

Continuous effect of pit and fissure sealing on *S. mutans* presence in situ

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Abstract

Purpose: The effect of sealants on *S. mutans* presence in situ was investigated.

Methods: Four intact, fully erupted first permanent molars in each of 74 children, aged 6-8 years were required for inclusion in the study. Baseline examination included deft and plaque index. *S. mutans* presence on occlusal surfaces of the molars was evaluated, using a microbial replica method. Immediately after sealing the first permanent molars on one side, *S. mutans* presence in situ was re-evaluated, as well as three and six months thereafter. Three months after the initiation of the study, *S. mutans* presence was evaluated on the molars of the unsealed side, which were consequently sealed and re-evaluated immediately, and three months later.

Results: Positive correlation was found between deft scores, plaque indices and microbial replica values, at baseline. Sealing caused a significant reduction in *S. mutans* levels on the treated occlusal surfaces, in vivo ($P < 0.001$), which lasted, in most cases, up to six months.

Conclusions: The data suggest that sealants enable a prolonged reduction of *S. mutans* presence in situ, indicating an additional prevention effect, by reducing one source of dissemination. (*Pediatr Dent* 21:164-168, 1999)

Early detection of incipient caries is essential for treatment planning strategy. It saves the need for operative treatment, which leads to irreversible loss of tooth structure and enables the design of a conservative prevention treatment plan by dietary control recommendations, fluoride protocol, and sealant performance.¹

The most prevalent diagnostic method for incipient occlusal caries is based on inspection and probing. This method is highly prone to subjective bias and may potentially cause further damage to tooth structure.²⁻⁵ Microbial monitoring has been considered as an alternative method for evaluating current caries activity and future caries risk. Longitudinal studies have shown a relative rise of *S. mutans* count in plaque samples from tooth surfaces that become carious at a later stage.⁶⁻⁹ *S. mutans* levels have also been measured in the saliva of children from different backgrounds, and were found to correlate with the patient's caries activity levels.^{10,11} These methods did not gain acceptance in routine clinical use because of technical complexity, lack of correlation to caries on specific tooth

surfaces, and the inability of salivary *S. mutans* levels to significantly define caries activity risk.¹²

Rosenberg et al developed a simple method based on a sucrose enriched impression matrix which replicates *S. mutans* from the actual site of an affected fissure on the matrix-medium interface.^{13,14} The method has the potential to provide tooth-specific diagnosis of caries prone sites and to enable clinical evaluation of occlusal restorations.

Although a continuous decline in overall caries activity was reported in western populations, pits and fissures remain susceptible to caries attacks. Consequently, sealants are accepted as a major attribute to preventive dentistry and are recommended as soon as teeth erupt.¹⁵ Sealants may also be effective in arresting initial occlusal caries by interfering with the vital conditions required for bacteria proliferation.¹⁶⁻¹⁸ Theoretically, sealing the occlusal surfaces should reduce bacterial dissemination. However, Carlsson et al.¹⁹ found no immediate influence of pit and fissure sealing on *S. mutans* counts in the saliva of children.

It is still unclear whether sealing pits and fissures has any effect on *S. mutans* presence on those surfaces. The purpose of this study was therefore to evaluate the immediate and continuous effect of sealing occlusal surfaces, on *S. mutans* presence, in situ, by using the microbial replica method.^{13,14}

Methods

Study population

A total of 312 children, aged 6-8 years, from three different educational institutions were initially screened. The criteria for inclusion in the study was the existence of four fully erupted intact first permanent molars without operculum involvement, caries, restorations, or anatomic abnormalities. Out of these, 75 children were selected to participate in the study: 25 children from Kibbutz Sdot Yam (a communal rural society-group 1), 28 elementary school children from the city of Tel Aviv (group 2), and 22 boarding school children from the peripheral city of Bnei Braq (group 3). Each child was examined and treated three times according to the clinical protocol described below. A total of 52 children, 25 from groups 1 and 27 from group 2 completed the entire clinical protocol (28 girls and 24 boys). Group 3 did not complete the clinical protocol as specified, due to lack of cooperation from the school's board of

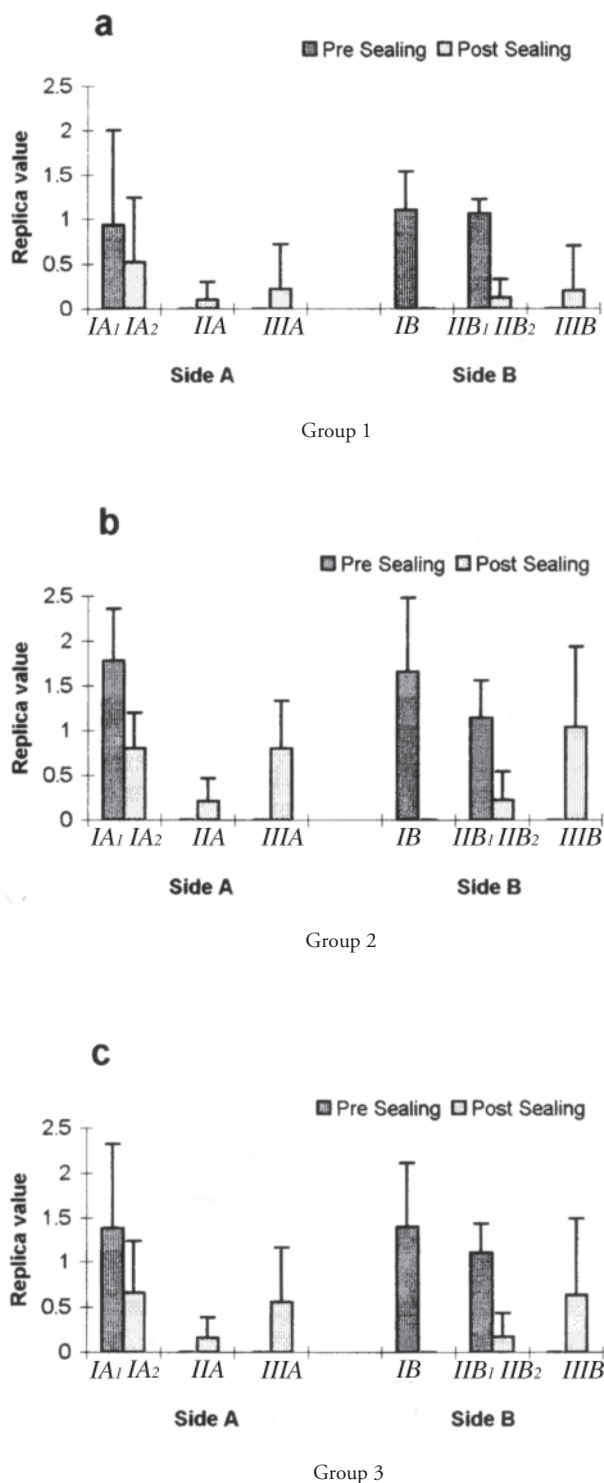


Fig 1. Pre- and post-sealing microbial-replica values of maxillary and mandibular first permanent molars. IA₁, IB=initial (baseline) of side A and B, IA₂=immediate post sealing, side A, IIA=side A in three months, IIIA=side A in six months, IIB₁=side B, in three months, IIB₂=immediate post-sealing, side B, IIIB=side B three months after sealing the molars of this side.

directors. Hence, the results for the entire clinical protocol are based on data from 52 children, and the results for group 3 are reported separately.

Microbial replica method

Microbial replica is a technique for localizing caries-associated bacteria on tooth surfaces.¹⁴ It is based on a semi-solid impression matrix with the ability to replicate the occlusal surface morphology along with adherent bacteria in situ. The impression matrix includes sucrose as carbon and energy source for bacterial growth. The liquid medium, in which the matrix and the replicated bacteria are subsequently incubated, contains tryptose, proteose peptone, trypan blue, gentian violet, potassium tellurite (Difco, Detroit, MI), and 0.5 units/ml bacitracin (Sigma, St. Louis, MO). The last two additives are selective agents that limit growth to caries inducing *S. mutans* serotypes c, e, f and *S. sobrinus* serotypes d and g. The sucrose is excluded from the liquid medium, in order to allow bacterial growth, exclusively at the site of bacterial adhesion, on the matrix-liquid interface.

Clinical sampling involves placing a strip of the impression matrix between the upper and lower teeth on the examined side. The patient is instructed to bite for three seconds to obtain an impression of the upper and lower occlusal surfaces. The matrix is then rinsed in tap water to remove saliva and loosely adherent bacteria. Then the matrix is placed in a petri dish containing the liquid growth medium. After overnight incubation at 35° C, the matrix is removed from the liquid medium and examined for presence of dark blue *S. mutans* colonies.¹⁴

Clinical protocol

The clinical protocol included three treatment and evaluation sessions, which are designated by Roman numerals I, II, and III. The sides (left or right) of the mouth where sealants were performed randomly, are designated by the letters A or B. Where applicable, microbial replica values are characterized by subscript numbers as follows: 1=before sealing and 2=immediately after sealing. For example: IIB₂ indicates replica values in the second meeting (II), on side B, immediately after sealing.

At the first meeting, each child was examined for deft levels according to Von der Fehr²⁰ and for plaque index (PI) according to O'Leary.²¹ The following procedures were also performed:

1. Two baseline microbial replicas were taken from every child; each replica included an imprint of the upper and lower molars from one side. These replicas were designated randomly as replicas IA₁ and IB (no sealing).
2. Fissure sealing of the upper and lower molars of side A of the mouth was performed by two experienced operators (EM, EIW), using Helioseal (Vivadent Schaan, Liechtenstein), according to the manufacturers instructions. The molars on the opposite side (B) were not treated at this session.
3. Another microbial replica was taken of the teeth on the sealed side, immediately after sealing (ca. 5 min) and designated as replica IA₂.

The second meeting took place three months later and included the following procedures:

1. The integrity of the sealants on side A was ascertained.

Table 1. Mean and SD of Clinical Indices and Microbial Replica Values Measured in Groups 1 and 2 at Baseline

	Group 1 N=25	Group 2 N=27	t-test	Mean N=52
def-t	2.40±2.00	6.60±2.78	<i>P</i> <0.001	4.62±3.22
PI	1.56±0.58	2.46±0.64	<i>P</i> <0.001	2.01±0.61
IA ₁	0.94±1.06	1.78±0.58	<i>P</i> <0.001	1.38±0.94
IB	1.10±0.43	1.66±0.82	<i>P</i> <0.003	1.40±0.72

def-t is according to Von der Fehr²⁰; PI-according to O'Leary²¹; IA₁ and IB-Microbial replica values of both sides of the mouth, at baseline.

2. Microbial replica imprints were performed on both sides of the mouth and were designated as replicas IIA on the side which has been sealed previously and IIB₁ on the untreated side.
3. Sealants were applied to occlusal surfaces of the upper and lower molars on side B.
4. Microbial replica imprints were taken of the newly sealed teeth and designated as replicas IIB₂.

The third meeting took place three months after the second meeting (six months from the first meeting) and included assessment of the sealants integrity, and replica impressions on both sides of the mouth (IIIA and IIIB, respectively).

Evaluation of replicated bacteria

Immediately after incubation, all replica matrices were photographed under standardized conditions on a colored HR100 Kodak film and enlarged to a 9x13 cm print. All photographs were examined under double-blind conditions. To obtain a bacterial growth value for each tooth (replica value), relevant occlusal surfaces were divided into four quadrants (mesiobuccal, distobuccal, mesiolingual, and distolingual) and scored according to the following scale:

- 0 - No bacterial growth
- 1 - Bacterial colonies on one quadrant only
- 2 - Bacterial colonies on two quadrants
- 3 - Bacterial colonies on three to four quadrants

Mean replica values for each side of the mouth were obtained by calculating the mean values of the upper and lower molars of the corresponding side for each child. Data were analyzed statistically using Pearson's correlation coefficients and paired *t*-tests.

Results

Baseline deft, PI, and replica values of the study population, who completed the entire clinical protocol (N=52) are presented in Table 1. Significant differences were found between groups 1 and 2 in the PI, deft, IA₁, and IB values (*P*<0.001). Based on these differences, group 1 was considered as a low caries-active group and group 2 as high caries-active. Significant Pearson's correlations were found between: (i) deft and PI (*r*=0.42; *P*=0.001), deft and each of the initial replica values at both sides of the mouth (IA₁: *r*=0.34; *P*=0.007) and (IB: *r*=0.25; *p*=0.036), and between PI and each of the initial replica values (IA₁: *r*=0.50; *P*=0.001) and (IB: *r*=0.31; *P*=0.026) (Table 2).

Microbial replica values obtained during the entire clinical protocol are presented in Figure 1. The clinical integrity of the sealants at three and six months was found adequate in all

teeth. Sealing fissures of the first permanent molars led to an immediate and sustained decrease in replica values in both groups. For the combined groups (52 children), the difference between baseline value on side A (IA₁) and the immediate replica value (IA₂) after sealing was significant at the 0.001 level (paired *t*-test) (Table 3). The differences between baseline value (IA₁), and

the value after three and six months (IIA and IIIA, respectively) were also significant (*P*<0.001). On side B, two baseline values were measured; one at the initiation of the study along with IA₁ and one after three months, before sealing side B. There was no significant difference between IA₁ and IB. However, a decrease in replica values was noted (in group 2 and in the combined groups, *P*<0.01), between the initiation of the study (IB) and three months thereafter (IIB₁). Sealing of side B led to a significant decrease (*P*<0.001) in values immediately after sealing (IIB₂) in both groups, as well as in the combined groups (Fig 1). Replica values recorded 3 months later (IIIB) showed a significant decrease (*P*<0.01) in the combined groups, compared to baseline IB or IIB₁. However, in group 2, this reduction was significant only between IB and IIIB.

In group 3 (N=22), which participated only in the first two meetings and exhibited remarkably high deft and PI values, similar trends were evident. Immediately following sealing teeth on side A had a significant decrease in bacteria on the sealed side. This significant decrease (*P*<0.0001) remained virtually the same three months later (Table 4).

Discussion

After fissure sealing, this study demonstrates a marked decline in *S. mutans* presence on the occlusal surfaces of permanent molars in situ, when evaluated by using a microbial replica method. The significant decrease in the replicated bacteria was immediate and sustained throughout the six months of this study.

This finding was valid for both the low and the high caries-active groups of children. Group 1 included children from a closed, homogenic, rural society where all children are raised together with similar nutrition, health care, and education. Group 2 is a heterogenic population of urban children from

Table 2. Pearson's Correlation Coefficients Between Clinical Variables of the Combined Groups Who Completed the Entire Clinical Protocol (N = 52)*

	def-t	PI	IA ₁
PI	<i>r</i> =0.42 <i>P</i> =0.001		
IA ₁	<i>r</i> =0.34 <i>P</i> =0.007	<i>r</i> =0.50 <i>P</i> =0.001	
IB	<i>r</i> =0.25 <i>P</i> =0.036	<i>r</i> =0.31 <i>P</i> =0.026	<i>r</i> =0.32 <i>P</i> =0.011

* Variables as defined in legend to Table 1.

Table 3. Mean Replica Values for the Combined Groups (N=52)

Meeting	Mean±SD*		Significance†		
			IA ₂	IIA	IIIA
1st	IA ₁ **	1.38±0.94	0.001	0.001	0.001
	IA ₂	0.66±0.58		0.001	(N.S)
2nd	IIA	0.16±0.23			0.001
3rd	IIIA	0.56±0.61			
			IIB ₁	IIB ₂	IIIB
1st	IB†	1.40±0.71	0.01	0.001	0.001
2nd	IIB ₁	1.10±0.33		0.001	0.001
	IIB ₂	0.17±0.27			0.001
3rd	IIIB	0.64±0.85			

*Mean replica values for the combined groups (Groups 1 and 2, N=52); **Replica values on side A as follows: IA₁-1st meeting prior to sealing; IA₂-1st meeting immediately following sealing; IIA-2nd meeting; IIIA-3rd meeting; †Replica values on side B as follows: IB-1st meeting; IIB₁-2nd meeting prior to sealing; IIB₂-2nd meeting immediately following sealing; IIIB-3rd meeting; ‡paired t-test

different socio-economic and cultural backgrounds. Nevertheless, the immediate and continuous effect of occlusal fissure sealants on the *S. mutans* population in situ was similar in both groups. This trend was also consistent for the other very high caries-active group (group 3), which represents a relatively homogenous population with exceptionally high deft rate, and which did not complete the entire clinical protocol. The fact that fissure sealing significantly reduced *S. mutans* growth on their sealed occlusal surfaces for at least three months, provides additional support for the findings in group 1 and 2. The decrease in baseline replica values (IIB₁) obtained in group 2, three months after the first baseline replica values were recorded (IB), may be coincidental and may reflect the effect of improvement in hygiene, because of the participation in the study.

Methods for estimating caries-associated bacteria in saliva^{15,22-24} have limited clinical use, since they can not locate and

predict the actual sites of carious activity.²⁴ Correlation between salivary *S. mutans* counts and caries activity is never definitive on an individual basis, or in epidemiological studies.²⁵ In the present study, the positive correlations between the initial replica values at both sides (IA₁, IB) and the deft and PI values of the three study populations suggest that this bacterial sampling method provides an additional valid diagnostic tool. It can be used to evaluate occlusal pits and fissures associated with cariogenic bacteria, as well as sealants and other restorations. It can be performed by semi-skilled personnel and can also be used in related lines of research.¹⁴

The effect of pit and fissure sealing on viable microorganisms between sealants and tooth structure has been reported.²⁶ Going et al¹⁶ also showed that five years after sealing carious lesions, only a limited number of cultivable microorganisms persisted, and they were not capable of continuing tooth destruction. The present study suggests reduction in colonizing microorganisms in the outer sealed surface, thus contributing an additional preventive aspect by eliminating some of the cariogenic bacterial reservoirs from the oral cavity.

Carlsson et al.¹⁹ reported that pit and fissure sealing does not affect salivary *S. mutans* levels. The finding of the present study implies that lowering *S. mutans* levels on the occlusal surfaces may not necessarily be manifested in whole saliva *S. mutans* counts, which in turn, might be affected by other reservoirs in the oral cavity. Our data do not appear to be in agreement with the observation of Skjorland and Sonju, who reported that composite restorations may increase bacterial colonization.²⁷ This may reflect differences in the materials used and the clinical procedures. We suggest that physical changes in tooth morphol-

Table 4. Mean and SD of Clinical Indices and Microbial Replica Values Measured in Group 3

Meeting	Replica	Mean ± SD	Significance*	
			IA ₂	IIA
I	IA ₁	2.29±0.57	0.001	0.001
	IA ₂	0.34±0.33		(N.S)
II	IIA	0.22±0.30		
def-t		8.54±3.23		
PI		2.36±0.49		

def-t is according to Von der Fehr²⁰; PI is according to O'Leary²¹; *paired t-test

ogy achieved by sealing, as well as the surface features of the restorative materials, affect the biological micro-environment which results in continuous reduction of surface associated potentially cariogenic bacteria. Further large scale studies are needed to validate this hypothesis.

Conclusions

1. A decline in the presence of potentially cariogenic bacteria on sealed surfaces may last up to six months
2. The elimination of *S. mutans* as a source for dissemination in the oral cavity is an additional beneficial effect of the sealing procedure
3. The replica method is a valid microbial sampling tool for monitoring bacteria in situ.

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