THE ROLE OF BIOFILMS IN OTITIS

SUE PATERSON

describes the role biofilms play in the condition in humans and animals and lists the various treatment options for veterinary surgeons to consider

OUR UNDERSTANDING OF THE MICROBIOLOGY of acute and chronic infections and the ways in which they should be managed has changed dramatically over the last 40 years.

In general it is now accepted that bacteria exist in two different forms during growth and proliferation. In one form, which accounts for most of the acute infective processes, the bacteria exist as single, independent cells and are referred to as planktonic. In chronic infections the bacteria are organised into sessile aggregates referred to as biofilms.

In acute disease, providing an accurate and speedy diagnosis is made, infections respond well to appropriate antibiotic therapy. Where a biofilm forms in chronic disease, the infection is far more difficult to treat.

The hallmarks of biofilm formation include extreme resistance to antibiotics and many other conventional anti-microbial agents and an extreme ability to evade the host's immune system.[1]

What is a biofilm?

Although he was unaware of the importance of his findings, biofilms were first discovered by Antonie van Leewenhoek in 1684, when he described the vast accumulations of bacteria found in dental plaque by saying: "The number of these animalcules in the scurf of a man's teeth are so many that I believe they exceed the number of men in the kingdom".[1]

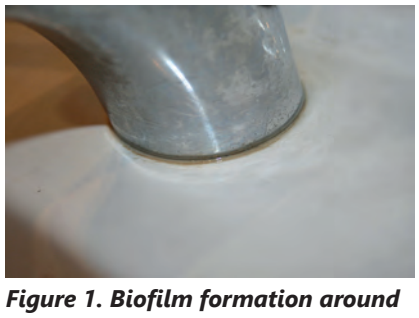

Figure 1. Biofilm formation around a hot tap.

It was not though until 1978 that the first theory of biofilm formation was postulated.[2] This early theory, which was derived mostly from observations of aquatic ecosystems, stated that the majority of bacteria grew in matrix-enclosed biofilms adherent to surfaces. It goes on to say that these sessile bacterial cells differ profoundly from their planktonic (floating) counterparts.

Since this time our understanding of biofilms has evolved considerably and a more modern definition of a biofilm now takes into consideration new data.

A biofilm may now be described as a microbially derived sessile community characterised by cells that are irreversibly attached to a surface or interface or to each other; are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.[1] Planktonic organisms do not have the ability to transcribe genes in this way.

Where do we find biofilms?

Biofilms are ubiquitous and seem to be able to form on virtually any non-shedding surface. They are found on rocks and pebbles at the bottom of most streams and they are also found on the surface of and inside plants.

In the home they are found in the shower, around the taps (**Figure 1**) or plug hole. They are present on the teeth of most animals as dental plaque (**Figure 2**), where they can go on to cause tooth decay and gum disease.

Biofilms have been found to be involved in a wide variety of microbial infections in humans. This includes urinary tract infections, endocarditis, periodontitis, pneumonia in cystic fibrosis and chronic bacterial prostatitis.[1,3,4]

The first paper to demonstrate the presence of polymicrobial biofilms in the middle ear of children with otitis media was published in 2006 by Hall-Stoodley *et al.*[5] Further papers have reinforced their importance in chronic otitis media in humans[6,7] and more recent work has suggested they may play a role in otitis media with effusion.[8]

Biofilms are recognised as being clinically important in veterinary medicine. They have been identified as causing many of the same problems they have been implicated with in human medicine. Notably urinary tract disease, gingivitis, wound infections, catheter and implant infections and otitis media.[9]

There is no doubt that biofilms in otitis are common and underdiagnosed.[10] All of the common bacterial and yeast pathogens found in otitis in the dog are capable of forming biofilms. Work by Han (2015) has shown that more than 90% of the isolates of both meticillin sensitive and resistant staphylococcus pseudintermedius from healthy dogs are capable of producing biofilms.[11] *Malassezia pachydermatis* has also been shown to form biofilms.[12]

Pathogenic isolates of Staphylococci[13] and *Pseudomonas aeruginosa*[14] from clinical cases of canine otitis (**Figure 3**) have also been shown to be capable of producing biofilms.

How do we diagnose the presence of a biofilm in otitis?

Biofilms have been implicated as a cause of chronic otitis in man.[15] Where infections have failed to respond to what appears to be completely appropriate antibiotic therapy, biofilms may be present.

Biofilms can be diagnosed on otoscopy and cytology.[10] Clinically they form an adherent, thick slimy discharge that is often dark brown or black. On cytology they appear as variably thick veil-like material that may obscure bacteria and cellular detail (**Figure 4**).

Why are biofilms resistant to antimicrobial agents?

Biofilms have an inherent resistance to antimicrobial agents whether they are antibiotics or disinfectants. It is because biofilm-associated cells grow more slowly than planktonic bacteria that they are less susceptible to antimicrobial agents.

Their secretion of an extracellular polymeric matrix produces a diffusional barrier to reduce antimicrobial penetration into the biofilm. This barrier leads to a range of different effects.

Where the significant level of antimicrobial agent penetrates the biofilm, bacteria will be exposed to a high dose of drug leading to their elimination, but where only low concentrations of the drug penetrate the biofilm they remain unaffected.

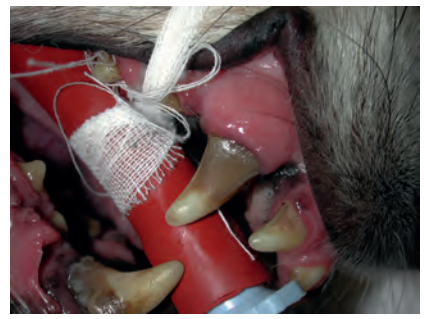

Figure 2. Dental plaque on a dog's teeth.

Problems occur where bacteria are exposed to an intermediate concentration of drug which may provide a mutant selection window, in which the more susceptible bacteria are eliminated but resistant mutants survive, leading to treatment failure and a recrudescence of a more resistant population of isolates.[10]

How do we treat biofilms?

Topical therapy is preferable in all cases of otitis because the levels of drug obtained in the ear are much higher than those achieved using systemic therapy.

Studies in both man[16] and dogs[17] have shown good levels of systemic drugs can be achieved in the middle ear[16] and external ear canal[17] after drugs are administered systemically. However, Cole's study (2009) suggested treatment with enrofloxacin could not be recommended for a bacterial organism with an intermediate susceptibility or resistance to enrofloxacin, since high enough levels of enrofloxacin would not be attained in the ear tissue to produce any antibacterial effects.[17]

By extrapolation it is safe to assume systemic levels of this drug would similarly not be high enough to treat biofilms. In order to assist penetration of topical drugs, a better strategy is to physically break the biofilms down and then remove them by flushing.

Several products have been shown to be useful in the management of biofilms, including topical formulations of tris EDTA[18], silver[19-22], lactoferrin[23], povidone iodine[24,25], honey[26] and topical and systemic N-acetyl cysteine.[27-31]

Tris EDTA

Tris EDTA damages bacterial cell walls to increase microbial penetration. It is well-tolerated and non-ototoxic.[32] It has been shown to have additive effects with a whole range of antibiotics including gentamicin[33], fluoroquinolones[33,34] as well as silver sulphadiazine[35] and chlorhexidine.[36]

More recently *in vitro* work by Pye (2014) has shown that tris EDTA may be a useful adjunctive treatment for chronic cases of *Pseudomonas otitis* where biofilms may have developed, if

Sue Paterson, MA, VetMB, DVD, DipECVD, MRCVS, RCVS and European Specialist in Veterinary Dermatology, gained her Certificate in Small Animal Dermatology in 1990, her British Diploma in Veterinary Dermatology in 1994 and her European Diploma in Veterinary Dermatology in 1996. She has written seven textbooks in addition to contributing chapters to a variety of other textbooks including the BSAVA *Manual of Small Animal Dermatology*, *Feline Internal Medicine*, *Equine Medicine* and *Therapeutics* and *Advances in Veterinary Dermatology*. She has lectured extensively in more than 40 countries thoughout Europe, Asia and the USA, including the VetsNorth congresses in Manchester. She sits on the Royal College Council and is currently president of the ESVD and chair of publications at the BSAVA.

gentamicin or neomycin is to be used as a topical treatment.[18]

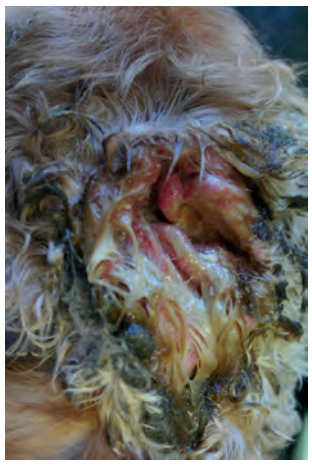

Figure 3. Mucoid discharge in severe chronic otitis externa.

N-acetyl cysteine

N-acetyl cysteine (NAC) is used in medical treatment of patients with chronic bronchitis. The positive effects of NAC treatment have primarily been attributed to the mucus-dissolving properties of NAC, as well as its ability to decrease biofilm formation, which reduces bacterial infections.

A recent systematic literature review of eight clinical trials involving NAC as an adjuvant treatment to eradicate pre-formed mature biofilms and to inhibit new biofilm production suggested a potential role for NAC as an adjuvant molecule in the treatment of bacterial biofilms, with an excellent safety and efficacy profile.

NAC, in combination with different antibiotics, significantly promoted their permeability to the deepest layers of the biofilm, overcoming the problem of the resistance to the classic antibacterial therapeutic approach.[28]

NAC is available as topical eye preparations and as injectable solutions that can be used topically in the ear. The author normally uses it systemically at a dose of 600mg per dog.

Conclusion

Biofilm formation appears to be common in all long-standing cases of otitis. The concurrent use of agents to help break down biofilms is useful where their presence is suspected.

NAC is a systemic drug that may be useful to help break down formation of the extracellular polymeric matrix that limits diffusion of antimicrobial agents into the area of infection. Topical drugs that may be useful include NAC, tris EDTA, honey, colloidal silver and povidone iodine.

References

1. Donlan, R. M., Costerton, J. W. (2002) Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* **15** (2): 167-193.
2. Costerton, J. W., Geesey, G. G., Cheng, G. K. (1978) How bacteria stick. *Sci Am* **238**: 86-95.
3. Bjarnsholt, T. (2013) The role of bacterial biofilms in chronic infections. *APMIS* **121** (136): 1-58.
4. Singh, P. K., Parsek, M. R., Greenberg, E. P., Welsh, M. J. (2002) A component of innate immunity prevents bacterial biofilm development. *Nature* **417** (6,888): 552-555.
5. Hall-Stoodley, L., Hu, F. Z., Gieseke A. (2006) Direct detection of bacterial biofilms on the middle ear mucosa of children with chronic otitis media. *JAMA* **296** (2): 202-211.
6. Akyildiz, I., Take, G., Uygur, K., Kizil, Y., Aydil, U. (2013) Bacterial biofilm formation in the middle-ear mucosa of chronic otitis media patients. *Indian J Otolaryngol Head Neck Surg* **65** (Suppl 3): 557-561.
7. Wessman, M., Bjarnsholt, T., Eickhardt-Sorensen, S. R., Johansen, H. K., Homoe, P. (2015) Mucosal biofilm detection in chronic otitis media: a study of middle ear biopsies from Greenlandic patients. *Eur Arch Otorhinolaryngol* **272** (5): 1,079-1,085.
8. Tawfik, S. A., Ibrahim, A. A., Talaat, I. M., El-Alkamy, S. S., Youssef, A. (2016) Role of bacterial biofilm in development of middle ear effusion. *Eur Arch Otorhinolaryngol* **273** (11): 4,003-4,009.
9. Gardner, A. J. (2011) Biofilms and role to infection and disease in veterinary medicine. In: Percival, S. L., Knottenbelt, D. C., Cochrane, C. A. (eds). *Biofilms and Veterinary Medicine 6*. Springer Berlin Heidelberg; pp111-128.
10. Nuttall, T. (2016) Successful management of otitis externa. *In Practice* **38**: 17-21.
11. Han, J. I., Yang, C. H., Park, H. M. (2015) Emergence of biofilm-producing *Staphylococcal pseudintermedius* isolated from healthy dogs in South Korea. *Vet Q* **35** (4): 207-210.
12. Cannizzo, F. T., Eraso, E., Ezkurra, P. A., Villar-Vidal, M., Bollo, E., Castella, G. *et al*, eds (2007) Biofilm development by clinical isolates of *Malassezia pachydermatis*. XXIV International Specialised Symposium on Yeasts; Oropesa del Mar, Spain.
13. Moreira, C. A., de Oliveira, L. C., Mendes, M. S., Santiago, Tde M., Barros, E. B., de Carvalho, C. B. (2012) Biofilm production by clinical staphylococci strains from canine otitis. *Braz J Microbiol* **43** (1): 371-374.
14. Pye, C. C., Yu, A. A., Weese, J. S. (2013) Evaluation of biofilm production by *Pseudomonas aeruginosa* from canine ears and the impact of biofilm on antimicrobial susceptibility *in vitro*. *Vet Dermatol* **24** (4): 446-449, e98-9.
15. Fusconi, M., Petrozza, V., Taddei, A. R., Vinciguerra, V., De Virgilio, A., Chiarini, F. *et al* (2011) Is biofilm the cause of chronic otitis externa? *Laryngoscope* **121** (12): 2,626-2,633.
16. Belfield, K., Bayston, R., Birchall, J. P., Daniel, M. (2015) Do orally administered antibiotics reach concentrations in the middle ear sufficient to eradicate planktonic and biofilm bacteria? A review. *Int J Pediatr Otorhinolaryngol* **79** (3): 296-300.
17. Cole, L. K., Papich, M. G., Kwochka, K. W., Hillier, A., Smeak, D. D., Lehman, A. M. (2009) Plasma and ear tissue concentrations of enrofloxacin and its metabolite ciprofloxacin in

continued overleaf

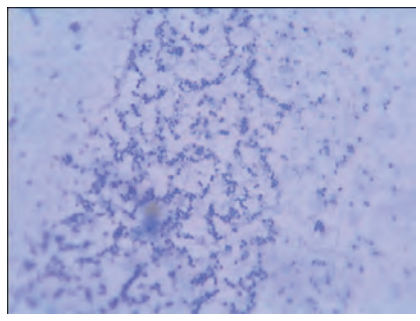

Figure 4. Cytology of a smear from a dog's ear with bacterial infection and probable biofilm formation. Note the lace-like filamentous pattern surrounding the bacterial organisms.