Recalcitrance of *Streptococcus mutans* biofilms towards detergent-stimulated detachment


The biofilm mode of growth protects the plaque microorganisms against environmental attacks, such as from antimicrobials or detergents. Detergents have a demonstrated ability to detach initially adhering bacteria from enamel surfaces, but the ability of detergent components to detach plaque bacteria is not always obvious from *in vivo* experiments and reports on their clinical efficacy are inconsistent. It is likely that antimicrobials or detergents are unable to penetrate the plaque and reach the bacteria that actually link the plaque mass to the substratum surface. Attenuated total reflectance/Fourier transform infrared spectroscopy was used to measure the transport of sodium lauryl sulphate (SLS) through *Streptococcus mutans* HG 985 biofilms. The transport of SLS to the base of the *S. mutans* biofilms was not hindered, while moreover an accumulation of SLS near the base of the biofilms was found, suggesting that SLS was adsorbed to biofilm components. X-ray photoelectron spectroscopy confirmed the ability of *S. mutans*, grown on sucrose supplemented medium, to adsorb SLS, and simultaneously indicated that exposure of cells to SLS might lead to a loss of surface proteins. Furthermore, experiments in a parallel-plate flow chamber demonstrated that initially adhering *S. mutans* HG 985 could be stimulated to detach by SLS, but that, depending on the growth stage of the biofilm, only maximally 27% of biofilm bacteria could be stimulated to detach by a 4% (w/v) SLS solution.

Conventional oral hygiene measures are based primarily on mechanical removal of plaque by toothbrushes, toothpicks and dental floss. In combination with a reduced and less frequent sugar intake, the development of dental caries can be almost completely prevented, and the incidence of periodontal diseases substantially reduced. However, only a limited number of people maintain the degree of motivation needed for effective oral hygiene (1). Alternative preventive measures, either as an adjunct to or as a replacement for mechanical cleansing, such as the use of mouthrinses to control dental plaque formation, are gaining popularity. The most effective anti-plaque agents currently available are Triclosan and chlorhexidine. However, prolonged use of chlorhexidine-based products can cause discoloration of teeth and soft tissues (2). Traditionally, mouthrinses are aimed at killing plaque microorganisms and seldom at the removal of the plaque. Recently, so-called pre-brushing rinses have been developed, with an emphasis on assisting removal of plaque microorganisms, but their clinical efficacy is not ubiquitously accepted (3).

Bacteria in dental plaque are usually embedded in a matrix of extracellular polymeric substances, which protects the adhering population against environmental attack. Biofilm bacteria are at least 500 times more resistant to antibacterial agents than planktonic cells (4). Therefore, topically
applied antimicrobial agents in dentifrices or mouthrinses will leave only a partially dead plaque, which will rapidly restore its original cariogenic capacity by growth of the surviving population and renewed, increased bacterial adhesion to the surface of the remaining biofilm (5).

Application of detergents in oral health care products may assist dental plaque control through detachment of plaque organisms, although in most formulations detergents are added with the sole aim of stimulating foam formation, which is considered emotionally desirable by the consumer popularity (6). A strategy to include detergents with the aim of detaching plaque organisms would avoid the risk of inducing a resistant microbial population within the oral cavity. Furthermore, the eradication of the entire oral community by antimicrobials might create an environment more susceptible to pathogens (7).

The detergents present in an oral health care product should fully penetrate the plaque to reach the salivary conditioning film and the initially adhering bacteria that link the oral biofilm to the enamel surface (8). If the linking film is disrupted by penetrating detergents, the entire adhering plaque mass may detach and a clean enamel surface will result. Under in vivo conditions penetration of detergents, antibiotics and other antimicrobials through biofilms on medical implants is a slow process (9–11). Complete penetration of components of dentifrices or mouthrinses through dental plaque is even less likely, as the contact time between a product and the biofilm is generally less than 2 min. After the time of usage of a product, the substantivity of its components determines its continued presence in the oral cavity, but this is often at a low concentration (12). Nevertheless, in vitro studies showed that a pre-brushing rinse or its detergent components stimulate substantial detachment from saliva-coated enamel of a variety of bacterial strains (13, 14), demonstrating the potential of a detachment-oriented approach provided that clinically the detergents are not hindered in their penetration through the biofilm.

The aim of this paper is to study the penetration of sodium laurel sulphate (SLS) by attenuated total reflection/Fourier transform infrared spectroscopy through artificial dental biofilms, consisting of the cariogenic oral microorganism Streptococcus mutans HG 985, grown with either glucose or sucrose as the main carbon source. As a second aim, the detachment of initially adhering cells and growing S. mutans HG 985 biofilms from enamel surfaces by SLS was studied in a parallel-plate flow chamber.

Material and methods

Bacterial strain and growth condition

S. mutans HG 985 was obtained from Dr. de Graaff (ACTA, Amsterdam, The Netherlands). For each experiment, S. mutans HG 985 was precultured from a frozen stock in a 10 ml Todd-Hewitt broth (THB; Oxoid, Basingstoke, UK) batch culture for 16 h at 37°C in ambient air. This culture was used to inoculate a second 75 ml culture in a homemade medium, which was left for 4 h. This medium contained per liter demineralized water, 10 g tryptone (Oxoid), 2 g phytone peptone (Becton Dickinson, Cockeysville, MD, USA), 2 g yeast extract (Oxoid), 2 g NaHCO3, 2 g NaCl, 3.75 g KH2PO4, 4 g K2HPO4, 2 mg MgSO4·7H2O, 0.2 mg MnSO4·H2O, and 0.6 g (NH4)2SO4, supplemented with either 2 g sucrose (Merck, Darmstadt, Germany), which stimulates extracellular polymeric substances formation, or 2 g D-glucose (Merck).

Detergent penetration through S. mutans biofilms by Fourier transform infrared spectroscopy (FTIR)

The measurement of antimicrobial penetration through biofilms by FTIR has been described before (15, 16) and has developed more or less as a standard technique. Nevertheless, the details of our experimental set-up will be briefly described. A schematic diagram of the FTIR flow system to study detergent penetration through biofilms is shown in Fig. 1. Three flow chambers with germanium internal reflection elements were inserted simultaneously into a Biorad FTS model 175 FTIR spectrometer (Richmond, VA, USA). For each experiment, biofilms were cultured on internal reflection elements in two of the three flow chambers. The third flow chamber was used as a control and background throughout all FTIR experiments and was perfused with sterile demineralized water.

Exponentially growing batch cultures (100 ml medium supplemented with the appropriate carbon source, 37°C) provided the inocula to the first two flow chambers through T-shaped connec-

![Fig. 1. Schematic presentation of the attenuated total reflection FTIR flow system. M, growth medium; DW, demineralized water; I, inoculum; A, air bubble trap; D, detergent; P, pump; W, waste.](image-url)
tions to the main lines. After 1 h, the inocula were turned off and fresh culture medium supplemented with either glucose (DMG medium) or sucrose (DMS medium) was supplied to the first two FTIR flow chambers for an additional 64 h. Then, the medium supply was switched off and the biofilm covered internal reflection elements were rinsed for 90 min with demineralized water. Subsequently, the three flow chambers were consecutively perfused for 90 min with a 4% (w/v) solution of SLS.

All fluids perfused the FTIR flow chambers at a flow rate of 5.5 ± 0.5 ml/h. The FTIR experiments were performed in a temperature controlled room at 22 ± 1°C.

The internal reflection elements were cleaned before each experiment by gently scrubbing with a 5% (v/v) solution of Extral MA 02 neutral detergent (Merck) with a cotton swab, and successively rinsing with tap water, acetone, methanol and demineralized water. All flow chambers containing the internal reflection elements and connecting tubes were sterilized immediately before filling the chambers and tubing with 70% ethanol and soaking them for 3 h. The complete experimental set-up without the flow chambers (see Fig. 1 for details), containing culture medium or demineralized water, when appropriate, was sterilized by autoclaving at 121°C for 20 min.

Before the internal reflection elements were inoculated with bacteria, a FTIR spectrum was collected from each flow chamber for use as a background to compute difference spectra during biofilm growth. IR spectra of biofilm growth were acquired by co-addition of 128 interferograms per spectrum once every 2 h, beginning at the time when the flow chamber inoculum was switched off. During perfusion of the flow chambers with SLS, FTIR spectra were collected by averaging 32 interferograms per spectrum every 60 s. A single FTIR spectrum was obtained from the flow chamber just before the SLS dosing was started and was used as the background to compute difference spectra during dosing.

Water vapour bands were removed from all IR spectra by subtraction of a pure water vapour spectrum. Areas of spectral features were computed for the relevant regions by the peak deconvolution option of the Win-IR software package. Amide II band of bacterial proteins, at 1550 cm⁻¹, was used as a measure for biofilm development on the germanium internal reflection elements. The strong sulphate absorbance band of SLS at 1220 cm⁻¹ was used to evaluate the transport characteristics of SLS to the interfacial region of the internal reflection elements.

In order to quantify the biofilms formed, separate experiments were carried out in which biofilms were grown on the internal reflection elements for 64 h as described above and after swabbing of the biofilms from the germanium surfaces, total colony forming units (CFU) were determined by plated serial dilutions.

**Analysis of SLS adsorption to bacteria by X-ray photoelectron spectroscopy (XPS)**

Since penetration of SLS through the *S. mutans* biofilms can be hampered by either diffusion problems or adsorption in the biofilm, XPS on bacterial cell surfaces (17, 18) was done prior to and after exposure of bacteria to a SLS solution. For XPS experiments, *S. mutans* HG 985 was grown for 4 h to the mid-exponential phase at 37°C in 200 ml cultures of defined medium supplemented with either glucose or sucrose. Cells were harvested by centrifugation (5 min at 4,000 g) and washed twice with demineralized water. Bacterial pellets were suspended in 30 ml of a 4% (w/v) SLS solution. After 30 min, SLS-treated cells were harvested by centrifugation (5 min at 4,000 g) and washed once or twice with demineralized water. Bacterial pellets of untreated, and once and twice washed, SLS-treated *S. mutans* cells were transferred to stainless steel troughs, frozen in liquid nitrogen, and subsequently freeze-dried. The freeze-dried samples were pressed into small stainless steel cups, and put into the XPS chamber (Surface Science Instruments, S-3000, Mountain View, CA, USA). X-ray production (10 kV, 22 mA) at a spot size of 250 × 1,000 μm occurred using a magnesium anode. Scans were made of the overall spectrum in the binding energy range of 1–1200 eV at a low resolution. The area under each peak, after Shirley background subtraction, was used to calculate peak intensities, yielding elemental surface concentrations for carbon, oxygen, nitrogen, and sulphur (indicative for SLS), after correction with sensitivity factors provided by the manufacturer.

**Detergent-stimulated detachment in a parallel-plate flow chamber**

The parallel-plate flow chamber (dimensions, 1 × w × h = 7.6 × 3.8 × 0.06 cm) and image analysis system have been described in detail before (19). An empty flask and flasks containing DMG medium, demineralized water or bacterial suspension were connected to the flow chamber via valves upstream of the flow chamber. One empty flask was connected to the outlet of the flow chamber to collect the bacterial waste (Fig. 2). The complete
set-up, but without the glass top and bottom plates of the flow chamber, was sterilized by autoclaving at 121°C for 20 min. For each experiment, a polished enamel chip with a thickness of 15–20 μm (13) was fixed to a clean glass bottom plate by a small drop of nail polish. The glass plates were immersed in 70% ethanol for 1 min, rinsed with sterile demineralized water, and subsequently inserted into the flow chamber.

In order to study detergent-stimulated detachment of initially adhering bacteria in the absence of growth (13, 14), S. mutans HG 985 cells grown to the stationary phase were deposited onto an enamel chip. An overnight grown culture in DMG medium was harvested by centrifugation (8 min at 9,600g), washed twice with demineralized water, and resuspended in 10 ml demineralized water. To break bacterial chains and aggregates, cells were sonicated for 30 s at 30 W (Vibra Cell model 375; Sonics and Materials, Danbury, CT, USA). Sonication was done intermittently while cooling in melting ice. These conditions were found not to cause cell lysis or death of the bacteria. Subsequently, the sonicated bacteria were suspended in 250 ml DMG medium. After the bacteria were deposited on the enamel surface in 2 h, flow was switched for 30 min to water in order to remove non-adhering cells from the tubes and chamber. Subsequently, the number of bacteria adhering to the enamel was determined. Then, 45 ml of a 4% (w/v) SLS solution, followed by 30 ml of demineralized water to clean the chamber, was perfused once through the flow chamber. Finally, the number of bacteria still adhering to the enamel surface was determined.

In order to study the detergent-stimulated detachment of growing bacteria adhering to the enamel, the flow chamber was inoculated with an exponentially growing 75 ml batch culture of S. mutans HG 985 in DMG medium. Just before inoculation, the batch culture was sonicated for 30 s at 30 W (Vibra Cell model 375), while cooled in an ice/water bath, to break bacterial chains and aggregates. After inoculation for 1 h, flow was switched to sterile DMG medium. The growth medium was supplied to the flow chamber for either 4 or 16 h, stimulating the development of early and late stage biofilms, respectively. Flow was then switched for 30 min to demineralized water to remove non-adhering bacteria from the tubes and chamber, and the biofilm development on the enamel surfaces were determined by measuring the degree of surface coverage. To this end, the flow chamber was put on the stage of a phase-contrast microscope (Olympus BH-2) equipped with a ×4 and ×40 ultra-long working distance objectives (Olympus ULWD-CD series), which enabled analysis of surface areas of 1.2 mm² or 0.012 mm², respectively, with a CCD-MX camera (High Technology, Eindhoven, The Netherlands) mounted on the microscope. The fractions of the enamel surfaces covered by the S. mutans biofilms were enumerated manually. After this, 45 ml of a 4% (w/v) SLS solution, followed by 30 ml of demineralized water to clean the chamber, was perfused through the flow chamber. Subsequently, the surface coverage by the biofilms was determined again.

S. mutans HG 985 cells grown on sucrose autoaggregated and did not adhere to enamel. Therefore, all detergent-stimulated detachment experiments in the parallel-plate flow chamber set-up were performed on S. mutans HG 985 biofilms grown with glucose as the main carbon source. All fluids perfused through the parallel-plate flow chamber at a flow rate of 1.5 ml/min. All
experiments in the flow chamber were performed in duplicate with separate bacterial cultures at room temperature.

Results

**S. mutans HG 985 biofilms on internal reflection elements**

Plated serial dilutions of swabbed biofilms showed that the *S. mutans* HG 985 biofilms grown on the germanium internal reflection elements consisted of up to $2.2 \times 10^9$ and $0.8 \times 10^9$ cells/cm$^2$, for glucose and sucrose grown biofilms, respectively. Assuming a bacterial cell size of $1 \times 1 \mu m$ and an optimal packing, this corresponds with biofilm thicknesses of between 8 and 22 bacterial cell layers for the sucrose and glucose grown biofilms, respectively.

**SLS penetration through *S. mutans* HG 985 biofilms**

Infrared radiation is absorbed by biofilm components in the first monolayer proximal to the surface of the internal reflection elements. Consequently, colonization of the surface by bacteria results in increased intensities of bacterial absorbance bands in the difference spectra. The area of the amide II protein absorbance band versus time represents the lateral colonization of the internal reflection elements by *S. mutans* HG 985. Examples of lateral growth curves obtained for both a glucose- and sucrose grown *S. mutans* HG 985 biofilm by FTIR are shown in Fig. 3.

Perfusion of the flow chamber with SLS resulted in a concomitant reduction of the amide absorption band areas of both the glucose and sucrose grown *S. mutans* biofilms (see Fig. 4A and B). The increase in the sulphate absorption band at 1220 cm$^{-1}$ reflects the rise of the interfacial concentration of the detergent. The transport curves for SLS to the base of both glucose and sucrose grown *S. mutans* biofilms are almost identical (compare Fig. 4A and B). Initially, the concentration of sulphate in the interfacial region rises rapidly to the dosing level. Without a biofilm on the internal reflection elements, the amount of detergent remains constant after this period. However, with *S. mutans* biofilms on the surfaces of the internal reflection elements, the concentration of sulphate at the base of the biofilm increases slowly, exceeding the dosing level of the detergent.

**SLS adsorption to *S. mutans* HG 985**

The elemental surface composition of the bacterial cell surfaces prior to and after exposure to SLS is compiled in Table 1. The surface of *S. mutans* HG 985 cells, grown with either glucose or sucrose as
Table 1
Percentage elemental surface composition of S. mutans HG 985 cells prior to and after exposure to SLS

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Treatment</th>
<th>Elementsa</th>
<th>C</th>
<th>O</th>
<th>N</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>None</td>
<td>57.3</td>
<td>35.9</td>
<td>5.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLS, followed by one rinse</td>
<td>57.6</td>
<td>35.0</td>
<td>6.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLS, followed by two rinses</td>
<td>58.3</td>
<td>34.0</td>
<td>6.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>None</td>
<td>57.4</td>
<td>36.2</td>
<td>5.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLS, followed by one rinse</td>
<td>61.5</td>
<td>33.2</td>
<td>3.6</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLS, followed by two rinses</td>
<td>58.8</td>
<td>36.2</td>
<td>3.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

a S. mutans cells were grown on defined medium supplemented either with glucose or sucrose as the main carbon source.
b Cells exposed to SLS were either rinsed once or twice with deionized water after exposure to the detergent.
c The surface composition was calculated assuming that carbon, oxygen, nitrogen and sulphur were the only elements present in significant amounts. All percentages reported are averages over duplicate experiments with separately cultured bacteria, which coincided within ±8% on average.
d Not detected.

the main carbon source, contains carbon, oxygen and nitrogen, in amounts typical for bacterial cell surfaces (18). Exposure to SLS of S. mutans cells grown in glucose did not alter the cell surface composition, but on sucrose grown S. mutans cells, however, traces of adsorbed sulfur were found, indicative of the detergent SLS. Furthermore, SLS exposure of the bacteria lowered the amount of nitrogen detected, which points to the removal of surface proteins by the detergent.

SLS-stimulated biofilm removal from enamel surfaces

Perfusing the parallel-plate flow chamber with a 4% (w/v) SLS solution stimulated a significant (P<0.05, Student’s t-test) detachment up to 65% of initially and in monolayer adhering, S. mutans HG 985 cells from enamel surfaces. However, when the biofilm was allowed to develop on the enamel surface, the efficacy of the detergent to stimulate bacterial detachment diminished rapidly. A so-called early stage (1–2 layers of bacterial cells) S. mutans biofilm, growing for only 4 h, was partially removed up to 27% (P<0.05, Student’s t-test), whereas an overnight (16 h, 3–4 layers of bacterial cells) grown, late stage S. mutans biofilm completely withstood the detergent action of the SLS solution (Table 2).

Discussion

The formation of dental plaque is a complex phenomenon involving microbial adhesion to the enamel and to other microorganisms, e.g., co-aggregation (20) and co-adhesion (21). In addition to microbial adhesion, however, it has been argued that microbial retention during periods of high shear (eating, speaking, drinking, swallowing, or mechanical tooth cleaning) is an important factor in dental plaque formation (22, 23). The ensemble, constituted by the salivary conditioning film on the enamel surface and the initially adhering bacteria is sometimes called the “linking film” (8). Once the linking film is disrupted by penetrating detergents alone or in combination with the occasionally high shear forces operative in the oral cavity, the entire plaque mass adhering on top of it may detach and a clean enamel surface can result. In vitro studies have demonstrated the potential of such a detachment-oriented approach (14). However, it remains unclear if in vivo the detergents are not hindered in their penetration through the dental plaque.

Only a few years ago, it was argued that a biofilm presented a diffusion barrier for antibiotics and other antimicrobial substances (24), but the use of scanning confocal laser microscopy has shown that many biofilms are, in effect, open structures (25, 26). Other hypotheses for biofilm recalcitrance towards antimicrobials are mainly physiologically

Table 2
SLS-stimulated detachment of initially adhering S. mutans HG 985 cells, and early and late stage, in DMG growing S. mutans biofilms from enamel surfaces in a parallel-plate flow chamber (all experiments were done in duplicate runs)

<table>
<thead>
<tr>
<th>Biofilm type</th>
<th>Bacteria on the surface before SLSc</th>
<th>Detachment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial adhesion</td>
<td>6.21 ± 1.78b</td>
<td>65</td>
</tr>
<tr>
<td>Early stage</td>
<td>0.30 ± 0.14c</td>
<td>27</td>
</tr>
<tr>
<td>Late stage</td>
<td>0.85 ± 0.16c</td>
<td>0</td>
</tr>
</tbody>
</table>

a mean value ± SD over 10 images from random positions on the enamel surface.
b numbers of bacteria (10⁶/cm²), from which the percentage detachment is derived for initial adhesion.
c Fractional surface coverage, from which the percentage detachment is derived for early and late stage biofilms.
related. A relatively slow growth rate of biofilm bacteria, the production of antimicrobial-degrading enzymes, and the derepression or induction of genes associated with a sessile existence can affect the susceptibility of the biofilm towards antimicrobial agents (9).

FTIR spectroscopic measurements indicated that the *S. mutans* HG 985 biofilms, grown either with glucose or sucrose as the main carbon source, did not inhibit the transport of the SLS to their bases. Moreover, the SLS concentration after perfusion of the system with detergent near a biofilm covered internal reflection element exceeded the SLS concentration near a clean internal reflection element (see Fig. 4A and B). Also, Vrány et al. (16) observed by FTIR that hindered transport played only a minor role in the recalcitrance of *Pseudomonas aeruginosa* biofilms towards fluoroquinolones, whereas Suci et al. (15) concluded the opposite, based on similar experiments. An earlier FTIR study performed in the authors’ laboratory on the penetration of SLS through a *Streptococcus oralis* biofilm showed that penetration of the detergent through the biofilm was severely hindered. However, in this particular study the detergent was applied in a much higher concentration, 10% (w/v) SLS versus 4% (w/v) SLS in water in the present study, respectively (14). Obviously, the mechanism of biofilm recalcitrance towards antibiotics or detergents can vary from strain to strain and compound to compound.

The accumulation of SLS in the base of the biofilm above the initial dosing level likely is a result of adsorption of the detergent to biofilm components. SLS is known to bind to membrane proteins (27). XPS measurements on glucose or sucrose grown *S. mutans* HG 985 cells showed that for the glucose grown bacteria the binding of the detergent was reversible, as no sulphur could be detected on the surface of bacteria exposed to SLS after one rinse with demineralized water. However, sucrose grown *S. mutans* cells exposed to SLS, which form extracellular polymeric substances while growing on sucrose, irreversibly adsorbed SLS, that could still be detected by XPS after two rinses with demineralized water due to the extracellular polymeric matrix.

Exposure of the *S. mutans* biofilms on the internal reflection elements with the 4% (w/v) SLS solution resulted in clear amide II absorption band area reductions of 60% and 80%, for the glucose and sucrose grown biofilms, respectively (see Fig. 4A and B). This could be caused by the removal of surface proteins from biofilm bacteria by the detergent or to detachment of biofilm bacteria. However, detergent-stimulated detachment in the parallel-plate flow chamber of *S. mutans* HG 985 biofilms was low and even absent for late stage biofilms. Since also the XPS results indicate that surface proteins may be removed upon exposure of the bacteria to SLS (37% reduction of surface nitrogen for sucrose grown bacteria), it is consequently concluded that the decrease in Amide II absorption band areas during exposure of biofilms on internal reflection elements must be due to removal of proteins and not to detergent-stimulated detachment of the biofilm.

Initially adhering *S. mutans* cells were removed in large numbers by the SLS solution, while an overnight developed biofilm completely withstood the detergent treatment and a 4-h biofilm already showed significant recalcitrance towards detergent-stimulated detachment. A number of studies suggest that slowly growing biofilm cells are less resistant towards antimicrobial agents than faster growing sessile or planktonic bacteria (28–30). Accordingly, the recalcitrance of *S. mutans* biofilms towards detergent-stimulated removal might parallel this physiologically based resistance of biofilms towards antimicrobials. Also, the isolated nature of the initially adhering bacteria, in the absence of any extracellular polymeric substances production, substratrum type and topography may have a profound influence on biofilm development (23, 31) and subsequent resistance to detergents (32). Clinically, such factors should be complemented with possible influences of the multispecies nature of dental plaque.

In conclusion, *S. mutans* HG 985 biofilms detach far less upon exposure to SLS than initially adhering bacteria in the absence of growth, presumably due to adsorption of the detergent by biofilm components during penetration to the base of the biofilm.

References


