Journal of Dental Research
http://jdr.sagepub.com/

Biofilm Formation on Dental Restorative and Implant Materials

H.J. Busscher, M. Rinastiti, W. Siswomihardjo and H.C. van der Mei

J DENT RES 2010 89: 657 originally published online 6 May 2010
DOI: 10.1177/0022034510368644

The online version of this article can be found at:
http://jdr.sagepub.com/content/89/7/657

Published by:

http://www.sagepublications.com

On behalf of:

International and American Associations for Dental Research

Additional services and information for *Journal of Dental Research* can be found at:

Email Alerts: http://jdr.sagepub.com/cgi/alerts

Subscriptions: http://jdr.sagepub.com/subscriptions

Reprints: http://www.sagepub.com/journalsReprints.nav

Permissions: http://www.sagepub.com/journalsPermissions.nav

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

Biofilm Formation on Dental Restorative and Implant Materials

H.J. Busscher[1], M. Rinastiti[1,2], W. Siswomihardjo[3], and H.C. van der Mei[1]*

[1]Department of Biomedical Engineering, University Medical Center Groningen, and University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands; [2]Department of Conservative Dentistry; and [3]Department of Biomaterials, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia; *corresponding author, h.c.van.der .mei@med.umcg.nl

J Dent Res 89(7):657-665, 2010

ABSTRACT

Biomaterials for the restoration of oral function are prone to biofilm formation, affecting oral health. Oral bacteria adhere to hydrophobic and hydrophilic surfaces, but due to fluctuating shear, little biofilm accumulates on hydrophobic surfaces *in vivo*. More biofilm accumulates on rough than on smooth surfaces. Oral biofilms mostly consist of multiple bacterial strains, but *Candida* species are found on acrylic dentures. Biofilms on gold and amalgam *in vivo* are thick and fully covering, but barely viable. Biofilms on ceramics are thin and highly viable. Biofilms on composites and glass-ionomer cements cause surface deterioration, which enhances biofilm formation again. Residual monomer release from composites influences biofilm growth *in vitro*, but effects *in vivo* are less pronounced, probably due to the large volume of saliva into which compounds are released and its continuous refreshment. Similarly, conflicting results have been reported on effects of fluoride release from glass-ionomer cements. Finally, biomaterial-associated infection of implants and devices elsewhere in the body is compared with oral biofilm formation. Biomaterial modifications to discourage biofilm formation on implants and devices are critically discussed for possible applications in dentistry. It is concluded that, for dental applications, antimicrobial coatings killing bacteria upon contact are more promising than antimicrobial-releasing coatings.

KEY WORDS: oral biofilm, restorative materials, antimicrobials.

DOI: 10.1177/0022034510368644

Received June 29, 2009; Last revision February 18, 2010; Accepted February 25, 2010

INTRODUCTION

Biofilms form on nearly all surfaces exposed to the natural environment (Moons *et al*., 2009). Biofilm formation in the oral cavity is undoubtedly the most well-known example. Controlling oral biofilm formation is an everlasting daily struggle for all of us. Biofilms form not only on dental hard and soft tissues, as the major cause of caries and periodontal diseases (Sbordone and Bortolaia, 2003), but also on the multitude of biomaterial surfaces used for the restoration of function in the oral cavity. Although, at first glance, biofilm formation on biomaterial surfaces in the oral cavity may appear relatively harmless, dependent on its location, its consequences may be severe. Similar to the development of periodontitis, biofilms on dental implants may lead to peri-implantitis (Grossner-Schreiber *et al*., 2009). An overhanging Class II restoration located in the gingival margin is prone to bacterial colonization, with an impact on gingival health (Jansson *et al.,* 1994; Joseph *et al.,* 1997; Cenci *et al*., 2006). Biofilm formation on composite resins not only degrades the material and roughens its surface (Beyth *et al.,* 2008), but also causes colonizing bacteria to invade the interface (Carvalho *et al.*, 1996) between the restoration and the tooth, leading to secondary caries (Collins *et al*., 1998) and pulp pathology (Pashley, 1990). Biofilms around brackets in orthodontic treatment may cause demineralization of the surrounding enamel as a negative side-effect of the treatment (Mitchell, 1992; Papaioannou *et al*., 2007). Consequently, the interest in new dental materials attracting less biofilm or releasing antimicrobial compounds is increasing.

The scope of this review is confined to the biomaterials properties affecting biofilm formation in the oral cavity, and the performance of different materials, including possible preventive, biomaterial-associated measures. At this point, it is important to emphasize that biofilm formation in the oral cavity always occurs on an existing conditioning film consisting of adsorbed salivary proteins preceding adhesion of the first colonizing micro-organisms, unless in cases of severe xerostomia. This review is not intended to deal with salivary protein adsorption as a separate step in biofilm formation, and it suffices to say that salivary protein adsorption equalizes differences in surface properties of different materials to an extent determined by the thickness (Hannig and Hannig, 2009), composition (Vroman, 2008), and conformation of proteins in the adsorbed layer (Norde, 2008).

MECHANISMS OF BIOFILM FORMATION

Despite many decades of research, a generally valid mechanism for bacterial adhesion to and biofilm formation on surfaces does not yet exist (Bos *et al*.,

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

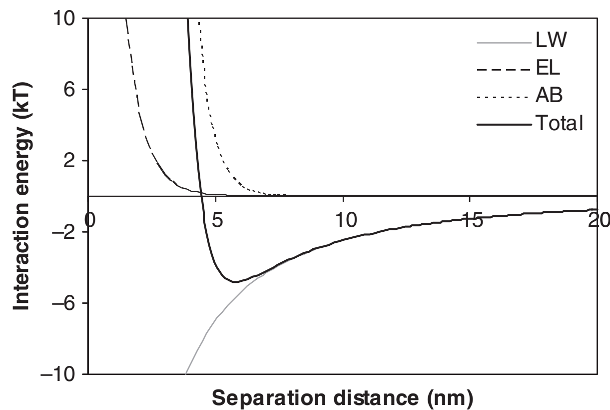

Figure 1. Example of the DLVO-interaction energy as a function of distance for a situation of electrostatic repulsion between bacteria and substratum surfaces and acid-base attraction. Lifshitz-Van der Waals component (LW); acid-base component (AB); electrostatic component (EL); total interaction energy (Total). Note that a negative-interaction energy indicates that adhesion is favorable.

1999; Hermansson, 1999). The forces that mediate bacterial adhesion to surfaces, and for that matter also the adsorption of selective salivary proteins (Hannig and Hannig, 2009), have been reasonably well identified in the past, and include ubiquitously present attractive Lifshitz-Van der Waals forces, electrostatic interactions, and acid-base bonding. According to the extended DLVO theory (Van Oss, 1995), named after Derjaguin-Landau-Verweij and Overbeek, these forces can be combined, and a distance-dependent interaction energy can be calculated (Fig. 1). Most naturally occurring surfaces are negatively charged and electron-donating, while possessing a small hydrogen-donating component. Due to the small hydrogen-donating surface component, the interaction energy at close approach is dominated by acid-base attraction, unless strong mono-polar repulsion exists.

In vitro, relationships have been described between substratum hydrophobicity, surface free-energy and charge, depth of DLVO-interaction energy minima, or surface roughness and numbers of adhering bacteria. However, the number of studies describing exceptions is equally as high as the number of studies establishing these relationships (Bos *et al.,* 1999; Bakker *et al*., 2004). Often these relationships cover a range of a factor of 2 to 3 in bacterial adhesion numbers. The differences between substrata with different properties rapidly disappear once the adhering bacteria are allowed to start growing into a more mature biofilm, and larger differences in adhesion numbers by at least 1 or 2 log-units are probably required for an effect on biofilm formation. *In vitro* relationships also depend heavily on experimental conditions, like the presence of shear, conditioning of substrata by an adsorbed salivary protein film, and the bacterial strain or combination of strains used (Bakker *et al*., 2004). Moreover, although having been a focal point in the past, it is not *a priori* obvious that the depth of any interaction energy minimum should relate to numbers of adhering bacteria. Possibly, bacterial binding forces or the ease with which adhering bacteria or biofilms can be detached from substrata is more likely to relate to the different features of DLVO-interaction energy curves.

In vivo, the situation may appear more complex than *in vitro*, but, surprisingly, *in vivo* research has turned out to be much more decisive in indicating the parameters that influence oral biofilm formation (Glantz, 1969). Whereas *in vitro*, bacteria adhere to virtually every surface, regardless of its properties, supragingivally hydrophobic surfaces in the oral cavity attract far less plaque than more hydrophilic ones, as has been established over a nine-day time period (Quirynen *et al*., 1989; Fig. 2). This observation has been confirmed in a study on oropharyngeal biofilm formation on voice prostheses (Everaert *et al*., 1997), encompassing a timescale of six weeks (Fig. 2). Interestingly, these timescales allow for the extensive adsorption of salivary conditioning films, interactions with dietary components, and adhesion of multiple bacterial strains and species (note that biofilms on silicone rubber voice prostheses also contain *Candida* species). However, a hydrophobic surface harvests far less biofilm than a more hydrophilic one. Most likely, this is due to the fluctuating shear conditions in the oral cavity, since *in vitro* bacteria also adhere to hydrophobic surfaces, at least under constant shear conditions (Boks *et al*., 2009). Hydrophobic surfaces placed subgingivally, for instance, do not harvest significantly less biofilm than hydrophilic surfaces (Quirynen *et al.*, 1990), which suggests that, under fluctuating shear, as exists supragingivally, biofilm is occasionally sloughed off during periods of high oral shear. This is notwithstanding the fact that bacteria do adhere to hydrophobic surfaces.

Nearly all studies indicate that, under *in vivo* conditions, smooth surfaces attract less biofilm than rough ones (Verran and Maryan, 1997; Teughels *et al*., 2006). From a series of split-mouth studies, it could be concluded that an increase in surface roughness above a threshold of 0.2 μm and/or an increase in surface free-energy facilitates biofilm formation on restorative materials. When both surface characteristics interact with each other, surface roughness was found to be dominant (Teughels *et al*., 2006).

BIOFILMS ON RESTORATIVE MATERIALS

Biofilms on Acrylic Resin Denture Base

Acrylic resin or polymethyl methacrylate has a wide variety of applications, such as impression trays, artificial teeth, and denture bases. The material was developed in 1928 in various laboratories and was brought to market in 1933 by the Rohm and Haas Company. Soon after its introduction to the market, the material was widely used by the dental profession, and by 1946, 98% of all dentures were constructed from methyl methacrylate polymers (Powers and Sakaguchi, 2006).

One of the main clinical problems associated with the use of acrylic dentures is the adhesion of *Candida,* which can lead to stomatitis (Ramage *et al.*, 2004), although bacteria also adhere to acrylic surfaces (Verran and Motteram, 1987).

Stomatitis is an inflammation of the mucosa, usually stimulated by the presence of (mainly maxillary) dentures in elderly and immunocompromised patients, causing a burning pain and altered

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

taste sensation. The predominant oral yeast isolated from dentures has been found to be *Candida albicans* (75%), but *Candida glabrata* has also been found (30%), and in higher proportions in patients with the highest grades of inflammation. However, *Candida dubliniensis*, *Candida parapsilosis*, *Candida krusei,* and *Candida tropicalis* have also been isolated (Coco *et al*., 2008).

C. albicans is a dimorphic fungus that is commensal in the gastro-intestinal and reproductive tracts of healthy individuals (Elguezabal *et al*., 2008) and is capable of initiating a variety of recurring superficial diseases, especially in the oral mucosa (Samaranayake and Samaranayake, 2001). *In vitro, C. albicans* has been described as able to form a biofilm on biomaterial surfaces (Chandra *et al*., 2005). *C. albicans* in the oral cavity is mostly detected in a mixed biofilm with bacteria (Holmes *et al*., 1995; Bamford *et al*., 2009). Different bacterial strains have been identified in denture biofilms as well (*Streptococcus*, *Veillonella*, *Lactobacillus*, *Prevotella,* and *Actinomyces* spp.) (Koopmans *et al*., 1988). It has been suggested that bacterial adhesion enhances subsequent adhesion of *Candida* (Verran and Motteram, 1987), which is likely, since bacteria can already be found within hours of exposure of an acrylic surface to the oral environment, while yeasts are observed only after days (Avon *et al*., 2007). Moreover, most commensal oral *Streptococcus* species possess antigen I/II, a cell-wall-anchored protein receptor mediating binding to specific partner micro-organisms, including *C. albicans* (Bamford *et al*., 2009).

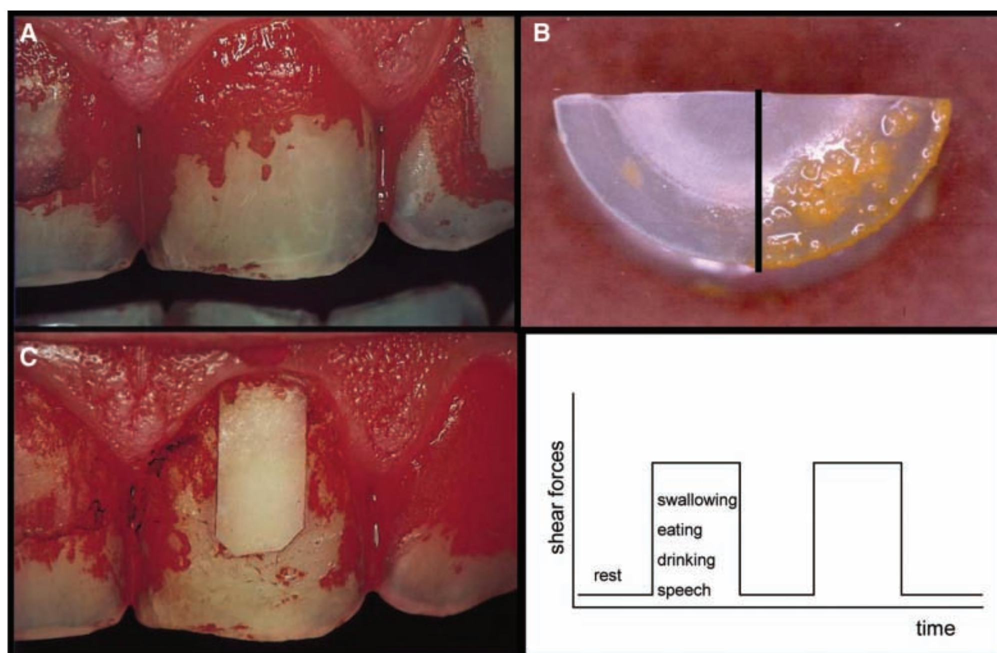

Figure 2. The role of substratum hydrophobicity and fluctuating shear in biofilm formation in the oral and oropharyngeal cavity. **(A)** Nine-day undisturbed plaque formation on a human front incisor (intermediate hydrophobicity) and a Teflon strip (hydrophobic) glued to a front incisor (from Quirynen *et al*., 1989). **(B)** Six-week biofilm formation on a dual-sided hydrophobic (left-hand side)-hydrophilic (right-hand side) voice prosthesis (from Everaert *et al*., 1997). **(C)** Shear forces in the oral cavity can vary during the day between wide ranges, creating periods of bacterial adhesion and detachment (from Busscher *et al*., 1992).

Mechanical or chemical removal of fungal biofilms represents a significant clinical problem (Chandra *et al*., 2005), since yeasts are known to adhere quite strongly to denture base materials (Nalbant *et al*., 2008), possibly as a result of the microporosity on the denture surface. Indeed, *C. albicans* adhesion is enhanced if the roughness of denture base materials is increased (Radford *et al.,* 1998). Denture-cleansing solutions can not only kill oral streptococci, but also condition the acrylic surface. Cleanser conditioning of acrylic surfaces *in vitro* encouraged the formation of a multilayer biofilm of *S. oralis,* which was easier to remove than the monolayer biofilm formed on water-treated acrylic (Morgan and Wilson, 2000). Considering that bacterial adhesion may be a necessary step for fungal biofilms to form, removal of a streptococcal biofilm may prevent the formation of a more pathogenic biofilm also involving yeast. Recently, it has been demonstrated that *C. albicans* biofilm formation on polyethylene-oxide-modified denture base materials is discouraged *in vitro* (Chandra *et al*., 2005). Whether these observations can be extrapolated to the clinical situation remains to be seen, since it has been suggested that the shift from a commensal bacterial biofilm to a more pathogenic biofilm, also involving yeasts, is more influenced by mucosal inflammation and the general condition of the patient than by the denture material and its surface properties (Avon *et al*., 2007).

Biofilms on Metallic Biomaterials

There is limited knowledge about possible differences in the mechanisms of bacterial adhesion to metal surfaces as compared with non-conducting polymer surfaces, but it has been suggested that electron-transfer plays a role in bacterial adhesion to conducting materials, like gold and amalgam (Poortinga *et al*., 1999). In addition, upon approach of a negatively charged bacterium to a conducting material, an oppositely charged image may develop in the conducting material, creating a strong electrostatic attractive force (Mei *et al*., 2009).

Five-day-old oral biofilms on gold and amalgam surfaces *in vivo* are known to be thick and fully covering the substratum surfaces, but, in contrast, were found to be barely (less than 8%) viable (Auschill *et al*., 2002). For comparison, the viability of oral biofilms on enamel *in vivo* was between approximately 41% and 56% (Van der Mei *et al*., 2006). Earlier work showed that pieces of amalgam placed intra-orally for 24 and 72 hrs attracted about half the number of viable bacteria than titanium oxide (Leonhardt *et al*., 1995). The low viability of oral biofilms on amalgam surfaces is probably due to the release of toxic

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

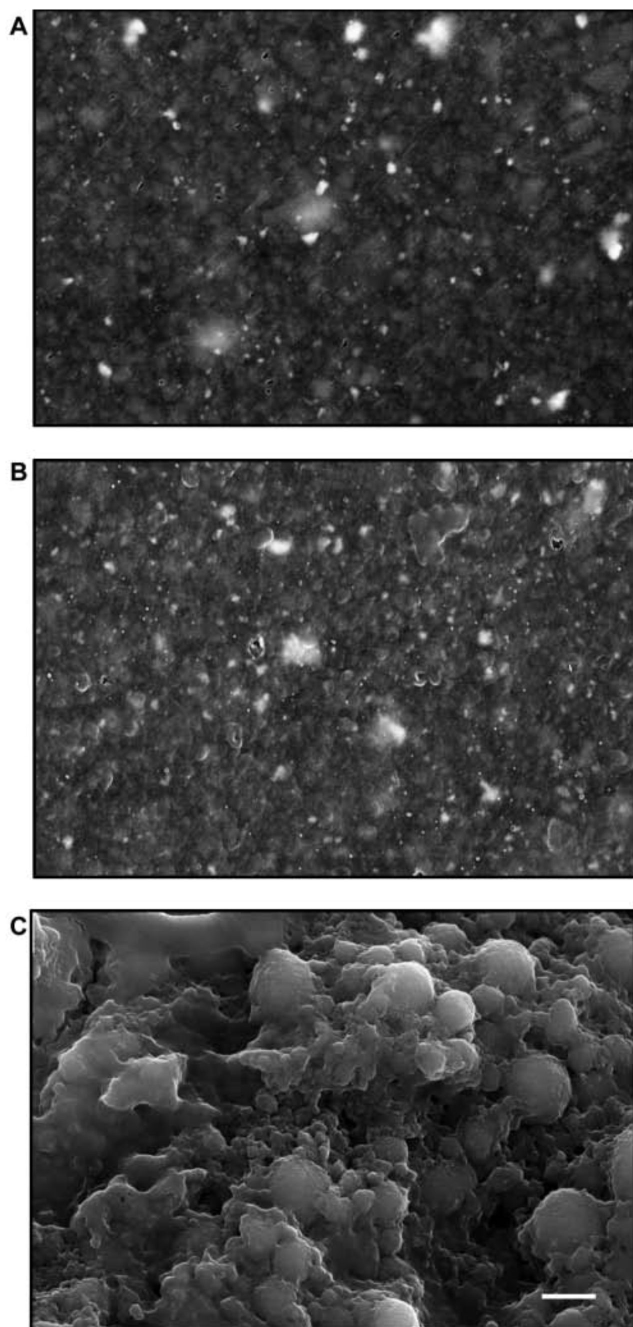

Figure 3. Scanning electron micrographs of nanohybrid-resin composite surfaces prior to **(A)** and after biofilm formation for 14 days *in vitro* **(B)** and after having been 180 days *in vivo* **(C).** Bar marker represents 2 µm.

compounds from the alloy. Amalgam consists of approximately 50% mercury and 35% silver, which may slowly diffuse from the amalgam into the biofilm. There is no evidence that sufficient Ag(0) is released from amalgam and oxidized to Ag(I) to have an antimicrobial effect (Silver, 2003), and antimicrobial effects of amalgam should be attributed to mercury Hg(II). Thus, it also becomes possible that bacteria develop resistance against mercury. *In vitro*, more bacteria resistant to mercury were found in microcosm oral biofilms grown on amalgam than on enamel. The levels of these mercury-resistant bacteria remained elevated for a period of 48 hrs, but after 72 hrs, the proportions returned to baseline levels. Of the 42 mercury-resistant bacterial strains isolated, 98% were streptococci, with *Streptococcus mitis* predominating (Ready *et al*., 2007). Interestingly, resistance to mercury was concurrent with resistance to several antibiotics, most notably tetracycline. The genes encoding for resistance to metals such as mercury were found on the same mobile genetic elements as resistance to antibiotics. Note that although the release of mercury from amalgam restorations may stimulate antibiotic resistance in oral bacteria, mercury-resistant bacteria were found in 71% of children without amalgam fillings and previous exposure to amalgam (Ready *et al*., 2003).

The low viability (less than 2%) of oral biofilms on gold cannot be due to the release of toxic compounds, since gold is completely inert (Auschill *et al*., 2002). Possibly, full coverage by a relatively thick biofilm hampers the supply of nutrients to the biofilm, leading to low viability.

Biofilms on Ceramics

Little is known about biofilms on ceramic surfaces. Inlays of two types of ceramic surfaces collected less plaque with reduced viability over a three-day period of no oral hygiene than did the natural tooth surface (Hahn *et al*., 1993). Compared with gold and amalgam, however, attracting 11- to 17-µm-thick biofilms, biofilms on ceramic biomaterials formed *in vivo* during 5 days (Auschill *et al*., 2002) were relatively thin (1-6 µm), but highly viable (from 34% to 86%). Note that this supports the suggestion above, that thick biofilms are less viable than thin ones, due to a hampered supply of nutrients to a thick biofilm.

Biofilms on Resin Composites and Glass-ionomer Cements

Biofilm formation on resin composites and glass-ionomer cements leads to a negative spiral of events (Beyth *et al*., 2008), in which the colonizing organisms cause severe deterioration of the surface (Fig. 3), which, in turn, promotes biofilm formation and therewith more extensive deterioration of the surface. The clinical manifestation of this downward spiral is the development of caries around or below a restoration (Sousa *et al*., 2009).

Surface deterioration of resin composites and glass-ionomer cements has been demonstrated by increased roughness, effects on filler particle exposure, and sometimes by a decreased microhardness of the materials upon exposure to biofilms *in vitro*. After one month's exposure to a *Streptococcus mutans* ATCC27351 biofilm, a bisphenol A glycidyl methacrylate, urethane dimethacrylate resin composite with filler particle sizes between 0.04 and 0.2 µm showed an increase in surface roughness from less than 10 to above 40 nm without affecting the microhardness, suggesting the removal of filler particles based on the roughness dimensions created. Resin composites with larger (0.01 to 3.5 µm) filler particles became significantly less

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

rough (around 15 nm) after biofilm growth (Beyth *et al*., 2008). In a similar study, one month's exposure to *S. mutans* biofilms showed an increase in surface roughness, from around 0.1 μm to well over 1 μm, of resin-modified and conventional glass-ionomer cements, while only the microhardness of the resin-modified cement was negatively affected (Fucio *et al*., 2008). Clearly, the *in vivo* presence of biofilm is just one of the factors that may stimulate surface degradation, other factors being acidic fluid intake, temperature fluctuations, or simply the presence of an aqueous environment.

The degree of conversion of resin composites is never complete, and approximately 5% to 10% of unpolymerized monomer can be extracted in water. It has been suggested that especially the release of ethyleneglycol dimethylacrylate and triethyleneglycol dimethacrylate from composite resins may enhance the growth of cariogenic bacteria, like mutans streptococci and lactobacilli, organisms found mostly along the margins of composite fillings (Hansel *et al*., 1998), and also enhance the glucosyltransferase activity in *Streptococcus sobrinus* (Kawai and Tsuchitani, 2000). More recently, Khalichi *et al*. (2009) found that triethyleneglycol, as the ether portion of triethyleneglycol dimethacrylate, modulates the expression levels of glucosyltransferase B involved in biofilm formation and yfiV as a putative transcription regulator gene in *S. mutans*. Interestingly, Takahashi *et al*. (2004) found that growth stimulation of *S. sobrinus* and *Streptococcus sanguis* by ethyleneglycol dimethylacrylate monomers as measured by optical density was not accompanied by an increase in the numbers of colony-forming units harvested. Consequently, the increase in optical density was ascribed to an increased size of the bacteria rather than to increased bacterial concentrations, which could be microscopically confirmed by the presence of a vesicular material surrounding the bacteria. Extraction and chemical analysis of this vesicular material showed a composition comparable with that of ethyleneglycol dimethylacrylate-polymer. Effects of monomer release became smaller when the light-curing time of the composites was increased (Brambilla *et al*., 2009; Khalichi *et al*., 2009). Also, components of dentin-bonding agents, such as hydroxyethyl methacrylate or triethyleneglycol dimethacrylate, have been shown to stimulate the growth of cariogenic organisms like *S. sobrinus* and *Lactobacillus acidophilus* (Schmalz *et al.*, 2004). Direct extrapolation of these *in vitro* studies to the clinical situation is difficult, since composite surfaces are usually polished, affecting the surface properties (Carlen *et al.,* 2001), while, most importantly, the volume in which monomers are released is large and continuously refreshed by salivary excretion and fluid intake.

The setting of glass-ionomer cements is *via* an acid-base reaction between fluoroaminosilicate glass particles and a polyacrylic acid solution, yielding a structure that is dimensionally more stable than composites. Hence, the use of glass-ionomer cements potentially reduces microleakage by adhering to tooth structure and enhances fluoride release with a potential impact on oral biofilm formation. Fluoride release occurs through an initially high burst release that may be between 1.6 and 1.8 μg/mm^2, after which a prolonged, long-term tail-release follows (Wiegand *et al*., 2007).

Fluoride can act as a buffer to neutralize acids produced by bacteria (Nicholson *et al.*, 2000) and suppresses the growth of caries-related oral bacteria (Nakajo *et al.*, 2009). Glass-ionomer cement indeed collects a thin biofilm with a low viability (2% to 3%), possibly as a result of fluoride release (Auschill *et al*., 2002). Levels of streptococci in particular, including *S. mutans* (Seppä *et al.*, 1995) and *S. sanguis* (Hengtrakool *et al*., 2006), appear reduced. However, an *in vitro* study (Al-Naimi *et al*., 2008) also showed that glass-ionomer cements containing fluoride did not reduce the amount of bacterial growth and biofilm formation on the surfaces bathed in saliva. This suggests that either fluoride is not a dominant factor in controlling biofilm formation, or that its concentration is too low to be effective, depending on the ratio between cement area and fluid volume in which the experiments were carried out. In the oral cavity, the large volume of saliva present, that is subject to wash-out (Wiegand *et al.*, 2007), makes the build-up of an effective fluoride concentration difficult. Nevertheless, 10x-elevated levels of fluoride were found with respect to baseline one year after the placement of one to six glass-ionomer restorations (Hatibovic-Kofman and Koch, 1991), but this may not be enough to anticipate antimicrobial effects. Hence, the benefits of fluoride release may be confined to the inhibition of demineralization.

Biomaterial-associated Infections Elsewhere in the Body and Their Prevention

Biofilms not only form on biomaterials in the oral cavity, but also constitute a general problem for biomaterial implants and devices throughout the human body. Mechanistically, however, there are major differences between biofilm formation in the oral cavity and elsewhere in the body. The oral cavity is designed to be colonized by a large variety of different microbial strains and species, and disease results only from an imbalance between the different strains and species (De Soete *et al*., 2005). Saliva is loaded with a high concentration of micro-organisms of about 10^9 colony-forming units *per* mL^{-1} (Petti *et al*., 2001), but biomaterial implants in, for example, the hips, knees, and vascular grafts are placed in an environment that is normally completely devoid of micro-organisms. Most oral biofilms are regularly removed by toothbrushing or other oral hygiene measures, and the sequence of spatio-temporal build-up of oral biofilm starts anew once or twice *per* day (Palmer *et al*., 2003), although not necessarily in fissures, interproximal spaces, and subgingival pockets. Mechanical removal of biofilm from biomedical implants and devices is clearly impossible without surgical intervention. Micro-organisms are able to colonize biomaterial implant surfaces after having gained access to the implant site during surgery ("peri-operative contamination"), during hospital treatment before complete wound closure ("post-operative contamination"), or through hematogenous spreading from infections elsewhere in the body (Busscher *et al*., 2009). Thus, whereas surfaces in the oral cavity are continuously challenged by high concentrations of micro-organisms, microbial threats to biomaterial implants usually occur by low-challenge concentration during a limited amount of time. Causative organisms are frequently commensals of the skin, like *Staphylococcus epidermidis* or *Staphylococcus aureus*.

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

Micro-organisms on biomaterial implants can remain in a dormant state for several years after entry into the body (Gorecki and Babiak, 2009) and develop into a biofilm with clinical signs of infections when the host is immunocompromised, such as during illness or extreme fatigue. As in the oral cavity, organisms in a biofilm on an implant surface are protected against the host immune system and antibiotic attacks. Therefore, infected implants and devices generally have to be replaced, which creates a significant burden on the treating physician and great discomfort to the patient. Moreover, replacement is often much more expensive than primary placement of an implant (Gorecki and Babiak, 2009).

Since the pioneering concept of "the race for the surface" was launched by the late orthopedic surgeon A.G. Gristina (1987), describing the fate of an infected biomaterial implant as a race for the surface between tissue integration and biofilm formation, preventive measures have aimed primarily at discouraging biofilm formation. To this end, a variety of approaches has been developed, including different anti-adhesive and antimicrobial coatings as well as antibiotic-release systems.

Antibiotic-releasing Systems

Antibiotic-releasing bone cement is widely used in total hip and knee arthroplasties to achieve high local concentrations of antibiotics that cannot be achieved by systemic administration (Buchholz and Engelbrecht, 1970). Bone cements are acrylic-based and can be loaded with antibiotics up to 2 wt%, without affecting their weight-bearing capacities. In non-weight-bearing applications, like the treatment of osteomyelitis, higher antibiotic loadings are used (Kűhn, 2000). Although gentamycin-loaded bone cements have proven efficacy in the control of artificial joint infections (Furnes *et al*., 2001), their use is not entirely without controversy. Antibiotic release occurs through an initially high, so-called "burst" release, which is needed to effectively kill micro-organisms introduced peri-operatively or early post-operatively, but antibiotic release is never complete. The low tail-release can continue for several years without yielding effective concentrations in the tissue and has been associated with the development of bacterial resistance (Van de Belt *et al*., 1999). In fact, gentamycin-loaded bone cement has been demonstrated to release antibiotics even five years after implantation (Neut *et al*., 2003). The pros and cons of antibiotic-releasing bone cements are valid for any system releasing an antimicrobial, including fluoride-releasing composites and cements. Particularly, the conditions in the oral cavity will make it difficult to create effective concentrations beyond the initial burst release.

Silver Coatings

Silver and its salts have been the most commonly used antimicrobial agent applied to urinary catheters and wound bandages. Solubilized silver ions constitute the bioactive form, and can be released in different ways from silver-containing coatings, yielding mixed clinical results (Wu and Grainger, 2006). Sustained release of bioactive Ag(I) silver cations over time periods of several days to weeks is difficult to obtain, and there is fear that silver resistance will lessen its usefulness (Silver, 2003). Moreover, both *in vitro* and *in vivo,* the antimicrobial efficacy of silver-releasing coatings depends on the Ag(I) concentration that can be achieved, which depends in turn on the fluid volume into which silver is released and whether fluid is refreshed. This is probably the reason that the antibacterial efficacy of a silver-based glass-ionomer cement (Ketac-Silver) has been limited to *in vitro* experiments (Herrera *et al*., 2000).

Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) are widely used as antimicrobial agents to inhibit microbial growth. The antimicrobial activity provided by QACs results from both ionic and hydrophobic interactions between the QAC and components of the microbial cell wall that leads to cell death or malfunction in cellular processes (Majumdar *et al*., 2009). A promising feature of QACs is that they continue to possess antibacterial properties when coupled to a surface (Murata *et al*., 2007) without release into the body. Contact killing by QAC coatings, however, leaves a layer of dead bacteria to which newly arriving bacteria can adhere. Therefore, on biomedical implants and devices, QAC coatings are applicable only for single bacterial challenges, or when the immune system helps to remove dead bacteria (Roosjen *et al*., 2006). For application in the oral cavity, removal of dead bacteria might be achieved by brushing, and QAC coatings may turn out to be useful.

Polymer Brushes

Polymer-brush coatings are currently the most promising non-adhesive coatings, since they reduce the initial adhesion of various bacterial strains and yeasts by several log-units, both in terms of adhesion numbers as well as in terms of adhesion forces (Roosjen *et al*., 2006). A polymer brush is formed when hydrophilic polymer chains are end-grafted to a surface in high density, forcing the polymer chains to stretch away from the surface into the adjacent medium. Compression of such a structure upon microbial approach gives rise to an osmotic pressure and decreased mobility (conformational entropy) of the polymer chains in the brush, which causes repulsion of approaching micro-organisms. Since polymer-brush coatings are usually also repulsive toward protein adsorption (Halperin *et al*., 2007), salivary proteins do not necessarily interfere with the non-adhesive functionality of a brush, dependent on the brush length and the grafting density (Fundeanu *et al*., 2010). Since polymer-brush coatings hitherto have presented the only coatings showing log-unit reductions in initial bacterial adhesion, it is anticipated that these differences will translate into reduced biofilm formation, with prospects for oral application.

Bi-functional Coatings

Most coatings for biomaterial implants and devices are mono-functional, *i.e.,* aimed solely at discouraging biofilm formation or enhancing tissue integration. New approaches include bi-functional coatings containing anti-adhesive functionalities, such as a polyethylene glycol polymer brush to discourage biofilm formation, while at the same time possessing functionalities like arginine-glycine-aspartic acid sequences to support tissue integration (Maddikeri *et al*., 2008). Bi-functional coatings on

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

titanium implants may be especially promising for the prevention of peri-implantitis, since the fate of dental implants also depends on a race for the surface (Gristina, 1987) between biofilm formation and tissue integration.

Dental-Material-Associated Preventive Measures

Enhanced fluoride-releasing composites have been developed to reduce biofilm formation and its effects on the surrounding enamel. Fluoride release can be established through the incorporation of water-soluble salts, fluoride-releasing filler systems, or matrix-bound fluoride (Wiegand *et al*., 2007) and requires penetration of water into the polymer matrix, which can be stimulated by increasing matrix hydrophilicity through the introduction of 2-hydroxyethyl methacrylate (Chan *et al*., 2006). The amount of fluoride released from composites is lower than that from glass-ionomer cement and decays over time (Yap *et al.*, 1999), as described above for antibiotic-loaded bone cements. However, clinical studies have reported conflicting data as to whether fluoride-releasing materials significantly prevent or inhibit secondary caries and affect the growth of caries-associated bacteria compared with non-fluoridated restoratives (Wiegand *et al*., 2007). As is often the case with antimicrobial release systems, these conflicting results may be due to experimental differences in area of the releasing material *vs.* the fluid volume into which the antimicrobial is released and possible wash-out.

Phosphate-based glasses have also been loaded with silver and found to reduce the viability of 300-µm-thick adherent biofilms of *S. sanguis* (Mulligan *et al*., 2003). Interestingly, these experiments were done in a constant-depth film fermenter with silver-containing glass discs placed on the bottom of each well, thus ensuring release of silver directly into the biofilm. Evidently, in the oral cavity, with a larger volume than a constant-depth film fermenter well, there will be a much more pronounced wash-out, and it is doubtful whether a similar release of silver would still show efficacy. This is a general drawback of antimicrobial-releasing materials, particularly in the oral cavity, although glass ionomers have a high fluoride rechargeability upon daily exposure to fluoride-containing dentifrices (Wiegand *et al*., 2007).

A more promising approach is to immobilize antibacterial components on biomaterials surfaces in a way that maintains antibacterial efficacy. For that reason, resin composites have been modified by the addition of antibacterial components, such as 12-methacryloyloxydodecylpyridinium bromide combined with quaternary ammonium and a methacryl group in the resin matrix. This material inactivated bacteria upon coming into contact with its surface, hence inhibiting biofilm formation on the composite surface (Imazato and McCabe, 1994; Imazato *et al.*, 1994, 1999), even after contact with saliva (Ebi *et al*., 2001). Whether these modifications yield clinically significant results under the dynamic conditions of the oral cavity remains to be seen (Imazato, 2003). For titanium implants, anodization by discharge in a NaCl solution has been suggested to reduce adhesion of viable bacteria, since peroxidation products of Ti-Cl were able to destroy surface structures of adhering bacteria (Omori *et al*., 2009), but here too, clinical efficacy remains to be demonstrated.

ACKNOWLEDGMENTS

This study was supported by the University Medical Center Groningen-University of Groningen, Groningen, The Netherlands.

REFERENCES

Al-Naimi OT, Itota T, Hobson RS, McCabe JF (2008). Fluoride release for restorative materials and its effect on biofilm formation in natural saliva. *J Mater Sci Mater Med* 19:243-1248.

Auschill TM, Arweiler NB, Brecx M, Reich E, Sculean A, Netuschil L (2002). The effect of dental restorative materials on dental biofilm. *Eur J Oral Sci* 110:48-53.

Avon SL, Goulet J-P, Deslauriers N (2007). Removable acrylic resin disk as a sampling system for the study of denture biofilms *in vivo*. *J Prosthet Dent* 97:32-38.

Bakker DP, Postmus BR, Busscher HJ, Van der Mei HC (2004). Bacterial strains isolated from different niches can exhibit different patterns of adhesion to substrata. *Appl Environ Microbiol* 70:3758-3760.

Bamford CV, d'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF (2009). *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. *Infect Immun* 77:3696-3704.

Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI (2008). *Streptococcus mutans* biofilm changes surface-topography of resin composites. *Dent Mater* 24:732-736.

Boks NP, Kaper HJ, Norde W, Van der Mei HC, Busscher HJ (2009). Mobile and immobile adhesion of staphylococcal strains to hydrophilic and hydrophobic surfaces. *J Colloid Interface Sci* 331:60-64.

Bos R, Van der Mei HC, Busscher HJ (1999). Physico-chemistry of initial microbial adhesive interactions—its mechanisms and methods for study. *FEMS Microbiol Rev* 23:179-230.

Brambilla E, Gagliani M, Ionescu A, Fadini L, García-Godoy F (2009). The influence of light-curing time on the bacterial colonization of resin composite surfaces. *Dent Mater* 25:1067-1072.

Buchholz HW, Engelbrecht H (1970). Über die depot wirkung einiger antibiotica bei vermischung mit dem kunstharz Palacos. *Chirurg* 40:511-515 [article in German].

Busscher HJ, Cowan MM, Van der Mei HC (1992). On the relative importance of specific and non-specific approaches to oral microbial adhesion. *FEMS Microbiol Rev* 88:199-209.

Busscher HJ, Ploeg RJ, Van der Mei HC (2009). SnapShot: biofilms and biomaterials; mechanisms of medical device related infections. *Biomaterials* 30:4247-4248.

Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J (2001). Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. *Biomaterials* 22:481-487.

Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH (1996). A review of polymerisation contraction: the influence of stress development versus stress relief. *Oper Dent* 21:17-24.

Chan WD, Yang L, Wan W, Rizkalla AS (2006). Fluoride release from dental cements and composites: a mechanistic study. *Dent Mater* 22:366-373.

Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, *et al*. (2005). Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Appl Environ Microbiol* 71:8795-8801.

Cenci MS, Lund RG, Pereira CL, de Carvalho RM, Demarco FF (2006). *In vivo* and *in vitro* evaluation of Class II composite resin restorations with different matrix systems. *J Adhes Dent* 8:127-132.

Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G (2008). Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol* 23:377-383.

Collins CJ, Bryant RW, Hodge KL (1998). A clinical evaluation of posterior composite resin restorations: 8-year findings. *J Dent* 26:311-317.

De Soete M, Dekeyser C, Pauwels M, Teughels W, Van Steenberghe D, Quirynen M (2005). Increase in cariogenic bacteria after initial periodontal therapy. *J Dent Res* 84:48-53.

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

Ebi N, Imazato S, Noiri Y, Ebisu S (2001). Inhibitory effect of resin composite containing bactericide-immobilized filler on plaque accumulation. *Dent Mater* 17:485-491.

Elguezabal N, Maza JL, Dorronsoro S, Ponton J (2008). Whole saliva has a dual role on the adherence of *Candida albicans* to polymethylmethacrylate. *Oper Dent J* 2:1-4.

Everaert EP, Mahieu HF, Wong Chung RP, Verkerke GJ, Van der Mei HC, Busscher HJ (1997). A new method for *in vivo* evaluation of biofilms on surface-modified silicone rubber voice prostheses. *Eur Arch Otorhinolaryngol* 254:261-263.

Fucio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppin-Rontani RM (2008). The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials—An *in vitro* study. *J Dent* 36:833-839.

Fundeanu I, Van der Mei HC, Schouten AJ, Busscher HJ (2010). Microbial adhesion to surface-grafted polyacrylamide brushes after long-term exposure to PBS and saliva. *J Biomed Mater Res* (in press).

Furnes O, Havelin LI, Espehaug B (2001). Effect of type of bone cement and antibiotic prophylaxis on early revision of cemented total hip replacement presentation from the Norwegian arthroplasty register 1987-1996. In: *Bone cement and cementing techniques*. Walenkamp GIHM, Murray DW, Henz U, Kock HJ, editors. Berlin: Springer, pp. 135-142.

Glantz PO (1969). On wettability and adhesiveness. *Odontol Rev* 20(Suppl 17):1-132.

Gorecki A, Babiak I (2009). Infection of joint prostheses and local drug delivery. In: *The infected implant*. Kienapfel H, Kűhn KD, editors. Heidelberg: Springer, pp. 19-26.

Gristina AG (1987). Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* 237:1588-1595.

Grossner-Schreiber B, Teichmann J, Hannig M, Dorfer C, Wenderoth DF, Ott SJ (2009). Modified implant surfaces show different biofilm compositions under *in vivo* conditions. *Clin Oral Implants Res* 20:817-826.

Hahn R, Weiger R, Netuschil L, Brüch M (1993). Microbial accumulation and vitality on different restorative materials. *Dent Mater* 9:312-316.

Halperin A, Fragneto G, Schollier A, Sferrazza M (2007). Primary versus ternary adsorption of proteins onto PEG brushes. *Langmuir* 23:10603-10617.

Hannig C, Hannig M (2009). The oral cavity—a key system to understand substratum-dependent bioadhesion on solid surfaces in man. *Clin Oral Investig* 13:123-139.

Hansel C, Leyhausen G, Mai UE, Geurtsen W (1998). Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms *in vitro*. *J Dent Res* 77:60-76.

Hatibovic-Kofman S, Koch G (1991). Fluoride release from glass ionomer cements *in vivo* and *in vitro*. *Swed Dent J* 15:253-258.

Hengtrakool C, Pearson GJ, Wilson M (2006). Interaction between GIC and *S. sanguis* biofilms: antibacterial properties and changes of surface hardness. *J Dent* 34:588-597.

Hermansson M (1999). The DLVO theory in microbial adhesion. *Colloid Surf B: Biointerfaces* 14:105-119.

Herrera M, Castillo A, Bravo M, Liebana J, Carrion P (2000). Antibacterial activity of resin adhesives, glass ionomer and resin-modified cements and a compomer in contact with dentin caries samples. *Oper Dent* 25:265-269.

Holmes AR, Cannon RD, Jenkinson HF (1995). Interactions of *Candida albicans* with bacteria and salivary molecules in oral biofilms. *J Ind Microbiol* 15:208-213.

Imazato S (2003). Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 19:449-457.

Imazato S, McCabe JF (1994). Influence of incorporation of antibacterial monomer on curing behavior of a dental composite. *J Dent Res* 73:1641-1645.

Imazato S, Torii M, Tsuchitani Y, McCabe JF, Russell RR (1994). Incorporation of bacterial inhibitor into resin composite. *J Dent Res* 73:1437-1443.

Imazato S, Tarumi H, Kato S, Ebisu S (1999). Water sorption and colour stability of composites containing the antibacterial monomer MDPB. *J Dent* 27:279-283.

Jansson L, Ehnevid H, Lindskog S, Blomlöf L (1994). Proximal restoration and periodontal status. *J Clin Periodontol* 21:557-582.

Joseph I, Blomster S, Forsgårdh A, Bergman E, Berglund E, Foss L, *et al*. (1997). Interactory effect between marginal plaque and subgingival restorations on periodontal pocket depth. *Swed Dent J* 21:77-83.

Kawai K, Tsuchitani Y (2000). Effects of resin composite components on glucosyltransferase of cariogenic bacterium. *J Biomed Mater Res* 51:123-127.

Khalichi P, Singh J, Cvitkovitch DG, Santerre JP (2009). The influence of triethylene glycol derived from dental composite resins on the regulation of *Streptococcus mutans* gene expression. *Biomaterials* 30:452-459.

Koopmans AS, Kippuw N, De Graaff J (1988). Bacterial involvement in denture-induced stomatitis. *J Dent Res* 67:1246-1250.

Kühn KD (2000). Bone cements. Berlin: Springer, pp. 149-225.

Leonhardt A, Olsson J, Dahlén G (1995). Bacterial colonization on titanium, hydroxyapatite, and amalgam surfaces *in vivo*. *J Dent Res* 74:1607-1612.

Maddikeri RR, Tosatti S, Schuler M, Chessari S, Textor M, Richards RG, *et al*. (2008). Reduced medical infection related bacterial strains adhesion on bioactive RGD modified titanium surfaces: A first step toward cell selective surfaces. *J Biomed Mater Res A* 84:425-435.

Majumdar P, Lee E, Gubbins N, Stafslien SJ, Daniels J, Thorson CJ, *et al*. (2009). Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS (Q-POSS) and polysiloxane coatings containing Q-POSS. *Polymer* 50:1124-1133.

Mei L, Van der Mei HC, Ren Y, Norde W, Busscher HJ (2009). Poisson analysis of streptococcal bond strengthening on stainless steel with and without a salivary conditioning film. *Langmuir* 25:6227-6231.

Mitchell L (1992). Decalcification during orthodontic treatment with fixed appliances—an overview. *Br J Orthod* 19:199-205.

Moons P, Michiels CW, Aertsen A (2009). Bacterial interactions in biofilms. *Crit Rev Microbiol* 35:157-168.

Morgan TD, Wilson M (2000). Anti-adhesive and antibacterial properties of a proprietary denture cleanser. *J Appl Microbiol* 89:617-623.

Mulligan AM, Wilson M, Knowles JC (2003). Effects of increasing silver content in phosphate-based glasses on biofilms of *Streptococcus sanguis*. *J Biomed Mater Res A* 67:401-412.

Murata H, Koepsel RR, Matyjaszewski K, Russell AJ (2007). Permanent, non-leaching antibacterial surfaces–2: how high density cationic surfaces kill bacterial cells. *Biomaterials* 28:4870-4879.

Nakajo K, Imazato S, Takahashi Y, Kiba W, Ebisu S, Takahashi N (2009). Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci. *Dent Mater* 25:703-708.

Nalbant AD, Kalkanci A, Filiz B, Kustimur S (2008). Effectiveness of different cleaning agents against the colonization of *Candida* spp and the *in vitro* detection of the adherence of these yeast cells to denture acrylic surfaces. *Yonsei Med J* 4:647-654.

Neut D, Van de Belt H, Van Horn JR, Van der Mei HC, Busscher HJ (2003). Residual gentamicin-release from antibiotic-loaded polymethylmethacrylate beads after 5 years of implantation. *Biomaterials* 24:1829-1831.

Nicholson JW, Aggarwal A, Czarnecka B, Limanowska-Shaw H (2000). The rate of change of pH of lactic acid exposed to glass-ionomer dental cements. *Biomaterials* 21:1989-1993.

Norde W (2008). My voyage of discovery to proteins in flatland…and beyond. *Colloids Surf B Biointerfaces* 61:1-9.

Omori S, Shibata Y, Arimoto T, Igarashi T, Baba K, Miyazaki T (2009). Microorganism and cell viability on antimicrobially modified titanium. *J Dent Res* 88:957-962.

Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M (2007). Adhesion of *Streptococcus mutans* to different types of brackets. *Angle Orthod* 77:1090-1095.

Palmer RJ, Gordon SM, Cisar JO, Kolenbrander PE (2003). Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. *J Bacteriol* 185:3400-3409.

Pashley DH (1990). Clinical considerations of microleakage. *J Endod* 16:70-77.

Petti S, Tarsitani G, D'Arca AS (2001). A randomized clinical trial of the effect of yoghurt on the human salivary microflora. *Arch Oral Biol* 46:705-712.

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011

Poortinga AT, Bos R, Busscher HJ (1999). Measurement of charge transfer during bacterial adhesion to an indium tin oxide surface in a parallel plate flow chamber. *J Microbiol Methods* 38:183-189.

Powers JM, Sakaguchi RL (2006). Craig's restorative dental materials. 12th ed. St. Louis, MO, USA: Mosby Elsevier, pp. 514.

Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL*, et al*. (1989). The influence of surface free-energy on planimetric plaque growth in man. *J Dent Res* 68:796-799.

Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, Van Steenberghe D (1990). The influence of surface free-energy and surface roughness on early plaque formation. An *in vivo* study in man. *J Clin Periodontol* 17:138-144.

Radford DR, Sweet SP, Challacombe SJ, Walter JD (1998). Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent* 26:577-583.

Ramage G, Tomsett K, Wickes BL, Lopez Ribot JL, Redding SW (2004). Denture stomatitis—a role for Candida biofilm. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98:53-59.

Ready D, Qureshi F, Bedi R, Mullany P, Wilson M (2003). Oral bacteria resistant to mercury and to antibiotics are present in children with no previous exposure to mercury amalgam restorative materials. *FEMS Microbiol Lett* 223:107-111.

Ready D, Pratten J, Mordan N, Watts E, Wilson M (2007). The effect of amalgam exposure on mercury- and antibiotic-resistant bacteria. *Int J Antimicrob Agents* 30:34-39.

Roosjen A, Norde W, Van der Mei HC, Busscher HJ (2006). The use of positively charged or low surface free energy coatings versus polymer brushes in controlling biofilm formation. *Prog Colloid Polym Sci* 132:138-144.

Samaranayake YH, Samaranayake LP (2001). Experimental oral candidiasis in animal models. *Clin Microbiol Rev* 14:398-429.

Sbordone L, Bortolaia C (2003). Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. *Clin Oral Investig* 7:181-188.

Schmalz G, Ergücü Z, Hiller KA (2004). Effect of dentin on the antibacterial activity of dentin bonding agents. *J Endod* 30:352-358.

Seppä L, Korhonen A, Nuutinen A (1995). Inhibitory effect on *S. mutans* by fluoride-treated conventional and resin-reinforced glass ionomer cements. *Eur J Oral Sci* 103:182-185.

Silver S (2003). Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 27:341-353.

Sousa RP, Zanin IC, Lima JP, Vasconcelos SM, Melo MA, Beltrão HC*, et al*. (2009). *In situ* effects of restorative materials on dental biofilm and enamel demineralisation. *J Dent* 37:44-51.

Takahashi Y, Imazato S, Russell RR, Noiri Y, Ebisu S (2004). Influence of resin monomers on growth of oral streptococci. *J Dent Res* 83:302-306.

Teughels W, Van Assche N, Sliepen I, Quirynen M (2006). Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res* 17(Suppl 2):68-81.

Van de Belt H, Neut D, Van Horn JR, Van der Mei HC, Schenk W, Busscher HJ (1999). . . . or not to treat? *Nat Med* 5:358-359.

Van der Mei HC, White DJ, Atema-Smit J, Van de Belt-Gritter E, Busscher HJ (2006). A method to study sustained antimicrobial activity of rinse and dentifrice components on biofilm viability *in vivo*. *J Clin Periodontol* 33:14-20.

Van Oss CJ (1995). Hydrophobicity of biosurfaces—Origin, quantitative determination and interaction energies. *Colloids Surf B Biointerfaces* 5:91-110.

Verran J, Motteram KL (1987). The effect of adherent oral streptococci on the subsequent adherence of *Candida albicans* to acrylic *in vitro*. *J Dent* 15:73-76.

Verran J, Maryan CJ (1997). Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *J Prosthet Dent* 77:535-539.

Vroman L (2008). Finding seconds count after contact with blood (and that is all I did). *Coll Surf B Biointerfaces* 62:1-4.

Wiegand A, Buchalla W, Attin T (2007). Review on fluoride-releasing restorative materials–Fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dent Mater* 23:343-362.

Wu P, Grainger DW (2006). Drug/device combinations for local drug therapies and infection prophylaxis. *Biomaterials* 27:2450-2467.

Yap AU, Khor E, Foo SH (1999). Fluoride release and antibacterial properties of new-generation tooth colored restoratives. *Oper Dent* 24: 297-305.

Downloaded from jdr.sagepub.com at UNICAMP BIBLIOTECA CENTRAL on February 16, 2011