Biofilms can be defined as a microbially derived sessile community of organisms 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 (Donlan and Costerton, 2002).

Biofilms are recognised as being clinically important in veterinary medicine, they are ubiquitous and seem to be able to form on virtually any non-shedding surface. They complicate many different diseases such as canine and feline urinary tract disease, gastrointestinal disease, periodontal disease, otitis, dermatitis and wounds. They have also been shown to be able to form on medical devices such as catheters and orthopaedic implants.

The initial phase of biofilm formation is attachment of the micro-organisms to a surface. After attachment, cell division starts and the micro-organisms start to form micro-colonies. The microbes then produce a protective, slimy extracellular polymeric substance (EPS) that provides a structural scaffold for the biofilm and binds it to the underlying surface. As biofilm maturation occurs multiple layers of cells build up and are incorporated into the matrix. The final structure is a complex three dimensional construction in which the matrix is interspersed with open water channels that provide nutrients and oxygen to developing micro-colonies; which remain protected against antibiotics, toxic chemicals and where necessary the body’s immune system within their EPS (Donlan, 2002). The final phase of biofilm development is when bacterial cells detach and disperse to colonise new sites.

Many of the bacteria and yeast that contribute to dermatological disease are capable of producing biofilms. This includes meticillin sensitive and resistant *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa* and *Escherichia coli*. *Malassezia pachydermatis* isolated from cases of canine seborrhoeic dermatitis has also been shown to be capable of producing biofilms.

Factors that increase the risk of biofilm formation and implant associated infections includes the presence of extensive soft tissue trauma and the location of the implant (oral cavity or a limb) In human medicine patient co-morbidities such as immunosuppression due to concurrent disease such as diabetes or medication (chemotherapy and corticosteroids) also appear to be important.

**DIAGNOSIS OF BIOFILM INFECTIONS**

Biofilm bacteria may not be easily isolated using standard culture swab techniques. In human medicine the presence of at least two positive cultures specimens with identical micro-organisms is usually required to confirm a periprosthetic joint infection. False negative cultures are not uncommon because of the difficulty in detaching and identifying bacteria from the biofilm surface. Molecular methods such as polymerase chain reactions and 16S ribosomal probes have been shown to have a higher degree of sensitivity than culture. Unfortunately they are still not widely available to the primary care clinician or the referral clinician outside academia. Other diagnostic criteria that have been proposed to help in human medicine to make a diagnosis of biofilm infection include:

1) Positive culture or molecular identification of microbes known to be associated with biofilm formation

2) Microscopic evidence of microbial aggregates and biofilm structure

3) History of a condition that predisposes to the development of biofilm formation e.g. presence of an orthopaedic implant
4) Recurrence of infection despite appropriate antimicrobial therapy
5) Evidence of antibiotic failure despite adequate antimicrobial therapy

These criteria can readily be applied to veterinary cases to raise the suspicion of the presence of a biofilm infection.

**THERAPY OF BIOFILM INFECTIONS**

Biofilm bacteria can be from 100 to 1000 fold more resistant to antibiotics than their planktonic, free living counterparts. Biofilm bacteria have several mechanisms that facilitate their resistance this includes restricted penetration of antibiotics; restricted growth at low oxygen tension; expression of biofilm specific genes and the presence of persisters.

Restricted penetration of antimicrobials through the extracellular matrix can contribute to antimicrobial resistance of biofilms. This is caused by a range of mechanisms which include both the physical barrier of the EPS through antibiotic binding to components of the biofilm matrix and also the inactivation of the antibiotics by matrix enzymes such as beta-lactamases. Bacteria exhibit different levels of physiological activity within a biofilm. Whilst metabolic activity is high in those on the outer part of the biofilm; it is low in those in the inner part. This reduction in activity is thought to be due to limited oxygen and nutrient penetration into the biofilm. As many antibiotics target growing bacteria, though their action against e.g. replication and cell wall synthesis, bacteria deep within the biofilm will have increased bacterial tolerance to these types of antibiotics. “Persistor” cells are is the term given to these cells within the biofilm that are either dividing very slowly or not at all. Although the number of persister cells is usually low (< 0.1%), cells in this dormant state have a diminished susceptibility to antibiotics.

Once biofilm infection has been diagnosed or there is a high level of suspicion of biofilm formation, a range of different treatment options may be explored. Mechanical removal of the biofilm is the most effective method; however this is not always feasible to do. Combinations of therapy that include antimicrobial drugs with other anti-biofilm agents have been shown to be successful. Possible treatment options includes:

1) Antimicrobial therapy
2) Mechanical removal of biofilms
3) Quorum sensing inhibitors
4) Antiadhesive agents
5) Bacteriophages
6) Miscellaneous

**ANTIMICROBIAL THERAPY**

Systemic antibiotics are often employed to treat canine otitis. Although studies in both man and dogs have shown good levels of antibiotics can be achieved in the middle ear and external ear canal after drugs are administered systemically; Cole’s work (2009) showed systemic treatment with enrofloxacin did not produce high enough level in middle ear tissue to treat bacteria with an intermediate susceptibility. One can postulate that it is therefore likely that levels of antibiotics administered systemically may fail to be effective against biofilm infection in both otitis externa and media. Topical administration of antibiotics has the advantage of providing high local concentrations of drug at the site of the infection. By achieving high local concentrations of drug the mean inhibitory concentration (MIC) of the bacteria can be exceeded several fold. Azithromycin possess high in vitro and in vivo anti-biofilm activity against *Pseudomonas aeruginosa* as it inhibits the formation of a key component of their matrix, alginate. The combination of this drug with a second antibiotic based on culture and sensitivity may offer an attractive option to treat chronic *Pseudomonas* spp. infections. Some antibiotics have been shown to have the ability to penetrate the extracellular matrix and therefore achieve high localised bactericidal levels of drug. In vitro studies have shown that rifampicin can penetrate staphylococcal biofilm and ciprofloxacin can penetrate the biofilm produced by *Klebsiella pneumoniae*. These drugs may therefore be useful respectively for Gram positive and negative biofilm infections Where used these types of drugs should only be prescribed on the basis of culture and sensitivity and in the case of rifampicin by clinicians who are experienced in the use of a potentially hepatotoxic antibiotic and the need for careful monitoring. Rifampicin is also best used in combination with other drugs because of the rapid development of resistance to it by bacteria. Some
antimicrobials such as colistin, sodium dodecyl sulphate (SDS, sodium lauryl sulphate),
ethylenediaminetetraacetic acid (EDTA) and chlorhexidine preferentially kill the non-growing bacteria
located in the inner part of the biofilm which means that such products have the potential to be used with
topical antibiotic therapy to target all of the physiological stages of bacterial growth within the biofilm.

MECHANICAL REMOVAL OF BIOFILMS.
Where wounds have biofilm infection debridement forms an important part of the treatment process.
Several different debridement techniques can be employed these include autolytic (hydrogels,
hydrocolloids, cadexomer iodine, honey), biosurgical (medical maggots), hydrosurgical (mechanical water
jet), mechanical (wet and dry dressings), sharp or surgical (surgical excision) and ultrasonic (low
frequency ultrasound. Although mechanical removal of biofilm in ear cases can be more challenging
thorough cleaning can help break down biofilms and ensure maximum penetration of topical drugs.

QUORUM SENSING INHIBITORS (QSI)
Quorum sensing inhibitors and antagonists are currently one of the most promising areas of therapy of
biofilm infections. Quorum sensing in biofilms is used to coordinate gene expression and regulate
numerous processes that are involved in virulence: by disrupting this process biofilms can be treated
more effectively. Many naturally occurring products have been shown to have QSI activity. These include
garlic, ginseng, pomegranate, vanilla extract and essential oils such as rose, lavender and rosemary. N-
acetyl cysteine (NAC) has been shown to have QSI properties to affects growth, extracellular
 polysaccharide production, and bacterial biofilm formation on solid surfaces. NAC is used in veterinary
medicine as a topical product in otitis both topically and systemically to help manage chronic ear disease.

ANTI-ADHESIVE AGENTS
Anti-adhesive agents should specifically interact with the adhesins of the pathogen to prevent the union
between the pathogen and the eukaryotic cell. Antiadhesive agents include mannosides, curlicides and
pilicides. Mannosides, which are molecules containing mannose sugar groups, are utilised in urinary
supplements to help treat and maintain animals prone to urinary tract disease. The incorporation of anti-
adhesive agents into veterinary ear cleaning solutions in the form of monosaccharides (D-galactose, D-
mannose, L-rhamnose) have been shown to reduce bacterial adherence to canine corneocytes

BACTERIOPHAGES
Bacteriophages are viruses that specifically infect bacteria. Phages have the ability to control biofilms in a
armage of different ways. They can multiply at the site of the infection to produce enzymes that degrade
the EPS of the biofilm matrix. They have also been shown to have the ability to propagate through the
biofilm. Some preliminary studies have suggested they may have the potential to treat Pseudomonas
infections in otitis

MISCELLANEOUS PRODUCTS WITH BIOFILM ACTIVITY
Honey has also been shown to prevent bacterial biofilms and eliminate established biofilms in vitro
However as there is little standardisation between trials for floral source, geographical location and the
level of the two principal antibacterial components (hydrogen peroxide and MGO) it is difficult to make
any specific recommendations for the use of honey.

EDTA has antibacterial activities. When combined with tromethamine (Tris) it has been shown to have
the ability to damage bacterial cell walls to increase microbial penetration. It is well tolerated and non-
 ototoxic. It has has additive effects with a wide range of antibiotics including gentamicin, fluoroquinolones,
silver sulphadiazine and chlorhexidine. Work by Pye (2014) has shown that Triz-EDTA may be a useful
adjunctive treatment for chronic cases of Pseudomonas otitis where biofilms may have developed, if
gentamicin or neomycin is to be used as a topical treatment (Pye et al., 2014).

Silver impregnated dressings have been shown to reduce the viability of biofilm bacteria in wounds and
increase their susceptibility to antibiotics. Silver impregnated coatings on orthopaedic implants and
catheter have been shown to reduce the risk of biofilm infections. Colloidal silver has also been used
topically to treat biofilms infections.
Bacterial biofilms are a major cause of infection in man and animals. They have the ability to form on implants and also on living tissue. They are an important reason for orthopaedic implant failure and contribute to a wide range of veterinary diseases including urinary tract infections, periodontal disease, wound infection and otitis. Conventional antimicrobial therapy often fails to eliminate biofilm infection and new novel therapies to tackle these infections are currently being developed. It is important that veterinarians are aware of the presence of biofilms in disease and the need to modify their therapeutic approach to deal with them.

REFERENCES