Biofilm Implication in Oral Diseases of Dogs and Cats

Csilla Zambori[1], Emil Tirziu[1], Ileana Nichita[1], Ciceronis Cumpanasoiu[1], Radu Valentin Gros[1], Monica Seres[1], Bianca Mladin[1], Daniela Mot[2]

[1]*University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 300645, Timisoara, 119 Calea Aradului, Romania*
[2]*University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotehechnologies, 300645, Timisoara, 119 Calea Aradului, Romania*

Abstract
The importance of biofilm in disease processes in humans and animals is now widely recognized. In animal species, the risk of infection is probably greater than the risk in humans. This is due to the difference in animal housing and living environments – animals naturally live in environments with a large and much more diverse microbial community. Most oral bacteria live symbiotically in biofilm. This symbiotic association gives the bacteria different communal properties than individual planktonic bacteria.
Bacteria that form biofilm live and develop in communities which are an important property for dental plaque formation that leads to dental calculus formation, periodontal diseases, dental caries and systemic diseases.
The objective of this study is to reveal the role of dental plaque (oral biofilm) in pathogenesis of dental calculus, periodontal disease and dental caries in dogs and cats.

Keywords: biofilm, dental plaque, symbiosis, oral bacteria.

1. Introduction

The new definition of a biofilm is a microbial derived sessile community characterized by cells that are irreversibly attached to a substratum or interface (biotic, non-biotic) or to each other, are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.
Biofilm can form on various surfaces, including biotic surfaces (e.g., teeth, mucosal membranes), medical devices, and household surfaces [1].
In the oral cavity, teeth provide constant humidity and adherent surfaces causing the attachment of extensive deposits of microorganisms. In dogs and cats mouth normal bacterial microflora is structured in a variety of aerobic, facultative or strictly anaerobic bacteria [2].

*Corresponding author: Csilla Zambori, Tel: 0722698706, Email: csillaza@yahoo.com

Therefore from the oral cavity in dogs were isolated:

- anaerobic bacteria: *Clostridium* spp., *Bacteroides sensu lato, Fusobacterium* spp., *Propionibacterium* spp., *Peptostreptococcus sensu lato, Lactobacillus* spp., *Actinomyces* spp. *(Actinomyces bovis, Actinomyces meyeri, Actinomyces odontolyticus, Actinomyces viscosus, Actinomyces hordeovulneris), Lactobacillus* spp., *Bifidobacterium* spp., *Eubacterium* spp., *Veillonella* spp. and *spirochetes*.
- aerobic and facultative anaerobic bacteria: *Streptococcus* spp. alpha hemolytic, non-hemolytic streptococci, *Enterococcus* spp., coagulase-negative staphylococci, *Micrococcus sensu lato*, *Neisseria* spp. (*Neisseria weaveri*), *Moraxella* spp. (*Moraxella canis*), *Acinetobacter* spp., *Alcaligenes* spp., *Pasteurella canis, Pasteurella multocida* subsp. *multocida, Pasteurella multocida* subsp. *septica*, *dagmatis Pasteurella, Pasteurella stomatis, Neisseria animaloris, Neisseria zoodegmatis, Escherichia coli, Enterobacter* spp., *Bacillus* spp., beta-

hemolytic streptococci (*Streptococcus canis*), *Proteus spp*., *Klebsiella pneumoniae, Pseudomonas* spp., *Corynebacterium* spp., *Capnocytophaga canimorsus,* coagulase-positive staphylococci, *Capnocytophaga cynodegmi*, *Actinobacillus actinomycetemcomitans, Bergeyella zoohelcum, Mycoplasma* spp.

In the oral cavity of healthy cats were isolated:

- aerobic and facultative anaerobic bacteria: *Streptococcus* spp., *Pasteurella multocida* subsp. *multocida*, *Pasteurella multocida* subsp. *septica*, *Pasteurella multocida* subsp. *gallicida, dagmatis, Pasteurella canis, Moraxella* spp., *Flavobacterium sensu lato, Pseudomonas* spp., *Corynebacterium* spp., *Neisseria animaloris, Neisseria zoodegmatis, Neisseria weaveri, Bergeyella zoohelcum, Capnocytophaga canimorsus, Capnocytophaga cynodegmi, Nocardia* spp., *Mycoplasma* feliminutum , *Ureaplasma* spp.
- anaerobic bacteria: *Bacteroides* spp., *Porphyromonas* spp., *Prevotella* spp., *Fusobacterium* spp., *Clostridium* spp., *Actinomyces* spp. (*Actinomyces viscosus*), *Peptostreptococcus anaerobius, Wolinella* spp. [3].

All these microorganisms existing in the mouth are harmless, benefiting from various pathogenic factors. Dynamic micro - macroorganisms relationship is dependent on the interaction between pathogenic capabilities of microorganisms and antimicrobial capacity of host. In relation to their pathogenic microbes can be non-pathogenic, moderately pathogenic and highly pathogenic [4].

2. Dental plaque

Dental plaque (biofilm) is a normal occurrence on the surface of teeth and particularly so where its mechanical removal by salivary flow is reduced, as encountered at stagnant sites such as in the interproximal regions or fissures of teeth.

The pattern of plaque biofilm development can be divided into five phases: pellicle formation; attachment, young supragingival plaque, aged supragingival plaque, subgingival plaque formation (Figure 1) [5].

Generally dental plaque is divided into two forms: subgingival and supragingival plaque. The supragingival plaque consists mainly of Gram positive facultative anaerobic (streptococci) and the subgingival plaque of anaerobic Gram-negative bacteria. After attachment and multiplication of anaerobic Gram-negative bacteria there will occur a release of acids that will promote tooth surface demineralization and dental caries.

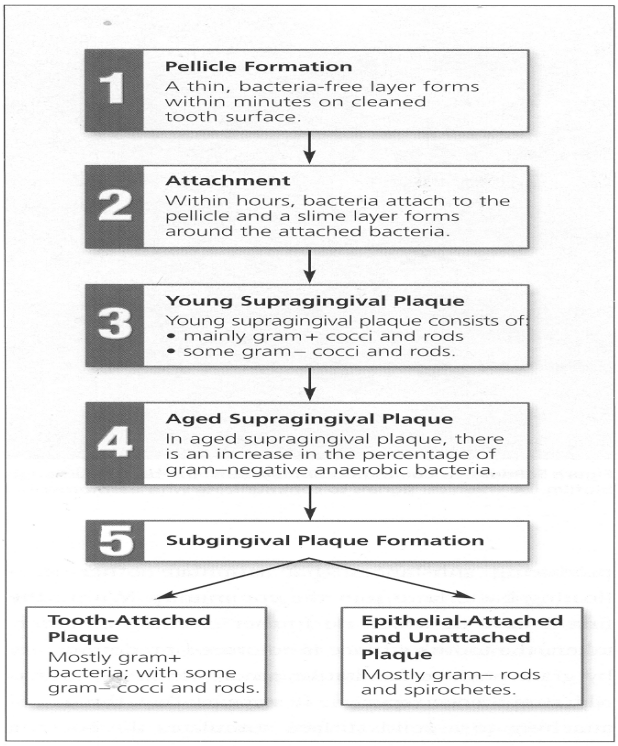

Figure 1. Phases of plaque formation (Nield, G.J.S., et al., 2003) [5]

The formation of plaque begins with the development of an acquired pellicle on the enamel of the teeth. This pellicle consists of glycoprotein, proline-rich peptides (PRPs), lipids and phosphoproteins and other components originating largely from saliva, although there may also be involvement of gingival crevicular fluid molecules. Pellicle formation is a rapid process, occurring within seconds of a clean tooth surface being exposed to a salivary conditioning film and results in the generation of a surface offering receptors suitable for initial attachment of primary colonizing bacteria [6].

These primary colonizers are Gram positive bacteria such as (*Streptococcus sanguis*, *Streptococcus mutans* and *Actinomyces viscosus*).

Microbial adherence initially involves relatively weak and reversible electrostatic and hydrophobic interactions. However, through the interaction of specific adhesins on the microbial cell surface with target receptors, an irreversible adherence is achieved. Subsequently, 'late colonizers' are recruited to the plaque community, often by aggregation to the primary colonizing organisms or to components of the EPS matrix produced by the developing biofilm [7].

The secondary colonizers are Gram negative bacteria such as *Fusobacterium nucleatum,*

Prevotella intermedia, Capnocytophaga spp. and the third frequent colonizers are *Porphyromonas, Campylobacter, Eikenella, Treponema.*

The effect of this recruitment to the plaque biofilm is that a complex and diverse population of microorganisms develops with interaction occurring between its members in terms of both metabolic co-operation and competition. The end result of all these interactions is that a relatively stable, climax microbial community occurs within the mature biofilm, where certain combinations of bacterial species will occupy the same locality or alternatively grow in separate locations within the biofilm.

Dental plaque formation is the primary cause of dental calculus, periodontal diseases and dental caries.

3. Dental calculus

Shortly after plaque formation there is a process of mineralization (calcification) of dental plaque that leeds to tartar formation [8].

Anaerobic bacteria that adhere to dental plaque encourage the intensification of fermentation processes, increasing acidity in the oral cavity and underlying enamel demineralization process. Enamel demineralization process will create favorable conditions for dental plaque development. Calcium, phosphorus and other minerals contained in saliva are absorbed in the existing dental plaque and dental plaque mineralization occurs with the formation of dental calculus.

Dental calculus differs from dental plaque because it structure contains crystallized calcium phosphate [9].

Dental plaque composition varies with position, age and individual deposit. Plaque is made up of nonorganic and organic substances. Inorganic components consist mainly of calcium phosphate as hydroxyapatite.

The main causes of dental calculus formation are: high concentrations of calcium and phosphate ions in dental plaque, local alkaline pH, increased levels of ammonia and urea in saliva and dental plaque, reduced alkaline phosphatase in saliva, which inhibits the formation of crystallized calcium phosphate enzyme [8].

In terms of location dental calculus was divided into supragingival and subgingival calculus.

4. Periodontal disease

Periodontal diseases are a collection of infections involving the degradation of the supporting tissues of the teeth, including the gum, periodontal ligament, alveolar bone and root cement of the tooth [6].

Periodontal diseases are the number one health problem in small animal patients. By just two years of age, 70% of cats and 80% of dogs have some form of periodontal diseases. Periodontal disease is described in two stages, gingivitis and periodontitis. Gingivitis is the initial, reversible stage of the disease process in which the inflammation is confined to the gum. This inflammation is created by plaque bacteria and may be reversed with a thorough dental prophylaxis and consistent home care. Periodontitis is the later stage of the disease process and is defined as an inflammatory disease of the deeper supporting structures of the tooth (periodontal ligament and alveolar bone) caused by microorganisms [10].

The development of periodontal disease is associated with deepening of the gingival crevice into a periodontal pocket that can be several millimeters in depth and bleeds after probing. Periodontitis is a chronic bacterial infection of the gingival crevice that is caused by mixed-species bacterial biofilm.

The metabolic action of early bacterial colonizers in the gingival crevice alters the environment and facilitates colonization by secondary organisms. These secondary colonizers tend to be more pathogenic and when they exceed threshold levels disease can occur [11].

At infected sites, the numbers of Gram negative and proteolytic bacterial genera such as *Porphyromonas* and *Tannerella* increase and these become the predominant members of the biofilm. Studies have indeed demonstrated higher numbers of these 'periodontopathogens' in periodontal infections compared with disease-free counterparts in animals, including dogs [12, 13, 14, 15] and cats [16, 17, 18].

Plaque is a microbial biofilm, a well-organized community of cooperating microorganisms (e.g. *Actinomyces* spp., *Streptococcus* spp., early colonizers) on the teeth surface, embedded in a matrix of polymers of bacterial and salivary origin [19]. In the subgingival plaque, which forms within the gingival sulcus, the microenvironment changes to facultative anaerobic with an increase in the number of Gram-negative, motile, anaerobic bacteria (e.g. *Prevotella* spp., *Porphyromonas* spp., *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, and *Treponema* spp.), resulting in the onset of periodontal inflammation [20].

Bacterial components and products promote the chemotactic attraction of neutrophils and vasodilatation, as well as the activation of host systems, such as the complement and kinin systems and the

arachidonic acid pathways [21]. Additionally, cellular components, including monocytes and fibroblasts, are stimulated by bacterial components such as lipopolysaccharides to produce cytokines. These cytokines stimulate inflammatory responses and catabolic processes, such as bone resorption and collagen destruction via the matrix metalloproteinases [22]. This complex immune-inflammatory response results in severe destruction of the periodontium [23], so the emphasis of ongoing investigations is on host/dental plaque interactions [24].

In addition to tooth loss, there are six proven local severe sequelae of severe periodontal disease.

The most common of these local consequences is an oral nasal fistula (ONF). ONFs are typically seen in older, small breed dogs (especially chondrodystrophic breeds); however, they can occur in any breed as well as felines.

Another potential severe consequence of periodontal disease can be seen in multi-rooted teeth, and is called a class II perio-endo abscess. This occurs when the periodontal loss progresses apically and gains access to the endodontic system, thereby causing endodontic disease via bacterial contamination.

The third potential local consequence of severe periodontal disease is a pathologic fracture. These fractures typically occur in the mandible (especially the area of the canines and first molars) because of chronic periodontal loss, which weakens the bone in affected areas.

The fourth local consequence of severe periodontal disease results from inflammation close to the orbit, which could potentially lead to blindness.

The fifth local consequence is described in recent studies that have linked chronic periodontal disease to oral cancer.

The sixth significant local consequence of periodontal disease is chronic osteomyelitis, which is an area of dead, infected bone [10].

While periodontal diseases are localized to the tissues supporting the teeth, evidence is emerging that periodontal infections and periodontal organisms are associated with serious systemic diseases such as liver, kidney, heart, respiratory diseases, diabetes, osteoporosis and other diseases.

5. Dental caries

Dental caries is an infection of teeth caused by plaque bacteria [6]. Caries results from bacterial decay of the tooth structure brought about by the release of acids from oral bacteria fermenting carbohydrates on the tooth surface. Therefore, a diet containing great amounts of highly refined and easily fermentable carbohydrates will favor the development of caries [25].

They are most common between teeth (interproximal) and on the occlusal surfaces of molar teeth.

The most common teeth involved are the maxillary fourth premolar as well as the first and second molar teeth, and are typically bilaterally symmetrical [26]. In order for caries to occur a number of factors have to be present. Firstly, the animal must have an accumulation of plaque that contains the necessary bacteria capable of both surviving in an acidic environment (aciduric bacteria) and also producing acid (acidogenic) under these conditions. Secondly, the diet of the animal has to contain carbohydrates in sufficient quantity and type to yield the necessary acid for enamel degradation [6].

Bacteria related to the dental caries that form biofilm are generally cocci and lactobacilli populations, mainly *Streptococcus mutans*, *Streptococcus mitis, Streptococcus salivarius, Lactobacillus acidophilus* and *Lactobacillus casei*. Nowadays it was demonstrated that *Streptococcus mutans* creates the most acidic conditions, thus reconsidering the role of acidophilus lactobacilli [9].

In dogs, naturally occurring caries has an estimated incidence of approximately 5% [27].

Among the reasons proposed for the lower incidence of caries in dogs compared with historical rates in humans are: conical tooth shape and wider inter-dental spacing with less area for food impaction and stagnation; diets which include little fermentable carbohydrate; higher salivary pH (in dogs, mean pH of 7.5 compared with 6.5 in humans) to buffer acids produced by bacterial fermentation of carbohydrates; in dogs, a relatively low level of salivary amylase to break down starches which are retained in and around the teeth. When caries develops, it does so in the following manner.

As bacteria on the tooth surface ferment the carbohydrates, acids (lactic, acetic, propionic) are released. These acids diffuse into and determine the enamel surface demineralization. Loss of mineral exposes the organic (protein) matrix of the enamel, which is digested by enzymes from the oral bacteria and/or leukocytes. As the process penetrates deeper, microcavitations develop under

the surface. As these expand, they coalesce and the undermined enamel collapses [25].

As with dogs, caries is a rare occurrence in cats and it is important to note that cats dental caries are not the underlying reason for the majority of dental cavities seen, which are often referred to as feline odontoclastic resorptive lesions. In such lesions saccharolytic acid producing bacteria are not involved [6].

Acknowledgements

This work was supported by the grant POSDRU /21/1.5/G/38347.

References

1. Tamilvanan, S., Biofilm, Eradication and Prevention: A Pharmaceutical Approach To Medial Device Infections. John Wiley & Sons, Inc., Hoboken, New Jersey, 2010.
2. Pavlica, Z., Biofilm, Microbial communities and periodontal disease, World Congress World Small Animal Veterinary Association, 2006.
3. Guerin, F.V., Euzeby, J.P., 2000, Etude de quelquer bacteries pathogenespour le cheval et ou les carnivores domestiques, 456-500. In: FRENEY J, RENAUD F, Hansen W, Bollet C., Precis de Bacteriologie Clinique. Eska, Paris.
4. Buiuc, D., Neguţ, M., Tratat de microbiologie clinică, Ed. Medicală Iasi, 2009.
5. Nield, G.J.S., Willmann, D.E., Foundations of Periodontics for the Dental Hygienist. Philadelphia: Lippincott Williams & Wilkins, 2003, 67-73.
6. Percival, S.L., Knottenbelt, D.C., Cochrane, C.A., Biofilm of Veterinary Medicine. Springer-Verlag Berlin Heidelberg, 2011.
7. Kolenbrander, P.E., Andersen, R.N., Kazmerak, K.M., Palmer, R.J., Coaggregation and coadhesion in oral biofilms. In: Allison DG, Gilbert P, Lappin-Scott HM, Wilson, M. (eds.), Community structure and cooperation in biofilms (Society for General Microbiology symposium no. 59). Cambridge University Press, Cambridge, 2000, pp 65–85.
8. Costinar, L., Cercetări privind microflora implicată în gingivite şi parodontite la câine. Editura Mirton, Timişoara, 2007.
9. Howard, F.J., Lamont, R.J., Oral microbial communities in sickness and in health. Trends in Microbiology. Volume 13, 2005, Issue 12, pp. 589-595.
10. Brook, A.N., Periodontal disease. Top Companion Anim. Med. 2008, 23(2):72-80.
11. Richard, J.L., Howard, F.J., Oral Microbiology at a glance. Wiley Blackwell, 2010.
12. Gorrel, C., Rawlings, J.M., The role of a 'dental hygiene chew' in maintaining periodontal health in dogs. J. Vet. Dent, 1996, 13:31–34.
13. Isogai, H., Kosako, Y., Benno, Y., Isogai, E., Ecology of genus *Porphyromonas* in canine periodontal disease. Zentralbl Veterinarmed B, 1999, 46:467–473.
14. Nordhoff, M., Rühe, B., Kellermeier, C., Moter, A., Schmitz, R., Brunnberg, L., Wieler, L.H., Association of *Treponema* spp. with canine periodontitis. Vet. Microbiol, 2008, 127:334–342.
15. Watson, A.D.J., Diet and periodontal disease in dogs and cats. Aust. Vet. J., 1994, 71:313–318.
16. Mallonee, D.H., Harvey, C.E., Venner, M., Hammond, B.F., Bacteriology of periodontal disease in the cat. Arch, Oral Biol,, 1988, 33:677–683,
17. Norris, J.M., Love, D.N., Associations amongst three feline *Porphyromonas* species from the gingival margin of cats during periodontal health and disease. Vet. Microbiol, 1999, 65:195–207.
18. Valdez, M., Haines, R., Riviere, K.H., Riviere, G.R., Thomas, D.D., Isolation of oral spirochetes from dogs and cats and provisional identification using polymerase chain reaction (PCR) analysis specific for human plaque *Treponema* spp., J. Vet. Dent, 2000, 17:23–26.
19. West-Hyde L., Floyd, M., Ettinger, S.J. Feldman, E.C., Editors Textbook of Veterinary Internal Medicine–Diseases of the Dog and Cat, vol. 2, (Fourth Ed.), W.B. Saunders, Philadelphia, USA, 1995, pp. 1102–1107.
20. Hardham, J., Dreier, K., Wong, J., Sfintescu, C., Evans, R.T., Pigmented-anaerobic bacteria associated with canine periodontitis. Veterinary Microbiology, 2005, 106, pp. 119–128.
21. Kinane, D.F., Berglundh, T., Lindhe, J., Host–parasite interactions in periodontal disease, Clinical Periodontology and Implant Dentistry, (Fourth Ed.), Blackwell Munksgaard, Oxford, UK, 2003, pp. 186–231.
22. Ishikawa, I., Host responses in periodontal diseases: A preview. Periodontology 2000. 2007, 43, pp. 9–13.
23. Teng, Y.T., Protective and destructive immunity in the periodontium: Part 1– Innate and humoral immunity and the periodontium. Journal of Dental Research, 2006, 85, pp. 198–208.
24. Hasturk, H., Kantarci, A., Goguet-Surmenian, E., Blackwood, A., Andry, C., Serhan, C.N., Van Dyke, T.E., Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis *in vivo*. Journal of Immunology, 2007, 179, pp. 7021–7029.
25. Fraser, A.H., Dental caries in the dog. Can. Vet. J., 2009, 50(12): 1301–1304.
26. Brook, A.N., Oral Pathology. Topics in Companion Animal Medicine, 2008, Volume 23, Issue 2, pp. 59-71.
27. Fraser, A.H., Dental caries in the dog. J. Vet. Dent., 1998, 15:79–83.