# The Effect of Substratum Properties on the Survival of Attached Microorganisms on Inert Surfaces

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**Abstract** Biofilm formation is dependent on the surrounding environmental conditions and substratum parameters. Once a biofilm forms many factors may influence cell survival and resistance. Cell adhesion to a surface is a prerequisite for colonization. However, attached microorganisms may not be able to multiply, and may merely be surviving on the surface, for example at a solid–air interface, rather than forming a biofilm. Retention of attached cells is a key focus in terms of surface hygiene and biofilm control. Factors that affect this retention may differ from those affecting biofilm formed at the solid–liquid interface: the nature of the substratum, presence of organic material, vitality of the attached microorganism, and of course the surrounding environment. The majority of publications focus on the solid–liquid interface; literature addressing the solid–air interface is considerably less substantial.

## 1 Introduction

Microbial attachment, adhesion, retention and subsequent biofilm formation are major concerns in many settings where biofilms play a key role in ensuring the survival of microorganisms and their resistance to a range of external "attacks" for example by protozoa, environmental conditions or chemical agents. Mechanisms of resistance to these external forces are diverse.

The literature concerning biofilms and resistance is significant, and the fact that biofilms demonstrate significantly enhanced resistance is well recognized. This paper focuses on the survival of attached cells rather than on biofilm. Donlan and Costerton (2002) define a biofilm as "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other and that are embedded in a matrix of extracellular polymeric substances

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that they themselves have produced. The cells in the biofilm may also exhibit an altered phenotype with respect to growth rate and gene transcription". Attached cells on the other hand may be surrounded by preformed extracellular polymeric substances (EPS), but will not produce more unless appropriate environmental conditions are present. Attached cells rather than biofilm are therefore found at the solid–air as well as the solid–liquid interface. Intermittent exposure of the substratum to moisture, for example during cleaning of hygienic surfaces or external surface exposure to rain, or at a meniscus (Fig. 1) generates a solid-liquid–air interface, at which fouling is apparent.

Adhesion is a prerequisite for colonization. Microorganisms can survive in very thin water films but attached microorganisms may not be able to multiply, particularly if there is little moisture available. Thus many of the factors affecting the survival of cells in a biofilm may not be applicable to cells retained on a surface in the absence of moisture, but in the presence of organic material. Thus external factors, such as the nature of the substratum and of the surrounding environment, will significantly affect survival and "biotransfer potential" (Verran and Boyd 2001).

In this chapter a range of examples showing the effect of surface features on cell survival and resistance will be discussed, focusing on the marine environment wherever possible/appropriate, and addressing any differences between the solid–liquid and solid–air interface.

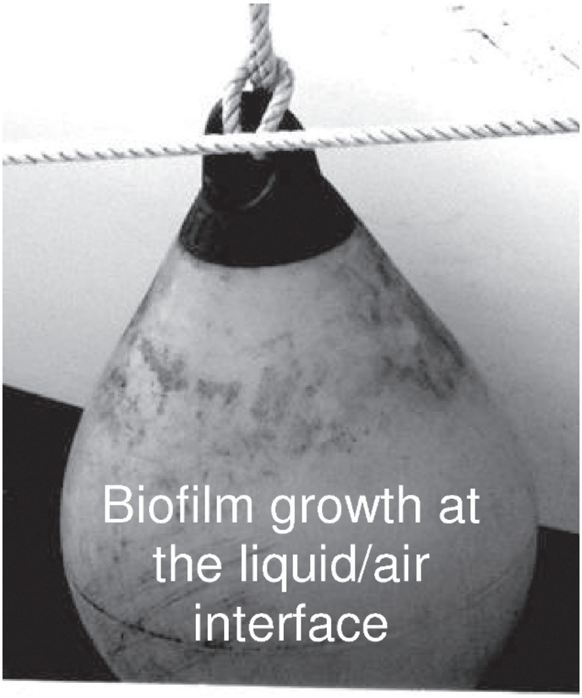

**Fig. 1** Plastic buoy that has biofilm growing on its surface, resulting in fouling of the material

## 2 Microbial Attachment to Surfaces

Viruses, bacteria, fungi, algae and protozoa may all be found in the marine biofilm community, (Fig. 2) along with “macroorganisms”, such as barnacles and seaweeds. Many studies have attributed microbial survival and resistance of attachment microorganisms to their cellular physiology but it is now thought that there are a number of contributory physical and chemical factors involved. Physicochemical parameters will affect initial attachment. Once the cells attach, the surface chemistry will influence cell adhesion, whilst topographic features allow maximum cell-surface binding, enhancing strength of attachment and thus retention.

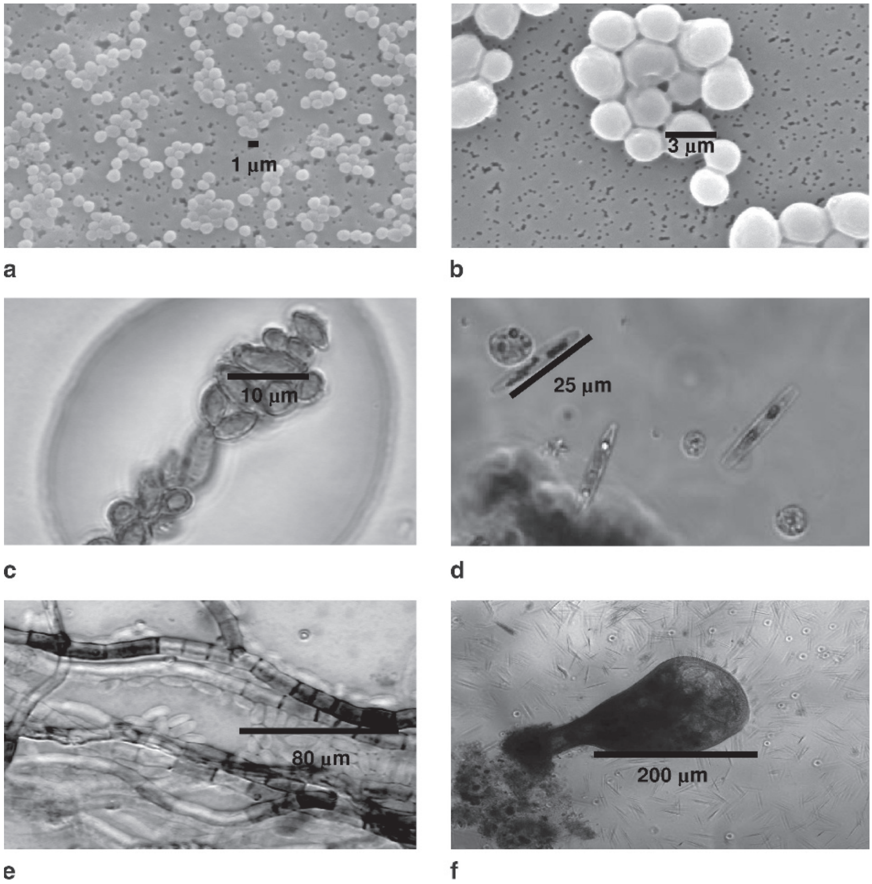

**Fig. 2** The scale of the surface roughness is important since the organisms that may be involved in the formation of an environmental biofilm may vary greatly in size and shape **(a)** *Staphylococcus aureus* (bacteria) around 1 µm; **(b)** *Candida albicans* (yeast) around 3–5 µm; **(c)** *Cladsporium sp.* (fungal species) 12–18 µm; d) planktonic algae around 25 µm; **(e)** *Aureobasidum pullulans* hyphae can grow to various lengths; **(f)** *Stentor coeruleus* (cilitae) >200 µm

In an aqueous environment bacterial attachment to a surface occurs rapidly, over a few seconds to a few minutes. Moreover, the binding of microorganisms to a surface can confer advantages to cell survival, for example the attachment of cells to solid surfaces has been reported to immediately upregulate alginate synthesis in a strain of *Pseudomonas aeruginosa* (Davies et al. 1993), therefore strengthening cell–substratum binding. Bright and Fletcher (1983) also gave evidence that supported the existence of a direct substratum influence on the assimilation of amino acids in a marine *Pseudomonas* sp. thus enhancing nutrient availability to the cell.

If cells are deposited on a surface in the absence of a solid–liquid interface, for example by direct contact, then the behaviour of the passively attached cells may differ from that described above. However, in either case, since the surface of the substratum is the primary contact for cell attachment, the study of cell–surface interactions is of utmost importance.

## 3 Primary Adhesion to Surfaces

Much of the literature discusses microbial resistance and cell eradication once cells have attached to a surface. However, it would seem that a more proactive approach is to target the organisms in order to prevent initial cell attachment and thus subsequent retention to a surface. Primary cell adhesion to surfaces is dictated by a number of parameters. In an aqueous environment (liquid–solid), cells will first approach a surface by natural forces such as diffusion, gravitation, and Brownian motion. However, once in the vicinity of a surface, physicochemical parameters will come into play and the influence of Lifshitz–van de Waals forces, electrostatic forces and hydrogen bonding will influence the cells approach and subsequent attachment to the surface. It would seem obvious that the physicochemical, chemical and the topography have an influence on the properties of the substratum. However, the properties of the cell surface also need to be considered. The cell is a complex arrangement of different chemical species and topographies (on the micro- and nanoscale), and hence comprises islands of different physicochemical and chemical properties. Further, these properties will alter with changes in a given environment. The substratum, once in an aqueous environment will become coated with organic material, known as a conditioning film, as will cells, in addition to the presence of EPS. The EPS also plays a paramount role in primary adhesion.

At the solid–air interface in an open environment, the initial transfer of cells to a surface may occur in one fouling event where surfaces are contaminated by direct contact with the fouling material. Despite the complexities of these initial attachment scenarios it would seem logical to attempt to reduce/delay/prevent this initial cell–surface interaction in preference to managing the subsequent attached biofilm, for example by surface modification. There are a number of approaches that are directed towards this phenomenon, including the modification of surface topography (macro-, micro- and nanoscale), chemistry (self-assembled monolayers) and physicochemistry (superhydrophobic surfaces). However, it is inevitable that practically all surfaces will be colonized sooner or later (Flemming, personal communication).

## 4 Substratum Physicochemical Properties

Many different physicochemical interactions between microorganisms and the solid surface have been described in the literature (Boonaert and Rouxhet 2000; Simoes et al. 2007). If the physicochemical properties of a surface can be defined and controlled, then cell attachment, survival and biofilm formation can in turn be more easily managed. There is conflicting literature concerning the complex effect of surface and/or microbial physicochemical properties on microbial attachment to surfaces (Bos et al. 1999; Chen and Strevett 2001). Adhesion of vegetative cells (Sinde and Carballo 2000), bacterial spores (Husmark and Ronner 1993) and freshwater bacteria (Pringle and Fletcher 1983) has been shown to increase with increasing surface hydrophobicity. Other organisms have also been shown to preferentially bind to hydrophobic surfaces: for example *Enteromorpha* spores (Callow et al. 2002). It has been suggested that cell attachment to hydrophobic plastics occurs very quickly (Carson and Allsopp 1980) whereas cell attachment to hydrophilic surfaces such as metallic oxides, glass and metals increases with longer exposure times (Dexter 1979).

The surface free energy of a substratum is believed to be important in initial cell attachment, but the interactions involved are complex. Biofilm formation coincides with increased inorganic positively charged elements at the surface (Carlen et al. 2001), but positive substratum surface charge has also been shown to impede bacterial surface growth despite initially promoting adhesion (Gottenbos et al. 2001). A maximum detachment rate for marine biofilms or bacteria has been demonstrated for surface free energies of 20–27 mN $m^{-1}$ (Becker 1998; Pereni et al. 2006).

The surface energy distribution on substrata will be dependent on the surface structure and will be affected by surface imperfections such as cracks or pores, and also on the conditioning layer of the substratum, which is in turn defined by the surrounding environment. It has been suggested that the differences observed between surfaces in in-vitro hydrophobicityassessments may be due to changes in the substratum characteristics that occur during the first few minutes of exposure to the surrounding fluid, where a primary film of organic molecules known as the conditioning film is adsorbed to the substratum (Pringle and Fletcher 1983). The presence of this film clearly affects microbial retention, and also contributes to cell interactions with the surface. It is likely that conditioning films may mask some substratum properties.

This interaction may not be relevant to the attachment of cells on open surfaces, where contact between the cell and the substratum may be achieved by transient wetting or transfer between surfaces involving direct contact, or airborne transmission. However, the retention of the cells will be affected by the cohesive forces and by the area of contact between the cell and the substratum, be it conditioned or otherwise.

In an aqueous environment the conditioning of a surface by smaller molecules and ions will occur before bacterial attachment, thus the film provides the linking layer between the cells and the surface. A clear understanding of all interactions is needed if a logical attempt at controlling surface fouling is to transpire. Antifouling surfaces are possible but, since each fouling environment is essentially unique, it may be that situations have to be addressed on an individual basis. The life span and cost of production for any antifouling product must also be considered alongside the expected antifouling benefit. It is unlikely that fouling can be completely prevented, but if soil is more easily removed or if fouling is delayed then clear economical, ecological or health-associated benefits may be derived.

## 5 Chemical Properties of Materials

The chemical properties of materials are defined by the elements that ultimately make up the molecules of a surface. The surface chemistry, i.e. the chemical properties of the materials, has been shown to directly affect microbial attachment (Verran and Whitehead 2005: Whitehead and Verran 2007) and survival. A range of inert substrata find use in environments where microbial attachment and biofilm formation are common. The chemistry of the surface inevitably affects these interactions. Thus the choice of material must be made depending on the intended properties of the surface (e.g. immersed/exposed, high cleanability/low fouling, low wear, non-toxic, low cost etc.).

### *5.1 Metals*

Cell attachment and thus biofilm formation can occur on metals, including aluminium (Nickels et al. 1981), stainless steel (Mittelman et al. 1990) and copper (Geesey and Bremer 1990). However, some metals such as aluminium or copper are considered toxic to bacteria (Avery et al. 1996). It has been suggested that microbial resistance to some metals, for example lead acetate, can be attributed to the high lead content of disinfectants and antiseptics, whilst resistance to copper sulfate may be due to its use as an algicide (Hiramatsu et al. 1997). However, even with concerns of increased resistance of microorganisms, and the frequent necessity of moisture to enable the antimicrobial action to occur, the incorporation of a range of metals into “antibacterial” surfaces has been reported (Kielemoes et al. 2000). The location of these surfaces, whether immersed, intermittently wet or dry, will clearly affect any intended antimicrobial effect. In particular, silver and copper have received significant attention. Antimicrobial silver and/or copper reagents have been occasionally applied to the water distribution system for inactivation of pathogens (Liu et al. 1998). However, bacterial resistance against silver and other metals may lead to limitations in the efficacy of these bactericide-releasing materials (Cloete 2003).

Copper has been shown to increase the growth rate of some bacteria (Starr and Jones 1957), whilst reduced growth in response to copper has been demonstrated for microbial populations (Jonas 1989). When compared to plastics and stainless steel surfaces, copper has been shown to have inhibitory effects on various microorganisms (De Veer et al. 1994; Keevil 2001). Copper-containing alloys have also shown increased antibacterial activity when compared to stainless steel and brasses, with increasing copper content reducing cell survival time (Wilks et al. 2005). It has been suggested that for biocidal purposes the use of copper alloyed surfaces should be restricted to regularly cleaned surfaces (Kielemoes and Verstraete 2001), since accumulation of non-microbial material and potential reaction of the cleaning agent with the copper and the fouling material may interfere with the antimicrobial effect, on open as well as closed surfaces (Airey and Verran 2007). Indeed, the ability of any antimicrobial agent in a surface to affect cells in the biofilm above will depend on the ability of the agent to diffuse through the biofilm from the substratum. Conversely, any antimicrobial agent whose effect relies on direct contact will only be active against those cells at the base of the biofilm. One might speculate that the effectiveness of an antifouling surface is only predictable for a given period of time, since once conditioning of the surface begins, surface properties will change. This will result in loss of direct contact of the surface with the foulant and consequently the loss of the surface antifouling effect. This has been demonstrated in the copper pipes containing disinfectant concentrate where biofilms have been found (Exner et al. 1983). As the copper surface becomes fouled, antimicrobial properties become diminished unless regularly cleaned (Airey and Verran 2007).

### *5.2 Polymers*

Synthetic polymers may contain many additive chemicals, such as antioxidants, light stabilizers, lubricants, pigments and plasticizers, added to improve the desired physical and chemical properties of the material (Brocca et al. 2002). However, these additives may leach into the surrounding environment and provide nutrient for microorganisms present: phosphorus has been shown to increase the formation of biofilms on polyvinyl chloride in phosphorus-limited water (Lehtola et al. 2002). Several studies have shown that plastic materials can support the growth of biofilms, but it has been suggested that growth in plastic pipes is usually comparable with that on iron, steel or cement (Niquette et al. 2000). However, Bachmann and Edyvean (2006) used *Aquabacterium commune* cells under continuous cultivation with stainless steel and medium density polyethylene (MDPE) surfaces and found that biofilm cell density on MDPE slides was four times greater than on stainless steel. When various pipe materials were tested with chlorine and monochloramine disinfection, it was found that cement-based materials supported fewer fixed bacteria than plastic-based materials (Momba and Makala 2004).

Again, most of these surfaces are exposed to liquid and, potentially microorganisms, at a solid–liquid interface, often in a closed system. On open surfaces, many different properties of polymers can be exploited, depending on the intended end use. The relative softness of these surfaces makes them susceptible to surface damage, which will affect surface topography, and hence fouling and cleanability (Verran et al. 2000). However, as with all surfaces, long-term studies are required to assess the effect of surface wear and the effect of fouling, e.g. by humic substances, oil or mineral particles.

### 5.2.1 Incorporation and Release of Antimicrobial Agents in Polymers

In attempts to prevent/reduce cell attachment and survival on surfaces, antimicrobial agents have been incorporated in and onto polymers. Clearly, the release of the biocide/metal ions will be determined by the matrix and properties of the bulk material and surrounding environment. Biocides can be encapsulated to facilitate "delayed release", thereby extending the intended antimicrobial effect (Lukaszczyk and Kluczka 1995; Coulthwaite et al. 2005). Coatings and molecules extracted from natural sources have been suggested for use to deter microbial survival on a surface. On open surfaces, incorporation of antimicrobial agents such as biocides (for example Microban) or metals (for example BioCote or Agion) are used to achieve "antibacterial" properties, but the mechanism of the biocide action (e.g. is moisture required), duration, spectrum, speed and magnitude of effect are all important determinants of eventual effectiveness at intended point of use.

There are a number of important factors that need to be considered with respect to the development of biocide-incorporated materials, including physical and environmental aspects. The effectiveness of a biocide-incorporated surface is dependent upon the ability of the biocide to be released from the bulk material into which it is incorporated. This is a delicate balance, since if the blending, dispersion and binding properties are incorrect then the biocide release rate may be too fast (shortened life span of material), too slow (not effective), or non-existent. There is always a limited lifetime to these materials since an infinite amount of biocide is not available.

The release of biocide into the environment should also be considered. The Biocidal Product Directive (European Parliament 1998) was designed to review existing substances and aimed to provide high levels of protection for humans, animals and the environment. Many antifouling paints used to reduce the attachment of living organisms to the submerged surfaces of ships, boats and aquatic structures have biocide-release mechanisms. Two common biocides in use are the triazine herbicide Irgarol 1051 (*N*-2-methylthio-4-tert-butylamino-6-cyclopropylamino-*s*-triazine), and diuron (1-(3,4-dichlorophenyl)3,3-dimethylurea), which are designed to inhibit algal photosynthesis. It has been shown that due to leaching, environmental concentrations of the compounds pose significant risks to the plant species *Apium nodiflorum* and *Chara vulgaris* (Lambert et al. 2006). With biocide-incorporated materials there are also problems encountered with the targeted organisms, e.g. increased tolerance and resistance to the active material. Resistance to many chemical compounds including benzalkonium chloride, benzisothiazolone, chloroallyltriazine-azoniadamantane, dibromodicyanobutane, methylchloro/methylisothiazolone, tetrahydrothiadiazinthione and trifluoromethyl dichlorocarbanilide has been detected (Chapman 1998).

By definition, biocides will not assist in the accumulation and removal of organic material present on the surface and in the surrounding aqueous environment. The result may be that, although micro- and macroorganisms that attach to a surface may be inhibited or killed, the transfer of organic matter to the surface will not be affected. Thus an organic material layer will gradually build up on the surface over time, potentially masking any biocide effect.

If the biocide is not uniformly dispersed in the bulk material, then there will be areas of the surface that may allow attachment of tolerant or resistant microorganisms. Once this attachment occurs, microbial colonization and thus biofilm formation can occur, potentially enveloping the surrounding areas of material that are higher in biocide concentration. Thus although biocide-releasing surfaces may be a practical solution for surfaces that are to be used in the short term, in the long term they may be of limited value, particularly at the solid–liquid interface.

### *5.3 Paints*

Coatings and paints intended for use on ships and underwater components or superstructures are a complex mixture of compounds that may include binders, pigments, extenders, solvents, thinners and additives (e.g. biocides) (Watermann et al. 2005). The purpose of antifouling paints is primarily to prevent development of macrofouling, particularly barnacles. Since microorganisms on a surface can increase the attachment of other organisms, inhibition of microbial biofilm development might decrease subsequent development of barnacles on the surface (Tang and Cooney 1998). Thus it is of importance to test new formulations for the survival and resistance of macro- and microorganisms.

As with blended polymers, the complex nature of the paint and its components will affect the activity of biocide/antimicrobial used and thus the final antimicrobial activity of the paint. To provide effective antifouling properties, organic biocides such as Irgarol, are often added in conjunction with copper to control copper-resistant fouling organisms (Voulvoulis et al. 1999). It has been shown that the release rate of copper depends not only on the copper compound and its dissolution properties, but also on the character of the paint matrix (Sandberg et al. 2007). The underlying substrata may also affect the antifouling properties of paint. Work by Tang and Cooney (1998) showed that coating surfaces with a marine paint decreased the numbers of *Pseudomonas aeruginosa* on stainless steel but had little effect on numbers of cells on fibreglass or aluminium. However, when they added copper or tributyltin (TBT) to the paint the initial development of biofilms was inhibited for 72–96 h. Biofilms that formed on surfaces coated with copper or TBT-containing paint did not synthesize greater amounts of EPS, thus the biofilms may have contained copper- or TBT-resistant cells.

There have been some attempts to use naturally extracted products as antifouling agents in paints. Four bacterial isolates from a marine environment were used to produce extracts that were formulated into ten water-based paints: nine showed activity against a test panel of fouling bacteria (Burgess et al. 2003). Five of the paints were shown to inhibit the settlement of barnacle larvae, *Balanus amphitrite*, and algal spores of *Ulva lactuca*, and for their ability to inhibit the growth of *Ulva lactuca* when grown on paint containing an extract from *Pseudomonas* sp. strain (Burgess et al. 2003).

It is interesting to note that manufacturers do not need to specify ingredients of the paint that are below 1% weight, thus antifouling paints may include significant amounts of metallic and non-metallic elements (Sandberg et al. 2007). Unfortunately, some materials used in paints (such as both organotins and copper) can be toxic to non-target marine species, such as the dog-whelk (Gibbs and Bryan 1986), oysters (Axiak et al. 1995) and juvenile carp (de Boeck et al. 1995; Tang and Cooney 1998). The use of biocidal antifouling paints has been prohibited in some European countries, such as Sweden, Denmark, Germany and France (Watermann et al. 2005). It should be noted that although copper is widely used in Europe, Sweden has prohibited its use in antifouling paints on pleasure crafts in fresh water and along the Swedish coast of the Baltic Sea (Sandberg et al. 2007). However, recent investigations have shown that newly developed, "toxin-free" antifouling paints that do not contain, e.g., copper, Irgarol or TBT may still be toxic towards marine organisms (Karlsson and Eklund 2004).

On surfaces that are not submerged but are externally exposed, a solid–liquid–air interface will form as rain droplets pass over the surface. The physical washing effect, coupled with release of any intended antimicrobial properties, will thus help reduce fouling on the surface. On internal surfaces, required properties might encompass easy cleanability rather than specifically antimicrobial properties – although in hygienic environments some biocidal effect would be desirable. Thus, photocatalytic paints are finding applications. UV radiation is an effective, but temporary photochemical method for disinfection, which requires a special irradiation source within the UV (185–254 nm) band. Photocatalysis is an alternative to direct UV disinfection and antimicrobial efficacy is possible with higher wavelengths, which are naturally present in ambient solar and artificial light (Erkan et al. 2006). Large band gap semiconductors, such as titanium dioxide ($TiO_2$), tin oxide and zinc oxide, are suitable photocatalytic materials with their higher wavelength UV absorption (320–400 nm) (Erkan et al. 2006). Titanium dioxide doped with metals has demonstrated photocatalytic activity, leading to an increased rate of destruction of organic compounds (Vohra et al. 2005) and microorganisms (Sunada et al. 2003). There has been some work carried out on the effectiveness of nanoparticle anatase titania on the destruction of bacteria (Allen et al. 2005; Verran et al. 2007). An example of photocatalytic paint currently on the market is Aoinn®. However, *in situ* information on the effectiveness of these materials is limited. The effectiveness of the activity of photocatalytic paint on microorganisms is further complicated by the interactions of the paint components interfering with the active chemicals (Caballero et al., 2008).

## 6 Substratum Roughness

There are a number of engineering terms used to define surface roughness, but the $R_a$, (the average of the peak and valley distances measured along a centre line) is the most universally used roughness parameter for general quality control (Verran and Maryan 1997) and in microbiological publications (Verran and Boyd 2001). An important consideration when describing surface topography is that there are several scales that can be used to characterize material surfaces in terms of surface waviness, roughness and topography (Table 1). Thus the surface feature dimension should be considered alongside the dimensions of the organism of concern.

Simplistically, an increase in surface roughness will increase the retention of microorganisms on a surface (Boulange-Petermann et al. 1997; Verran and Whitehead 2005; Whitehead and Verran 2006). However, there is some debate over the phenomenon (Duddridge and Prichard 1983; Taylor and Holah 1996), which may be accounted for by a consideration of the scale of topography, the "patterning" of the features on the surface and of the testing methodology used.

Electropolishinghas shown to be advantageous in minimizing initial bacterial adhesion (Arnold et al. 2001) but, in the long-term, surface roughness has been shown not to affect the development of mature biofilms (Hunt and Parry 1998)

**Table 1** Descriptions of the different scale of surface topographies

| | Size of surface features | Description |
|---|---|---|
| Macro-topography | $R_a > 10$ µm | Will include surface finishes produced by industrial processes, e.g. the use of cutting tools (uniform spacing of surface features with a well-defined direction) or grinding processes (usually directional in character with generally of irregular spacing). Roughening of a surface will increase the area available for microbial adhesion and retention; however, if the surface roughness is greatly increased, this may result in wash out of microorganisms |
| Micro-topography | $Ra \sim 1$ µm | Surfaces with features of micron dimension are of importance if hygiene is of concern, e.g. in food processing |
| Nano-topography | $Ra < 1$ µm | Procedures such as polishing, whereby fine abrasives are used to produce a smooth shiny surface, nevertheless, all surfaces have a nanotopography. Nanotopographies are likely to have little effect on the $R_a$ or other roughness values as usually measured, but may affect retention of organic material |
| Angstrom-scale topography | Surface features 1–10 nm | Angstrom-sized surface features involve the configuration and mobility or functional groups, which may be of importance for both the cell and the substratum, especially where dynamic surfaces are being investigated |
| Molecular topography | Molecules | The charge on surface molecules ultimately make up the overall charge on the microbial or substratum surface and will affect the initial cell–surface binding |

– a property already noted in the impact of biocides. For surfaces deemed “hygienic”, usually encompassing microorganisms at the surface–air interface where “surface features” are smaller than the microorganisms, topography does not affect the retention of microorganisms on a surface (Verran et al. 2001a), although the cells tend to be immobilized on the features. Work by Hilbert et al. (2003) on stainless steel that was smoothed to $R_a$ of 0.9–0.01 µm also found that the adherence of microorganisms was not affected by differences in the surface roughness, but they did conclude that surface roughness was an important parameter for corrosion resistance of the stainless steel.

At the “macro” level it has been suggested that surface roughness may not pose a major problem in terms of bacterial adherence: because the surface features are so much larger than the bacterial cells, they can have no role in retention. However, some fungal spores, algae, protozoa and larger organisms, such as those found in marine environments and implicated in fouling, may be of significance. Thus an effect of surface roughness on attachment has been demonstrated for algal spores (Fletcher and Callow 1992) and invertebrate larvae (Crisp 1974). However, such macrofeatures may well encompass a micro- or nanotopography, which can retain smaller cells (Fig. 3). *In situ*, this means that the surface may become colonized with smaller cells such as bacteria prior to eukaryotic colonization. Not only might this provide anchorage points for the larger organisms but also for possible nutrients, thus increasing the chances of survival of the larger cells (Pickup et al. 2006). This succession of surface conditioning, micro- and macrofouling is a well-described phenomenon in immersed aquatic systems. Both viable and non-viable cells will contribute to this succession.

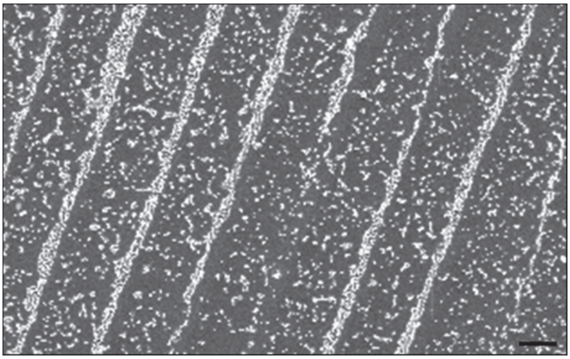

**Fig. 3** Surface grooves with a macrotopography (30 µm). However, these large scale features exhibit a micro- or nanotopography in the peaks between grooves. Cells are washed from large grooves but are piled up on the top of the groove peaks $R_a$ = 0.35 µm (image courtesy of A. Packer, MMU)

## 6.1 Stainless Steel: Surface Topography and Microbial Retention

Stainless steel is the most commonly used material for a number of industrial applications. Grade 316 stainless steel contains molybdenum, which increases resistance to surface pitting in aggressive environments, therefore it is widely used in the environment (Little et al. 1991). For stainless steel, different finishes will produce surfaces with differing topographies, whilst retaining low $R_a$ values below 0.8 µm, the value used for describing "hygienic" surfaces (Flint et al. 1997). As noted above, features of appropriate dimension will retain and protect microorganisms (Fig. 4), and reduce surface cleanability and hygienic status.

On open surfaces that are regularly cleaned, biofilm formation is unlikely (Verran and Jones 2000), but in closed environments, increased retention of viable microorganisms may accelerate development of biofilm, even if more mature biofilm is unaffected by the underlying surface topography (Verran and Hissett 1999). Larger surface defects will potentially entrap accumulations of microorganisms in both open and closed systems. Work by Boyd et al. (2002) demonstrated that on stainless steel surfaces, lateral changes of 0.1 µm were sufficient to increase the strength of bacterial attachment. Such surfaces should ideally be free from defects and chemical inhomogeneity in order to minimize microbial attachment. However, Bachmann and Edyvean (2006) suggested that electropolishing of stainless steel pipes for drinking water installations was not necessary, although at joints, welds, dead ends and other features on pipelines, polishing may be necessary since microbial accumulation is more likely at these sites.

## 6.2 Controlling Topography to Manage Fouling

Recently, it has been noted that the shape of surface features is of importance in microbiological binding to a surface (Edwards and Rutenberg 2001; Whitehead et al. 2005, 2006). Since surface topography affects the amount and strength of attachment and retention, several groups have produced surfaces with defined topographies in order to truly assess the interactions. Callow et al. (2002) showed that when using textured surfaces consisting of valleys or pillars, the swimming spores of the

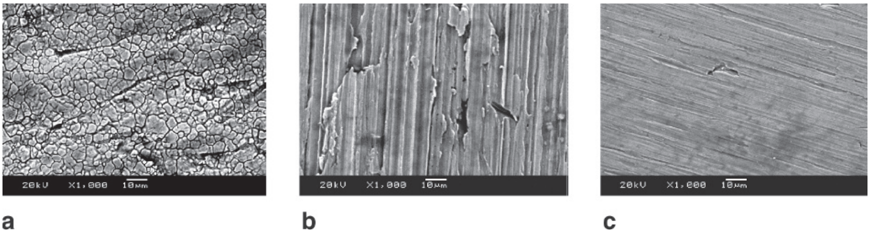

**Fig. 4** SEM of a brushed finish stainless steel surface demonstrating microbial retention within linear features

green fouling alga *Enteromorpha* settled preferentially in valleys and against pillars, and that the number of spores that settled increased as the width of the valley decreased. Surface textural features of 50–100 µm have been shown to be significantly less fouled by barnacles (Andersson et al. 1999); features smaller than the diameter of the barnacle prevented attachment. The attachment of barnacle larvae has similarly been shown to be enhanced or reduced according to the scale, shape and periodicity of surface roughness (Hills and Thomason 1998; Berntsson et al. 2000). Surface roughness has also been shown to influence the settlement behaviour of fouling larvae (Howell and Behrends 2006).

On a smaller scale, a range of engineered surfaces with controlled topographical features, i.e. pits (Whitehead et al. 2004) and grooves (Packer et al. 2007), has been developed to demonstrate the effect of surface topography on cell binding. Using microbial retention assays, Whitehead et al. (2005) demonstrated that with a range of differently sized unrelated microorganisms, the dimensions of the surface feature are important with respect to the size of the cell and its subsequent retention, with maximal retention occurring when features were of a diameter comparable with the microorganisms. This observation was supported when assessing the force of adhesion of the cells on the surfaces using atomic force microscopy (Whitehead et al. 2006). Edwards and Rutenberg (2001) have further recognized that the cross-sectional shape of a groove will have a large effect on binding potential, which is especially important where flow is concerned. Likewise, the orientation of features with respect to the flow or direction of cleaning will affect retention. It should also be noted that, in order to simplify calculations, cells are treated as rigid bodies whereas actually a living cell has a flexible wall and can deform to fit surface features (Beach et al. 2002). The study of the interactions occurring between cells and substratum features of defined dimensions is thus contributing to our understanding of surface fouling at the earliest stages of biofilm formation at both the solid–liquid and solid–air interface. Wear of materials may occur on the nanometer scale (Verran and Boyd 2001). Nanoscale surface features have been shown to affect both bacterial retention (Bruinsma et al. 2002) and cell behaviour (Dalby et al. 2002; Fan et al. 2002; Curtis et al. 2004). It may be speculated that surface nanofeatures will also invariably affect organic soil retention.

## 7 Substratum Conditioning

The first event that occurs when a surface comes into contact with a fluid is the adsorption of molecules to the surface; the molecules attach to the surface more rapidly than the cells, and the composition of the conditioning film is dependent on the composition of the bulk fluid (Hood and Zottola 1995) and of the substratum. Retained soil in surface features may facilitate the attachment of microorganisms to the surface, provide a nutrient source for microorganisms, be indicative of poor hygiene/cleaning processes (Verran et al. 2001b), affect the susceptibility of microorganisms to sanitising agents (Holah 1995), physically protect cells retained in surface defects (Kramer 1992) or provide attachment foci for re-colonization (Storgards et al. 1999).

Considering the effect of initial surface “conditioning” on attachment, retention and survival, adsorbed proteins have been found to either increase or decrease attachment (Carballo et al. 1991; Helke et al. 1993). The specificity of adhesion–receptor interaction is more relevant at solid–liquid interfaces, where the microorganisms can move towards a more advantageous location. At the solid–air interface, the immobilized cells tend to require another surface to facilitate transfer.

The presence of organic material may result in complexation and reduction in activity of some antifouling agents. Previous investigations have shown the majority (>80%) of the total copper in natural water to be complexed to organic matter (Bruland et al. 2000). Once natural sediments bind to a surface and reduce the effect of the antifouling agent, the surface becomes freely available for cell attachment to take place. *In-situ* field measurements on ships hulls on both pleasure crafts and navy vessels have shown lower release rates compared to laboratory tests on panels, most probably as a result of biofilm formation (Valkirs et al. 2003)

## 8 Microbial Resistance, Tolerance and Persistence

To help prevent the development of bacterial resistance, it is essential to understand the ramifications of the use of antimicrobial surfaces and/or cleaning and disinfection products, and to maintain excellent cleaning or management/maintenance protocols. If a cell is able to survive on a surface, resisting cleaning treatment, it can then be a source for biotransfer potential. A number of research reports have expressed concern that use of biocides may contribute to development of antibiotic resistance (Levy et al. 2000; McDonnell et al. 1999). Several workers have reported that the number of mercury-resistant bacteria in soil and aquatic environments varied according to the mercury content of the environment, where in these strains heavy metal-resistance properties were associated with multiple drug resistance (Misra 1992).

There is a vast difference between the magnitude of resistance, tolerance and persistence (RTP) of microorganisms dependent on whether the cells are found in as single units or as colonies, or if the cells are in the protective matrix of a biofilm. When *Pseudomonas aeruginosa* was tested in suspension or following deposition onto metallic or polymeric surfaces to determine the effectiveness of disinfectants (Cavicide, Cidexplus, Clorox, Exspor, Lysol, Renalin and Wavicide) and non-formulated germicidal agents (glutaraldehyde, formaldehyde, peracetic acid, hydrogen peroxide, sodium hypochlorite, phenol and cupric ascorbate) it was found that cells were on average 300-fold more resistant when present on contaminated surfaces than in suspension (Sagripanti and Bonifacino 2000). Further, it was also shown that the surface to which bacteria were attached influenced the effectiveness of disinfectants.

The development of tolerance and resistance to antimicrobial agents is not the focus of this chapter. However, although different challenges face cells at a solid–air interface in comparison with biofilms at solid–liquid interfaces, in either case the potential exists for survival, development of resistance and dissemination.

## 9 Conclusions

The attachment of microorganisms on inert substrata is a key to the development of biofilm at solid–liquid interfaces, and also to the potential for transfer on open surfaces at solid–air interfaces. Although the means for deposition of cells at the surface in these two systems will vary, properties of the substratum such as surface chemistry, surface topography, and the presence of organic (or inorganic) material conditioning the surfaces are essentially common to both systems.

The chemical and physicochemical properties of the substratum are important in initial cell attachment and adhesion, but once biofilm has formed, the underlying substratum has little effect on development – although surface roughness can have a significant effect on cell retention, especially under conditions of flow.

Surface modification designed to produce antifouling surfaces as an independent entity needs to focus on management of initial organic material and cell deposition in order to prevent, control or delay subsequent cell retention and multiplication. Forces used in the cleaning need to overcome those interactions that are active in adhesion of primary organic material and pioneer cells.

A variety of surface modification strategies are being explored, coupled with more fundamental investigations of factors affecting interactions occurring between cells and inert substrata. A multidisciplinary approach between biologists, chemists, physicists, engineers and modellers will facilitate the development of well-engineered and designed surfaces and systems, which are economically viable and environmentally acceptable, to enable optimum control of microbial fouling of surfaces. Promising approaches include those based on superhydrophobic surfaces. At these surfaces, the interplay of surface topography and chemistry results in contact angles approaching 180°. The development of chemically modified surfaces may be advantageous, but the use of chemical species that are detrimental to the surrounding environmental should be avoided. Mass-produced generic “solutions” may not be realistic; antifouling surface design needs to be tailored to individual applications. Although initially time-consuming, this would result in successful application and long-term cost savings.

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