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ORIGINAL ARTICLE

Effects of aging on surface properties and adhesion of *Streptococcus mutans* on various fissure sealants

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Abstract The aim of the present study was the quantification of *Streptococcus mutans* adhesion on ten widely used pit and fissure sealant materials and the correlation of these findings to surface roughness (R_a) and surface free energy (SFE). Additionally, changes in streptococcal adhesion and surface parameters after water immersion and artificial aging have been investigated. Circular specimens of ten fissure sealants (seven resin-based composites, two glass ionomers, and one compomer) were made and polished. Surface roughness was determined by perthometer and SFE by goniometer measurements. Sealant materials were incubated with *S. mutans* suspension (2.5 h, 37°C), and adhering bacteria were quantified by using a biofluorescence assay in combination with an automated plate reader. Surface properties and *S. mutans* adhesion were measured prior to and after water immersion after 1 and 6 months and after additional thermocycling (5,000 cycles; 5°C/55°C). The tested sealants showed significant differences in *S. mutans* adhesion prior to and after the applied aging procedures. Aging resulted in slight increases (mostly <0.2 µm) in surface roughness, as well as in significant decreases in SFE and in significantly lower quantities of adhering bacteria. Ketac Bond and UltraSeal XT plus revealed the lowest adhesion potential after artificial aging. In general, the amount of adhering *S. mutans* was reduced after aging, which may be related to the decline in SFEs.

Keywords Aging · Surface properties · Adhesion · *Streptococcus mutans* · Fissure sealants

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Introduction

In pedodontics, dental pit and fissure sealants have been used for the prevention of occlusal tooth surfaces from dental caries since the 1960s. The effectiveness of these sealants in managing tooth decay in children at various levels of risk has been demonstrated in many studies [1, 3, 8, 11, 21]. Sealant materials provide a physical barrier between the oral habitant and deep pits and fissures, which are highly vulnerable to the initiation of caries [15].

The bacterium *Streptococcus mutans* has been identified as the main etiological agent of caries, which represents the most common chronic childhood disease [15]. Within the complex formation of a dental biofilm, *S. mutans* is primarily responsible for the initiation of tooth decay as well as for the progression of an established lesion [9, 16]. The initial adhesion of specific bacteria to tooth surfaces or artificial dental substrata is both the primary and the essential prerequisite for the formation of a cariopathogenic biofilm within the oral cavity [25, 40]. Therefore, sealants and dental materials in general should reveal a low susceptibility to adhere to oral bacteria. Ideally, sealants should also exhibit antibacterial properties that may amplify their potential to prevent caries [19]. It comes as a surprise that—to our knowledge—the adhesion of microorganisms to sealant materials has not yet been investigated. In fact, only few investigations into the antibacterial properties of these materials are available so far [19, 22].

Currently, three types of sealant materials are available: resin-based composites, compomers, and glass ionomer cements [1]. It is still unknown if one of these sealants is superior in preventing the development of caries lesions in vivo [34, 3]. The acid etch technique, the current bonding systems, and the invention of modern resin materials with adequate mechanical properties have made resin-based composites the most popular sealing materials in contemporary dentistry [14]. Resin-based composites are highly esthetic and exhibit the best retention length of all sealant materials [3, 14, 21]. Although new composites offer a fluoride-releasing effect, Menon et al. could not find any antibacterial property, neither for Helioseal (without fluoride) nor for Helioseal F (containing fluoride), by using the disc diffusion method [22]. The second type of material—glass ionomer cements—was introduced as a sealing material in the 1970s [20]. Because of their high release of fluoride, the pivot of preventive dentistry, glass ionomer cements are preferred in clinical situations in which the field of work cannot be kept moisture-free [3, 22, 35]. Compomers, also referred to as polyacid-modified resin composites, were marketed to provide the mechanical and esthetic benefits of composites as well as the fluoride-releasing advantages of glass ionomers [23, 24]. Matalon et al. [19] demonstrated in an in vitro agar diffusion testing that—when compared against three resin-based sealants—the compomer-based sealant Dyract Seal featured the most potent and long-lasting antibacterial activity. Besides the type of sealant material, physicochemical surface characteristics such as surface roughness, hydrophobicity, and surface free energy (SFE) have been shown to significantly influence bacterial adhesion [2, 37]. It has been demonstrated that the SFE of a solid substratum has a crucial influence on the adhesion of oral bacteria [6, 28, 29, 39]. In the thermodynamic model of microbial adhesion, bacterial strains with high SFE (such as *S. mutans*) have a negative interfacial free energy of adhesion ($\Delta F_{adh}<0$) at substratum surfaces with high SFE and are, therefore, expected to preferentially adhere to such substrata [28, 29, 39]. Surprisingly, little information is available on sealants with regard to changes of SFE through aging and the potential effects on microbial adhesion.

Thus, the aim of the present in vitro study was to investigate the adhesion of *S. mutans* on various pit and fissure sealant materials ($n=10$) before and after artificial aging and to correlate these findings to changes in surface roughness and SFE.

Materials and methods

Specimen preparation

A total of ten widely used pit and fissure sealants (seven resin-based composites, two glass ionomers, and one compomer) were assessed in this study (cf. Table 1). All materials were applied in exact accordance with their respective manufacturer's instructions. Uniform cylindrical specimens (measuring 10.0 mm in diameter and 2.0 mm in thickness) were prepared by carefully dispensing the sealants in a custom plastic mold with calibrated circular holes in order to avoid air bubble entrapment. The light-curing materials were covered immediately from top to bottom with two glass slides (Alfred Becht, Offenburg, Germany) followed by light polymerization for 1 min from

Table 1 Ten fissure sealants and two reference materials

Type of material	Material	Manufacturer	Lot no.
Resin-based composite	Clinpro Sealant	3M ESPE, St. Paul, MN, USA	20051202
	Delton FS+	Dentsply DeTrey, Konstanz, Germany	051007
	Embrace WetBond	Pulpdent, Watertown, MA, USA	060227
	Grandio Seal	Voco, Cuxhaven, Germany	V 31185
	Guardian Seal	KerrHawe, Orange, CA, USA	447015
	Helioseal F	Ivoclar Vivadent, Ellwangen, Germany	6127844
	UltraSeal XT plus	Ultradent Products, South Jordan, UT, USA	B1TDX
Glass ionomer	Ketac Bond	3M ESPE, St. Paul, MN, USA	3079498
	Fuji II LC	GC, Leuven, Belgium	0604191
Compomer	Dyract Seal	Dentsply DeTrey, Konstanz, Germany	0605000708
Reference materials	Sinfony (veneering composite)	3M ESPE, St. Paul, MN, USA	237870
	Glass	Marienfeld, Koenigshofen, Germany	–

each side (Heliolux DLX1; Vivadent, Schaan, Liechtenstein/ 100 W, 2 cm distance from the tip). Every specimen was accurately polished to high gloss by using a polishing machine (Motopol 8; Buehler, Coventry, UK) and wet abrasive paper (grain 4000; Buehler, Duesseldorf, Germany). Glass slides (Marienfeld, Koenigshofen, Germany) and specimens of a veneering composite (Sinfony; 3M ESPE, St. Paul, MN, USA) served as reference substrata. A total of 240 specimens, meaning 20 samples of each material, were made. All specimens were stored in distilled water for 5 days to reduce the potential antibacterial influence of residual monomers or other cytotoxic constituents.

Surface roughness and surface free energy

The arithmetic average of surface roughness (R_a) was determined on three spots of six specimens for each material by using a profilometric stylus instrument (Perthometer S6P; Perthen, Goettingen, Germany). The total SFE/γ as well as its dispersion and polar components were calculated from automated contact angle measurements (OCA 15 plus; Dataphysics Instruments, Filderstadt, Germany). Therefore, three liquids with differing surface tension were used: deionized water, diiodomethane (Sigma-Aldrich, St. Louis, MO, USA), and ethylene glycol (Merck KgaA, Darmstadt, Germany). Four drops for each liquid (2 µL) were examined on five randomly selected specimens for each material. The left and right contact angles of each drop were averaged. SFE was calculated according to the Owens, Wendt, Rabel, and Kaelble method [26].

Adhesion testing

Adherent *S. mutans* were quantified as described before [5]. Briefly, the *S. mutans* strain 20523 (DMSZ, Braunschweig, Germany) was used as a test microorganism in this study. The day prior to the experiment, 1 mL of a bacterial suspension was inoculated with 250 mL of sterile trypticase soy broth (BD Diagnostics) and incubated at 37°C for 12 h. The optical density of the suspensions was adjusted with a spectrophotometer (Genesys 10S; Thermo Spectronic, Rochester, NY, USA) to 0.3 at 540 nm. The oxidation–reduction fluorescence dye Alamar Blue/Resazurin (Sigma-Aldrich, 0.75 g/mL *aqua dest*) was used to determine the quantity of bacterial adhesion. Fluorescence intensities were recorded by an automated multidetection reader (Fluostar optima; BMG Labtech, Offenburg, Germany) at wavelengths of 530 nm excitation and 590 nm emission. High relative fluorescence intensities indicated high streptococcal adhesion.

Aging regimes (water immersion and thermocycling)

All test and reference materials were similarly allotted to the aging regime. Surface roughness values, SFEs, and quantification of adhering streptococci were determined at the following time points: at baseline, after 1 month of immersion in distilled water, after 6 months of water immersion, and after additional aging in a thermal cycler (Regensburger Kausimulator, EGO, Germany) with 5,000 cycles (5°C/55°C).

Statistics

Continuous data were summarized by using medians and interquartile ranges (25th to 75th percentile). Global between-group comparisons were done by the Kruskal–Wallis test. The Mann–Whitney *U* test in combination with the Bonferroni adjustment was performed to detect differences in surface roughness, SFE, and relative fluorescence intensities. Spearman's rank correlation coefficients were calculated to assess correlations between the variables relative fluorescence intensity, surface roughness, and SFE. Calculations were done using the statistical software SPSS 15.0 for Windows (SPSS, Chicago, IL, USA).

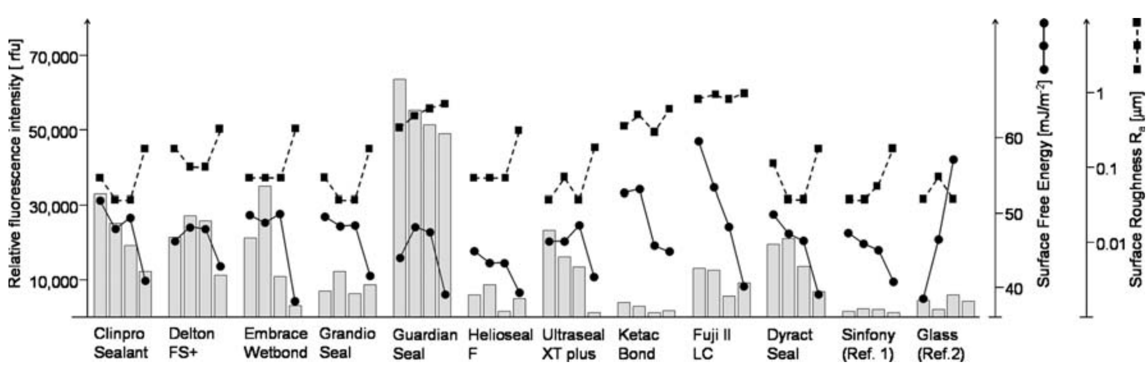

Fig. 1 Relative fluorescence intensities indicating streptococcal adhesion (*bars*), total SFE (*circles and continuous lines*), and arithmetic average of surface roughness (*squares and dashed lines*) at baseline, after 1 month of water immersion, after 6 months of water immersion, and after additional thermocycling (medians) of ten fissure sealants and two reference materials

Results

Arithmetic surface roughness (R_a)

The pairwise comparison by Mann–Whitney *U* test revealed statistically significant differences in arithmetic surface roughness between the various assessed materials (66 pairs; α=0.0008). The significantly highest roughness values, both at baseline as well as after thermocycling, were found for Guardian Seal (0.23/0.53 µm), Ketac Bond (0.29/0.44 µm), and Fuji II LC (0.90/0.96 µm). Except for Fuji II LC that displayed steady roughness values, surface roughness of all test materials increased significantly after the particular aging regimes, especially after thermocycling (six pairs; α=0.0083) (cf. Table 2 and Fig. 1).

Total surface free energy

Significant differences were observed in SFE at baseline. Fuji II LC showed significantly higher SFE values (59.8 mJ/m^{-2}) and the reference material Glass significantly lower SFE values (38.8 mJ/m^{-2}) than all other assessed materials (66 pairs; α=0.0008). After the various aging protocols, statistically significant differences were no longer detectable (p>0.0008 for all pairwise comparisons). In general, SFE values of all test materials significantly declined after the various aging procedures (six pairs; α=0.0083). The most significant declines were calculated after thermocycling (cf. Table 2 and Fig. 1).

Streptococcus mutans adhesion

The comparative adhesion of *S. mutans* is displayed in Fig. 1 and Table 3 as relative fluorescence intensities for all sealant and reference materials. The pairwise comparisons between baseline and after thermocycling showed significant decreases of fluorescence for Clinpro Sealant (p=0.001), Delton FS+ (p=0.002), Embrace WetBond (p<0.001), Guardian Seal (p=0.002), UltraSeal XT plus (p<0.001), Ketac Bond (p<0.001), and Dyract Seal (p<0.001). No statistically significant differences between baseline and after thermocycling were found for Grandio Seal (p=0.650), Helioseal F (p=0.880), Fuji II LC (p=0.059), and reference materials Sinfony (p=0.070) and Glass (p=0.762). The Kruskal–Wallis rank analysis of variance revealed statistically significant differences between test and reference materials for all four assessed stages of aging (p<0.001).

At the starting point of the investigation, a significantly lower fluorescence intensity was found on reference material Sinfony than on all tested sealant materials. The comparisons between Glass and all tested materials revealed significant differences for all sealants, except for Grandio Seal (p=

Table 2 Arithmetic average of surface roughness R_a (median; 25%/75%; µm) and SFE (total SFE, dispersion component and polar component; mJ/m^{-2}) of ten fissure sealants and two reference materials before and after water immersion and artificial aging

Material	Starting point		After 1month		After 6months		After thermocycling	
	R_a	SFE	R_a	SFE	R_a	SFE	R_a	SFE
Clinpro Sealant	0.08 (0.04/0.08)	51.9 (27.9/24.0)	0.04 (0.04/0.04)	48.1 (30.6/17.5)	0.04 (0.04/0.04)	49.6 (26.0/23.6)	0.15 (0.15/0.19)	40.8 (26.3/14.5)
Delton FS+	0.15 (0.11/0.15)	46.6 (27.3/19.3)	0.10 (0.08/0.15)	48.4 (31.0/17.4)	0.10 (0.08/0.23)	48.2 (26.8/21.4)	0.21 (0.19/0.27)	43.9 (31.4/12.5)
Embrace WetBond	0.08 (0.08/0.08)	49.9 (29.8/20.1)	0.08 (0.08/0.08)	48.8 (29.9/18.9)	0.08 (0.09/0.11)	50.0 (28.3/21.7)	0.21 (0.19/0.27)	38.3 (31.3/7.0)
Grandio Seal	0.08 (0.04/0.08)	49.7 (27.9/21.8)	0.04 (0.04/0.08)	48.4 (26.4/22.0)	0.04 (0.04/0.04)	48.7 (22.1/26.6)	0.15 (0.15/0.19)	42.1 (35.8/6.3)
Guardian Seal	0.23 (0.15/0.46)	44.9 (35.2/9.7)	0.38 (0.19/0.53)	48.1 (30.0/18.1)	0.46 (0.31/0.53)	47.6 (26.9/20.7)	0.53 (0.38/0.72)	39.3 (31.9/7.4)
Helioseal F	0.08 (0.08/0.08)	45.1 (25.2/19.9)	0.08 (0.08/0.08)	43.3 (21.9/21.4)	0.08 (0.08/0.11)	43.6 (26.6/17.0)	0.19 (0.19/0.23)	39.4 (32.2/7.2)
UltraSeal XT plus	0.04 (0.04/0.04)	46.5 (26.6/19.9)	0.08 (0.04/0.08)	46.5 (29.4/17.1)	0.04 (0.04/0.04)	48.3 (26.3/22.0)	0.15 (0.11/0.15)	41.6 (28.9/12.7)
Ketac Bond	0.29 (0.23/0.50)	52.9 (27.8/25.1)	0.38 (0.27/0.65)	53.3 (24.5/28.8)	0.19 (0.15/0.31)	45.7 (22.8/22.9)	0.44 (0.31/0.61)	45.0 (31.1/13.9)
Fuji II LC	0.90 (0.61/1.11)	59.8 (22.6/37.2)	0.92 (0.69/1.07)	53.3 (24.8/28.5)	0.88 (0.65/1.14)	48.2 (25.3/22.9)	0.96 (0.61/1.30)	40.1 (31.5/8.6)
Dyract Seal	0.11 (0.11/0.15)	50.0 (26.6/23.4)	0.04 (0.04/0.04)	47.3 (26.6/20.7)	0.04 (0.04/0.04)	46.5 (29.1/17.4)	0.15 (0.11/0.15)	39.1 (28.9/10.2)
Sinfony (ref. 1)	0.04 (0.04/0.04)	47.5 (29.5/18.0)	0.04 (0.04/0.08)	46.0 (31.1/14.9)	0.06 (0.04/0.08)	45.3 (28.8/16.5)	0.15 (0.11/0.15)	40.9 (33.2/7.7)
Glass (ref. 2)	0.04 (0.04/0.08)	38.8 (17.3/21.4)	0.08 (0.04/0.08)	46.8 (20.2/26.6)	0.04 (0.04/0.04)	57.6 (19.7/37.9)	[a]	[a]

[a] All glass slides were broken during thermocycling

Table 3 Relative fluorescence intensities [no units] on ten fissure sealants and two reference materials before and after water immersion and artificial aging (median; 25%/75%)

Material	Baseline	After 1month	After 6months	After thermocycling
Clinpro Sealant	32,398 (29,000/35,099)	25,363 (18,125/28,889)	18,874 (17,025/22,383)	12,072 (8,908/14,658)
Delton FS+	21,527 (16,081/28,856)	27,299 (24,460/30,085)	26,101 (22,321/32,297)	10,629 (7,906/12,612)
Embrace WetBond	21,206 (14,434/40,632)	34,829 (31,670/37,077)	10,691 (9,192/13,060)	3,487 (2,937/5,069)
Grandio Seal	7,735 (6,256/9,557)	12,150 (9,966/14,055)	6,684 (4,214/7,323)	8,360 (6,563/9,293)
Guardian Seal	63,586 (63,524/63,695)	55,692 (54,640/57,870)	51,249 (48,511/57,572)	48,446 (42,902/55,053)
Helioseal F	5,909 (4,082/9,412)	8,228 (5,595/8,751)	1,409 (900/2,172)	5,461 (4,890/7,205)
UltraSeal XT plus	23,144 (19,426/29,164)	16,010 (15,476/19,245)	13,552 (11,619/15,294)	708 (587/749)
Ketac Bond	3,900 (2,042/4,739)	2,879 (1,621/4,486)	493 (381/1,177)	1,003 (492/1,382)
Fuji II LC	13,129 (8,676/19,642)	12,529 (10,143/15,263)	5,577 (4,699/9,641)	9,273 (8,973/9,980)
Dyract Seal	19,497 (18,430/23,277)	20,699 (17,728/27,246)	13,480 (10,525/16,318)	6,505 (5,615/9,438)
Sinfony (ref. 1)	1,410 (1,232/1,771)	2,182 (1,935/3,322)	1,619 (982/1,804)	1,205 (1,074/1,276)
Glass (ref. 2)	4,288 (3,839/5,707)	2,030 (290/3,419)	6,028 (3,135/7,595)	4,455 (1,962/5,708)

High fluorescence intensities indicate high amounts of adhering *S. mutans*

0.013), Helioseal F (p=0.226), and Ketac Bond (p=0.364). In general, high relative fluorescence intensities (rfu>20,000) could be observed for Clinpro Sealant, Delton FS+, Embrace WetBond, Guardian Seal, and UltraSeal XT plus. Moderate fluorescence intensities (rfu between 10,000 and 20,000) were found for Fuji II LC and Dyract Seal, and low fluorescence values (rfu<10,000) for Grandio Seal, Helioseal F, Ketac Bond, Glass, and Sinfony.

After 6 months of water immersion and thermocycling, Sinfony still revealed significantly lower fluorescence intensities than all tested sealants, except for Ketac Bond with comparable values (p=0.496). UltraSeal XT plus showed a significantly lower fluorescence intensity than both reference materials, Sinfony and Glass (p=0.001). Significantly higher fluorescence intensities than for reference material Glass were found for Clinpro Sealant, Delton FS+, Guardian Seal, Grandio Seal, Ketac Bond, and Fuji II LC. No statistical difference to Glass was revealed by Embrace WetBond (p=0.705), Helioseal F (p=0.151), and Dyract Seal (p=0.023). After aging, high relative fluorescence intensities were still found for Guardian Seal and moderate fluorescence intensities for Clinpro Sealant and Delton FS+. All other tested materials showed low fluorescence intensities.

Correlations between fluorescence, R_a, and SFE

Spearman's rho coefficients (SR) were calculated to detect possible correlations between the assessed surface properties and the quantity of bacterial adhesion. No significant correlations were found between fluorescence and R_a (SR= −0.015). Medium correlations were found between fluorescence and SFE (SR=+0.301) and between R_a and SFE (SR= +0.203).

Discussion

In modern dentistry, pit and fissure sealants represent a state-of-art method for preventing the initiation of occlusal caries in children [1, 3, 21]. Thus, the potential of a pit and fissure sealant material to reduce microbial adhesion and prevent the colonization of cariopathogenic bacteria may contribute to the prevention of caries [1, 18, 19]. The present study aimed at assessing changes in surface properties of sealant materials after artificial aging and at attempting to correlate these findings to the in vitro adhesion of *S. mutans*.

The resazurin reduction test employed in this study is a well-established fluorometric assay for the in vitro quantification of viable bacteria [12, 33]. The comparison of antibacterial properties in vivo seems to be preferable but must be regarded as impracticable in consideration of the high number of materials tested (n=12). Surprisingly, most advice from in vitro studies on the suitability of a sealant material for clinical settings has predominantly been based on retention rates and mechanical properties rather than on antibacterial or antiadhesion properties [3, 22]. However, these biological parameters are indicators for the effectiveness of a specific material to prevent caries, which, after all, is the main reason for the application of a sealant.

High surface roughness values are known to promote extensive plaque accumulation on both oral tissues and dental materials [37]. Bollen et al. [4] found a threshold value of 0.2 μm. Below this value, surface roughness had no further influence on the quantity of bacterial adhesion. Therefore, all specimens in this study were polished to high gloss. Most specimens had significantly lower roughness values than this threshold value, although aging generally resulted in increasing roughness values. Increasing surface roughness values on various dental materials after artificial aging have been reported by other authors, which corresponds to the findings of this study [36, 41, 42]. In resin-based composites, aging is generally known to cause erosion of the resin matrices and exposure of filler particles [27]. In this study, only the resin-based composite Guardian Seal and the glass ionomers Ketac Bond and Fuji II LC revealed significantly higher R_a values than 0.2 µm. When compared to all other resin-based composites, Guardian Seal also had the significantly highest bacterial adhesion, which—in this case—may result from its above-average roughness values. In contrast to Guardian Seal, Fuji II LC did not reveal a high streptococcal adhesion in spite of the highest surface roughness values of all materials tested. Therefore, other surface properties besides surface roughness seem to have an influence on *S. mutans* adhesion. Despite the significant differences in arithmetic surface roughness values, no general correlation to adhesion quantities could be observed in this study.

The crucial influence of hydrophobicity and SFE on the bacterial adhesion process is widely accepted, but there is still conflicting evidence if high SFE surfaces reduce or enhance the quantity of adhering microorganisms [2, 6, 7, 28, 29, 31, 32, 37]. In this study, significant differences in total SFE between the assessed materials were found at baseline (SFE values ranged from 44.9 to 59.8 mJ/m^{-2}). Because of the specific chemistry of cements, the two glass ionomers tested had significantly higher SFE values than the resin-based composites. After the assessment of all test materials, aging caused a leveling of these originally distinct SFE values (38.3 to 45.0 mJ/m^{-2} after water immersion and thermocycling). This leveling effect and the general tendency for declining SFE values after the various aging protocols may result from the absorption of water. In turn, this absorption may lead to soaking and to a rearrangement of monomer chains of the composite with disperse parts oriented to the surface. Differences in the contribution of polar and dispersion components were poor (data not shown).

All surfaces in the oral cavity are rapidly covered with a layer of salivary proteins [6, 28, 29, 39]. This proteinaceous pellicle crucially affects the adhesion process of microorganisms to solid surfaces, but a considerable direct influence of the underlying substratum on the quantity and quality of bacterial accumulation remains [28, 39]. Specific substratum properties such as SFE are transferred from the material–protein interface to the protein–bacterium interface [28, 29]. As we intended to evaluate the bare material-specific influences of different fissure sealants on streptococcal adhesion in the present study, no pellicle precoating of specimens was conducted. By eliminating the influence of the pellicle, changes in streptococcal adhesion could be reduced to the influence of material properties only, which in turn led to a simplified interpretation of the results. In general, the salivary pellicle reduces the material-specific effects on bacterial adhesion by leveling differences in SFE between high-SFE and low-SFE substrata [28, 39].

The microbial adhesion process is not only dependent on the SFE of the substratum but also on the SFE of the bacterial strain [6, 7, 28, 39]. It has been assumed (in the thermodynamic model of bacterial adhesion) that microorganisms with low SFEs prefer low-SFE surfaces and that high-SFE bacteria adhere more strongly to substrata with high SFE values [2, 7, 32, 37, 39]. Several authors found high SFEs (>100 mJ/m^{-2}) for different *S. mutans* strains [28, 38, 39] and Pratt-Terpstra et al. demonstrated that increased quantities of *S. mutans* adhered on pellicle-free substrata with high SFE [28]. Consequently, fissure sealant surfaces with lower SFE values would be expected to be thermodynamically unfavorable for *S. mutans* adhesion in the present study. The declining number of adhering *S. mutans* after aging and the correlation to diminishing SFE values are in strong accordance with these findings. A single streptococcal strain, *S. mutans*, was used as a test bacterium in this study. Because of the complexity of the individual bacterial cell surface and the unpredictable influence of different bacterial strains (with differing SFE) on the adhesion process, our findings must not be transferred to the in vivo clinical situation without any restrictions [7, 30].

It has been suggested that the formulation of the compound has a significant effect on the antibacterial property of sealant materials and hence on the quantity of adhering microorganisms [22]. This assumption has been affirmed by the present study because significant differences have been found in the bacterial adhesion to the various sealant materials. The resin-based composite Guardian Seal revealed the highest adhesion potential both at baseline as well as after thermocycling. At the starting point, the glass ionomer Ketac Bond and the composites Grandio Seal and Helioseal F had the significantly lowest adhesion values. After the various aging procedures, Ketac Bond and UltraSeal XT plus showed low adhesion potentials. This shifting emphasizes the importance of artificial aging in the interpretation of in vitro microbial adhesion studies. The observation of aging effects is particularly interesting in the present study because anti-adhesion properties in sealants might be helpful in preventing caries in the long-term [19]. Matalon et al. [19] found the compomer Dyract Seal to have the most potent and longest-lasting antibacterial activity when compared against Helioseal F and UltraSeal XT plus. These findings have not been consistent to our data where Dyract Seal did not show a significantly lower streptococcal adhesion than these two resin-based composites. Although, in our study, adhesion testing on glass ionomers tended to result in rather low adhesion values, it remains equivocal if any type of sealant material is superior when comparing resin-based composites, compomers, and glass ionomers.

The fluoride release by sealant materials has been suggested to reduce the adherence of *S. mutans* and may, therefore, contribute to the prevention of caries [13, 17, 19]. Previous studies have reported that the fluoride level in dental plaque on glass ionomers is significantly higher than that on resin-based composites [10], which might serve as a valid explanation for the low adherence of *S. mutans* to the two glass ionomers used in this study.

In consideration of the limitations of this in vitro study, the following conclusions may be drawn:

1. The tested sealant materials demonstrated a significantly different *S. mutans* adhesion with a tendency for reduced adhesion values after artificial aging. Ketac Bond and UltraSeal XT plus revealed the lowest adhesion potentials after artificial aging.
2. Except for Guardian Seal, which yielded high surface roughness as well as high streptococcal adhesion, no general correlation was found between surface roughness and the quantity of adhering *S. mutans*.
3. Artificial aging and thermocycling resulted in increased surface roughness values and declining SFEs.
4. Declining SFE values corresponded to declining adhesion potentials during aging, a medium correlation between SFE and bacterial adhesion could be observed.
5. The correlation between these in vitro findings and the clinical performance of the various sealants has to be established in further studies.

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Conflict of interest The authors declare that they have no conflict of interest.

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