Science Progress (2001), 84 (3), 235–254

Bacterial biofilms and human disease

MICHAEL WILSON

The term biofilm is used to denote a polymer-encased community of microbes which accumulates at a surface. Biofilms are responsible for a number of diseases of man and, because of the intrinsic resistance of these structures to antibiotics and host defence systems, such diseases are very difficult to treat effectively. The application of new microscopic and molecular techniques to biofilms has revolutionised our understanding of their structure, composition, organisation and activities. This review will describe the role that biofilms play in human disease and will outline our new millennial view of these complex and fascinating bacterial communities.

What is a biofilm?

Traditionally, bacteriologists have studied most aspects of bacterial structure and behaviour using cells that have been grown suspended in a liquid medium. Organisms grown in this way are described as being "planktonic". However, it is increasingly being realised that, in their natural habitat, most bacteria grow attached to surfaces *i.e.* they are "sessile"[1]. Furthermore, the growth of many sessile bacteria results in the formation of large aggregates and these are known as "biofilms" [2]. Now, while it is true that most bacteriologists would recognise a biofilm if they saw one, it has proved to be very difficult to come up with a definition of the term that is satisfactory to all researchers in the field. Nevertheless, most would not be too critical of a definition along the lines of the following – a biofilm is a community of bacteria (or other microbes) and their extracellular polymers that is attached to a surface. In man, the surfaces available for attachment are many and varied and all surfaces exposed to the external environment (*i.e.* the skin, teeth, respiratory and intestinal

Michael Wilson is Professor of Microbiology in the Faculty of Clinical Sciences at University College London and Head of the Department of Microbiology at the Eastman Dental Institute, University College London, 256 Grays Inn Road, London WC1X 8LD, UK. His main research interests are bacterial virulence factors, biofilms, antibiotic resistance and the development of new antimicrobial strategies.

mucosa etc.) support populations of sessile bacteria. However, as the majority of such surfaces are continually being shed (along with the bacteria attached to them) and renewed, the opportunities for biofilm formation are more limited. The only natural, non-shedding surfaces in man exposed to the environment are those provided by the teeth and here we find excellent examples of true biofilms (as opposed to sessile bacteria) – dental plaques[3]. Despite the fact that the other externally-exposed surfaces of man are continually being shed, biofilm formation is possible on some of these as a consequence of a slow rate of shedding, anatomical factors or some abnormality/disfunction in the individual. The vagina, for example, has a thick biofilm composed mainly of lactobacilli while the crypts of the tongue and the follicles of the skin can also support biofilms. Although man has few natural non-shedding surfaces, advances in medical and surgical techniques have resulted in the use of a wide range of implantable medical devices (discussed in the next section) which provide non-shedding surfaces on which biofilms can form[4,5]. Biofilm formation on these devices, and the adverse consequences of this, constitute a major problem.

Which bacteria form biofilms?

The simple answer to this question is that most bacteria, given the right conditions, can grow as a biofilm. However, certain species appear to have a predilection to form biofilms and examples of these are given in Table 1.

Most of these species are members of the normal microflora of man and form biofilms at sites where they are found naturally. Hence, streptococci, cariogenic bacteria and periodontopathogenic bacteria form biofilms on the surfaces of teeth[6], while lactobacilli form biofilms in the vagina[7]. Staphylococci, which are members of

Table 1 *Examples of organisms that frequently form biofilms*

Organism	Site of biofilm formation
Staphylococcus aureus	Implantable medical devices
Staphylococcus epidermidis and other coagulase-negative staphylococci	Implantable medical devices
Pseudomonas aeruginosa	Lungs of cystic fibrosis patients
Escherichia coli and other enterobacteria	Urinary catheters
Escherichia coli	Intestinal tract
Streptococcus spp.	Teeth
Actinomyces spp.	Teeth
Lactobacillus spp.	Vagina, teeth

the normal microflora of the skin, often form biofilms on implantable medical devices which penetrate the skin (e.g. central venous catheters) or they gain access to totally-implanted devices such as hip, knee and other joint prostheses[4,8] *Escherichia coli* can form biofilms on urinary catheters (as can a variety of other members of the intestinal microflora) and also in the intestinal tract[9,10]. Of the organisms listed in Table 1, *Pseudomonas aeruginosa* is exceptional in that it is not a member of the normal microflora but is an environmental species which is a notorious opportunistic pathogen of individuals whose defence systems are impaired in some way. Hence, it often causes infections of burns and wounds and is a major problem for immunocompromised individuals[11]. It is very adept at biofilm formation and readily forms such structures in the lungs of individuals with cystic fibrosis – this often results in the death of the patient[12,13].

Biofilms and disease

While much emphasis is placed on the adverse effects of biofilms and the difficulty in treating diseases which they cause (to be discussed later), it must be emphasised that some biofilms have a protective role. Hence, biofilms (composed mainly of lactobacilli) in the vagina prevent colonisation by exogenous pathogens (a phenomenon known as "colonisation resistance") and, indeed, their presence is usually synonymous with vaginal health. The ability of the biofilm to prevent colonisation by pathogens is attributable to the production of acids, bacteriocins, hydrogen peroxide, and biosurfactants[14]. Disruption, or disappearance of this protective biofilm, is used as an indication of the presence of vaginal pathogens such as *Gardnerella vaginalis* or other anaerobes[15]. Indeed, re-colonisation of the vagina with lactobacilli is advocated by many as an appropriate means of treating vaginal infections[16]. Dental plaque, the biofilm that forms on the surface of teeth, also protects against colonisation by exogenous pathogens. While this biofilm consists mainly of streptococci and *Actinomyces* spp., many other species may be present and, under certain conditions (to be discussed later), such species can proliferate resulting in a biofilm that is not compatible with health[17,18]. These biofilms can induce diseases such as caries, gingivitis and periodontitis which are among the most common infections of man[19].

Apart from oral infections, which will be discussed later, human diseases due to biofilms are usually associated with the presence of some implantable medical device (e.g. catheters, joint prostheses) or are the consequence of some impairment of the host defence systems e.g. lung infections in cystic fibrosis patients[20].

Table 2 summarises the types of disease due to biofilms and also gives some idea of their prevalence.

An important feature of the biofilms responsible for the infections listed in Table 2 is that in the majority of cases they consist of a single bacterial species. The exceptions to this generalisation are the biofilms associated with urinary catheters and voice prostheses which are more like oral biofilms in that they often consist of a variety of organisms. When it comes to species complexity in biofilms, oral biofilms are the example par excellence as more than 350 different bacterial species have been isolated from dental plaques – though not all from the same sample[21]. However, that is not the end of the story as molecular techniques (such as 16S rRNA sequencing) have revealed that there may well be a similar number of organisms present that are currently uncultivable. This finding that at least half of the microflora of dental plaques cannot be cultured in the laboratory is not really surprising as the application of molecular identification methods to other microbial habitats (*e.g.* soil, sea water *etc.*) has revealed that fewer than 1% of the bacteria present can be grown in the laboratory[22]. Although the range of organisms that can be found

Table 2 *Diseases caused by biofilms (AGNB = aerobic Gram-negative bacilli; CNS = coagulase-negative staphylococci)*

Infection	Causative organism	Prevalence
Caries	*Strep. mutans*, *Lactobacillus* spp. *Actinomyces* spp.	60% of children/ adolescents
Gingivitis	uncertain aetiology, possibly involves *Fusobacterium nucleatum*, *Veillonella parvula*, *Campylobacter* spp., *Treponema* spp.	almost 100% of population
Periodontitis	*Porphyromonas gingivalis*, *Bacteroides forsythus*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans* Spirochaetes	15% of population
Prosthetic heart valves	CNS, *Staph. aureus*, oral streptococci	1.5% of patients
Prosthetic hip/knee joint	CNS, *Staph. aureus*, *Peptococcus* spp., AGNB	1–3% of patients
Central venous catheters	CNS, *Staph. aureus*, AGNB, *Candida* spp.	3–10% of patients
Hydrocephalus shunts	CNS, *Staph. aureus*, *Corynebacterium* spp., AGNB	3–20% of patients
Voice prostheses	*Candida* spp., *Staph. aureus*	100% of patients
Urinary catheters	AGNB, enterococci, CNS, *Candida* spp.	100% of patients
Lung infections accompanying cystic fibrosis	*Ps. aeruginosa*, *Staph. aureus*, *H. influenzae*, *Burkholderia cepacia*	100% of patients
Contact lenses	*Ps. aeruginosa*, CNS	Unknown

in dental plaque is extremely large (approximately 700 including uncultivable species), the number of different species found in a particular sample from an individual is, fortunately, usually more restricted. Hence, it is usual to culture between 20 and 30 organisms from an individual plaque sample so that the total number of species present is likely to be approximately 50. Despite this complexity, some generalisations can be made about the composition of oral biofilms. Streptococci and *Actinomyces* spp. are invariably the numerically-dominant organisms, with members of the following genera usually being present in smaller numbers – *Veillonella, Haemophilus, Neisseria* and Gram-negative anaerobic bacilli (*Fusobacterium* spp., *Porphyromonas* spp., *Prevotella* spp.). It was mentioned previously that oral biofilms lead a Jekyll and Hide existence in that they have a protective role in exerting "colonisation resistance" but they are also responsible for some of the most prevalent infections of man – caries, gingivitis and periodontitis. This dual behaviour is best understood in terms of the "ecological plaque hypothesis" postulated by Marsh[23]. The complex mixture of organisms present in an oral biofilm constitutes a community whose composition can remain stable (termed a climax community) due to a variety of interactions (both beneficial and antagonistic) between the constituent species. Such interactions involve food webs, competition for nutrients, bacteriocin production and co-aggregation. However, this "microbial homeostasis" is dependent on the external environment remaining constant. Consider what happens when an individual chooses to imbibe large quantities of sucrose-containing food or drink. In this case, the biofilm is flooded with a huge excess of an easily-metabolisable carbohydrate which is converted by the constituent organisms to acidic end-products of metabolism. This creates an environment favouring acidogenic and aciduric species (such as *Streptococcus mutans* and *Lactobacillus* spp.) which come to dominate the community and lower the pH of the biofilm so inducing the dissolution of the enamel layer of the tooth[24]. This results in a carious lesion. In the case of the other major group of oral infections – the periodontal diseases – the environmental perturbation responsible for precipitating disease is less clearly defined. The biofilm present at the gap (known as the gingival crevice) between the tooth and the gums if not continually removed (by brushing and flossing) eventually stimulates an inflammatory response in the host resulting in the increased secretion of a serum-like exudate (gingival crevicular fluid). This proteinaceous fluid can act as a source of nutrients for certain organisms (*e.g. Porphyromonas gingivalis, Bacteroides forsythus, Treponema* spp.) initially present in very low

numbers. The proliferation of such organisms (known as periodontopathogens) changes the biofilm community to one that is dominated by Gram-negative anaerobes. Such a community is able to induce the host to over-produce a range of inflammatory mediators (*e.g.* cytokines and prostaglandins) which results in the breakdown (*i.e.* self-destruction) of the tooth-supporting tissues[25].

How do biofilms form?

Several stages can be recognised in the formation of biofilms[26]. First of all, the bacteria must reach the substratum to which they will ultimately adhere (Figure 1). In the case of non-motile organisms such transport can result from random, Brownian, motion or the organism may be carried there by the flow of the suspending fluid. In contrast, motile organisms may actually "seek out" the surface guided by some chemotactic, aerotactic or phototactic response. Once it has reached the substratum an organism may then adhere to it. It is important to emphasise at this point that, in natural environments, bacteria rarely adhere to the substratum itself – invariably this is coated with a layer of adsorbed molecules known as a "conditioning film", and it is to this film that the organism usually adheres. In the oral cavity, this conditioning film (which can be up to 1.0 μm thick) is formed mainly from the glycoproteins and other molecules present in saliva whereas implanted medical devices are invariably coated with serum proteins. Not all bacteria reaching the conditioning film-coated substratum actually adhere permanently, some return to, and

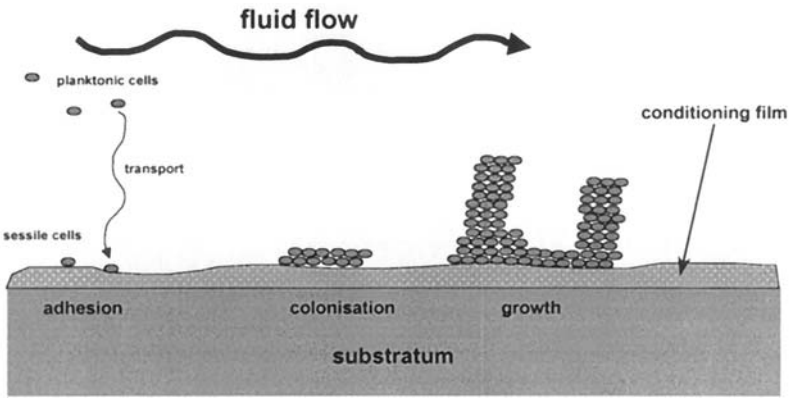

Fig. 1. *Diagram showing the main sequence of events leading to the formation of a biofilm. Planktonic bacteria adhere to the conditioning film of the substratum and then grow and synthesise extracellular matrix molecules. Further growth and cell replication lead to the formation of a biofilm.*

remain in, the fluid phase. However, some cells irreversibly adhere to the substratum as a result of specific interactions between bacterial adhesins and their complementary receptors present on molecules on the substratum's surface[27].

It has been known for many years that adhesion of a bacterium to a surface alters its phenotype. Hence, all of the following activities of bacteria have been shown to be affected once a planktonic cell has become sessile: respiration rate, rate of oxygen uptake, electron transport activity, synthesis of extracellular polymers, substrate uptake rates, rate of substrate breakdown, heat production and growth rate. Details are now emerging of the molecular basis for some of these changes. Hence, it has been shown in *Ps. aeruginosa* that one of the genes (*algC*) required for the expression of alginate (the predominant polysaccharide in the matrix of biofilms formed by this organism) is up-regulated five-fold when the organism changes from the planktonic to the sessile life style[28]. At the same time, a key flagellar biosynthetic gene is down-regulated[29]. The dramatic effect that adhesion can have on an organism can be appreciated by the results of a study of biofilm formation by *E. coli* in which it was found that attachment of the organism to a surface altered the transcription of 38% of its genes[30].

Adhesion of bacteria is then followed by a colonisation stage which involves the synthesis of extracellular matrix molecules (usually polysaccharides), multiplication of the attached organisms and/or attachment of other bacteria (similar or different species) to the already-adherent cells – a phenomenon known as co-adhesion. The synthesis of matrix molecules is crucial to this stage of biofilm development and has been extensively studied in organisms such as *Ps. aeruginosa* and *Staphylococcus aureus*. As has been mentioned, alginate sysnthesis is important in the former organism while in the latter, the synthesis of a polysaccharide intercellular adhesin has been shown to be essential for binding the cells together so enabling biofilm formation[31].

The stage is now set for further growth of the attached organisms resulting in the formation of the dense bacterial aggregates characteristic of mature biofilms. The latter structures are often exposed to strong mechanical and hydrodynamic forces that can result in detachment of the biofilm or parts of it[32]. The detached sections can, of course, re-adhere to the substratum and this could constitute an effective means of colonising a large area of the substratum.

It is important to realise that biofilm formation can alter the local environmental conditions quite substantially and that this has important ecological consequences. If we consider, for example, a tooth

surface. This is an aerobic environment that is subjected to strong mechanical shearing forces (due to chewing and tongue movements) so that the only organisms capable of colonising this region will be aerobic or facultatively-anaerobic species able to adhere to the salivary glycoproteins coating the tooth surface. Such organisms – known as primary colonisers – include *Neisseria* spp. and streptococci. These organisms will then proceed to alter the local environment *e.g.* by utilising oxygen and producing acids as end-products of metabolism. This creates conditions suitable for organisms such as *Veillonella* spp. which are obligate anaerobes that can utilise acids as a carbon and energy source – such species are known as secondary colonisers. The resulting community will, in turn, alter the conditions within the growing biofilm so creating environments suitable for yet other physiological types of bacteria. Furthermore, ingress of oxygen and nutrients present in saliva will be hindered by the growing bacterial aggregate thereby creating gradients through the biofilm. Gradients in metabolic products will also be created, hence an enormous number of micro-habitats will be generated within the biofilm allowing the survival of species with very different nutritional and physico-chemical requirements (Figure 2). As mentioned previously, antagonistic and beneficial interactions between members of this complex community will eventually exert a homeostatic effect so creating a stable "climax community".

What do biofilms look like?

First of all, their gross anatomy. An apparently simple question such as "how thick is a biofilm?" is in reality very difficult to answer with any certainty. While the thickness of biofilms such as those found on tooth surfaces can be measured with no great difficulty (because they are readily accessible and are relatively thick structures), biofilms on many implantable medical devices present a much greater problem as they are not so easy to access (and may be damaged during removal of the device from the patient) and tend to be much thinner. Furthermore, it is now well established that living biofilms are highly hydrated and that bacteria occupy only between 10 and 50% of the total volume of a biofilm[1]. This means that the staining and dehydration techniques used to prepare biofilms for examination by light and/or electron microscopy will grossly distort their structure leading to errors in estimates of their thickness and organisation. Fortunately, the advent of confocal laser scanning microscopy (CLSM) which enables the examination of biofilms in their native, hydrated state has overcome these drawbacks and enables more

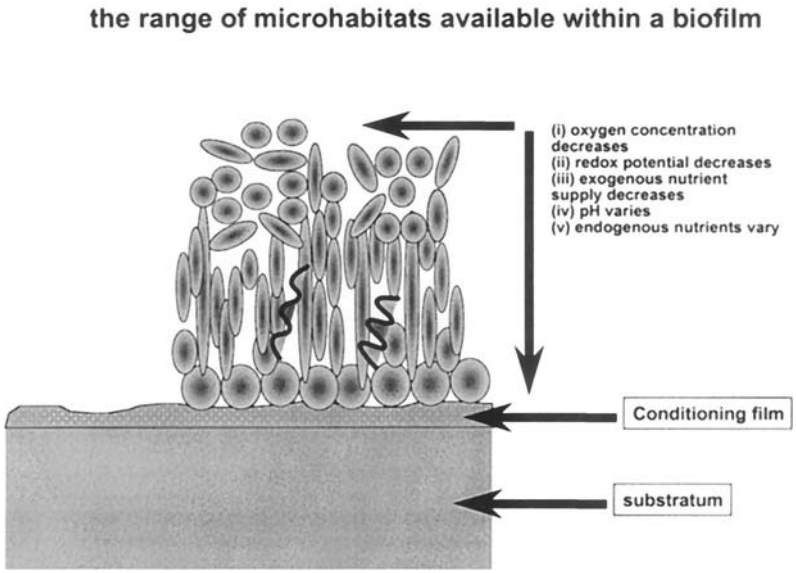

Fig. 2. *The range of habitats available within a biofilm because of the formation of nutrient, gaseous and physico-chemical gradients from the biofilm/liquid interface through to the substratum and from the outside of a "stack" (see Fig. 3) through to its centre.*

accurate estimation of their structure and dimensions[33,34]. Despite the abovementioned difficulties, it is possible to make some general comments about the dimensions of biofilms.

Biofilms growing in or on humans exhibit tremendous variation with regard to their thickness. Oral biofilms, for example, may be up to 1 mm thick in protected regions of the mouth such as between the teeth. In contrast, biofilms on implantable medical devices tend to be considerably thinner. For example, CLSM has shown that *Staphylococcus epidermidis* biofilms formed on CAPD catheters are approximately 30 μm thick [35]. In a study of biofilms present on 50 indwelling bladder catheters, their thickness was found to vary from 3 to 490 μm, layers of bacterial cells up to 400 cells deep were seen[9].

The key elements in the structure of a biofilm are the bacteria, the extracellular matrix and water. The extracellular matrix (often abbreviated to EPS – extracellular polymeric substances) of many biofilms appears to consist mainly of polysaccharide(s) although other polymers (proteins, nucleic acids, phospholipids) are increasingly being detected[36]. Unfortunately, chemical analysis of the polymers present in many biofilms has not been carried out. Table 3 shows some examples of the matrix molecules found in the biofilms produced by a variety of organisms.

Table 3 *Nature of the extracellular matrix of biofilms*

Organism	Extracellular matrix polymer(s)
Pseudomonas aeruginosa	Alginate
Streptococcus mutans	Mutan, fructan
Staphylococcus epidermidis	Beta-1,6-linked glucosaminylglycan
Escherichia coli	Colanic acid

It must be remembered that the matrix is highly hydrated and may consist of up to 97% water[37].

Until CLSM began to be used for studying biofilm structure, there was little evidence that biofilms displayed any organised structure – bacteria were thought to be more-or-less randomly distributed throughout the matrix. However, CLSM (and other modern microscopic techniques such as differential interference contrast microscopy) have enabled us to view biofilms in their living, hydrated state and this has revealed structures that are both complex and beautiful[34]. As a number of factors can affect biofilm structure, there is no single, unifying structure that can be said to characterise all biofilms. The key variables involved include: the nature of the organism (or community), the concentration of nutrients present, the hydrodynamic properties of the environment and the presence (and nature) of any mechanical forces[38]. Hence, the structure of a biofilm can range from the relatively-featureless, flat type to one consisting of a more complex organization involving mushroom-like aggregates separated by water channels (Figure 3). The latter are characteristic of biofilms formed under the following conditions – low nutrient concentration, high hydrodynamic shear stress and the absence of mechanical, abrasive and compressive forces. Depending on the bacterial composition of the particular biofilm, the mushroom-shaped stacks may consist of a single species or of microcolonies of a number of different bacterial species. A microcolony forms at the particular location within a stack that has the appropriate combination of environmental factors (due to diffusion gradients as mentioned previously) suitable for the survival and growth of that organism. The water channels may function as a primitive circulatory system, bringing fresh supplies of nutrients and oxygen while removing metabolic waste products.

What are bacteria doing inside biofilms?

In a suspension containing planktonic cells of a single bacterial species, all of the cells will be behaving in an identical fashion as all are exposed

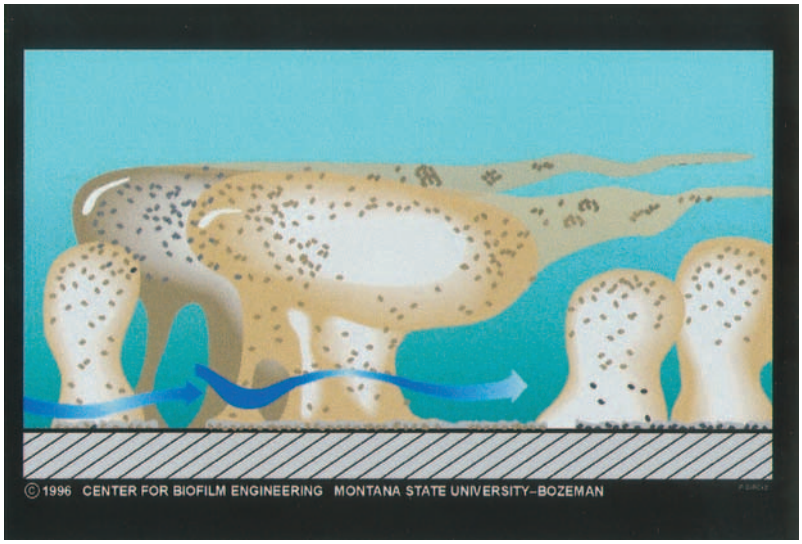

Fig. 3. *Diagrammatic representation of the overall structure of a typical biofilm showing mushroom-like stacks containing bacteria embedded in an extracellular matrix. The stacks are separated by water channels. The arrow shows the direction of fluid flow. Image kindly supplied by Peg Dirckx, Center for Biofilm Engineering at Montana State University–Bozeman, Bozeman, MT 59717-3980, USA.*

to the same set of environmental conditions. However, even if we take the simplest possible biofilm (that consisting of a single species), the situation is very different. Hence, because of gradients in nutrients and physico-chemical factors, cells at different depths within the biofilm (or within one of the mushroom-shaped stacks of a biofilm) will be exposed to different conditions and so will display different patterns of gene expression and so different phenotypes[38]. These changes in the pattern of gene expression and/or physiology have been investigated by a number of means (see Table 4).

Table 4 *Techniques used to investigate gene expression and physiological activities of bacteria in biofilms*

Technique	Examples	Reference
Microelectrodes	Monitoring concentration of nutrients, waste products and gases	39,40
Reporter gene fusions	Induction of gene expression	41,42
Quantification of mRNA Transcripts	Induction of gene expression	42,43
Fluorescent probes	Monitoring physico-chemical changes (pH, E_h) and bacterial viability	44, 45, 46

Application of these techniques has revealed remarkable differences in gene expression and/or physiology at different locations within even the simplest biofilms. For example, the expression of alkaline phosphatase in *Ps. aeruginosa* biofilms can be seen from Figure 4a to take place almost exclusively in the surface layers whereas in Figure 4b, respiratory activity can be seen to be located mainly in the surface layers but also in discrete regions in the depths of the biofilm.

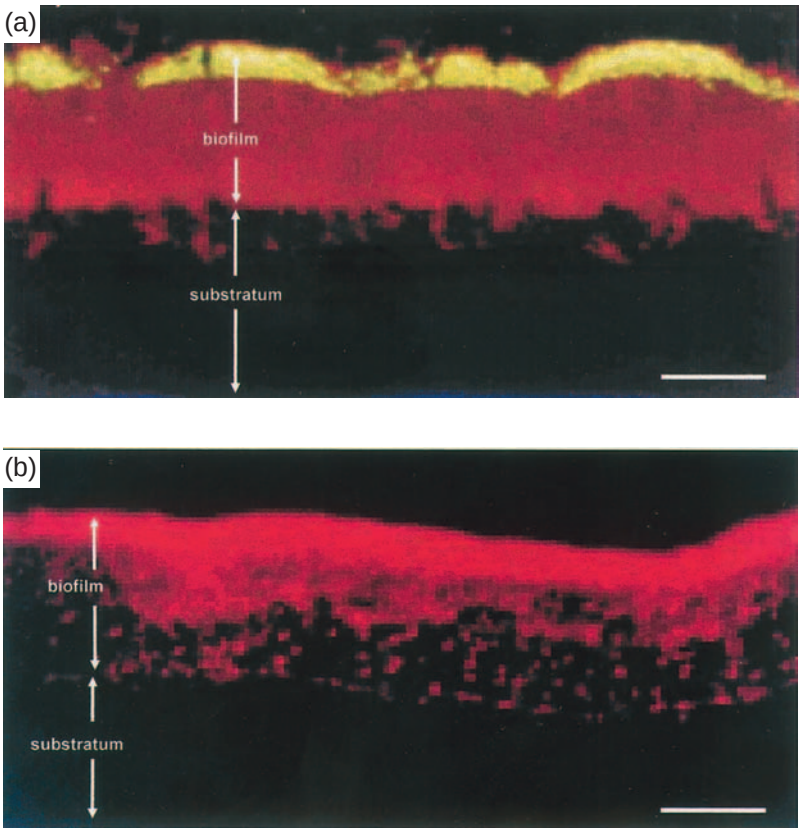

Fig. 4. *Cross-section through a Pseudomonas aeruginosa biofilm – substratum at bottom. (a) Stained with a probe for alkaline phosphatase activity (yellow-staining areas). Bar = 50 μm. (b) Stained with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) to detect respiratory activity. The red-staining areas denote regions of respiratory activity Bar = 50 μm. Images kindly supplied by Philip Stewart, Center for Biofilm Engineering at Montana State University–Bozeman, Bozeman, MT 59717-3980, USA.*

Can bacteria in biofilms communicate?

Although it has been known for a long time that bacteria can sense, and respond to, their external environment, it has only recently been discovered that many species are also able to sense the presence of other bacteria[47,48]. This phenomenon (known as "quorum sensing") involves the production of a low molecular mass "auto-inducer" which diffuses out of the cell but which, on reaching a threshold concentration (due to the presence of a critical population density), can activate the transcription of certain genes[49]. The nature of the auto-inducer depends on the particular species – in Gram-negative bacteria it is usually an acyl-homoserine lactone (AHL). In Gram-positive organisms, the system is more complex and involves the active export of the auto-inducer (which is usually a small peptide) and a two-component signal transduction system[50].

In effect, therefore, what this means is that bacteria have the ability to regulate the expression of certain genes in a population-dependent manner – a phenomenon of undoubted relevance to biofilms with their high bacterial density. Genes controlled by quorum sensing include those encoding many virulence factors, as well as competence and conjugation. The ability to limit gene expression until a large population has been reached is advantageous to the organism in a number of ways. Bacteria generally derive their nutrients from complex polymers and the degradation of such polymers requires the concerted secretion of enzymes from large number of cells. An individual cell, or a population in which only some of the members are secreting the appropriate enzymes, would not constitute an effective means of utilising the available nutrient resources. This applies to the quorum-dependent secretion of proteases by *Ps. aeruginosa*. The advantage of competence and conjugation being regulated by a population-dependent process is obvious – DNA transfer is not possible in the absence of other cells. The ability to limit virulence factor secretion until a large number of bacteria are present could be a protective measure against host defence systems. Hence, if only a few bacteria were to secrete a particular virulence factor (small concentrations of which would be unlikely to cause serious damage) this could alert the host which may then be able to dispose of this threat effectively – something it is less likely to be able to do if a large population is present.

Cell-cell communication also appears to be important in controlling biofilm structure. Hence, it has been shown that a mutant of *Ps. aeruginosa* which was unable to synthesise AHL was also unable to produce biofilms consisting of characteristic stacks and water

channels – instead it produced only a thin, homogeneous layer of cells [51].

Can gene transfer take place in biofilms?

The transfer of genes between bacteria is known to be an important means by which antibiotic resistance and virulence factors are spread between members of the same, and different, species[52,53]. Intuitively, one would expect that gene transfer would be facilitated in biofilms because of their high population density. Furthermore, as described above, both competence and conjugation in a number of species are regulated by quorum sensing – a process known to operate in biofilms. However, few studies have actually demonstrated gene transfer in biofilms and only a very limited number of these have involved organisms associated with human diseases. One such study showed that a plasmid encoding resistance to a number of antibiotics could be transferred from one strain of *E. coli* to another when the organisms were either in a biofilm or in the intestinal tract of a mouse[54]. The transfer of a transposon (carrying a gene encoding resistance to tetracycline) from an environmental organism (*Bacillus subtilis*) to a *Streptococcus* sp. in an oral biofilm has also been reported[55].

Why are biofilms such a problem?

As has been described above, biofilm-related infections are a major cause of morbidity and mortality both for individuals with an implantable medical device and for those who have a defective antibacterial defence system. Even before the current problem of widespread resistance to antibiotics, the treatment of such infections proved to be very difficult. Indeed, in the case of infections of implanted devices, often the only course of action is to remove the device – this is a great inconvenience to the patient and, often, a very expensive procedure. For example, the estimated cost of a hip replacement in the UK is £3,500 but the hospital costs associated with a subsequent infection can be as high as £30,000[56]. So what is the problem? Why are biofilm-associated infections so difficult to deal with? The difficulty arises from two major problems: (i) our defence systems cannot cope very well with biofilms – the infection, therefore, tends to persist for long periods of time; and (ii) biofilms display remarkable resistance to antimicrobial agents.

With regard to the former, phagocytic cells (our first line of defence), find it very difficult to ingest bacteria within biofilms because of the anti-phagocytic properties of the biofilm matrix[57,58].

Furthermore, in the absence of specific antibodies, the polysaccharide also blocks complement activation. Even when antibodies are produced, they may well be rendered ineffective by the matrix. This is because the Fc region of an antibody molecule that binds to a bacterium within the biofilm is very unlikely to be exposed at the surface of the biofilm and so cannot function as an opsonin. Studies have also shown that the polysaccharides of the matrix are themselves able to interfere with host defence systems. Hence, they are able to inhibit chemotaxis and degranulation by polymorphs and macrophage phagocytosis and also to depress the lymphoproliferative response of monocytes to polyclonal activators[59,60]. Not only are host defences unable to deal effectively with biofilms, but their continuous, ineffectual efforts actually cause tissue damage. This is clearly seen in relation to the biofilms that form on the tooth surface adjacent to the gums. Here, the persistent attack of polymorphs *etc.* on dental plaque produces an inflammatory response resulting in gingivitis – an inflammatory condition affecting most of the world's population.

Since the dawn of the antibiotic era we have relied heavily on the use of these chemotherapeutic agents to treat infectious diseases. It came as some surprise, therefore, to find that biofilm-related infections did not succumb so easily to this approach. A considerable amount of research has been carried out over the years to establish why biofilms are so resistant to antimicrobial agents and a number of hypotheses have been formulated to explain this phenomenon. The work in this field has been extensively reviewed and a detailed discussion of the topic is beyond the scope of this review[61–65]. Some of the factors that are thought to contribute to the ability of biofilms to tolerate high concentrations of antimicrobial agents include: (i). binding of the antimicrobial agent to the extracellular matrix of the biofilm, thereby limiting its penetration; (ii) inactivation of the antimicrobial agent by enzymes trapped in the biofilm matrix; (iii) the reduced growth rate of bacteria in biofilms renders them less susceptible to the antimicrobial agent; (iv) the altered micro-environment within the biofilms (*e.g.* pH, oxygen content) can reduce the activity of the agent; and (v). altered gene expression by organisms within the biofilm can result in a phenotype with reduced susceptibility to the antimicrobial agent.

How do we get rid of biofilms?

Antibiotics by themselves are often unable to kill all of the bacteria in a biofilm so one area of active research is concerned with ways of

enhancing the activity of these agents. It has been shown that exposing biofilms to electric currents or to ultrasound in the presence of antibiotics has a synergistic effect that can achieve killing of all of the organisms in the biofilm[66,67]. However, as is often the case, "prevention is better than cure" and considerable effort has gone in to developing means of preventing biofilm formation on medical devices[68–71].

Four main approaches have been studied: (i) administering prophylactic antibiotics during insertion of the device; (ii) incorporating antimicrobial agents into the material used; (iii) coating the material with an antimicrobial agent; (iv) altering the surface of the device (chemically or physically) to try and prevent bacteria adhering to it. Of these strategies, prophylactic antibiotics are thought to have contributed significantly to the prevention of infections associated with joint replacements. With regard to the other approaches, while many have demonstrated their effectiveness *in vitro*, few have been found to work very well *in vivo*. One factor that may account for these disappointing failures is that many investigators have not incorporated a conditioning film into their *in vitro* models. The conditioning film formed *in vivo* may well negate any of the beneficial effects observed *in vitro* by, for example, neutralising antimicrobial agents or by masking changes made to the surfaces of the device.

So, what is a biofilm?

Although as already pointed out, no really adequate definition of the term "biofilm" exists, we can recognise the main features of a biofilm as being the following:

(a) a three-dimensional structure containing one or more bacterial species;
(b) it forms at interfaces – solid/liquid, liquid/air, solid/air;
(c) it exhibits spatial heterogeneity due to physico-chemical and chemical gradients which develop within it;
(d) it is often permeated by water channels;
(e) the organisms within it exhibit a marked decrease in susceptibility to antimicrobial agents and host defence systems compared to their planktonic counterparts .

Biofilms, therefore, are cellular communities with an ordered structure and a circulatory system, they display different physiologies within different regions, have a form of intercellular communication and can resist noxious chemicals and other threats from their environ-

ment. This description should strike a few chords, and give us food for thought, as it sounds remarkably like a description of a multicellular organism!

References

1. Costerton, J.W., Lewandowski, Z., Ca ldwell, D.E., Korber, D.R. & Lappin-Scott, H.M. (1995) Microbial biofilms. *Ann. Rev. Microbiol.*, 49, 711–745.
2. Watnick, P. & Kolter, R. (2000) Biofilm, city of microbes. *J. Bacteriol*., **182**, 2675–2679.
3. Newman, H.N. & Wilson, M. (1999) Dental Plaque Revisited: oral biofilms in health and disease. BioLine, Cardiff.
4. Habash, M. & Reid, G. (1999) Microbial biofilms: their development and significance for medical device-related infections. *J. Clin. Pharmacol.,* **39,** 887–898.
5. Khardori, N. & Yassien, M. (1995) Biofilms in device-related infections. *J. Ind. Microbiol.,* **15**,141–147.
6. Listgarten, M. (1999) Formation of dental plaque and other oral biofilms. In: Newman, H.N. & Wilson, M. (eds), *Dental Plaque Revisited: oral biofilms in health and disease*, pp. 187–210. BioLine, Cardiff.
7. Domingue, P.A., Sadhu, K., Costerton, J.W., Bartlett, K. & Chow, A.W. (1991) The human vagina: normal flora considered as an *in situ* tissue-associated, adherent biofilm. *Genitourin Med.*, **67,** 226–231.
8. Bayston, R. (1999) Medical Problems due to Biofilms: Clinical Impact, Aetiology, Molecular Pathogenesis, Treatment and Prevention. In: Newman, H.N. & Wilson, M. (eds), *Dental Plaque Revisited: oral biofilms in health and disease*, pp. 111–124. BioLine, Cardiff.
9. Ganderton, L., Chawla, J., Winters, C., Wimpenny, J. & Stickler, D. (1992) Scanning electron microscopy of bacterial biofilms on indwelling bladder catheters. *Eur. J. Clin. Microbiol. Infect. Dis.*, **11**, 789–796.
10. Cassels, F.J. & Wolf, M.K. (1995) Colonization factors of diarrheagenic E. coli and their intestinal receptors. *J. Ind. Microbiol.,* **15**, 214–226.
11. Lyczak, J.B., Cannon, C.L. & Pier, G.B. (2000) Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect*, **2**, 1051–1060.
12. Pier, G.B. (2000) Peptides, *Pseudomonas aeruginosa*, polysaccharides and lipopolysaccharides – players in the predicament of cystic fibrosis patients. *Trends Microbiol.,* **8**, 247–250.
13. Høiby, N., Johansen, H.K., Moser, C., Song, Z., Ciofu, O. & Kharazmi, A. (2001). *Pseudomonas aeruginosa* and the *in vitro* and *in vivo* biofilm mode of growth. *Microbes Infection*, **3,** 23–35.
14. Reid, G. (2001) Probiotic agents to protect the urogenital tract against infection. *Am. J. Clin. Nutr.,* **73**, 437S–443S.
15. Wang, J. (2000) Bacterial vaginosis. *Prim. Care Update Ob. Gyns.*, **7**, 181–185.
16. McLean, N.W. & Rosenstein, I.J. (2000) Characterisation and selection of a Lactobacillus species to re-colonise the vagina of women with recurrent bacterial vaginosis. *J. Med. Microbiol*, **49**, 543–552.
17. Kolenbrander, P.E. (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Ann. Rev. Microbiol.,* **54**, 413–437.

18. Marsh, P.D & Bradshaw, D.J. (1995) Dental plaque as a biofilm. *J. Ind. Microbiol.*, **15**,169–175.
19. Rosan, B. & Lamont, R.J. (2000) Dental plaque formation. *Microbes Infect.*, **2**, 1599–1607.
20. Costerton, J.W., Stewart, P.S. & Greenberg, E.P. (1999) Bacterial biofilms: a common cause of persistent infections. *Science*, **284**,1318–1322.
21. Moore, W.E. & Moore, L.V. (1994) The bacteria of periodontal diseases. *Periodontol., 2000*. **5**, 66–77.
22. Amann, R.I., Ludwig, W. & Schleifer, K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev*., **59**,143–169.
23. Marsh, P.D. (1994) Microbial ecology of dental plaque and its significance in health and disease. *Adv. Dent. Res.*, **8**, 263–271.
24. Marsh, P.D & Bradshaw, D.J. (1997). Physiological approaches to the control of oral biofilms. *Adv. Dent. Res.*, **11**, 176–185.
25. Wilson, M., Reddi, K. & Henderson, B. (1996) Cytokine-inducing components of periodontopathogenic bacteria. *J. Periodont. Res.*, **31**, 393–407.
26. Palmer, R. Jr. & White DC. (1997). Developmental biology of biofilms: implications for treatment and control. *Trends Microbiol.*, **5**, 435–440.
27. Whittaker, C.J., Klier, C.M. & Kolenbrander, P.E. (1996) Mechanisms of adhesion by oral bacteria. *Ann. Rev. Microbiol.*, **50**, 513–552.
28. Davies, D.G., Chakrabarty, A.M. & Geesey, G.G. (1993) Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by Pseudomonas aeruginosa. *Appl. Environ. Microbiol.*, **59**, 1181–1186.
29. Garrett, E.S., Perlegas, D. & Wozniak, D.J. (1999) Negative control of flagellum synthesis in Pseudomonas aeruginosa is modulated by the alternative sigma factor AlgT (AlgU). *J. Bacteriol.*, **181**, 7401–7404.
30. Prigent-Combaret, C., Vidal, O., Dorel, C. & Lejeune, P. (1999) Abiotic surface sensing and biofilm-dependent regulation of gene expression in *Escherichia coli*. *J. Bacteriol.*, **181**, 5993–6002.
31. Cramton, S.E., Gerke, C., Schnell, N.F., Nichols, W.W. & Gotz, F. (1999) The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect. Immun.*, **67**, 5427–5433.
32. Moore, G.F., Dunsmore, B.C., Jones, S.M., Smejkal, C.W., Jass, J., Stoodley, P. & Lappin-Scott, H.M. (2000) Microbial detachment from biofilms. In: Allison, D.G., Gilbert, P., Lappin-Scott, H.M. & Wilson, M. (eds), *Community Structure and Co-operation in Biofilms*. Cambridge University Press.
33. Lawrence, J.R. & Neu, T.R. (1999) Confocal laser scanning microscopy for analysis of microbial biofilms. *Methods Enzymol.*, **310**, 131–144
34. Palmer, R.J. & Sternberg, C. (1999) Modern microscopy in biofilm research: confocal microscopy and other approaches. *Curr. Opin. Biotechnol.*, **10**, 263–268.
35. Mawhinney, W.M., Adair, C.G. & Gorman, S.P. (1990) Confocal laser scanning microscopic investigation of microbial biofilm associated with CAPD catheters. *Pharmacotherapy*, **10**, 241.
36. Sutherland, I.W. (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology*, **147**, 3–9.
37. Zhang, X.Q., Bishop, P. L. & Kupferle, M. J. (1998). Measurement of polysaccharides and proteins in biofilm extracellular polymers. *Water Sci. Technol.*, **37**, 345–348.

38. Wimpenny, J., Manz, W. & Szewzyk, U. (2000) Heterogeneity in biofilms. *FEMS Microbiol. Rev.*, **24**, 661–671.
39. Stewart, P.S., Roe, F., Rayner, J., Elkins, J.G., Lewandowski, Z., Ochsner, U.A. & Hassett, D.J. (2000) Effect of catalase on hydrogen peroxide penetration into Pseudomonas aeruginosa biofilms. *Appl. Environ. Microbiol.*, **66**, 836–838.
40. Rasmussen, K. & Lewandowski, Z. (1998) Microelectrode measurements of local mass transport rates in heterogeneous biofilms. *Biotechnol. Bioeng.*, **59**, 302–309.
41. Korber, D.R., Wolfaardt, G.M., Brozel, V., MacDonald, R. & Niepel, T. (1999) Reporter systems for microscopic analysis of microbial biofilms. *Methods Enzymol.*, **310**, 3–20.
42. Prigent-Combaret, C. & Lejeune, P. (1999) Monitoring gene expression in biofilms. *Methods Enzymol.*, **310**, 56–79.
43. Hodson, R.E., Dustman, W.A., Garg, R.P. & Moran, M.A. (1995) In situ PCR for visualization of microscale distribution of specific genes and gene products in prokaryotic communities. *Appl. Environ. Microbiol.*, **61**, 4074–4082.
44. Lisle, J.T., Stewart, P.S. & McFeters, G.A. (1999) Fluorescent probes applied to physiological characterization of bacterial biofilms. *Methods. Enzymol.*, **310**, 166–178.
45. Yu, F.P. & McFeters, G.A. (1994) Physiological responses of bacteria in biofilms to disinfection. *Appl. Environ. Microbiol.*, **60**, 2462–2466.
46. Vroom, J.M., De Grauw, K.J., Gerritsen, H.C., Bradshaw, D.J., Marsh, P.D., Watson, G.K., Birmingham, J.J. & Allison, C. (1999) Depth penetration and detection of pH gradients in biofilms by two-photon excitation microscopy. *Appl. Environ. Microbiol.*, **65**, 3502–3511.
47. de Kievit, T.R. & Iglewski, B.H. (2000) Bacterial quorum sensing in pathogenic relationships. *Infect. Immunol.*, **68**, 4839–4849.
48. Bassler, B. L. (1999). How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.*, **2,** 582–587.
49. Hardman, A.M., Stewart, G.S. & Williams, P. (1998) Quorum sensing and the cell-cell communication dependent regulation of gene expression in pathogenic and non-pathogenic bacteria. *Antonie Van Leeuwenhoek,* **74**,199–210.
50. Dunny, G.M. & Leonard, B.A.B. (1997). Cell-Cell communication in Gram-positive bacteria. *Annu. Rev. Microbiol.*, **51**, 527–564).
51. Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. & Greenberg, E.P. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*, **280**, 295–298.
52. Stefani, S. & Agodi, A. (2000) Molecular epidemiology of antibiotic resistance. *Int. J. Antimicrob. Agents*, **13**, 143–153.
53. Marcus, S.L., Brumell, J.H., Pfeifer, C.G. & Finlay, B.B. (2000) Salmonella pathogenicity islands: big virulence in small packages. *Microbes Infect.*, **2**, 145–156.
54. Licht, T.R., Christensen, B.B., Krogfelt, K.A. & Molin, S. (1999) Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. *Microbiology*, **145**(9), 2615–2622.
55. Roberts, A.P., Pratten, J., Wilson, M. & Mullany, P. (1999) Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. *FEMS Microbiol. Lett.*, **177**, 63–66.

56. Bayston, R. (2000) Biofilms and prosthetic devices. In: Allison, D.G., Gilbert, P., Lappin-Scott, H.M. & Wilson, M. (eds), *Community Structure and Co-operation in Biofilms.* pp. 295–307. Cambridge University Press.
57. Williams, P. (1994) Host immune defences and biofilms. In: Wimpenny, J., Nichols, W., Sickler, D. & Lappin-Scott, H. (eds), *Bacterial biofilms and their control in medicine and industry.* pp.93–96. BioLine, Cardiff.
58. Kharazmi A. (1991) Mechanisms involved in the evasion of the host defence by *Pseudomonas aeruginosa*. *Immunol. Lett.*, **30**, 201–205.
59. Johnson, G.M., Lee, D.A., Regelmann, W.E., Gray, E.D., Peters, G. & Quie, P.G. (1986) Interference with granulocyte function by *Staphylococcus epidermidis* slime. *Infect. Immun,* **54**, 13–20.
60. Shiau, A.L. & Wu, C.L. (1998) The inhibitory effect of *Staphylococcus epidermidis* slime on the phagocytosis of murine peritoneal macrophages is interferon-independent. *Microbiol. Immunol.*, **42**, 33–40.
61. Schierholz, J.M., Beuth, J., Konig, D., Nurnberger, A. & Pulverer, G. (1999) Antimicrobial substances and effects on sessile bacteria. *Zentralbl. Bakteriol*., **289**, 165–177.
62. Xu, K.D., McFeters, G.A. & Stewart, P.S. (2000) Biofilm resistance to antimicrobial agents. *Microbiology*, **146**, 547–549.
63. Allison, D.G., McBain, A.J. & Gilbert, P. (2000) Biofilms: problems of control. In: Allison, D.G., Gilbert, P., Lappin-Scott, H.M. & Wilson, M. (eds), *Community Structure and Co-operation in Biofilms,* (309–327). Cambridge University Press.
64. Mah, T.C. & O'Toole, G.A. (2001) Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.*, **9**, 34–39.
65. Wilson, M. (1996) Susceptibility of oral bacterial biofilms to antimicrobial agents. *J. Med. Microbiol.*, **44,** 79–87.
66. Wellman, N., Fortun, S.M. & McLeod, B.R. (1996) Bacterial biofilms and the bioelectric effect. *Antimicrob. Agents Chemother.*, **40**, 2012–2014.
67. Rediske, A.M., Roeder, B.L., Nelson, J.L., Robison, R.L., Schaalje, G.B., Robison, R.A. & Pitt, W.G. (2000) Pulsed ultrasound enhances the killing of Escherichia coli biofilms by aminoglycoside antibiotics in vivo. *Antimicrob. Agents Chemother.*, **44**, 771–772.
68. Kohnen, W. & Jansen, B. (2000) Changing material surface chemistry for preventing bacterial adhesin. In: An, Y.H. & Friedman, R.J. (eds), *Handbook of bacterial adhesion,* pp. 581–590. Humana Press, Totowa.
69. Rosch, W. & Lugauer, S. (1999) Catheter-associated infections in urology: possible use of silver-impregnated catheters and the Erlanger silver catheter. *Infection,* **27**, Suppl 1:S74–77.
70. Costerton, J.W., Khoury, A.E., Ward, K.H. & Anwar, H. (1993) Practical measures to control device-related bacterial infections. *Int. J. Artif. Organs,* **16,** 765–770.
71. Bach, A. (1999) Prevention of infections caused by central venous catheters-established and novel measures. *Infection,* **27**, Suppl 1:S11–15.