes numerous interactions between cells, mediators of
the extracellular matrix and soluble molecules that have an important role in reconstruction of the skin. Some factors such as moisture, heat and nutritional resources from the infected wounds together with a weak immune system stimulate the development and formation of favorable conditions such as (necrotic, ischemic, devitalized tissue and other foreign materials), all favoring bacterial growth. Belatedly process is achieved by the fact that biofilms stimulate an inflammatory process to get rid of the biofilm forming microorganisms (Figure 1). The answer is an increase of number of inflammatory cells such as neutrophils and macrophages surrounding the biofilm. These cells secrete large amounts of reactive oxygen, proteases (matrix metalloproteinases and elastase). Proteases favor the destruction of biofilm by breaking down the attachments between biofilm and tissue [5, 6]. This chronic inflammatory response is not always successful in removing the biofilm and it is supposed that the response is in the benefit of the biofilm. The process of wound healing is delayed by an ineffective inflammatory response, in which the biofilm protects the microorganisms it contains. Therefore the process of biofilm formation increases exudate production, creates favorable conditions for providing sources of nutrition and maturation of the biofilm [7].

Dogs are responsible for about 80% of bite wounds, of which 15-20% are estimated to be infected. Typically, infection occurs in 8-24 hours after the bite, with pain on the site of the injury, cellulitis, accompanied by a purulent discharge, sometimes foul-smelling.

It can appear septic arthritis and osteomyelitis if the dog tooth penetrated synovia or bone. While most infections caused after dog bite injury are located in the lesion, many microorganisms involved are capable of producing systemic infection, including fever, bacteremia, brain abscess, meningitis, endocarditis, lymphadenopathy and lymphangitis may also occur.

These infections mainly occur in hosts with edema or compromised lymphatic drainage of the extremity involved and immunocompromised patients as a result of medication administration or illness (e.g., glucocorticoid use, systemic lupus erythematous, acute leukemia or cirrhosis). In addition, dog bites and scratches can cause systemic diseases such as rabies and tetanus.

Microbiology of infected wounds produced from dog bites are polymicrobial containing both aerobic and anaerobic bacteria. Microbiology of infected wounds produced after dog bite is similar to those in oral cavity of the dog. Very rarely infected wounds contain bacteria that originate from skin or other environmental sources of the person bitten [8, 9, 10].

For example Bailie et al. [11] examined oral and nasal fluids of 50 dogs in order to determine the prevalence of aerobic bacteria frequently associated with animal bite wounds. The most frequently isolated microorganisms included: \( \text{IIj} \), \( \text{EF-4} \), \( \text{Pasteurella multocida} \), \( \text{Staphylococcus aureus} \), \( \text{Staphylococcus epidermidis} \), group D streptococci, \( \text{Corynebacterium} \) sp., \( \text{Enterobacteria} \), \( \text{Neisseria} \) sp., \( \text{Moraxella} \) sp., and \( \text{Bacillus} \) sp. They recovered also species and genera that were infrequently isolated but may represent transient flora. \( \text{IIj} \), \( \text{EF-4} \), \( \text{Pasteurella multocida} \), and \( \text{Staphylococcus aureus} \) were isolated in high incidence and this species of bacteria are all known as human pathogens and they should be considered as possible contaminants in bite wounds infections [11].

Microbiology of infections in dog bite wounds is usually mixed and includes \( \text{Staphylococcus} \) species, alpha-hemolytic streptococci, \( \text{Pasteurella multocida} \), \( \text{Eikenella corrodens} \) and
Researches made by Talan et. al. [9] state that the most common aerobic bacteria isolated from 50 infected dog bite wounds were: Pasteurella (50%), Streptococcus (46%), Staphylococcus (46%), Neisseria (32%) and Corynebacterium (12%) (Table 1) [9].

Less frequently were isolated: Moraxella (10%), Enterococcus (10%), Bacillus (8%), Pseudomonas (6%), Actinomyces (6%), Brevibacterium (6%), Gemella morbillorum (6%), Escherichia coli (6%), Weekella zoohelcum (4%), Klebsiella (4%), Lactobacillus (4%), Citrobacter (4%), Flavobacterium (4%), Micrococcus (4%), Proteus mirabilis (4%), Stenotrophomonas maltophilia (4%), Capnocytophaga ochracea (2%), Eikenella corrodens (2%), Flavimonas oryzihabtans (2%), Dermabacter hominis (2%), Oerskovia (2%), Pediococcus damnosus (2%), and Stomatococcus mucilaginosus (2%).

Most anaerobic bacteria isolated from wound infections were: Fusobacterium nucleatum (16%), Bacteroides tectus (14%), Prevotella heparinolytica (14%), Propionibacterium acnes (14%), Prevotella intermedia (8%), Peptostreptococcus anaerobius (8%), Porphyromonas macacae (6%), and Porphyromonas canis (6%) [9].

<table>
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<tr>
<th>Aerobic bacteria</th>
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<tbody>
<tr>
<td>Pasteurella</td>
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<td>Streptococcus</td>
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<td>Staphylococcus</td>
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<tr>
<td>Enterococcus</td>
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<td>Bacillus</td>
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Table 1. Common aerobic and anaerobic bacterial genera isolated from 50 infected dog bite wounds [9]

The frequency of isolation of Pasteurella sp. from the infected wound after dog bites is as follows: P. canis 27%, 13% P. multocida, P. septica 13%. Isolation of Pasteurella dagmatis is rare. Dog bites are reported in 80% of the medical emergencies and more than 20-30% of dog bites are infected with Pasteurella. Pasteurella meningitis was isolated from human dental caries that have regularly kissed their dog. Pasteurella may cause respiratory tract infections with tonsillitis, sinusitis, epiglotiditis. Submandibular cellulitis (Ludwig’s angina) can develop after 10 days in people who have played with an infected dog.

Immunocompromised patients or those predisposed to certain diseases such as diabetes or liver dysfunction may develop more quickly bacteremia and die. Even neonatal dogs may be susceptible to certain virulent strains of Pasteurella probably present in the oral cavity. P. canis virulent strain of biotype I was considered to be the cause of death in neonatal puppies with multisystem infection [12].

The most frequently Staphylococcus species that were isolated from the mouth of dogs from the supragingival and subgingival plaque were: S. epidermidis, S. aureus, S. intermedius, S. warneri. In dog bite injuries are found with the same frequency as the staphylococcal species in the oral cavity of dogs: S. aureus, S. intermedius, S. epidermidis and S. warneri and rarely S. auricularis, S. hominis, S. cohnii and S. xylosus [13].

Streptococcus species frequently isolated from wounds caused by dog bite are: Streptococcus mitis (22%), Streptococcus mutans (12%) and Streptococcus pyogenes (12%), Streptococcus sanguis biotype II (8%), Streptococcus intermedius (6%), Streptococcus constellatus (4%), Streptococcus equinus (2%), Streptococcus sanguis biotype I (2%), Streptococcus agalactiae (2%), Streptococcus sanguis (2%), beta-hemolytic Streptococcus group G (2%), and Streptococcus dysgalactiae (2%) [9].

Group A streptococci and sometimes other species of streptococci can cause a range of infections processes that can affect the skin, subcutaneous tissue, muscle and fascia. General syndromes should be considered as a general guide in predicting the level of tissue damage in a patient and the possible need for surgery or intensive supportive therapy. Impetigo (pyoderma) is a superficial skin infection caused by group A streptococci and sometimes other streptococci or Staphylococcus aureus.

Inoculation of microorganisms may be followed by skin infection that affects the skin and subcutaneous tissue, or can cause cellulitis. A particular form of streptococcal cellulitis is known as erysipelas and is characterized by red skin...
lesions in the form of stars, well separated from surrounding normal skin. Streptococcal infection of the wound or localized cellulitis may also be associated with lymphangitis, red tracks that extend proximal to the site of infection, along lymphatic superficial vessels.

In dogs the most common species of Neisseria isolated from mouth were: Neisseria Weaver (14%) (formerly known as CDC group M-5), Neisseria zoodegmatis (10%) (formerly known as the EF-4b), Neisseria animaloris (6%) (formerly known as the EF-4a), and Neisseria subflava (2%) as well as Neisseria canis. Neisseria Weaver is a commensal of the oral cavity in dogs. Sources of human infections are dog bites [14, 15].

The lack of research into the bacterial composition and antimicrobial susceptibility of the bacteria within dog bite wounds and the increased prevalence of resistance to bacteria in our patients, the possibility of these microorganisms to form biofilms, the need to understand the process of colonization, resistance and the judicious use of antimicrobials has become a priority.

Studies have shown that a lot of commercial topical agents and wound dressings in use are ineffective against the biofilm matrix. Mechanical debridement becomes essential in the eradication of a wound biofilm. Many antibiotics and antimicrobial agents may be effective in the treatment of the wound after debridement in the prevention of biofilm reformation. Therefore, because the prevalence of antimicrobial resistance in pathogens is increasing there is a need to use antimicrobials in such a way that they have positive effect on the recovery of animals from life threatening diseases to humans without increasing bacteria resistance to antimicrobials [16, 17].

References