

Introduction to Biofilms

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Abstract In the seventeenth century, a dry-goods merchant named Antonie van Leeuwenhoek first observed “animalcules” swarming on living and dead matter. Leeuwenhoek’s curiosity and inventiveness were remarkable; he discovered these “animalcules” in the tartar on his own teeth and even after meticulous cleansing, the remaining opaque deposits isolated between his teeth were still “as thick as if it were batter”. These deposits contained a mat of various forms of “animalcules” that we now know were the bacteria of dental plaque. It is reasonable to suggest that this early study of dental plaque was the first documented evidence of the existence of microbial biofilms. Today, we generally define such biofilms as microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves. Biofilms may form on a wide variety of surfaces, including natural aquatic systems living tissues, indwelling medical devices and industrial/potable water system piping. The vast majority of microbes grow as biofilms in aqueous environments. These biofilms can be benign or pathogenic, releasing harmful products and toxins, which become encased within the biofilm matrix. Biofilm formation is a phenomenon that occurs in both natural and man-made environments under diverse conditions, occurring on most moist surfaces, plant roots and nearly every living animal. Biofilms may exist as beneficial epithilic communities in rivers and streams, wastewater treatment plant trickling beds or in the alimentary canal of mammals. Given the prevalence of biofilms in

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natural environments, it is not surprising that these growth forms are responsible for infection in humans and animals. In humans, biofilms have been linked with numerous conditions and equally in animals equivalent infections may occur.

1 Introduction and Historical Perspectives

In the seventeenth century, a dry-goods merchant named Antonie van Leeuwenhoek first observed “animalcules” swarming on living and dead matter. Leeuwenhoek’s curiosity and inventiveness were remarkable; he discovered these “animalcules” in the tartar on his own teeth and even after meticulous cleansing, the remaining opaque deposits isolated between his teeth were still “as thick as if it were batter”. These deposits contained a mat of various forms of “animalcules” that we now know were the bacteria of dental plaque. It is reasonable to suggest that this early study of dental plaque was the first documented evidence of the existence of microbial biofilms. Today, we generally define such biofilms as microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves.

After van Leuwenhoek’s early work, it was not until 1940 that the so-called “bottle effect” in marine microorganisms was first observed (Heukelekian and Heller 1940). This showed that the growth of bacteria was substantially increased when they were attached to a surface. Further advancements in our knowledge of biofilms were made by Zobell in 1943 when he noted that bacteria on surfaces were greater in number compared with the surrounding seawater. From his studies, Zobell also postulated that the adhesion of bacteria consisted of a two-stage process of reversible and then irreversible adhesion.

Despite the above studies being the first documented ones on biofilms, the extensive physical and chemical analysis of bacterial biofilms did not begin until the late 1960s and early 1970s, when a few investigators recognised the prevalence of bacterial biofilms (Jones et al. 1969; Characklis 1973; Costerton et al. 1978). Jones et al. (1969) used scanning and transmission electron microscopy to examine biofilms on trickling filters in a wastewater treatment plant. From this work it was shown that biofilms were composed of a variety of different microorganisms and revealed that the matrix material or EPS was primarily composed of polysaccharides. The investigation of biofilms around this time was greatly aided by the use of electron microscopy, which provided information, not only on biofilm structure, but also on the presence of EPS. In 1973, Characklis who investigated microbial slimes in industrial water revealed that biofilms were both tenacious and highly resistant to the antimicrobial effects of chlorine (Characklis 1973).

The first true analysis of biofilms per se was not recognised until 1978 (Costerton et al. 1978), when studies showed that many bacteria spent the majority of their existence within surface-attached, sessile communities.

Work on dental plaque and sessile communities in mountain streams enabled Costerton et al. (1978) to hypothesise the mechanisms by which microorganisms

adhered to living and non-living materials and derived benefit from this ecologic niche.

Since the 1970s, the study of biofilms in industrial, ecological and medical settings has followed similar paths. Initial biofilm studies generally concentrated on composition, especially of the polymer matrix or “glycocalyx” that was thought to conserve and concentrate the digestive enzymes released by the bacteria, thus increasing the metabolic efficiency of the cells. Research by Costerton and Geesey (1979) indicated that this glycocalyx also acted as an ionic exchange matrix, trapping nutrients that were then transported into cells by highly efficient permeases.

In 1981 (Costerton et al. 1981), glycocalyx was characterised as a hydrated polyanionic polysaccharide matrix produced by polymerases affixed to the lipopolysaccharide component of the bacterial cell wall. In aqueous environments, biofilm production of glycocalyx is prevalent with organic and inorganic nutrients being concentrated at the solid/liquid interface. Additionally, the glycocalyx provides a physical/chemical barrier that offers partial protection against antibacterial agents.

Since biofilms form under diverse conditions, and may be composed of single or multiple species, the structures of various biofilms will necessarily have distinct features. Nevertheless, biophysical, structural and chemical studies have led to a useful basic concept of a “biofilm model” (Costerton et al. 1995). In this model, microorganisms form microcolonies surrounded by copious amounts of expolysaccharide. Between the microcolonies are water-filled channels, and it has been proposed that these promote the influx of nutrients and the efflux of waste products. This biofilm model will be discussed in detail both in this chapter and later on in the book.

In 1998, important advances to our understanding of the development and behaviour of biofilms were made, when studies that combined molecular genetic approaches with confocal laser scanning microscopy (CLSM) emerged.

Bacteria are remarkably adept at surviving “feast and famine”, and also adjusting their needs to accommodate highly diverse environments. Scientific inquiry has discovered a number of the microbial characteristics that facilitate the way bacteria adapt to changing environments. The capacity to form and maintain biofilms is key to these adaptations. Traditionally, microbiologists have performed physiological experiments with microorganisms grown in liquid monocultures where the cells are “free swimming” or planktonic. Whilst it may seem that microbiologists are always striving for pure cultures, most of the bacteria in the world live in these polymicrobial ecosystems called biofilms; a complex community of microorganisms that are not “free swimming”, but are instead attached to surfaces.

2 Prevalence and Importance of Biofilms in Animals and Humans

Biofilms may form on a wide variety of surfaces, including natural aquatic systems living tissues, indwelling medical devices and industrial/potable water system piping. The vast majority of microbes grow as biofilms in aqueous environments.

These biofilms can be benign or pathogenic, releasing harmful products and toxins, which become encased within the biofilm matrix.

Biofilm formation is a phenomenon that occurs in both natural and man-made environments under diverse conditions, occurring on most moist surfaces, plant roots and nearly every living animal. Biofilms may exist as beneficial epithelial communities in rivers and streams, wastewater treatment plant trickling beds or in the alimentary canal of mammals (Costerton et al. 1981).

Biofilms are not, however, confined to solid/liquid interfaces, and can also be found at solid/air or liquid/liquid interfaces (e.g. airborne pathogens and deteriotogens have been shown to be important factors in the biodeterioration of surface coatings; biofilms at liquid/liquid interfaces have been implicated in hydrocarbon degradation, including fuels, oils and industrial coolants).

In humans, an estimated 65% of all hospital infections are of biofilm origin (Costerton et al. 1999; Donlan 2001; Donlan and Costerton 2002; Douglas 2003; Ramage et al. 2006). Once established, biofilm infections are very difficult to eradicate due to their resilience to removal by host defence mechanisms and antimicrobials. Table 1 outlines several examples of clinically significant biofilm infections. Of particular note are biofilm infections in patients with medical devices, *Pseudomonas aeruginosa* infection in cystic fibrosis patients, tooth decay and periodontal disease, and chronic wound infections. As well as increasing patient morbidity and mortality, there is also an economic burden to biofilm infections with

Table 1 Comparison of biofilm and planktonic lifestyles

Characteristic	Lifestyle
<i>Physiological</i>	
Free swimming, often in an aqueous environment	Planktonic
Metabolic products continuously removed from the milieu, stresses of physical, chemical and biological nature	Planktonic
Slow growth	Biofilm
Found ubiquitously	Biofilm
Generally higher tolerance to antibiotics, hydrogen peroxide, phagocytosis	Biofilm
Stress conditions: lower metabolic activity (e.g. decreased nutrient accessibility, limitation of oxygen, increased osmotic pressure, pH variation, problems with accumulation of waste metabolites)	Biofilm
Bacteria develop stress responses	Biofilm
Source of infections	Planktonic and biofilm
Display unique gene expression patterns	Biofilm
Attachment can influence metabolic activities	Biofilm
Express virulence factors	Biofilm and planktonic
<i>Structural</i>	
Attached to a solid substrate	Biofilm
Many cells are dormant, likely to be smaller, not actively engaged in cell division	Biofilm
Flagella function in transport and initiation of cell-to-surface interactions and detachment	Biofilm

those involving medical devices estimated to cost \$20 billion in the USA alone. Furthermore, biofilms in air-conditioning and other water retention systems can cause human infection if ingested or inhaled, and examples include those biofilms caused by *Legionella* species, which persist in water storage tanks despite chlorination (Percival and Walker 1999).

3 Why Do Microorganisms Form Biofilms?

There are a number of possible advantages gained when a microorganism is in a biofilm compared to a planktonic existence. These include the increased expression of beneficial genes, phenotypic changes in colony morphology, acquisition of antibiotic resistance genes by plasmid transfer, the production of copious amounts of extracellular polymers (Costerton et al. 1987), enhanced access to nutrients and closer proximity between cells facilitating mutualistic or synergistic associations and protection (Costerton and Lappin-Scott 1989). This list is by no means complete but helps to highlight the possible advantages of survival in biofilms.

Microbiology research has traditionally focused on the in vitro analysis of single species in liquid culture. The study of planktonic microorganisms has undoubtedly been of value in expanding our knowledge on microbial physiology and biochemistry. However, in recent years, the importance of biofilm growth has been recognised (Percival and Bowler 2004; Wilson 2001) with microorganisms in a biofilm shown to differ markedly from their planktonic counterparts in terms of behaviour, structure and physiology (Table 2). These differences have significance both to the pathogenic potential of microorganisms together with their susceptibility to antimicrobials.

Table 2 Clinically significant examples of biofilm infections

Infection	Typical biofilm organism
Dental caries	<i>Streptococcus</i> spp.
Periodontitis	<i>Fusobacterium nucleatum</i> , <i>Porphyromonas gingivalis</i> , <i>Bacteroides forsythus</i> , <i>Prevotella intermedia</i>
Otitis media	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Moraxella catarrhalis</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i>
Cystic fibrosis	<i>P. aeruginosa</i> , <i>Burkholderia cepacia</i>
Chronic wounds	Staphylococci, streptococci, enterococci, facultative Gram-negative bacilli, anaerobic bacteria such as <i>Fusobacterium</i> spp. and peptostreptococci
Foreign body/medical device infection	
Urinary catheters	<i>Proteus mirabilis</i> , <i>Morganella morganii</i> , <i>P. aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Proteus vulgaris</i>
Native valve endocarditis (NVE)	Streptococci, staphylococci, Gram negative bacteria, fungi

4 Definition of a Biofilm

Biofilms are not easily defined as they vary greatly in structure and composition from one environmental niche to another. Microbial biofilms are extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin. As well as microbial components, non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, may also be found in the biofilm matrix. Biofilms, particularly in water systems, can be highly complex, whilst others such as those on medical devices, may be simpler, and composed of single, coccoid or rod-shaped organisms. Given these differences, it does not seem plausible to suggest that a true “biofilm model” can be defined that is applicable to every ecological, industrial and medical situation. Therefore the definition of a biofilm has to be kept general and thus may be redefined as “microbial cells immobilised in a matrix of extracellular polymers acting as an independent functioning ecosystem, homeostatically regulated” (Percival et al. 2000).

5 The Biofilm Model

Despite ongoing discussions on the so-called “biofilm model” the diversity of biofilms suggests that strict phraseology for a constantly changing dynamic ecosystem is not possible. It is feasible to suggest that biofilms in distinct settings form different structures comprising different microbial consortia dictated by biological and environmental parameters. These biofilms can quickly respond and adapt both phenotypically, genetically and structurally to constantly changing internal and external conditions (Percival et al. 2000). Whilst a so-called “biofilm model” has been proposed and will be discussed in detail throughout the book, it must be borne in mind that applying laboratory-based observations to an otherwise chaotic, totally unpredictable biofilm in the environment may well aid in clouding independent thought of a “true biofilm model”, if in fact one exists. From the authors’ experiences and research it is clear that environmental/industrial and medical biofilms cannot be truly replicated and reproduced to the conditions that prevail in the field. Therefore one must endeavour to move away from the single “gold standard” and possibly concede to the fact that a “true biofilm model” does not exist and that nature determines the complexities of a biofilm that cannot be reproduced in vitro. However, we need to start somewhere, which is where laboratory-based analysis is warranted. Therefore to appreciate the biofilm and to address the “biofilm model” concept we need to fully understand what a biofilm is and how it forms. This will be discussed in detail below.

The development of a mature biofilm is a multistage process and is dependent on a number of variables including the type of microorganism, the surface to which attachment occurs, environmental factors and expression of biofilm essential genes

(Carpentier and Cerf 1993; Dunne 2002). Aspects of biofilm development are discussed below and elsewhere in this book.

Primary adhesion between bacteria and abiotic surfaces is often non-specific (e.g. mediated by hydrophobic interaction), whereas adhesion to living tissue tends to be through specific molecular (lectin, ligand or adhesin) mechanisms (Carpentier and Cerf 1993; Dunne 2002). Primary adhesion is reversible and in biological systems occurs between a conditioned surface and a planktonic microorganism. The conditioning film is provided by body fluids bathing the surface and its composition may alter the affinity of a microorganism to the surface (Dunne 2002).

Bacteria and host surfaces express multiple adhesins and receptors to facilitate specific adherence. For example, *Staphylococcus epidermidis* and *Staphylococcus aureus* produce a polysaccharide intercellular adhesion (PIA), which is associated with cell-to-cell adhesion and subsequent biofilm formation (Crampton et al. 1999; Heilmann et al. 1996; Mack et al. 1994, 1996).

After microorganisms have successfully adhered to a surface, they form aggregates and produce an extracellular polysaccharide matrix (Costerton et al. 1999), which serves to encase the initial colonisers and biofilm formers. Coaggregation is a specific means of cell-to-cell adherence (Kolenbrander et al. 2006) which can be highly specific and plays a role in the development of multi-species biofilms in many different environments such as dental plaque (Rickard et al. 2002, 2003a, b), urogenital system (Reid et al. 1988), crops of chickens (Vandevoorde et al. 1992) and in aquatic biofilm-forming bacteria (Buswell et al. 1997; Rickard et al. 2000).

The maturation of biofilms involves recruitment of additional microorganisms from the local environment. Biofilms are heterogeneous environments and in addition to aggregates of microbial cells, interstitial voids and channels develop within the matrix, which separate micro-colonies (Sutherland 2001). The purpose of these channels is to enable the delivery of nutrients and gasses as well as the removal of waste products.

6 Stages in the Formation of Biofilms

The process of biofilm formation is complex, but generally recognised as consisting of five stages (Palmer and White 1997):

1. Development of a surface conditioning film
2. Movement of microorganisms into close proximity with the surface
3. Adhesion (reversible and irreversible adhesion of microbes to the conditioned surface)
4. Growth and division of the organisms with the colonisation of the surface, microcolony formation and biofilm formation; phenotype and genotype changes
5. Biofilm cell detachment/dispersal

Each of these processes will be considered in turn.

6.1 Development of Conditioning Films and Substratum Effects

Within the natural environment, microorganisms do not adhere directly to a substratum per se, but actually adhere to a conditioning film, which is known to form on most substrata. The composition of the conditioning film is complex and results in chemical modification of the original surface, thus influencing the rate and extent of microbial adhesion (Mittelman 1996).

The first documented evidence that a “conditioning” film existed was by Loeb and Neihof (1975), although whether a conditioning film is a pre-requisite for bacterial attachment is still debateable with evidence of this still elusive in the scientific literature.

In aquatic or terrestrial environments, the conditioning layer has been shown to consist of complex polysaccharides, glycoproteins and humic compounds (Chamberlain 1992; Marshall et al. 1971; Baier 1980; Rittle et al. 1990). In comparison human host conditioning films may also be complex and determined by the site being conditioned.

An area of “biofilmology” that has been studied extensively is the role of dental plaque in oral disease. In this regard, the enamel of teeth is conditioned by a proteinaceous “pellicle” composed of albumin, glycoproteins, lipids, lysozyme, phosphoproteins and other components of saliva and gingival crevicular fluids (Marsh 1995). Other types of conditioning films have also been reported, particularly on biomaterials used for human use. In these situations the components of blood, tears, urine, saliva, intravascular fluid and respiratory secretions may all contribute to the conditioning film.

The role of the conditioning film in biofilm development is in its ability to modify the physico-chemical properties of the substratum, as well as providing a concentrated nutrient source and important trace elements. It is important to note that conditioning films may actually inhibit rather than promote the adhesion of certain bacteria.

The topography of the surface to which a microbial cell attaches is also fundamental to biofilm formation. Generally, as the roughness of a surface increases, bacterial adhesion will also increase (Characklis et al. 1990a). There are several possible reasons for this, including most specifically the provision of shelter for the microbes from the effects of shear forces. Apart from increasing the available interfacial area, a rough surface enhances mass transfer coefficients and allows cells to “anchor” to micro-irregularities, where they are better protected from possible desorption (Characklis et al. 1990b). Regardless of surface roughness, the attachment of living particles is energetically favourable if the change in the free energy during the process is negative. Despite metallic surfaces being energetically favourable to the attachment of the pioneer colonisers, the chemical composition of surfaces may interfere with adhesion, cellular metabolism and production of exopolymers (Beech and Gaylarde 1989). The surface effect of certain metals on bacterial adhesion was reported by Vieira et al. (1992), who found that *Pseudomonas fluorescens* preferentially fouled aluminium surfaces within a few hours, followed by copper and brass.

In addition to surface roughness, the physicochemical properties of the surface affects bacterial adhesion in that microorganisms attach more rapidly to hydrophobic, non-polar surfaces such as Teflon and other plastics than to hydrophilic materials such as glass or metals (Pringle and Fletcher 1983; Bendinger et al. 1993; Percival and Thomas 2009). However, such results need to be addressed with care, as many studies have proved contradictory, primarily due to non-availability of standardised methods for surface hydrophobicity measurements.

6.2 *Transport Mechanisms Involved in Adhesion of Microorganisms to a Surface*

The transport of microbial cells and nutrients to a surface is generally achieved by a number of well-established fluid dynamic processes. These include mass transport, thermal (Brownian motion, molecular diffusion) and gravitational effects (differential settling, sedimentation; Characklis 1981).

Within fluid transport pipes, two main flow conditions exist: laminar and turbulent flow. In the blood stream and urinary system, laminar flow is evident and characterised by parallel smooth flow patterns with little or no lateral mixing with the fastest flow in the centre (Fletcher and Marshall 1982; Lappin-Scott et al. 1993). During laminar flow, microorganisms and nutrients maintain a straight path and remain in a stabilised position dictated by flow rate (Lappin-Scott et al. 1993). In contrast, turbulent flow is random and chaotic, ultimately increasing the mixing of bacteria and nutrients (Characklis et al. 1990a, b) and microbial adherence (Percival et al. 1999).

Eddying currents (random and unpredictable flow) are evident in turbulent flow and these cause up- and down-sweep forces, which propel bacteria to within short distances of the surface thereby increasing the chance of adhesion.

Adhesion in static or quiescent environments is aided by a number of factors including Brownian diffusion, gravity and microbial motility (Byers 1987). Motility of a bacterium is known to increase the chances of adhesion (Fletcher 1977; Marmur and Ruckenstein 1986). This is possibly due to the provision of enough potential energy to overcome any repulsive forces known to operate between the bacterial surface and the substratum in question. To reinforce this, it is generally found that a reduction in motility decreases adhesion (Fletcher 1977). Another mechanism known to be a factor affecting surface colonisation is gravitational cell sedimentation, often only of relevance in flowing systems when co-aggregation occurs (Walt et al. 1985).

6.3 *Adhesion*

Following surface conditioning and transport of bacteria into an area close to the substratum surface, adhesion of the microorganism usually takes place. Adhesion,

as previously mentioned, was first proposed in 1943 (Zobell 1943), as consisting of a two-step sequence involving reversible and irreversible processes. Reversible adhesion is the initial weak attachment of microbial cells to a surface (Rittman 1989) and irreversible adhesion and permanent bonding of the microorganisms to a surface, generally follows. Bacteria influence this process, through expression of specific bacterial adhesins (Whittaker et al. 1996), which bind to receptors on the substratum and in the extracellular polymeric matrix (Marshall et al. 1971).

Bacterial adhesion appears to be related to the distance between the microorganism and the surface (Busscher and Weerkamp 1987). At distances greater than 50 nm, *Van Der Waals* forces occur, whilst at distances of 10–20 nm, both *Van Der Waals* and electrostatic interactions occur. When a microorganism is less than 1.5 nm from a surface *Van Der Waals*, electrostatic and specific interactions occur between the cell and the surface.

The surface of a microbial cell has a major impact on adhesion to a substratum. Cell surface hydrophobicity, the presence of fimbriae and flagella, and particularly the extent and composition of generated EPS, influence both the rate and extent of microbial adhesion. A possible role of proteins for bacterial adhesion has been proposed with treatment of adsorbed cells by proteolytic enzymes found to cause a marked detachment of bacteria (Bashan and Levanony 1988; Danielsson et al. 1977).

Fimbriae contain a high proportion of hydrophobic amino acid residues (Rosenberg and Kjelleberg 1986), which can affect cell surface hydrophobicity and therefore attachment to a surface. It is probable that such fimbriae are able to overcome the initial electrostatic repulsion barrier that exists between the cell and substratum (Corpe 1980; Bullitt and Makowski 1995).

Korber et al. (1989) used motile and non-motile strains of *P. fluorescens* and demonstrated that the former attached in highest numbers and against flow (back growth). In addition, non-motile strains do not recolonize or seed vacant areas on a substratum as evenly as motile strains, resulting in comparatively slower biofilm formation.

6.4 Extracellular Polymeric Substances

If cells reside at a surface for a certain time, irreversible adhesion forms through the production of extracellular cementing substances. As mentioned earlier, this extracellular material, associated with the cell has also been referred to as glycocalyx (Costerton et al. 1978), a slime layer, capsule or a sheath.

The EPS of biofilms may account for 50–90% of the total organic carbon of biofilms (Flemming et al. 2000). Glycoproteins, proteins and nucleic acids are also found within this organic matrix (Humphrey et al. 1979).

EPS varies in chemical and physical properties, but in the case of Gram-negative bacteria, is primarily composed of neutral or polyanionic polysaccharides. Uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked

pyruvates are known to constitute part of the EPS matrix. These give anionic properties to the biofilm allowing cross-linking of divalent cations such as calcium and magnesium (Flemming et al. 2000; Sutherland 2001). Biofilms that primarily contain Gram-positive bacteria produce an EPS that is primarily cationic.

The involvement of extracellular polymers in bacterial attachment has been documented for both fresh and marine water bacteria (Corpe 1970; Marshall et al. 1971). Analysis of bacteria isolated from these environments has shown such polymers are largely composed of acidic polysaccharides (Fletcher 1980). The extent to which polysaccharides are involved in the adhesion process remains open to question. Some evidence suggests that excess polymer production may even prevent adhesion, although trace amounts of polysaccharide might be required initially for adhesion (Brown et al. 1977).

EPS is highly hydrated, and can be both hydrophilic and hydrophobic with varying degrees of solubility. The polysaccharide content of EPS has a marked effect on the biofilm (Sutherland 2001) as the composition and the structure will determine their primary conformation. Bacterial EPS contains backbone structures of 1,3- or 1,4- β -linked hexose residues, which are rigid and generally poorly soluble or insoluble, whereas other EPS molecules are more readily soluble in water.

EPS provides many benefits to a biofilm (Characklis and Cooksey 1983) including the promotion of cohesive forces, increased absorption of nutrients and heavy metals (Bryers 1984; Marshall 1992), the sequestration of microbial products and other microbes, protection of immobilised cells from environmental changes and the provision of a medium for intercellular communication and transfer of genetic material.

Extending polymers on cell surfaces interact with vacant bonding sites on the surface by polymer bridging and as a result, the cell is held near the surface. Possible mechanisms for polymer bridging have been suggested (Characklis and Cooksey 1983) but remain to be fully elucidated. Bacteria can be connected to the substratum via exopolymer–substratum interactions, which are predominately covalent bonds. Research into the ecology of sessile microbial populations often focused on the extracellular polymers produced by the cells (Corpe 1970; Costerton et al. 1981; Uhlinger and White 1983).

In aquatic habitats, microbial exopolymers commonly occur as discrete capsules firmly attached to the cell surface or as slime fibres loosely associated with, or dissociated from, the cells. While it is now believed that many of the capsular polymers serve as holdfasts, anchoring cells to each other and to inert surfaces, the extent to which they facilitate other interactions between sessile bacteria and their environment is less well understood.

EPS influences the physical properties of the biofilm, including diffusivity, thermal conductivity and rheological properties. EPS, irrespective of charge density or its ionic state, has some of the properties of diffusion barriers, molecular sieves and adsorbents, thus influencing physiochemical processes such as diffusion and fluid frictional resistance. The predominantly polyanionic, highly hydrated nature of EPS also means that it can act as an ion exchange matrix, serving to increase local concentrations of ionic species such as heavy metals, ammonium, potassium,

etc., whilst opposite effects are generated on anionic groups. EPS has little effect on uncharged molecules including potential nutrients such as sugars (Hamilton and Characklis 1989). However, biofilm bacteria are thought to concentrate and use cationic nutrients such as amines, suggesting that EPS can act as a nutrient trap, especially under oligotrophic conditions (Costerton et al. 1981). Conversely the penetration of charged molecules such as biocides and antibiotics may be at least partly restricted by this phenomenon (Costerton and Lashen 1984).

7 Microcolony and Biofilm Formation

The adsorption of macromolecules and attachment of microbial cells to a substratum are only the initial stages of biofilm development. These are followed by microbial growth, development of microcolonies and recruitment of additional microorganisms. As attachment of microorganisms occurs, the colonising bacteria grow with the production and accumulation of extracellular polymers. The microorganisms eventually become embedded in this hydrated polymeric matrix and immobilised. As a result, the cells are dependent upon substrate flux from the liquid phase and/or exchange of nutrients with neighbouring cells in the biofilm. An important feature is that the microorganisms are immobilised in relatively close proximity to one another. Additional microorganisms may be located within or on top of the biofilm matrix. Specific functional types of microorganisms may, through their activities, create conditions that favour other complementary functional groups of microorganisms. This leads to the establishment of spatially separated, but interactive, functional groups of organisms, which exchange metabolites at group boundaries achieving physiological cooperation (Blenkinsopp and Costerton 1991).

As biofilm communities tend to be complex, both taxonomically and functionally, there is the potential for synergistic interaction among constituent organisms. Homeostatic mechanisms can develop that protect the biofilm microorganisms from outside perturbations and these are extremely important in natural communities exposed to disturbances, such as episodes of pollution. As biofilm heterogeneity increases, chemical and physical microgradients develop including those of pH, oxygen and nutrients.

Generally, when the biofilm reaches a thickness of 10–25 μm , conditions at its base become anaerobic and indicate that the biofilm is approaching a state of maturity, with high species diversity and stability (Hamilton 1987). The thickness of a biofilm is actually very hard to define, as it is not universal and dependent on the local environment. Medical biofilms tend to be thinner than those found within industrial environments. As already mentioned, biofilms are highly hydrated with microorganisms known to only occupy a small component (Costerton et al. 1995). With the use of staining and dehydration techniques used to prepare biofilms for examination for light and electron microscopy, the structure of the biofilm can become distorted leading to errors in determining biofilms thickness and structure.

This, however, has been overcome with the introduction of CLSM which allows biofilms to be studied in their native, hydrated state providing an accurate picture of structure and dimensions (Lawrence and Neu 1999; Palmer and Sternberg 1999). It is now possible to make some general comments about biofilms based on this form of analysis. Oral biofilms have been reported to be up to 1 mm thick, those in CAPD catheters 30 μm thick, whilst a study of biofilms on 50 indwelling bladder catheters found that their thicknesses varied from 3 to 490 μm with layers of cells documented to be 400 cells deep.

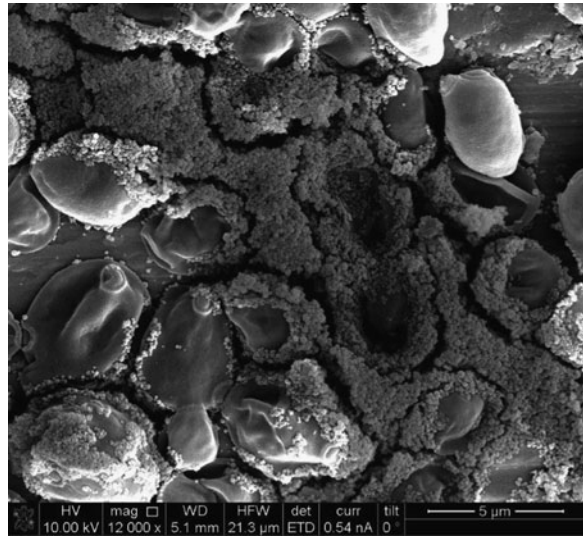
Biofilm structure has now been described in the literature for both mixed and pure cultures in many different environments. Most of these biofilms demonstrate heterogeneity, often evident as a patchy biofilm configuration. With the use of CLSM together with microelectrode measurements, researchers have established that the biofilm consists of cell clusters located in an EPS matrix. These clusters have been shown to vary in shape often ranging from cylinders to filaments forming a “mushroom” structure (Lewandowski et al. 1995; Caldwell et al. 1992; Costerton et al. 1994; de Beer et al. 1994; Keevil et al. 1993). Open channels are evident in biofilms (particularly within potable water systems) and are also referred to as voids and pores, and represent further evidence of biofilm spatial and temporal complexity. Voids facilitate mass transfer, which favours higher nutrient concentrations in the void spaces and also allows cellular metabolites and byproducts to be more concentrated under cell clusters. These stack systems, which are evident within oligotrophic environments, have been replicated in simple computer simulations (Wimpenny and Colasanti 1997).

Overall, the development of a biofilm is generally governed by a number of parameters including ambient and system temperatures, which are in turn related to season, day length, climate and wind velocity; hydrodynamic conditions (shear forces, friction drag and mass transfer); nutrient availability (concentration, reactivity, antimicrobial properties); surface roughness, hydrophobicity and electrochemical characteristics of the surface; pH (an approximately neutral pH of the water is optimal for the growth of most biofilm-forming bacteria); the presence of particulate matter (which can become entrapped in the developing biofilm and thus provide additional attachment sites); and the effectiveness of biofilm control measures. Of these, the four major influencing factors are the surface or interface properties, hydrodynamics, nutrients and biofilm consortia (Stoodley et al. 1997). The controversial factor known to affect biofilm structure is the hydrodynamic forces, which operate within flowing conditions. It is now well established that biofilms exposed to high turbulent flow develop a phenomenon known as “streaming”. The significance of this is still unclear.

7.1 Microbial Interaction in Biofilms

In biofilms in natural environments, there is evidence of a high level of cellular interaction and competitive behaviour (Connell and Slatyer 1977; Fredrickson

Fig. 1 *Candida albicans* and *Streptococcus mutans* attached to titanium



1977) arising as a consequence of resource availability. As a result of competition strategies by microorganisms, the biofilm system is consistently under a state of flux (Connell and Slatyer 1977; Baier 1984; Wahl 1989) with microbial succession being a common feature. During adhesion, pioneer colonisers have defined requirements, dictated by the conditioning film. The succession of the biofilm community is then governed by a number of physiological and biological events initiated by this initial coloniser (Fletcher and Loeb 1979; Characklis 1981; Baier 1984).

Since microorganisms in a biofilm are in close proximity, they invariably interact and this may be beneficial to one or more of the microorganisms involved, or may be antagonistic (James et al. 1995; Stewart et al. 1997). Figure 1 demonstrates evidence that *Streptococcus mutans* and *Candida albicans* interact together demonstrating a mutualistic relationship. Synergistic mechanisms are considered important in mixed species biofilm development (Hofstad 1992) and a good example of this is the consumption of oxygen by aerobes, which locally increases the redox potential allowing anaerobes to also survive. Other synergistic effects include the sharing of biochemical pathways to fully exploit available nutrients as well combining approaches to protect against host immune defences and antimicrobial agents. In terms of pathogenic potential, polymicrobial interaction may well play a key role for biofilms in delayed wound healing. For example, microorganisms with low invasive capacity may act synergistically with more virulent ones.

Quorum sensing (QS) is microbial cell-to-cell communication through chemical messages (Jones 2005) and is considered key in regulating colonisation and virulence factor expression within the biofilm (Yarwood and Schlievert 2003). QS chemicals are often referred to as microbial pheromones or autoinducers and their concentration often correlates to the population density. Until relatively recently,

such inter-bacterial signalling was thought to only occur in a few microorganisms, namely *Vibrio fischeri* and *Vibrio harveyi*, *Enterococcus faecalis*, *Myxococcus xanthus* and *Streptomyces* spp. Research into inter-bacterial signals uncovered the use of acyl homoserine lactones (AHLs) as QS molecules in Gram-negative bacteria. These small signal molecules are excreted by cells and accumulate in cultures as a function of cell density, termed the quorum; the accumulated AHLs interact with receptors on the bacterial cell surface and signal to control gene expression. In this way coordinated expression of sets of genes can be achieved in response to local cell density. The dense populations of cells in biofilms led to speculation that AHLs may have specific functions in these communities. AHLs were subsequently detected in natural biofilms growing on submerged stones taken from a river and in biofilms that had formed *in vivo* on urethral catheters. AHLs in a developing biofilm cause the transformation of individual cells from the planktonic to the biofilm phenotype and coordinate their behaviour in some way so that they build the complex structures of the multicellular communities.

Gram-positive bacteria have not yet been reported to use the autoinducer-based signalling systems; in some, however, an oligopeptide pheromone has been involved in inter-bacterial signalling. Pheromone-based inter-bacterial regulatory systems are thought to regulate bacterial cell density, bioluminescence, conjugative transfer of bacterial plasmids, genetic competence, production of hydrolytic enzymes and secondary metabolism. Several of the systems regulated by QS are involved in the regulation of the virulence determinates of bacterial pathogens. The best understood example of a human pathogen involved in quorum sensing is *P. aeruginosa*. Xie et al. (2000) showed that certain dental plaque bacteria modulate the expression of the genes encoding fimbrial expression (*fimA*) in *Porphyromonas gingivalis*. *Porphyromonas gingivalis* would not attach to *Streptococcus cristatus* biofilms grown on glass slides whilst *P. gingivalis*, readily attached to *S. gordonii*. *Streptococcus cristatus* cell-free extracts substantially affected expression of *fimA* in *P. gingivalis*, as determined by a reporter system. *Streptococcus cristatus* is therefore able to modulate *P. gingivalis* *fimA* expression and prevent its attachment to the biofilm.

8 Detachment and Dispersal of the Biofilm

Characklis et al. (1990a, b) suggested that the detachment of biofilms could be categorised into three areas, namely erosion, sloughing and abrasion. Characklis referred to detachment as an interfacial transfer process, which involved the transfer of cells and other components from the biofilm compartment to the bulk liquid, with the detachment of microbial cells and related biofilm material occurring from the moment of initial attachment.

Many different parameters known to affect biofilm detachment have been examined, including pH, temperature and the presence of organic macromolecules either absorbed on the substratum or dissolved in the liquid phase (McEldowney

and Fletcher 1988). The effects these have on bacterial detachment are generally species specific. Surface roughness of the substratum may also be a significant factor in biofilm detachment, with early events in biofilm formation being controlled by hydrodynamic forces (Powell and Slater 1982). As detachment increases with increasing fluid shear stress at the substratum surface, macro and micro roughness may significantly influence the detachment rates of the biofilm due to a sheltering effect from hydrodynamic shear. The detached cells may be transported close to the surface (in a viscous sublayer) resulting in collisions with the surface providing more opportunity for reattachment.

To date, detachment still remains a poorly researched and understood phenomenon, which therefore complicates the formation of satisfactory models. This is surprising considering that the detachment of biofilms from surfaces into surrounding environments does have important implications to public health. In microbiological terms, detachment from surfaces may at first be seen to be a disadvantage to the biofilm. However, it has been found that biofilms with greater detachment rates have larger fractions of active bacteria. It has also been reported that detachment can occur as a result of low nutrient conditions indicating a survival mechanism, which may be genetically determined. Therefore, detachment is not just important for promoting genetic diversity, but also for escaping unfavourable habitats aiding in the development of new niches.

Biofilm dispersal/detachment of biofilm cells are routinely detached from biofilms and have very important implication in medical settings. Raad et al. (1992) determined a relationship between biofilm detachment and catheter-related septicaemia, whilst the detachment of biofilm aggregates from native heart valves has implications in infective endocarditis. It is these clumps of cells from biofilms, which also may contain platelets or erythrocytes, that lead to the production of emboli with serious complications to the host. Furthermore, biofilms in hospital water systems containing potentially pathogenic organisms might also detach as aggregates and, especially for those organisms with a low infective dose, consumption or exposure to water containing these organisms might result in infection. This may be a cause of the increasing incidences of nosocomial infections.

8.1 Resistance of Biofilms to Host Defence Mechanisms

Microorganisms within a biofilm grow in a protected microenvironment largely through production of a biofilm matrix composed of extracellular polysaccharides, proteins and nucleic acids (Davey and O'Toole 2000). The fact that biofilm-based infections are rarely resolved, even in individuals who have a competent innate and adaptive immune response, highlights the high degree of resistance exhibited by biofilms (Stewart and Costerton 2001).

8.2 Tolerance of Biofilms to Antimicrobial Agents

Biofilm structure and the physiological attributes of microorganisms within the biofilm also provide an intrinsic tolerance to antimicrobial agents (antibiotics, disinfectants, germicides or antifungals). Indeed, biofilms can be up to 1,000 times more tolerant to antibiotics than equivalent planktonic cultures (Hoyle and Costerton 1991; Mah and O'Toole 2001). Whilst biofilm cells may employ a variety of mechanisms to resist the action of antimicrobial agents (Mah et al. 2003), studies have also shown that a number of key factors contribute to reduced antimicrobial susceptibility of biofilms (Percival and Bowler 2004).

A reduced ability of the antimicrobials to gain access to the microorganisms located within the matrix of the biofilm is thought to be important in biofilm resistance against certain antimicrobials. This could arise through chemical interaction with extracellular biofilm components or adsorption to anionic polysaccharides. For example, it has been shown that penetration of positively charged aminoglycosides into a biofilm is retarded by binding to negatively charged matrices, such as the alginate produced in *P. aeruginosa* biofilms (Walters et al. 2003). Another study showed that extracellular matrix from coagulase-negative staphylococci reduced the effect of glycopeptide antibiotics, even in planktonic cultures (Fux et al. 2005; König et al. 2001). In addition, Mah et al. (2003) showed that bacteria within a biofilm may actively employ distinct mechanisms including antibiotic sequestration in the periplasm to prevent them from reaching their target sites. The enzyme β -lactamase, produced by several bacterial species can inhibit the activity of β -lactam ring structured antibiotics such as penicillins and cephamycins. β -Lactamase can accumulate in the biofilm matrix due to secretion or cell lysis, and deactivate β -lactam antibiotics at biofilm surface layers more rapidly than they diffuse into the biofilm (Anderl et al. 2003). It is, however, known that the biofilm matrix does not form a completely impenetrable barrier to antimicrobial agents (Percival and Bowler 2004) and other factors are likely to be involved.

8.3 Growth Rate of Biofilm Bacteria and Its Effect on Antimicrobial Tolerance

Antibiotics are generally more effective at killing actively reproducing cells. Hence, reduced activity of microorganisms could render these cells less susceptible. It has been known for sometime that non-dividing bacteria escape the killing effects of antibiotics targeted at growth-specific factors (Davies 2003). Antibiotics, such as ampicillin and penicillin, which inhibit cell wall synthesis, are unable to kill non-dividing cells (Costerton et al. 1999; Stewart and Costerton 2001).

The most likely location of slower growing bacteria in a biofilm is in the lower region of the biofilm (Davies 2003) and these cells are more likely to be metabolically inactive because of reduced access to essential nutrients and gaseous

exchange. Biofilm embedded cells may be metabolically dormant and in this state phenotypically equipped to persist in hostile environments (Anwar et al. 1992b; Rhoads et al. 2007). These so-called persister cells represent a small and slow-growing sub-population of the biofilm which have differentiated into an inactive, but highly protected state (Roberts and Stewart 2005; Percival et al. 2011). It has been estimated that these cells constitute 0.1–10% cells in a biofilm and it has been hypothesised after antimicrobial challenge that it is this subpopulation of cells that “re-seed” the biofilm (Harrison et al. 2005; Roberts and Stewart 2005).

8.4 Induction of a Biofilm Phenotype Tolerant to Antimicrobials

Once microorganisms attach to a surface, they may express a general and more virulent biofilm phenotype compared with planktonic counterparts (Mah and O’Toole 2001; Saye 2007). Gilbert et al. (1997) suggested that cells with a biofilm-specific phenotype may be induced. These phenotypes may express active mechanisms such as the expression of bacterial periplasmic glucans to bind to, and physically sequester antibiotics to reduce the efficacy of antibiotics (Gilbert et al. 1997; Maira-Litran et al. 2000; Percival and Bowler 2004).

Altered gene expression by organisms within a biofilm or a general stress response of a biofilm could reduce susceptibility to antimicrobial agents (Brown and Barker 1999). In theory, bacterial cells have a small number of target sites for antibiotics. It could therefore be that biofilm cells use specific genes to phenotypically alter these target sites to protect themselves (Saye 2007). Exact details about the physiological changes that occur during the transition from a planktonic to a biofilm state are not known. It has been suggested that multidrug resistance (MDR) efflux pumps may substantially contribute to the resistance of biofilms (De Kievit et al. 2001; Mah and O’Toole 2001).

8.5 Clinical Importance of Biofilms

Given the prevalence of biofilms in natural environments, it is not surprising that these growth forms are responsible for infection in humans and animals. In humans, biofilms have been linked with numerous conditions and equally in animals equivalent infections may occur.

Native valve endocarditis (NVE) is a condition that results from the interaction of bacteria, the vascular endothelium and pulmonic valves of the heart. The causes of NVE are varied, but it is frequently associated with streptococci, staphylococci, Gram-negative bacteria and also fungi (Braunwald 1997). These microorganisms gain access to the blood and the heart via the oropharynx, gastrointestinal and urinary tract. Once the intact endothelium is damaged, microorganisms adhere to it and non-bacterial thrombotic endocarditis (NBTE) develops at the injury. At the

point of injury, the thrombus develops, which is an accumulation of platelets, fibrin and red blood cells (Donlan and Costerton 2002). Treatment is less effective due to a combination of mass transfer limitations and inherent resistance of biofilms (Donlan and Costerton 2002).

Microbial biofilms often develop on, or within indwelling, medical devices, e.g. contact lenses, central venous catheters, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, urinary catheters and voice prostheses (Donlan 2001; Percival and Kite 2007) and a number of microorganisms can produce biofilms on these surfaces. *Staphylococcus* spp. (*S. aureus*, *S. epidermidis*) are members of the commensal microflora of the skin and can form biofilms on implantable medical devices, such as intravenous catheters, and hip and knee joint prostheses (Bayston 1999; Habash and Reid 1999; Khardori and Yassien 1995).

Implantation of mechanical heart valves causes tissue damage, and circulating platelets and fibrin tend to accumulate where the valve has been attached. Once the tissue is damaged, it facilitates a greater tendency for microorganisms to colonise these locations (Anwar et al. 1992a). The resulting biofilms develop on the heart tissue (Carrel et al. 1998; Illingworth et al. 1999) surrounding the prosthesis or the sewing cuff fabric used to attach the device to the tissue (Donlan 2001; Karchmer and Gibbons 1994).

In humans, urinary catheters are used to remove and monitor urine production from impaired patients. Urinary catheters also facilitate repair of the urethra after surgical procedures and manage urinary retention and incontinence in the elderly and disabled patients (Moore and Lindsay 2001; Stickler 2005). Yeasts such as *Candida parapsilosis* are very adapt at growing on urinary catheters when these are composed of silicone (Fig. 2). Biofilms associated with urinary catheters are particularly important because they cause infections in 10–50% of patients who undergo catheterisation (Mulhall et al. 1988; Stickler 2002). *Proteus mirabilis*,

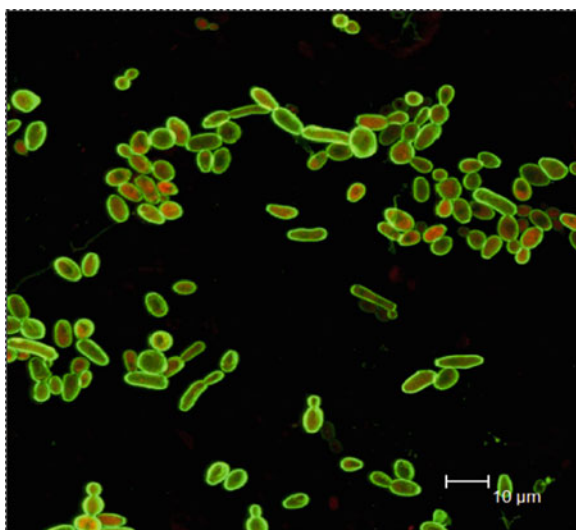


Fig. 2 Evidence of *Candida parapsilosis* on silicone tubing after 2 h exposure to urine

Morganella morganii, *P. aeruginosa*, *K. pneumoniae* and *Proteus vulgaris* are commonly found in urinary catheter biofilms. A number of these bacteria (e.g. *P. mirabilis*) express urease, an enzyme which hydrolyses urea found in the urine, resulting in the production of ammonia. Ammonia causes an increase in the pH of the urine, which in turn allows mineral precipitation including that of calcium and magnesium phosphates, leading to blockage of the catheter and infection (Stickler et al. 1999; Tunney et al. 1999). The problem is compounded because these organisms tend to be antibiotic resistant and as a biofilm forms within the catheter, they are less susceptible to host defences and antimicrobial treatments (Trautner and Darouiche 2004).

Unfortunately, urinary catheters also provide a passageway for bacteria from a heavily contaminated external skin site to a vulnerable body cavity. Polymicrobial communities will eventually develop, but initial infections are usually by single bacterial species (Stickler 2005).

9 Management of Biofilm Infections

Viewing bacteria from the perspective of multicellular behaviour is altering our view of microbiology and of Koch's postulates (Percival et al. 2010). It is evident that 99.9% of organisms prefer attachment, and that bacterial cells have the ability to aggregate into particular three-dimensional assemblages (Davey and O'Toole 2000). Biofilms have been recognised as being important in human disease and the number of biofilm-associated diseases seems to be increasing (Davies 2003). It is important to understand the characteristics of the biofilm mode of growth and the various aspects of biofilm formation. To successfully treat biofilm infections, knowledge of the phenotype of the bacterial population is required. This is important, as antibiotic treatment may not be totally effective if more than one phenotype exists. Some of these cells might remain intact serving to re-infect the host once the antimicrobial treatment has finished (Brooun et al. 2000; Davies 2003; Spoering and Lewis 2001).

A key factor to combating biofilm infections is to understand the physiology of biofilm development. Davies (2003) suggested that chemotherapeutic agents could be developed to promote or prevent transition from one stage of biofilm maturation to the next by targeting unique biofilm regulatory or signalling molecules. Specific agents might be discovered or developed which will interfere with the production of virulence factors, or promote or inhibit the shedding of biofilm bacteria (Davies 2003).

As mentioned before, biofilm resistance depends on aggregation of bacteria into multicellular communities. Therefore, one antimicrobial strategy might be to develop therapies to disrupt the multicellular structure of the biofilm. It could be that host defences might be able to resolve the infection once the multicellularity of the biofilm is reduced, and then the effectiveness of antibiotics might be restored (Stewart and Costerton 2001). Other potential therapies include enzymes that

dissolve the matrix polymers of the biofilm, chemical reactions that block biofilm matrix synthesis and analogues of microbial signalling molecules that interfere with cell-to-cell communication, required for normal biofilm formation (Nemoto et al. 2000; Parsek and Greenberg 2000; Yasuda et al. 1993). Already a number of QS inhibitors have been identified such as the inhibitory peptide RNAIII, which inhibits the *agr* system of Gram-positive bacteria (Rhoads et al. 2007). In *P. aeruginosa*, furanones derived from plants have been demonstrated to block AHL pathways (Heurlier et al. 2006).

For in vivo indwelling device-associated infections, effective, preventive and therapeutic strategies still need to be developed. One such therapy could be the production of materials with anti-adhesive surfaces, for example, heparin (Tenke et al. 2004). Tenke et al. (2004) showed that on heparin-coated catheter stents, no biofilm formation was evident between 6 and 8 weeks, whereas uncoated tubes were obstructed within 2–3 weeks. Heparin coating seems one possible solution, but further development of materials resisting bacterial colonisation is needed (Tenke et al. 2004).

Progress has already been made, but the future of biofilm research and management relies upon collaborative efforts to fully explore these complex systems of the microbial world.

10 Conclusion

Infectious disease processes due to bacteria associated with biofilms such as otitis media, periodontitis, cystic fibrosis, native valve endocarditis and chronic prostatitis all appear to be caused by biofilm-associated microorganisms. In addition, indwelling medical devices have been shown to harbour biofilms, which have been implicated in infections.

In hot water and also potable water distribution systems, biofilms have been shown to harbour pathogens, such as *Mycobacterium avium*, *Legionella pneumophila* and now *Helicobacter pylori* and *Arcobacter* spp. (Percival et al. 2001; Percival and Thomas 2009). How the interaction and growth of pathogenic organisms in a biofilm result in an infectious disease process is presently unknown and warrants extensive research.

To date we can appreciate that biofilms are important in infectious disease processes. The principles by which this is evident is when we consider detachment of cells or biofilm aggregates resulting in the production of emboli, bacteria may exchange resistance plasmids within biofilms, cells in biofilms have dramatically reduced susceptibility to antimicrobial agents, biofilm-associated Gram-negative bacteria may produce endotoxins and biofilms are resistant to host immune system clearance.

Biofilms are highly resistant to most antimicrobial agents and disinfectants. Sessile bacteria within a biofilm are able to acquire resistance through the transfer of resistance plasmids. This acquisition of resistance is particularly important in the

healthcare environment for patients with colonised urinary catheters and orthopaedic patients. Many organisms are shown to carry plasmids encoding resistance to multiple antimicrobial agents, particularly in the medical setting.

Microorganisms are capable of growing in both a free form (planktonic) or as biofilms attached to solid surfaces. Biofilms are communities of microorganisms, often adhered to a surface and encased within an extracellular polysaccharide matrix (Kumamoto and Marcelo 2005). Examples of surfaces supporting biofilm growth include inanimate environmental materials, biomaterials interfacing with host tissue and systems or the host tissue itself. The behaviour and phenotype of microbes existing in either planktonic and biofilm states are known to differ significantly and this is perhaps best exemplified by studies on antimicrobial efficacy against the different growth phases (Hill et al. 2003).

In the oral environment, candidal biofilms on prostheses and the oral mucosa have been associated with infection (Kumamoto and Marcelo 2005). Intra-oral biofilms can develop on the tooth enamel, oral mucosa or on introduced oral prostheses and these can all provide a reservoir of potentially pathogenic organisms promoting dental caries and periodontal disease. Furthermore, in the case of oral tissue, certain microorganisms such as yeast of the genus *Candida* have been shown to actually invade the tissue, invoking a pathogenic effect. Similarly, within a chronic wound, it may well be the case that the occurrence of a biofilm results in clinical problems due to the existence of the microbes in a more persistent state. Examples of clinically important biofilms include *P. mirabilis* on urinary tract catheters (Stickler 2005), *Candida* spp. on denture surfaces and bacteria (polymicrobial) within chronic wounds (Hill et al. 2003). Significantly, when biofilms are present on the surface of medical devices, a failure of the device can occur, as encountered in the blocking of urinary catheters and the obstruction of airways within artificial voice box prostheses (Douglas 2003; Van der Mei et al. 2000; Percival et al. 2009). Furthermore, biofilms tend to exhibit heightened resistance to antibiotics, possibly by diffusion limitation or by the presence of biochemically inert microbes within the biofilm (Donlan and Costerton 2002).

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Biofilms and Veterinary Medicine

Percival, S.L.; Knottenbelt, D.C.; Cochrane, C.A. (Eds.)

2011, XIV, 258 p., Hardcover

ISBN: 978-3-642-21288-8