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Detachment of Linking Film Bacteria from Enamel Surfaces by Oral Rinses and Penetration of Sodium Lauryl Sulphate through an Artificial Oral Biofilm

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Abstract—The biofilm mode of growth protects plaque micro-organisms against environmental attacks, such as from antimicrobials or detergents. Dental plaque is linked to enamel through the adhesion of initial colonizers. Once this link is disrupted, the entire plaque mass adhering to it detaches. Experiments in a parallel-plate flow chamber demonstrated that bacteria adhering to saliva-coated enamel could not be stimulated to detach by perfusion of the flow chamber with two traditional mouthrinses (Corsodyl® and Scope®), whereas perfusion with a pre-brushing rinse (Plax®) or its detergent components stimulated detachment from saliva-coated enamel of a wide variety of bacterial strains. Following perfusion of the flow chamber with the mouthrinses, little additional detachment of adhering bacteria by the passage of a liquid-air interface occurred. After perfusion with the pre-brushing rinse, however, significant numbers of still-adhering bacteria could be stimulated to detach by passage of a liquid-air interface, indicating that Plax® had weakened their adhesive bond. The ability of Plax® or its detergent components to detach plaque bacteria is not always obvious from *in vivo* experiments, and reports on its clinical efficacy are inconsistent. Likely, antimicrobials or detergents are unable to penetrate the plaque and reach the linking film bacteria, as demonstrated here by Fourier transform infrared spectroscopy.

Key words: Pre-brushing rinse, mouthrinse, bacterial detachment, detergent, enamel, penetration, FTIR.

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Plaque control is fundamental to the prevention of dental caries and periodontal diseases. Conventional oral hygiene procedures are mostly based on mechanical removal of plaque. Alternative preventive measures, such as the use of oral rinses as an addendum to conventional oral hygiene, are gaining more and more popularity. The modes of action of oral rinses differ widely. Traditional mouthrinse formulations are predominantly vehicles of antimicrobial agents (Adams and Addy, 1994; Mandel, 1994). The most effective mouthrinses are chlorhexidine-based formulations, such as Hibident® and Peridex® (Jenkins *et al.*, 1994a,b). Pre-brushing rinses, however, are aimed at loosening the plaque, thereby facilitating its removal during toothbrushing. Reports on the clinical efficacies of pre-brushing agents are inconsistent (Lobene *et al.*, 1990; Singh, 1990; Kozlovsky and Zubery, 1993). However, an *in vitro* study recently performed in our laboratory indicated that the pre-brushing rinse Plax® stimulated up to 65% detachment of adhering *Streptococcus sobrinus* HG 1025 from enamel with a salivary conditioning film (Landa *et al.*, 1996), whereas two other, traditional mouthrinses did not stimulate any significant detachment of adhering streptococci.

Microbial detachment stimulated by an oral rinse is more likely due to a proper detergent system rather than an antimicrobial component. An efficient detergent system to detach adhering bacteria as a means to control dental plaque may turn out to be favorable over antimicrobials, because antimicrobials only leave a dead biofilm, the surface of which is prone to renewed bacterial adhesion. Banks and Bryers (1992), for instance, demonstrated that the efficiency of bacterial deposition to biofilm surfaces was two to five times higher than that to a glass surface.

The formation of dental plaque is instantly initiated after tooth cleaning by the adsorption of salivary components to the enamel surface, followed by adhesion of initial colonizers, to which, eventually, the climax community of matured dental plaque will adhere (Socransky *et al.*, 1977; Scheie, 1994). The initially adhering strains are mainly Gram-positive bacteria and include *Actinomyces naeslundii*, *Streptococcus sanguis*, *Streptococcus oralis*, and several mutans streptococcal strains (Marsh and Martin, 1992). As plaque matures, the number of Gram-negative bacteria increases steadily (Nyvad and Kilian, 1987). Bacteria present in dental plaque are embedded in a matrix of salivary and microbial components (Marsh and Martin, 1992). Consequently, the biofilm mode of growth protects the

TABLE 1

STRAINS INVOLVED IN THIS STUDY, TOGETHER WITH THE GROWTH CONDITIONS USED AND THEIR SOURCES

Strain	Growth Condition, Medium	Source
Actinomyces naeslundii T14V-J1	Anaerobic, Schaedler's broth	Dr. Cisar, NIH, Bethesda, MD, USA
Actinomyces naeslundii 5951	Anaerobic, Schaedler's broth	*idem*
Actinomyces naeslundii 147	Anaerobic, Schaedler's broth	*idem*
Streptococcus sobrinus HG 1025	Aerobic, Todd-Hewitt broth	Dr. de Graaff, ACTA, Amsterdam, The Netherlands
Streptococcus sobrinus ATCC 33478	Aerobic, Todd-Hewitt broth	American Type Culture Collection, Rockville, MD, USA
Streptococcus mutans NS	Aerobic, Todd-Hewitt broth	own isolate
Streptococcus mutans ATCC 25175	Aerobic, Todd-Hewitt broth	American Type Culture Collection, Rockville, MD, USA
Streptococcus oralis 34	Aerobic, Todd-Hewitt broth	Dr. Cisar, NIH, Bethesda, MD, USA
Streptococcus oralis J22	Aerobic, Todd-Hewitt broth	Dr. Kolenbrander, NIH, Bethesda, MD, USA
Streptococcus oralis ATCC 35037	Aerobic, Todd-Hewitt broth	American Type Culture Collection, Rockville, MD, USA
Streptococcus sanguis PK 1889	Aerobic, Todd-Hewitt broth	Dr. Kolenbrander, NIH, Bethesda, MD, USA
Streptococcus sanguis ATCC 10556	Aerobic, Todd-Hewitt broth	American Type Culture Collection, Rockville, MD, USA
Lactobacillus acidophilus JP	CO_2-incubator, MRS broth	own isolate

adhering bacteria against environmental attacks, and the active components of an oral rinse must penetrate the plaque to reach the initially adhering bacteria that link the entire plaque mass to the enamel surface. Under *in vivo* conditions, this may well be impossible. Penetration of antibiotics through biofilms on medical implants, for instance, is an extremely slow process (Brown and Gilbert, 1993; Vorachit *et al.*, 1993). Penetration of oral rinse components through dental plaque is even less likely, since the contact time between a product and the oral tissues is generally less than two minutes. After the actual time of usage of a product, the substantivity of its components determines its presence in the oral cavity, but this is often at a low concentration (Goodson, 1989). The ensemble constituted by the salivary conditioning film on the enamel surface and the initially adhering bacteria is sometimes called the "linking film" (Busscher *et al.*, 1995). Once the linking film is disrupted by penetrating detergents alone or in combination with the occasionally high shear forces operative in the oral cavity, such as during eating, speaking, drinking, swallowing, or mechanical tooth cleaning, the entire plaque mass adhering to it detaches, and a clean enamel surface will result.

With an emphasis on the role of initially adhering bacteria in oral linking films, the aim of this paper is to compare the detachment of a collection of initial colonizers from enamel surfaces as stimulated by two traditional mouthrinses, a pre-brushing rinse and its detergent components. To this end, experiments were carried out in a parallel-plate flow chamber. Detachment of bacteria adhering to enamel with a salivary conditioning film was stimulated by perfusion of the system with oral rinses, Corsodyl®, Scope®, Plax®, or its detergent components. In addition, the penetration of sodium lauryl sulphate (SLS) through an artificial dental biofilm, consisting of the initial colonizing oral organism *S. oralis* J22, by Fourier transform infrared spectroscopy (FTIR) is studied.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and harvesting

The bacterial strains used in this study are listed in Table 1, together with their growth conditions and sources. *Actinomyces naeslundii* T14V-J1, *Actinomyces naeslundii* 5951, and *Actinomyces naeslundii* 147 were pre-cultured from a frozen stock in a 10-mL batch culture of Schaedler's broth supplemented with 0.01 g/L hemin for 24 hrs at 37°C in 10% H_2, 85% N_2, and 5% CO_2. All streptococci were pre-cultured from a frozen stock in a 10-mL Todd-Hewitt (TH) broth batch culture for 24 hrs at 37°C in ambient air. *Lactobacillus acidophilus* JP was pre-cultured from a frozen stock in a 10-mL MRS broth batch culture for 24 hrs at 37°C in an atmosphere with 5% CO_2. All pre-cultures were used to inoculate 200-mL cultures of the appropriate broth, which were grown for 16 hrs prior to being harvested.

For each experiment, bacteria were harvested by centrifugation (5 min at 4000 *g*), washed twice with demineralized water, and re-suspended in adhesion buffer (2

TABLE 2

ORAL RINSES USED IN THIS STUDY, THEIR MAIN COMPONENTS AND OBJECTIVES AS INDICATED BY THE MANUFACTURERS

Name	Main Components	Objective	Manufacturer
Corsodyl®	Chlorhexidine digluconate (0.2%)	Anti-bacterial	SmithKline Beecham, UK
Scope®	Cetylpyridinium chloride	Anti-bacterial	Procter & Gamble, USA
	Sodium benzoate		
	Sodium saccharin		
	Domiphen bromide		
	Benzoic acid		
	Glycerin		
	Alcohol (18.9%)		
	Flavor		
Plax®	Triclosan (0.03%)	Anti-plaque	Colgate-Palmolive, USA
	Sodium fluoride (0.025%)		
	Tauranol (0.2%)		
	Sodium lauryl sulphate (0.25%)		
Detergent-free Plax®[a]			Colgate-Palmolive, USA
Plax® detergents in water	Tauranol (0.2%)		
	Sodium lauryl sulphate (0.25%)		

[a] Also containing no Triclosan or sodium fluoride.

mM potassium phosphate, 50 mM potassium chloride, and 1 mM calcium chloride, pH 6.8). To break bacterial chains and aggregates, suspensions were sonicated for 30 sec at 30 W (Vibra Cell model 375, Sonics and Materials Inc., Danbury, CT, USA). Sonication was done intermittently while suspensions cooled in an ice/water bath. These conditions were found not to cause cell lysis in any strain. Subsequently, the bacteria were suspended in adhesion buffer to a density of 1 x 10^8 cells/mL for the actinomyces strains and to a density of 3 x 10^8 cells/mL for all other strains.

Saliva, oral rinses, and enamel preparation

Human whole saliva from 20 healthy volunteers of both sexes was collected into ice-chilled cups after the volunteers' salivary flow was stimulated by the chewing of Parafilm®. After the saliva was pooled and centrifuged at 12,000 *g* for 15 min at 4°C, phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM as a protease inhibitor. The solution was again centrifuged, dialyzed for 48 hrs at 4°C against demineralized water, and freeze-dried for storage. Reconstituted, human whole saliva was prepared from the lyophilized stock by dissolution of 1.5 mg/mL in adhesion buffer.

Table 2 lists the oral rinses used for this study together with their main components, primary objectives, and manufacturers. All products were purchased commercially, except detergent-free Plax® and the detergent Tauranol (sodium methyl cocoyl taurate), which were kindly provided by Dr. A. Gaffar and Dr. M.I. Williams (Colgate-Palmolive, Piscataway, NJ, USA). Sodium lauryl sulphate was obtained from Biorad (Richmond, VA, USA).

Enamel substrata were prepared from the labial surfaces of bovine dental incisors. First, an incisor was ground under running tap water with abrasive paper (1200 grit) and polished with a slurry of Al_2O_3 powder (particle diameter 0.05 μm) in distilled water. The polished surface was cleaned in an ultrasonic bath filled with demineralized water for 3 x 1 min. Subsequently, the polished labial surface was cut off the tooth to a slice with a thickness of approximately 300 μm, and fixed to a glass slide by means of a drop of nail polish. The exposed side of the enamel slice was ground with abrasive paper (1200 grit) to a thickness of 15-20 μm, polished with a slurry of Al_2O_3 powder, and again cleaned ultrasonically. Subsequently, the enamel chip was detached from the glass and stored at 8°C until further use. For flow chamber experiments, enamel chips were fixed to a clean glass plate by a small drop of nail polish. To check whether the preparation procedure had affected the enamel surface characteristics, we performed x-ray photoelectron spectroscopy (XPS) and water contact angle measurements. Water contact angles of thus-prepared enamel chips were 46 ± 10° (n = 6), which is in accordance with literature data for bovine (Glantz, 1971) and also human (Van der Mei *et al.*, 1990) enamel. The elemental surface concentration ratios of the enamel chips, as determined by XPS, were C/Ca = 1.9 ± 0.4, O/Ca = 3.3 ± 0.4, N/Ca = 0.1 ± 0.0, P/Ca = 0.7 ± 0.0, O/C = 1.77 ± 0.3, N/C = 0.06 ± 0.0, and P/C = 0.38 ± 0.1 (n = 6), which correspond well with values reported by Kuboki *et al.* (1987) and by Van der Mei *et al.* (1990) for ground and polished enamel.

Flow chamber, deposition protocol, and image analysis

The parallel-plate flow chamber (dimensions, l x w x h = 7.6

x 3.8 x 0.06 cm) and image analysis system have been described in detail before (Sjollema *et al.*, 1989). To be able to evaluate bacterial detachment stimulated by three oral rinses and a control (adhesion buffer) in one experiment, therewith reducing biological variations, we connected four flow chambers in series with the enamel chips glued on the bottom plates. Each flow chamber was connected separately to a flask containing an oral rinse solution *via* a valve upstream of each chamber. Prior to each experiment, all tubes and flow chambers were filled with buffer, while care was taken to remove air bubbles from the system. Flasks containing bacterial suspension, buffer, reconstituted human whole saliva, and an oral rinse, when appropriate, were connected to the flow chambers. All fluids circulated through the chamber under the influence of hydrostatic pressure at a shear rate of 30 s^{-1} (flow rate, 0.068 mL/s).

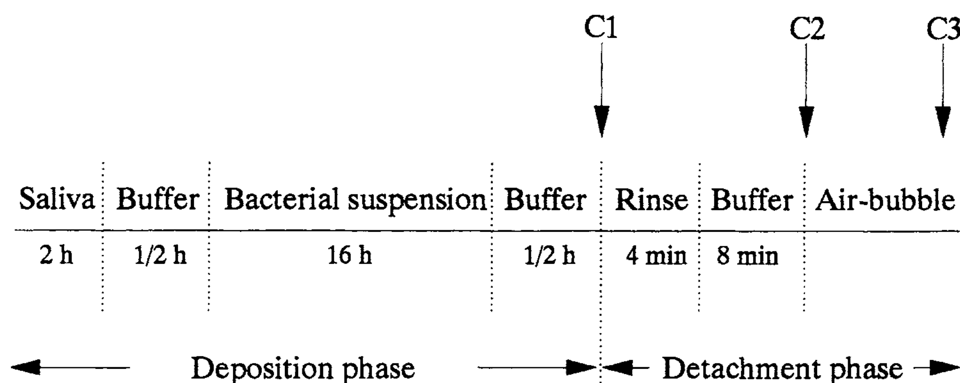

Fig. 1—Schematic presentation of the deposition protocol. C1 denotes the first count of the number of bacteria adhering to the saliva-coated enamel after the 16-hour deposition period, while C2 denotes the second count after perfusion of the chamber with an oral rinse. The third count, indicated C3, enumerates the number of bacteria that could withstand the passage of a liquid-air interface through the parallel-plate flow chamber.

The deposition protocol is schematically summarized in Fig. 1. First, flow was switched to saliva for 2 hrs to create a salivary conditioning film. Thereafter, flow was switched for 30 min to buffer for removal of all remnants of saliva from the tubes and chambers, and flow was switched to the bacterial suspension. The bacterial suspension was circulated through the system overnight for 16 hrs. Then, flow was switched for 30 min to buffer to remove non-adhering bacteria from the tubes and chambers, and the numbers of bacteria adhering to the substrata were determined (denoted as C1 in Fig. 1). To this end, each flow chamber was put on the stage of a phase-contrast microscope (Olympus BH-2) equipped with a 40 x ultra-long working distance objective (Olympus ULWD-CD Plan 40 PL). The numbers of bacteria adhering to the various enamel chips were observed over a surface area of 0.012 mm^2 with a CCD-MX camera (High Technology, Eindhoven, The Netherlands) mounted on the microscope and enumerated manually.

After this deposition period, one dosage (15 mL) of an oral rinse or buffer (control), followed by 30 mL of buffer to clean the chamber from rinse components, was perfused once through each flow chamber *via* the valve upstream of each chamber. As the rinse or buffer exited a flow chamber, another valve led it outside the flow circuit; hence each chamber was perfused with only one rinse. Rinses and cleaning buffer were applied at approximately the same flow rate as during the deposition period. The time required to pass one dosage through each chamber was approximately 4 min. Sub-sequently, the numbers of bacteria still adhering to the substrata were determined (C2 in Fig. 1). Then, the chambers were emptied by hydrostatic pressure, so that a liquid-air interface could pass over the surface, as occasionally occurs in the oral cavity during eating, speaking, drinking, or swallowing. Finally, the numbers of adhering organisms withstanding this extremely high removal force (Leenaars and O'Brien, 1989) were counted again (C3 in Fig. 1).

For each experiment, the percentages of bacteria removed from the enamel chips in each flow chamber, after perfusion with the different oral rinses, were determined. Furthermore, the percentages of bacteria subsequently removed from the substrata in each flow chamber by the passage of a liquid-air interface were determined. Percentages were calculated with respect to the number of organisms adhering prior to the introduction of a rinse in a flow chamber.

All experiments in the parallel-plate flow chamber were performed in duplicate with separate bacterial cultures at room temperature.

Penetration of SLS through an artificial biofilm

The measurement of antimicrobial penetration through biofilms by FTIR has been described before (Suci *et al.*, 1994) and has developed more or less as a standard technique. Nevertheless, the details of our experimental set-up will be briefly described.

Three FTIR flow chambers with germanium IRE's were inserted simultaneously into a Biorad FTS model 175 FTIR spectrometer equipped with a KBr beam-splitter and a DTGS detector, as shown schematically in Fig. 2. Prior to each experiment, all tubes and the flow chambers were filled with water, while care was taken to remove air bubbles from the system. In one FTIR flow chamber, only water was perfused; in a second chamber, an aqueous 10% SLS solution was flown through in the absence of a biofilm; while in the third chamber only, a three-day-old biofilm was grown on the IRE prior to perfusion with an SLS solution.

To create a biofilm on a germanium internal reflection element, we grew *S. oralis* J22 overnight at 37°C in defined medium, containing, *per* liter of demineralized water, 10 g tryptone, 2 g phytone, 2 g yeast extract, 2 g $NaHCO_3$, 2 g NaCl, 3.75 g KH_2PO_4, 4 g K_2HPO_4, 2 mg $MgSO_4$, 0.2 mg $MnSO_4$, 0.6 g $(NH_4)_2SO_4$, and 2 g glucose. Subsequently, a

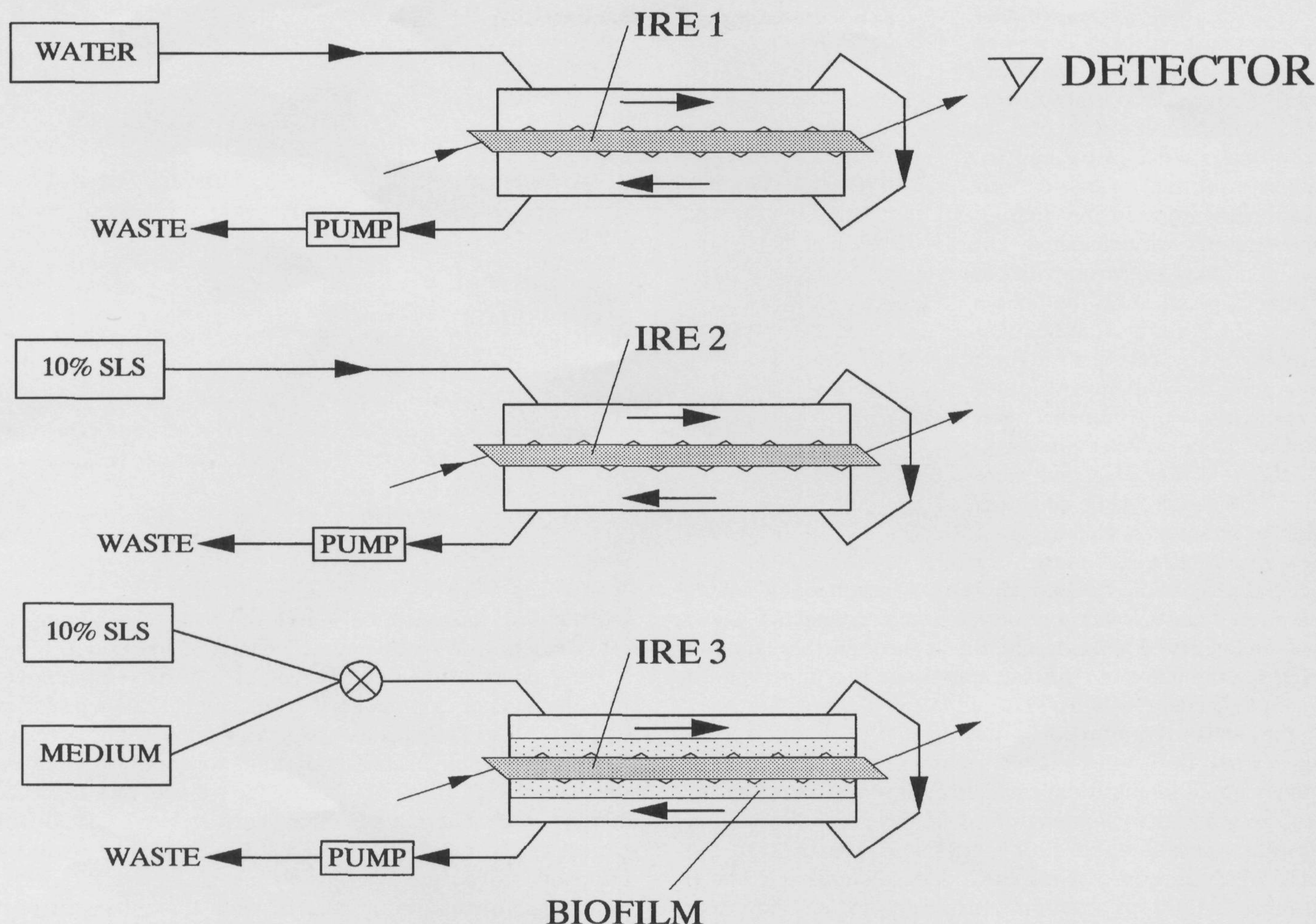

Fig. 2—Schematic presentation of the measurement of SLS penetration through an artificial dental plaque by Fourier transform infrared spectroscopy, with the biofilm growing on a germanium internal reflection element. Three FTIR flow chambers were inserted into the spectrometer simultaneously. Spectra were recorded from water (IRE 1), 10% SLS in water (IRE 2), and a 10% SLS solution in water in the presence of a biofilm on the germanium IRE (IRE 3).

1% inoculum was added to 75 mL of medium and when cells were in their mid-exponential phase perfused through the FTIR flow chamber, to allow the bacteria to adhere to the IRE. After 4 hrs, the FTIR flow chamber was perfused with medium at a flow rate of 5.2 mL/hr during 3 days to grow a confluent biofilm, consisting of approximately 10 monolayers.

We took absorbance spectra as a function of time by collecting and averaging 16 double-sided interferograms at a resolution of 4 cm^{-1}. Water spectra were used as a background for the aqueous SLS spectra and for the *S. oralis* J22 biofilm spectrum. Spectra of SLS penetrating the biofilm were obtained by subtraction of a biofilm spectrum from spectra of the biofilm taken during perfusion of the chamber with SLS, by means of the Win_IR software package.

RESULTS

Table 3 shows the number of linking film bacteria on saliva-coated enamel chips after the 16-hour deposition period. The number of adhering bacteria differs widely *per* strain, varying from fewer than 1 x 10^6 bacteria *per* cm^2 for *S. mutans* ATCC 25175 up to almost 30 x 10^6 *per* cm^2 for *S. sanguis* ATCC 10556. Although adhesion will be the main factor contributing to the number of bacteria on the enamel chips, growth of bacteria adhering to a salivary conditioning film over a 16-hour time interval may occur as well and cannot be excluded.

Fig. 3 summarizes the effects of perfusing oral rinses through the parallel-plate flow chamber on the numbers of adhering linking film bacteria, and their subsequent detachment stimulated by the passage of a liquid-air interface. Both mouthrinses, Corsodyl® and Scope®, stimulate minor bacterial detachment, and the percentages seen are close to those observed for buffer. However, the pre-brushing rinse Plax® stimulated up to 85% bacterial detachment for *A. naeslundii* 147, while most other strains

were also stimulated to detach in significant numbers. Interestingly, the other actinomyces strains and *S. mutans* ATCC 25175 were little stimulated to detach by Plax®.

In addition, Fig. 3 shows the subsequent effect of passing a liquid-air interface through the flow chambers. In the control experiment, *i.e.*, when the flow chamber was perfused only with buffer, sizeable percentages of various adhering strains were detached by the liquid-air interface. Also, for some strains, after the chamber was perfused with Corsodyl® or Scope®, small percentages of adhering bacteria were found to detach. However, after the chamber was perfused with Plax®, and despite the fact that the pre-brushing rinse alone had already detached large numbers of adhering bacteria, sizeable percentages of linking film bacteria were still stimulated to detach by the passage of a liquid-air interface, indicating that the pre-brushing rinse Plax® had weakened their bonding with the saliva-coated enamel.

Fig. 4 demonstrates that perfusing the chamber with the detergent components present in Plax® stimulates bacterial detachment from saliva-coated enamel surfaces in percentages approximately equal to those stimulated by Plax®. When the chamber was perfused with a detergent-free Plax® variant, bacterial detachment was absent, as for the two traditional mouthrinses and the control buffer (compare Figs. 2 and 3). However, when a liquid-air interface was subsequently passed through the chamber, eight strains detached in fair amounts, indicating that not only can detergents weaken the adhesive bond, but also other components of the pre-brushing rinse may be able to do so.

Fig. 5 presents infrared absorption spectra obtained by ATR-FTIR of a 10% solution of SLS in water and of an artificial *S. oralis* J22 biofilm on the germanium IRE. Note that the spectrum of the biofilm is dominated by the amide I and II bands around 1650 and 1550 cm^{-1}, respectively, and by phosphate (1230 cm^{-1}) and polysaccharide (1030 cm^{-1}) absorption bands. The infrared absorption spectrum of SLS is clearly different and distinguishes most from the spectrum of the biofilm by a strong absorption band around 1220 cm^{-1}, due to the sulphate.

The time required for the concentration of SLS in the vicinity of the germanium IRE to reach an equilibrium in the absence of a biofilm at the flow rate applied amounts to approximately 20 min, as can be seen from the time-dependent increase of the 1220 cm^{-1} infrared absorption band in Fig. 6. In the presence of the *S. oralis* J22 biofilm on the IRE, the SLS absorption band appears slower and less intense, obviously due to the difficulties involved in penetrating the biofilm.

TABLE 3

THE NUMBER OF LINKING FILM BACTERIA ADHERING TO ENAMEL WITH A SALIVARY CONDITIONING FILM AFTER A DEPOSITION PERIOD OF 16 hrs
(± denotes the SD over 4 experiments with separately prepared substrata and separately cultured micro-organisms)

Strain	$n_{(16h)}$ (10^6 cm^{-2})
A. naeslundii T14V-J1	3.9 ± 2.9
A. naeslundii 5951	6.4 ± 1.1
A. naeslundii 147	5.3 ± 2.7
S. sobrinus HG 1025	1.5 ± 0.2
S. sobrinus ATCC 33478	7.6 ± 1.1
S. mutans NS	7.0 ± 1.8
S. mutans ATCC 25175	1.0 ± 0.5
S. oralis J22	22.9 ± 2.5
S. oralis 34	16.6 ± 5.4
S. oralis ATCC 35037	15.1 ± 1.9
S. sanguis PK 1889	11.7 ± 2.4
S. sanguis ATCC 10556	29.4 ± 1.6
L. acidophilus JP	3.3 ± 1.6

DISCUSSION

In this paper, we compare the effects of two mouthrinses, Corsodyl® and Scope®, a pre-brushing rinse, Plax® , and its detergent components, on the detachment of a collection of linking film bacteria from saliva-coated enamel. All experiments were performed in parallel-plate flow chambers with *in situ* observation of the bacterial detachment as stimulated by the rinses and the passage of a liquid-air interface, yielding full control of the hydrodynamic conditions and detachment forces prior to enumeration (Busscher and Van der Mei, 1995), unlike in most *in vitro* adherence assays involving "rocking" (Steinberg *et al.*, 1993), "rolling" (Roger *et al.*, 1994), "pouring" (Wirtanen *et al.*, 1996), and "washing" (Vacca-Smith *et al.*, 1994). Consequently, the use of the parallel-plate flow chamber gives good correspondence between duplicate runs, especially considering that biological variations occur both from culturing as well as from the use of enamel as a substratum.

Microbial adhesion to enamel coated with a salivary conditioning film is most often described in terms of specific or stereochemical interactions between bacterial surface receptors and components in the salivary conditioning film (Scannapieco, 1994), which eventually determine the overall charge and hydrophobic (cell)-surface properties involved in adhesion (Busscher *et al.*, 1992a). Different strains and species have their own characteristic surface properties facilitating interactions with selected components of the salivary conditioning film on the enamel surface, like the salivary mucins, glycoproteins, agglutinins, and various other salivary proteins (Gibbons, 1989; Douglas, 1994; Scannapieco, 1994). Interestingly, as can be seen in Table 3, those species considered to be typical initial colonizers of tooth surfaces in the oral cavity, *i.e.*, *S. oralis* and *S. sanguis*, adhere in higher numbers after 16 hrs to saliva-coated enamel than, *e.g.*, the acidogenic *S. mutans* or *S. sobrinus*.

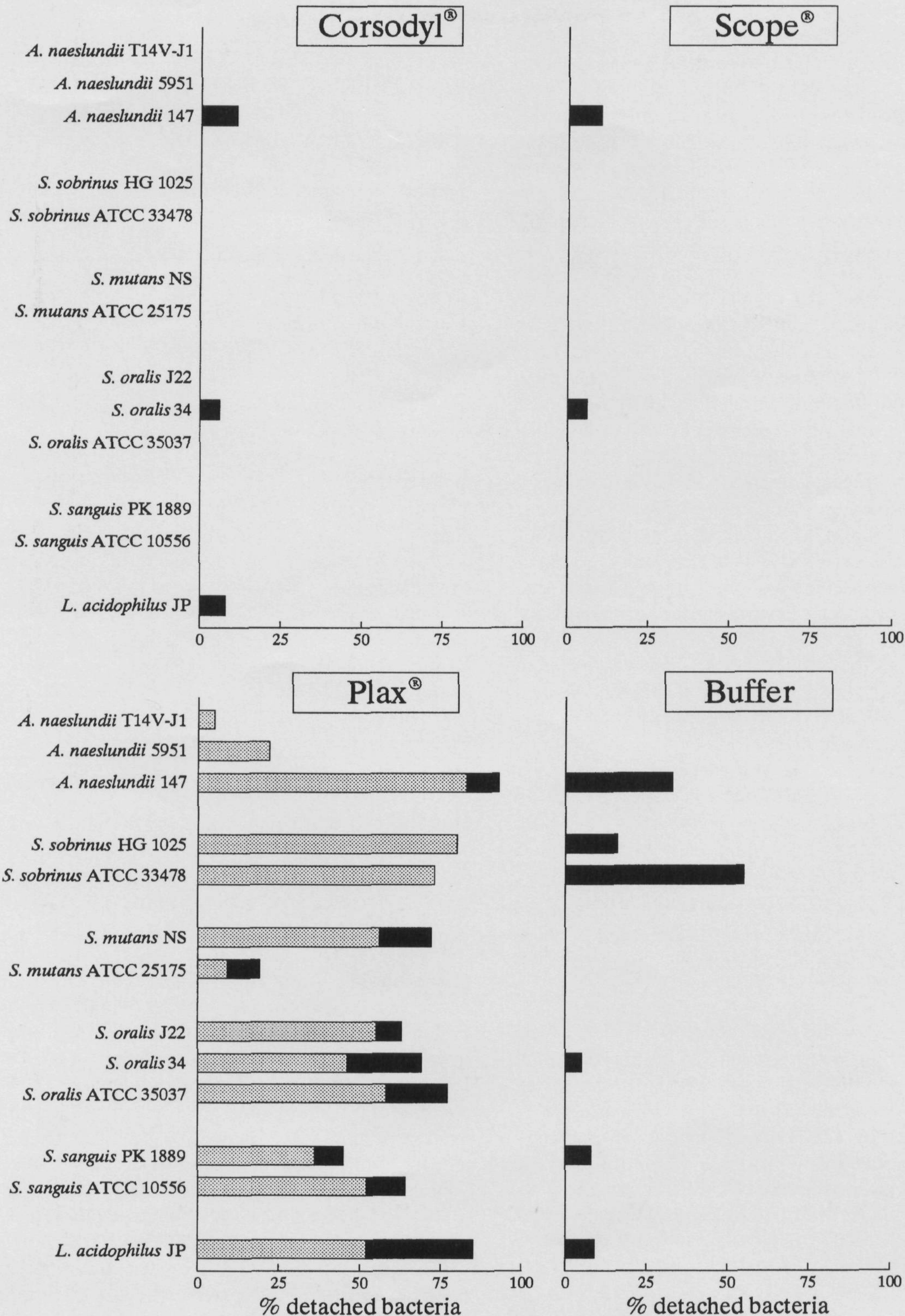

Fig. 3—Percentages of linking film bacteria detached from enamel with a salivary conditioning film, stimulated by perfusion of an oral rinse through a flow chamber (results of duplicate runs coinciding within 5%) and the subsequent passage of a liquid-air interface (coincidence of duplicate runs 7%). All percentages reported are calculated with respect to the number of organisms adhering prior to the introduction of a rinse in the flow chamber, while percentages less than 5 % were taken as zero. Shaded bar = detachment stimulated by perfusion with an oral rinse; black bar = detachment stimulated by the subsequent passage of a liquid-air interface.

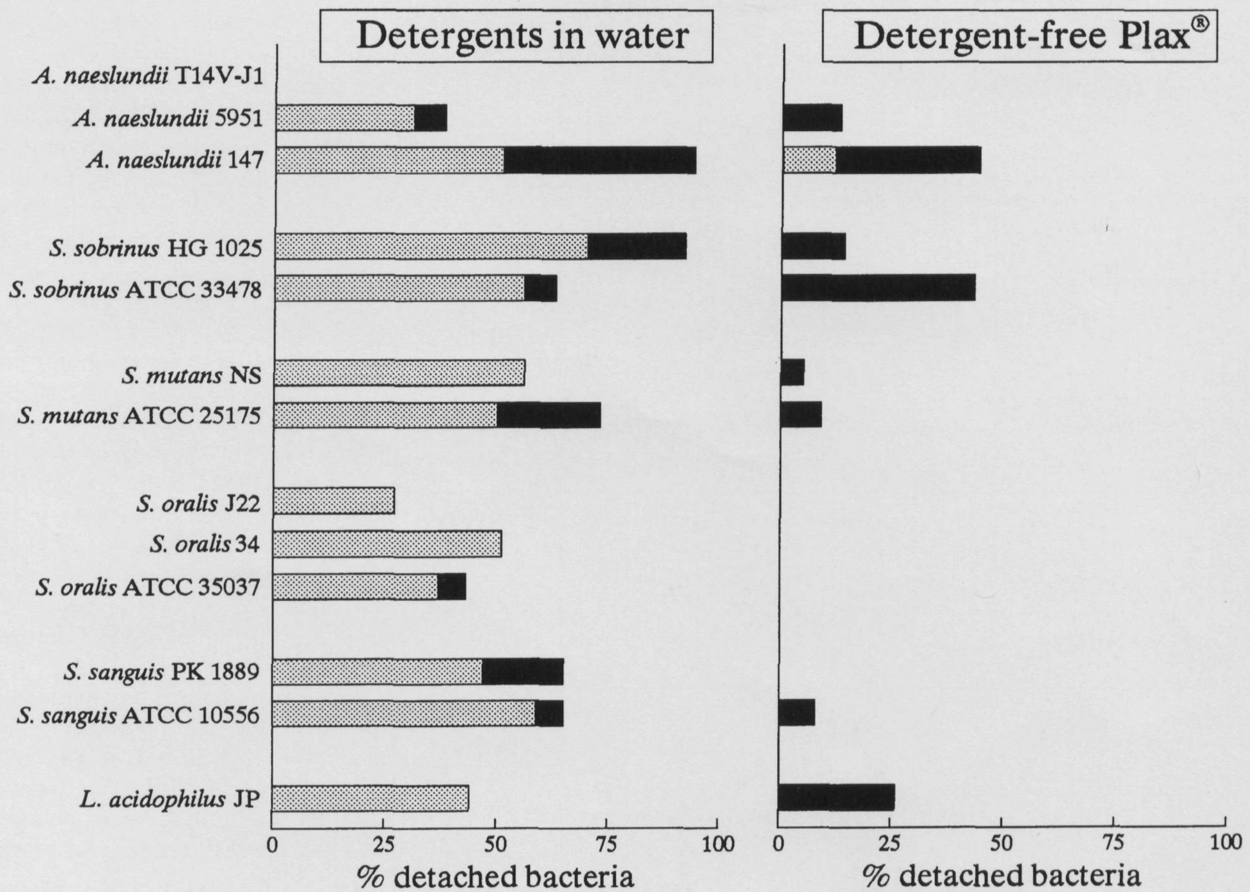

Fig. 4—Percentages of linking film bacteria detached from enamel with a salivary conditioning film, stimulated by perfusion of pre-brushing rinse components through a flow chamber (results of duplicate runs coinciding within 8%) and the subsequent passage of a liquid-air interface (coincidence of duplicate runs 7%). All percentages reported are calculated with respect to the number of organisms adhering prior to the introduction of a rinse in the flow chamber, while percentages less than 5% were taken as zero. Shaded bar = detachment stimulated by perfusion with an oral rinse; black bar = detachment stimulated by the subsequent passage of a liquid-air interface.

The formation of dental plaque is a complex phenomenon involving microbial adhesion to the enamel and to other micro-organisms, *e.g.*, co-aggregation (Kolenbrander, 1992) and co-adhesion (Bos *et al.*, 1994). 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 also an important factor in dental plaque formation (Christersson *et al.*, 1989; Busscher *et al.*, 1992b). Prakobphol *et al.* (1995) have shown that the force required to remove bacteria adhering to a salivary conditioning film on the bottoms of polyvinyl chloride microtiter wells by centrifugation ranged from 1.6 x 10^{-13} N for *Streptococcus sanguis* 72-40 to over 1.1 x 10^{-12} N for *Actinomyces viscosus* T14V, which is clearly within the range of the surface tension forces exerted by the passing of a liquid-air interface over adhering micron-sized particles and which can be estimated to be 10^{-8}-10^{-7} N, according to Leenaars and O'Brien (1989). Indeed, from the experiments done with buffer (see Fig. 2), it can be seen that the retentive capacities of bacteria are strain- and species-dependent. Also, the retentive capacities of the three actinomyces strains involved in this study to withstand perfusion of the flow chamber with Plax® and its detergent components differ greatly. Whereas *A. naeslundii* T14V-J1 and *A. naeslundii* 5951 were little stimulated to detach, *A. naeslundii* 147 was almost completely removed, possibly due to the lack of type 1 fimbriae, which supposedly mediate the binding to the salivary pellicle (Cisar *et al.*, 1988; Nesbitt *et al.*, 1996).

The two traditional mouthrinses, Corsodyl® or Scope®, do not stimulate detachment of adhering bacteria from enamel coated with a salivary conditioning film in line with the design properties of mouthrinses, aimed at killing the adhering bacteria rather than at stimulating their detachment. The pre-brushing rinse Plax® and its detergent components, Tauranol (0.2% w/v) and SLS (0.25% w/v), appear to be effective in disrupting the bond between adhering linking

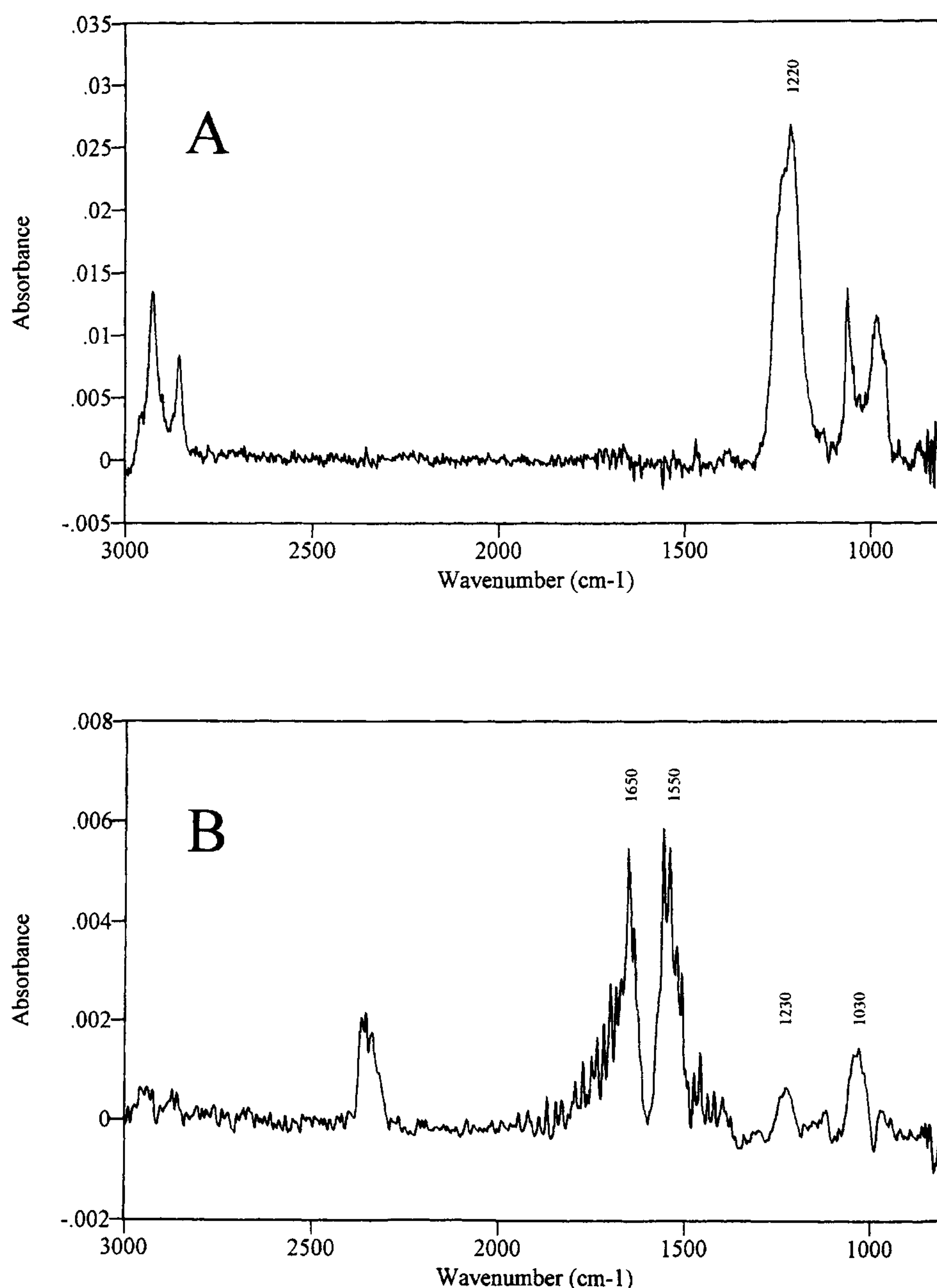

Fig. 5—(A) FTIR spectrum of an aqueous 10% solution of SLS in the FTIR flow chamber, with a strong sulphate absorption band at 1220 cm^{-1}. (B) FTIR spectrum of an S. oralis *J22 artificial biofilm on a germanium internal reflection element, obtained after three days of growth with amide I and II bands around 1650 and 1550 cm^{-1}, respectively, and phosphate (1230 cm^{-1}) and polysaccharide (1030 cm^{-1}) bands.*

film bacteria and the enamel. Whether this disruption is between the bacterium and the salivary conditioning film, in the conditioning film, or by desorption of the entire conditioning film cannot be decided upon from the present experiments. However, it has been reported that Plax® can desorb up to 90% of albumin bound to synthetic hydroxyapatite (Haikel *et al.*, 1994), which suggests that the detergent system of Plax® causes at least a partial desorption of the salivary conditioning film.

The clinical efficacy of Plax® as a pre-brushing rinse is not ubiquitously accepted (Kozlovsky and Zubery, 1993), most notably because its detergent components cannot reach the adhering linking film bacteria through the plaque. Even in the high concentration applied in this study, SLS was able to penetrate an artificial biofilm of *S. oralis* J22 on a germanium IRE to only a minor extent, clearly demonstrating that SLS applied in clinically relevant concentrations will practically not penetrate the plaque. Clinically, application of higher concentrations of SLS is impossible, since it has been demonstrated that SLS concentrations as low as 2% may already yield desquamation of oral mucosal tissues (Herlofson and Barkvoll, 1996). However, in the present study, a 10% SLS solution was applied only to facilitate demonstration by FTIR of the poor penetration of the detergent through a biofilm.

Several years ago, it was argued that a biofilm presented a diffusion barrier for antibiotics and other antimicrobial substances (Hoyle *et al.*, 1992), but the use of scanning confocal laser microscopy has shown that most biofilms are in effect open structures, and that the inability of antibiotics to penetrate the biofilms is mostly due to adsorption to microbial cell surfaces. Also, SLS is known to adsorb to microbial cell surfaces (Pader, 1985), which could be a reason for the lack of clinical efficacy of SLS-containing pre-brushing rinses. Possibly, combination of SLS with other detergents in a pre-brushing formulation, having a greater tendency to adsorb to microbial cell surfaces than SLS alone, may yield better penetration of SLS through dental plaque and subsequent detachment of linking film bacteria.

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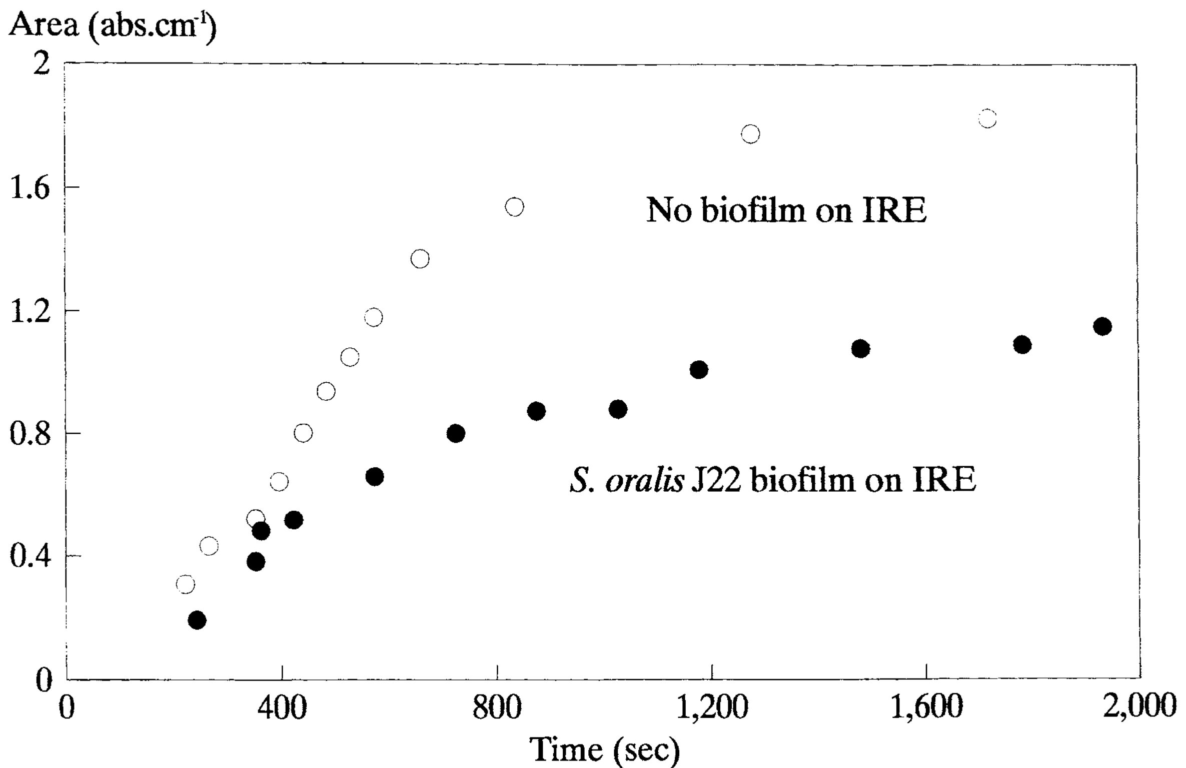

Fig. 6—The infrared absorption band area of the 1220 cm^{-1} band in the spectrum of a 10% SLS solution in water as a function of the perfusion time through the chamber in the absence (○) and presence (●) of a three-day-old S. oralis *J22 biofilm on the germanium internal reflection element. t = 0 denotes the moment the flow was switched to the SLS solution.*

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