The immediate and long-term effects of invasive and noninvasive pit and fissure sealing techniques on the microflora in occlusal fissures of human teeth

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Abstract

The purpose of this study was to evaluate the effect of acid conditioning and occlusal sealant on microbial colonization of pit and fissures submitted to ameloplasty or left intact. Human enamel blocks containing fissures prepared from the occlusal surfaces of unerupted third molars were implanted in occlusal fillings in molars of 12 patients for seven, 30, 60, and 120 days. After seven days of exposure to the oral environment, the pit and fissure blocks were removed and found to be colonized, mainly with gram-positive coccal flora. The acid-etching procedure itself reduced the number of cultivable microorganisms by about 95%. Subsequent application of occlusal sealant caused a gradual decrease of the remaining viable microorganism throughout the experiment. Despite the decrease of 100% after acid etching in most of the fissures submitted to ameloplasty, the occlusal sealant did not lead to a subsequent significant reduction. (Pediatr Dent 15: 108–12, 1993)

Introduction

Occlusal pits and fissures are the most susceptible sites for the development of dental caries, and present areas where prevention is very difficult.[1–3] Early attempts to control occlusal caries involved techniques which eradicated the pit and fissures, and removed sound tooth structure.[4,5] In recent years, the effectiveness of pit and fissure sealants in preventing occlusal caries has been well documented by controlled clinical studies.[6,7]

The clinical diagnosis of incipient caries lesions in posterior teeth frequently is complicated by the depth, narrowness, and anatomic complexity of the pits and fissures.[8–11] Consequently, such lesions may remain undetected and, inadvertently, be sealed with a dental sealant. It is inevitable that clinicians will differ in their restorative decisions and in the way they manage questionable or borderline lesions. In the literature, the choice between the invasive and noninvasive techniques remains a matter of debate.[11–14]

The fate of bacteria under restorations and under sealants has been investigated for decades. Most investigators observed a diminishing number or elimination of viable microflora as well as a decrease in cariogenic pathogenicity when bacteria were separated from the oral environment.[15–19] Sealants apparently seal off residual bacteria from their principal nutrient supply, thus preventing the production of acid in cariogenic concentrations. Further demonstration that sealing caries in pits and fissures is a safe and beneficial procedure would extend the clinical usefulness of such adhesives to areas other than prevention.

Dentists show a conservative pattern toward cavity preparation for initial carious lesions. After reviewing different procedures used for treating occlusal fissures, De Craene and coworkers[13] concluded that narrow, sticky fissures in which caries might develop should be treated with the invasive pit and fissure sealing technique. Meiers and Jensen[11] reported that, microbiologically, this technique is the most rational of all the invasive methods. The clinician removes the questionably carious portion of the fissure, and at the same time protects the clinically sound fissures from future caries. (No extension for prevention is carried out.)

According to Hicks,[20] Russo and coworkers,[21] and De Craene and coworkers,[13] the most important advantage of the invasive technique is the possibility of diagnosing the extent of the carious lesions. Furthermore, some studies suggested that microleakeage is reduced following mechanical preparation of the fissure area.[22–24]

The present investigation was conducted to compare the effect of acid conditioning and occlusal sealant on microbial colonization of natural human fissures submitted to ameloplasty or left intact.

Methods and materials

Blocks of human teeth measuring 5 x 4 x 3 mm, containing a well-defined occlusal fissure, were obtained from unerupted, surgically removed third molars. The fissure blocks were cemented using phosphate cement into the occlusal surface of pre-existing amalgam fillings in first permanent molars of 12 patients, according to the method described by Fejerskov and coworkers.[25] Each patient received four fissure blocks, one in each molar. Patients at the Pedodontic Clinic of the School of Dentistry of the University of São Paulo volunteered to participate in this study. Although each patient had several restorations reflecting various degrees of caries experience, clinical ex-

amination disclosed no active carious lesions. These patients were selected because implantation of the fissures required large occlusal amalgam fillings. Informed consent was obtained according to accepted procedures.

The 24 fissure blocks cemented in six patients were submitted to ameloplasty (invasive technique) with a miniaturized diamond bur at high speed over all pits and fissures, immediately after cementation. This procedure made it possible to reach the bottom of the fissure with a probe. Care was taken not to penetrate into the dentin, or to enlarge the fissure more than was necessary. All 48 fissure blocks were maintained in the patient's mouth for seven days to accumulate plaque. The volunteers continued their usual diet and tooth-brushing.

On the seventh day, two fissure blocks, one of which had been etched immediately before sampling with phosphoric acid (etching solution; Delton®, Johnson and Johnson) for 1 min (Table 1), were removed from each patient. One of two fissure blocks remaining in each patient received an occlusal sealant (Delton) according to the manufacturer's instructions. These two fissure blocks were removed after 30, 60, or 120 days (Table 2).

After removal from the amalgam filling with a small, round bur (no. 00), the fissure blocks were split open along the fissure using a chisel placed in the fissure opening; the fragments were dropped into a tube containing 2 ml of phosphate-buffered saline. The fissure contents were suspended by agitation (vortex) for 2 min.

Viable counts were performed by plating 0.1 ml of serial dilutions of the suspension on the following media:

1. Nonselective blood agar medium (B.H I./Difco®). These plates were incubated two days at 37°C, one set in air for aerobic viable count and another set in anaerobic jars (Gas-Pak® system) for anaerobic viable counts.
2. Mitis salivarius agar (Difco) incubated for two days at 37°C in candle jar for streptococcal viable counts.
3. MSB agar (Mitis salivarius agar containing 0.2 U/ml bacitracin and 20% sucrose, Gold et al.[26]) incubated for two days at 37°C in candle jar for *Streptococcus mutans* viable counts.
4. Rogosa selective *Lactobacillus* agar (Difco) incubated for two days at 37°C in pour-plate.
5. Rogosa selective *Veillonella* agar (Difco) incubated for two days at 37°C in anaerobic jars (Gas-Pak system).

The microorganisms were characterized by cell morphology and gram-stain. Although the dimensions of the fissures samples may have varied, all counts were calculated as number per fissure.

Results

The integrity of the seal was checked visually with an explorer, and it appeared sound in all instances.

Table 1. Viable counts of seven-day-old fissure plaque without acid-etching (NAC) and immediately after acid-etching (AE)

Subject	*Total Anaerobic*			*Total Aerobic*		*Total Streptococcal*		*Streptococcus mutans*		*Lactobacillus*		*Veillonella*	
	NAC	*AE*	*%Reduction*	*NAC*	*AE*	*NAC*	*AE*	*NAC*	*AE*	*NAC*	*AE*	*NAC*	*AE*
Noninvasive													
SD	1500	2	99. 87%	740	1	460	0	0. 20	0	0	0	5	0
JF	1300	43	96. 69	456	9	848	12. 20	62	5. 60	22	10. 40	69	21
HD	258	0. 02	99. 99	245	0	88	0	7	0	0	0	6	0
PK	818	25	96. 94	135	6. 20	82	2. 20	0	0	0	0	0. 40	0. 20
AY	1980	3	99. 85	610	2	320	22	68	0	1	0	12	0
JN	960	121	87. 40	740	96	285	34	0	0	0	0	1	0. 20
Range	258–1980	0.02–121	87.40–99.99	135–740	0–96	82–848	0–34	0–68	0–5.60	0–22	0–10.40	0.40–69	0–21
Mean	1136	32	96. 79%	488	19	347	12	23	1	4	2	16	4
Invasive													
CL	962	0	100. 00%	628	0. 02	8.70	0	0. 40	0	8	0	12	0
FA	538	0. 40	99. 93	988	1	248	0.02	0. 60	0. 02	0	0	2	0
MN	82	8	90. 24	44	2	20	1.60	1	0	0	0	2	0
BT	380	0	100. 00	272	0.04	36	0.02	0. 60	0	0	0	2	0
TZ	174	0	100. 00	60	2	22	0.04	1	0	22	0	3	0
KI	126	0	100. 00	58	0. 80	96	1	33	0. 20	0	0	0	0
Range	82–962	0–8	90.24–100.00	44–988	0.02–2	8.70–248	0–1.60	0.40–33	0–0.20	0–22	0	0–12	0
Mean	377	1	98. 36%	342	1	72	0. 45	6	0. 04	5	0	4	0

Counts per fissure = $N \times 10^{(3)}$.

Table 1 lists the numbers of colony-forming units isolated from occlusal fissures submitted to ameloplasty and exposed for seven days to the oral environment. It also shows the effect of acid-etching on the different types of microorganisms studied.

Table 2 lists the numbers of colony-forming units isolated from occlusal fissures submitted to ameloplasty or left intact and exposed for 30, 60, or 120 days to the oral environment. It also shows the effect of occlusal sealing on the different types of microorganisms throughout the experiment.

The acid etching caused an immediate reduction in the bacterial count, similar for both fissures. Occlusal sealing caused a gradual decrease in the remaining viable microorganisms only in fissures not submitted to ameloplasty. The acid etching and the occlusal sealing affected all bacterial types evaluated similarly.

There were no significant changes in the proportion of the various morphological types; gram-positive cocci predominated during the experiment. Regardless of plaque age, *S. mutans* and *Veillonella* were detected in most fissures. *Lactobacilli* were scarce and could be detected only in a few samples in fissures.

Discussion

The results of this investigation indicated that natural human fissures become colonized within seven days after exposure to the oral environment (Table 1). Although slight fluctuations in the relative proportion of the microflora were observed at the different sampling intervals, no significant changes in the relative distribution of bacterial types were detected. The microbial flora over the study period consists mainly of gram-positive cocci (Table 2). This outcome agrees with the findings of Theilade and coworkers.[27,28] Thus, the changes in proportions of various morphological types noted with increasing age of dentogingival plaque (Theilade et al.[29]) were not found in fissure plaque.

Svanberg and Loesche[30] proposed that once a fissure is colonized, it may be a closed system that prevents any future colonization by microorganisms. Therefore, only shifts in the number of bacteria from the initial inoculum would occur during the life of the fissure. Consequently, a fissure's future status could be determined conceivably at the time of its first exposure to the salivary bacterial flora.

This study also demonstrated that *S. mutans* was detected in all patients and in most fissures at some time

Table 2. Viable counts of 30-, 60-, and 120-day-old fissure plaque without sealing (NS) and after sealing (S)

Subject	*Total Anaerobic*			*Total Aerobic*		*Total Streptococcal*		*Streptococcus mutans*		*Lactobacillus*		*Veillonella*	
	NS	*S*	*% Reductions*	*NS*	*S*	*NS*	*S*	*NS*	*S*	*NS*	*S*	*NS*	*S*
Noninvasive													
SD	6200	62	99. 00%	2640	28	1350	14	18. 40	1	2. 60	9	166	18
JF 30	3200	45	98. 59	510	20	2630	120	1930	1	0	0	8	1
Mean	4700	54	98. 80	1575	24	1190	67	974	1	1	5	87	10
HD	628	1. 20	99. 81	284	9. 90	482	0	348	0	0	0	2	0
PK 60	428	0. 20	99. 96	132	0. 20	110	0. 02	1	0	0	0	1	0
Mean	528	1	99. 89	208	5	296	0. 01	175	0	0	0	2	0
AY	372	0. 02	100. 00	143	0	164	0	65	0	0	0	30	0
JN 120	1280	0	100. 00	628	0	520	0	0. 04	0	0	0	63	0
Mean	826	0. 01	100. 00%	386	0	342	0	33	0	0	0	47	0
Invasive													
CL	1	0. 80	20. 00%	154	14	0. 80	0. 04	0	0	0. 60	0. 20	0	0
FA 30	485	36	92. 58	984	8	128	2	12	0	0. 40	6	0. 60	0
Mean	243	18	56. 29	569	11	64	1	6	0	1	3	0. 30	0
MN	88	6	93. 20	98	5	9	0. 02	32	0. 40	0	0	0	0
BT 60	162	2	98. 77	68	1. 60	2. 40	1. 80	3	1. 60	0	0	0	0
Mean	125	4	95. 99	83	3	6	1	18	1	0	0	0	0
TZ	196	0. 80	99. 59	84	1. 40	20	0. 02	14	1	36	2	0	0
KI 120	186	0. 60	99. 68	86	2	124	0. 20	98	2	0	0	0	0
Mean	191	1	99. 64%	85	2	72	0. 11	56	2	18	1	0	0

Counts per fissure = $N \times 10^{(3)}$.

during the observation period (Table 2). These findings agree with those of Meiers and coworkers,[31] who demonstrated that *S. mutans* was found in both incipient caries and clinically caries-free fissures. This indicates that other microbial, physical, and physiological factors also must determine the health of a fissure rather than the mere presence and total number of *S. mutans*. *Lactobacillus* was detected sporadically in the fissure plaque during the experiment. This agrees with the hypothesis that this microorganism is associated with the further development of lesions.[32] *Veillonella* was detected in most fissures. According to Menaker,[33] it is an indigenous member of the fissure plaque flora (Table 2).

The enlargement of the fissure creates difficulties for implanting a stable microflora and establishing a closed system (Table 2). Theoretically, a cleanable occlusal fissure is produced, thereby preventing future bacterial colonization.

The acid-etching procedure itself reduces the number of viable microorganisms by as much as 96.79% (Table 1). However, all fissures contained viable microorganisms. Theilade and coworkers[16] reported similar results after acid etching and sealing, suggesting that the occlusal sealant does not have a direct effect on the immediate reduction of viable microorganisms. The subsequent application of occlusal sealant resulted in a decrease in the remaining viable microorganisms throughout the experiment (Table 2), as also reported previously by Jeronimus and coworkers,[34] Handelman and coworkers,[35] and Jensen and Handelman.[18]

From this study, one might conclude that the phosphoric acid may, to a large extent, be responsible for the antibacterial effect of sealing. As the seal is maintained, nutrients are prevented from entering the fissure, leading to a continued reduction in the viability of the remaining microorganisms.

It was particularly interesting that the decrease in bacterial population similarly affected all bacterial types studied (Table 2). These findings disagree with the descriptions by Theilade and coworkers[16] and Going and coworkers,[17] in which *S. mutans* and *Lactobacillus* seemed to survive better than other microorganisms in sites where availability of nutrients is limited, such as beneath sealants.

When the fissure is enlarged, the mean total viable counts decreased 100% after acid-etching in most of the fissure blocks (Table 1). According to Handelman,[18] Theilade and coworkers,[16] Going and coworkers,[17] and Jensen and Handelman,[18] as long as the sealant is intact over the pit and fissure and the bonding is effective, bacteria have great difficulty surviving. Consequently, the immediate decrease in the number of viable microorganisms in this fissure seems not to be of great importance.

Moreover, the lowest relative reduction in the remaining microorganisms after occlusal sealing was observed in fissures submitted to ameloplasty (Table 2). However, according to Le Bell and Forsten,[22] Hicks,[20] Shapira and Eidelman,[23] and De Craene and coworkers,[24] the risk of microleakage is reduced when the fissure is enlarged. This procedure eliminates organic material and plaque and a very thin layer of enamel which allows a plug of resin to be formed instead of a thin layer of varying thickness. This plug would adhere better to an etched surface.[22]

Our study does not suggest that mechanical preparation of the fissures results in higher retention rates of sealants or reduces the risk of microleakage. However, it partly agrees with Hicks,[20] Russo and coworkers,[21] and De Craene and coworkers,[13] that the most important advantage of the invasive technique is its ability to determine the extent of discoloration and eventually to detect an incipient caries lesion. On the other hand, the invasive technique may be responsible for unnecessary treatment.

Although our study used unerupted teeth, which, theoretically, would not have incipient lesions, the results of this investigation, coupled with previously reported clinical research data, suggest that the absolute diagnosis of incipient dental caries in the occlusal surfaces of teeth otherwise indicated for sealing may not be very important. As long as the sealant is intact, the number of viable bacteria progressively decrease, and finally, the lesion becomes inactive.

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