Microleakage of three different sealants on sound and questionable occlusal surfaces of permanent molars: An in vitro study

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Abstract

AIM: This was to evaluate the microleakage of 3 different sealants, applied on sound and questionably carious occlusal surfaces with and without a bonding agent. **METHODS:** A total of 120 human molars were selected, photographed with a digital video microscope, and assigned by 3 independent examiners, according to the criteria of ICDAS II, in 2 groups of 60 teeth each. *Group A:* teeth with deep, clear, sound occlusal surfaces (ICDAS II, code 0). *Group B:* teeth with questionable occlusal surfaces, having deep, stained pits and fissures with probable incipient, but non-cavitated, carious lesions (ICDAS II, codes 1 and 2). Each group was divided into 2 subgroups of 30 teeth each (bonding or no bonding) and then into 3 subgroups of 10 teeth each according to the type of sealant used: one conventional (Conseal®) and 2 fluoridated (Conseal F® and Teethmate F®). After the application of the bonding agent and the sealant to the appropriate teeth, all specimens were subjected to thermal cycling and immersed in a 10% methylene blue dye solution for 4 hours. Average and summed microleakage for each sample were estimated from dye penetration scores on 3 mesiodistal sections of the tooth across the sealed occlusal surface. Nonparametric Friedman's 2-way ANOVA by ranks and Conover-Inman pair wise comparisons were used for differences at the 0.05 level of significance. **RESULTS:** According to Friedman's 2-way ANOVA by ranks analysis, although there were no significant differences between the different sealants ($\chi 2= 0.048$, df = 2, $P = .976$), there were significant differences between the sound and questionably carious occlusal surfaces ($\chi 2 = 24$, df = 3, .000). Conover-Inman pair wise comparisons showed no differences between the groups using and not using bonding agents, on sound (SNB-SWB, $P = .4561$) or questionable occlusal surfaces (QNB-QWB, $P = .0842$). **CONCLUSIONS:** Sealant microleakage on questionably carious occlusal surfaces was statistically significantly higher than that of sound occlusal surfaces. Using a bonding agent or fluoridated FS did not influence microleakage significantly, either on sound or on questionable fissured surfaces.

Introduction

Fluoride is considered to be the most significant factor contributing to caries reduction on smooth surfaces, while the occlusal surfaces of primary and permanent teeth continue to be the most vulnerable ones; the incidence of caries on these surfaces is still rising [Locker et al., 2003]. Fissure sealants (FS) have recently been proven to be effective in preventing caries when they are used on an individual as well as on a public-health basis; their efficacy is dependent on, or related to, the general caries prevalence of the population. Reduction of caries incidence in children after sealant application ranges from 86% after 1 year to 78.6 % and 58.6% after 2 and 4 years, respectively [Ahovuo-Saloranta et al., 2004]. Their cost-effectiveness increases as the prior experience (dmft) of the patients increases [Quinonez et al., 2005].

However, although the effectiveness of FS in preventing occlusal caries is dependent on the caries risk of individuals [Leskinen et al., 2008], FS applied on patients with high dmft scores have either shown poor retention [Bravo et al., 1996], or they have not been effective in preventing pit-and-fissure caries in these high-risk children [Tickle et al., 2007]. One of the explanations given by the authors for the higher FS failures was that "more incipient fissure caries might have been sealed in the occlusal surfaces of these individuals..." meaning that some of the surfaces sealed were not sound [Bravo et al., 1996]. In a study by us [Oulis and Berdouses, 2009] the effect of prior caries experience on the behaviour of FS on retention, the need for resealing, and extent of caries development was evaluated. A group of children were stratified according to their caries baseline risk and twice as many of the teeth were found in the high-risk group, after resealing, developed caries compared with the low-risk group. These results are in agreement with the findings of Semecek [2005] in young adults, as well as that by Makhija et al., [2006]. According to these other investigators, baseline caries status was associated with FS failure and caries development and that those with dft = 0 at the time of FS placement had a higher success rate compared with those having dft > 0.

It seems that, although FS are more effective on high-risk teeth, this beneficial effect counts for only half the surfaces, while the other half fails because of questionable tooth surfaces. These are the surfaces for which, under clinical conditions, most dentists would rather place a FS and treat them as sound surfaces, though their tooth structure, content, and tissue quality might be different, However, the evidence for effective nonsurgical management strategies for early occlusal caries is, at best, weak [Bader and Shugars, 2006].

Key words: fluoridated sealant, questionable surfaces, bonding agent, microleakage
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The diverging and conflicting results found in the literature on the effectiveness of FS might be explained by the fact that the terminology and the criteria used appear to be unclear, as the same terms are used to describe a variety of lesions undoubtedly having different tissue composition and behaviour. Within in the literature a variety of terms are to be found, such as ‘suspicious,’ ‘suspected,’ ‘dubious,’ ‘doubtful,’ ‘questionable surfaces,’ ‘incipient,’ ‘noncavitated,’ and ‘early carious lesions.’ This is probably because clinical diagnosis of early, noncavitated fissure caries is complicated and frequently unreliable owing to the problems and shortcomings found in clinical conditions [Lussi, 1991]. Also, the presence of defects and stains, along with coverage of the floor of most fissures with organic plug, makes diagnosis and sealing over undetected, early enamel caries uncertain [Foreman, 1994]. This uncertainty becomes more complicated when one reads the findings of an in vivo study by Hamilton et al., [2001], in which 44% of occlusal surfaces initially diagnosed as questionable were found, after their preparation with air abrasion, to have caries extending into dentine.

Attempts to improve such problematic tooth surfaces and increase retention have been made by better cleaning methods (air polishing, air abrasion), different preparation techniques (widening of the fissure), and application of fluoridated FS or using a bonding agent in an attempt to increase enamel resistance and decrease microleakage with better adaptation of those materials to the enamel surface [Heifetz et al., 2004; Duangthip and Lussi, 2003]. However, these attempts have either not been successful or are applied in a different way, contrary to the preferred conservative approach in treating these fissures.

It seems, therefore, that the effectiveness of FS on these high-risk surfaces and on the people who need them the most is questionable, so that a new era in research must begin. In other words, our efforts must move from materials and techniques to the type, condition, and behaviour of these questionable occlusal surfaces as well. Microleakage of FS on questionable surfaces in vitro has not been studied so extensively as it has on sound enamel surfaces and answers to why microleakage is higher and retention is lower on these surfaces have not been given. It would be helpful, therefore, to target research efforts on these stained and questionably carious, pit-and-fissure surfaces and try to find out whether it is the materials and technique used or it is the condition of the surface and the quality of the tissue that make the retention of sealants problematic.

Based on the above, the aim of the present in vitro study was to evaluate the microleakage of FS on questionably carious occlusal surfaces (compared with sound enamel) and to determine whether microleakage is affected when fluoridated materials and bonding agents are used prior to sealant application.

Materials and methods

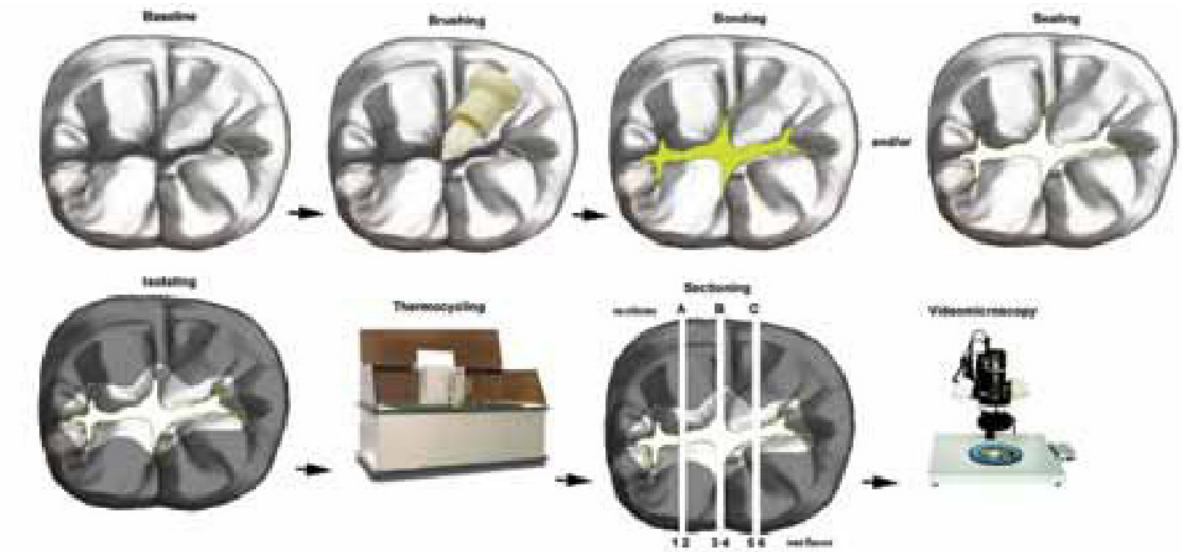
Sample selection and classification: Mandibular human molars (140) were selected, cleaned with a bristle brush on a low speed headpiece and a non-fluoride prophylaxis paste for complete organic debris removal, and stored in a 10% thymol solution until time of use. All teeth with fractures, opacities, calcified remains, or open cavities on pits and fissures (ICDAS II, codes 3, 4, and 5) were rejected from the study. Of the remaining 120 teeth, the occlusal surfaces were examined in a dry condition by 3 independent examiners after training (kappa coefficients for inter-examiner agreement were between 0.72 and 0.85). Teeth were photographed with a digital video microscope having a 25× magnifying lens and randomly assigned to 2 groups of 60 teeth according to the criteria of ICDAS II, as follows: Group A, teeth with deep, clear, or stained but sound pits and fissures (ICDAS II, code 0), Group B, teeth with questionable occlusal surfaces, having deeply stained and ‘catching’ (the probe’s ball end) pits and fissures with probable incipient but non-cavitated carious lesions (ICDAS II, codes 1 and 2) and surfaces with ‘first detectable changes and distinct changes of the enamel without loss of surface continuity’ [Marthaler, 1975].

Study design: The two groups were randomly divided into 2 subgroups of 30 teeth each, according to whether a bonding agent was applied or not (group A: SWB or SNB and group B: QWB, QNB) and then were further classified into 3 subgroups of 10 teeth each according to the sealant used, as follows: (a) 10 teeth were sealed with Conseal® (SDI, Victoria, Australia), (b) 10 teeth were sealed with Conseal F® (SDI), and (c) 10 teeth were sealed with Teethmate F-1 (Kuraray, Osaka, Japan). Table 1 shows how the teeth were classified into experimental groups.

Table 1: Study design with all experimental groups

	Sound (S)		**Questionable for caries (Q)**	
	With bonding agent **SWB**	Without bonding agent **SNB**	With bonding agent **QWB**	Without bonding agent **QNB**
Conseal® (clear)	10	10	10	10
Teethmate-F®	10	10	10	10
Conseal F®	10	10	10	10
Total	30	30	30	30

Figure 1. Schematic representation of methodology stages. Selection, brushing, bonding and/or sealing, coating, thermocycling, sectioning and imaging.



Tooth preparation and observation: Cleaned occlusal surfaces were etched with the manufacturer's etchant (37% orthophosphoric acid) for 30 seconds, washed with air-water spray for 10 seconds, and air-dried for 10 seconds. A bonding agent (Prime & Bond NT®/ Dentsply Caulk, Milford, Del, USA) was applied on 30 of the 60 teeth of each major group and light-cured for 20 seconds according to manufacturer's recommendations. Pit-and-fissure FS were also applied on 60 teeth of the 2 major groups, following the manufacturer's instructions.

After application of the bonding agent to the assigned groups of teeth (SWB, QWB) and the appropriate FS, the teeth were stored in distilled water for 24 hours. Then all specimens were subjected to thermal cycling for 500 cycles at 5°C and 55°C, with a 30-second dwell time. After thermocycling, the apices of the teeth were covered with melted utility wax. A nail varnish was then used to coat the teeth, including the apices, but not their occlusal surfaces up to 1 mm of enamel around the periphery of the sealant. The teeth were dried and immersed in a 10% methylene blue dye solution for 4 hours at room temperature.

In order to evaluate the penetration of dye, the teeth were prepared as follows: the roots were cut from the crowns using a water-cooled diamond blade and then the crowns were embedded in polyester blocks. Three buccolingual sections were made. The first was cut in the middle of the occlusal surface, and the other two were cut 2 mm to the left and 2 mm to the right of the central section, respectively (Figure 1)

The depth of dye penetration was assessed microscopically (Nikon Eclipse, Model ME 600L, 100-240V, 50-60 Hz, Japan) using the criteria of Overdo and Raadal [1990] as follows:

0: No dye penetration

1: Dye penetration to the outer half of the sealant

2: Dye penetration to the inner half of the sealant

3: Dye penetration to the full material depth

One calibrated examiner measured the degree of microleakage of each section (kappa coefficients for intra-examiner agreement, 0.87). The two opposing surfaces from each section were evaluated, but only the surface with the higher microleakage was counted. As a result of this, value 1 represented the microleakage in each section, while the sum of the 3 sections was determined to represent the overall microleakage of each sample.

Statistical analysis: Statistical analysis was performed by using Friedman's 2-way ANOVA by ranks. For the analysis, the statistical package SPSSv (12.0, SPSS Inc., Chicago Ill, USA). was used. Differences between teeth groups were found with the use of Conover-Inman pair wise comparisons with the statistical package StatsDirect v.2.5.6 (StatsDirect Ltd, Chesire, UK).

Results

Table 2: Mean microleakage values of all experimental groups

	SWB x ± sd	SNB x ± sd	QWB x ± sd	QNB x ± sd
Conseal	0.1 ± 0.3	0.3 ± 0.5	1.8 ± 1.1	1.7 ± 1.2
Teeth-mate-F	0.7 ± 0.6	0.5 ± 0.8	1.2 ± 1	1.3 ± 1.3
Conseal F	0.7 ± 1.2	0.6 ± 0.9	2.0 ± 0	0.6 ± 0.7

SWB =Sound surfaces With Bond, SNB=Sound surfaces No Bond , QWB=Questionable surfaces With Bond, QNB=Questionable surfaces No Bond

In Table 2, the average leakage scores and standard deviations between the two groups of teeth (sound vs. questionable) and the 3 subgroups of sealants (Conseal®, Teethmate®, and Conseal F®) are given. In the group with sound surfaces, the lower leakage values were associated with the high-viscosity sealant Conseal® and the higher leakage with the low-viscosity Conseal F®, bonding agent or not. The microleakage values for Teethmate F® were in between, with the subgroup without the bonding agent (SNB) to be better than the 1 with bonding agent (SWB). In the questionable group, the lowest microleakage values were realised by Conseal F® without bonding agent, followed by Teethmate-F® with bonding agent.

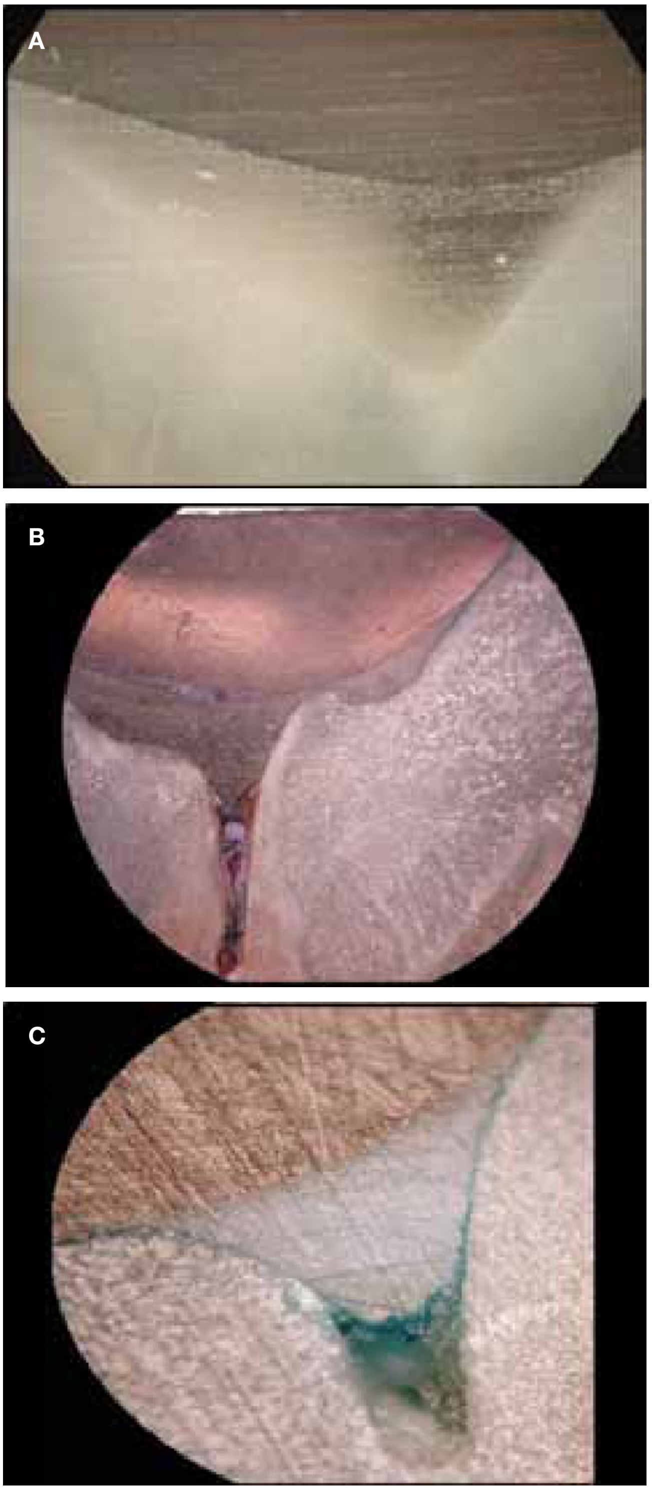
The highest microleakage values were turned in by Conseal® with (QWB) and Conseal F® without bonding agent (QNB). Characteristic leakage and scores in sections are given in Figures 2a, b and c. Friedman's 2-way ANOVA by ranks analysis, although not showing significant differences between groups of different sealants, ($\chi 2 = 0.048$, df = 2, P = .976), did show significant statistical differences between sound and questionably carious occlusal surfaces ($\chi 2 = 24$, df = 3, P = .000), with or without the use of a bonding agent.

Conover-Inman pair-wise comparisons showed no differences between the groups using bonding agents vs. those not using bonding agents, either on sound (SNB-SWB, P = .4561) or on questionable occlusal surfaces (QNB-QWB, P = .0842). The statistical results are best described by the equation, SNB = SWB ≠ QNB = QWB, meaning that the only parameter influencing the microleakage of FS was the condition of the surface, with 'questionable surfaces' showing statistically higher leakage values than sound surfaces, regardless of the sealant type or use of a bonding agent.

Discussion

The results of this study showed that FS applied to teeth with questionable surfaces presented significantly higher microleakage than those applied on sound surfaces. This increased leakage of FS might be the result of poor material adaptation to the enamel of questionable fissures. It might

Figure 2. Photographs of sectioned teeth showing: **A**. Score 0: No dye penetration; **B**. Score 1. Dye penetration to the outer half of the fissure sealant; **C**. Score 3 Dye penetration to the full depth of fissure sealant material.



also explain to some degree why (1) FS applied on high-risk children have shown problematic retention, (2) higher risk patients show higher FS failures [Oulis and Berdouses, 2009], and (3) the low retention of FS in these patients might be due to having sealed several questionable fissures as well [Bravo et al., 1996].

In other words, questionable surfaces showing poorer adaptation of the FS are different in terms of tissue characteristics and should not be treated as sound surfaces. One explanation for this difference could be that, by the time the FS are applied to high-risk, caries-active children, the occlusal pits and fissures might already have undergone structural changes during eruption that altered the properties of the enamel surface. In support of this is the finding by Carvalho et al., [1989] that, in permanent first molars, many incipient lesions due to favourable conditions for plaque accumulation initiated during eruption had been arrested by the time the teeth had come into full occlusion. The difference between sound enamel and questionable surfaces, which could have been demineralised and have an arrested enamel lesion, might be the organic material incorporated into the demineralised enamel during repeated cycles of the de-re-mineralisation process. Such a higher content of organic material might hinder the acid penetrating into the deeper layers and at the same time prevent the homogeneous etching of the enamel; thus, resin impregnation is hampered and shorter resin tags result [Irinoda et al., 2000]. So it is highly possible that we are etching presumably sound enamel, which apparently it is not.

In an attempt to increase the resistance of questionable occlusal surfaces, we have been using fluoridated materials, expecting the released fluoride to improve enamel structure and increase its resistance to carious attack [Hicks and Flaitz, 1992]. In the present study 2 fluoridated FS were tested because of their different mode of fluoride incorporation into the material and to determine whether they have different behaviours on microleakage, The difference between them is that in the first material (Conseal F®), fluoride is added to resin in the form of a soluble fluoride salt; in order for the fluoride ions to be released, the material has to be dissolved.

In the second material (Teethmate F®), organic fluoride is added chemically to the resin, and fluoride is released by exchange with other ions [Ripa, 1993]. According to the National Institute of Dental Research, in the first method the FS might be weakened because of fluoride ion release, therefore reducing its effectiveness on caries. In the second method, fluoride is replaced from other ions and is not lost. Thus there is no decrease in the strength of FS material.

However, the results of this study showed that although fluoridated FS (Conseal F®, Teethmate F®) have a tendency to show higher microleakage values, the differences between them were not significant. These results might be due to the FS high viscosity, which prevents their adapting well to pits and fissures [Hatibovic-Kofman et al., 1998] and becomes worse on questionable surfaces owing to the problem of proper enamel etching. In line with the above are the findings of Stavridakis et al., [2003], who showed that low viscosity Teethmate F-1® exhibited better adaptation to the enamel walls of the occlusal fissures than did similar high-viscosity materials tested. On the other hand, other studies have shown that the greatest influence on the ability of FS to penetrate occlusal grooves is the type of fissure [Duangthip and Lussi, 2004].

Our in vitro findings might also explain the findings of other studies showing no difference in occlusal caries prevalence between teeth sealed with fluoridated vs. conventional sealants [Morphis and Toumba, 1998; Heifetz et al., 2004]. In other words, the expected beneficial action of fluoridated FS in caries reduction is counteracted by their tendency to have the same or higher leakage values compared with conventional ones. Another problem with most clinical trials of fluoridated FS is that most of them are applied on sound, rather than questionably carious occlusal surfaces, on which the slow fluoride release can re-harden demineralised enamel and inhibit caries progression. Therefore, more studies, especially clinical trials, should be undertaken to evaluate the effectiveness of a fluoridated FS with subject variability (teeth with questionably carious occlusal surfaces) that will support the possible advantages of fluoridated FS.

As far as the use of a bonding agent is concerned, the results of this study showed that regardless of the FS material used or the quality of occlusal surfaces, the use of a bonding agent did not prevent microleakage. These findings are in disagreement with previous in vitro and in vivo studies, which showed that the application of a bonding agent before the sealant decreases microleakage [Duangthip and Lussi, 2003] and enhances bond adherence to saliva-contaminated or wet enamel [Feigal et al., 2000]. The reason for not seeing differences in microleakage within subgroups of the questionable surfaces is possibly the existence of a layer of arrested or carious lesions on the cuspal slopes of the pits and fissures that equalises whatever differences might exist. This layer of unhealthy hydroxyapatite between FS and enamel [Thylstrup and Fegerskov, 1981] is difficult to properly etch because of its increased organic material and it leads to compromised FS retention, even with the use of a bonding agent. However, more in vitro and in vivo research is needed to justify the above before it can be decided on the significance of using a bonding agent before any FS application on questionable occlusal surfaces in vivo.

Despite the differences between in vivo and in vitro studies and until a more effective technique for managing these questionably carious occlusal surfaces is developed, they should be sealed with caution, especially in high-risk patients, and followed up closely for resealing if necessary.

Conclusions

Microleakage of FS applied on questionably carious occlusal surfaces was statistically significantly higher than sealant microleakage on sound occlusal surfaces. The use of a bonding agent before sealant application did not significantly reduce the possibility of microleakage of the sealant on either sound or questionable occlusal surfaces. The use of fluoridated sealants on questionable occlusal surfaces showed increased microleakage compared with that on sound enamel surfaces, although not significantly. More research is needed to elucidate whether the differences in behaviour of the questionable occlusal surfaces are due to tissue characteristics or to something else and how we can improve etching and sealant adaptation.

References

Ahovuo-Saloranta A, Hiiri A, Nordblad A, Worthington H, Makela M. Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2004(3): CD 001830.

Bader JD, Shugars DA. The evidence supporting alternative management strategies for early occlusal caries and suspected occlusal dentinal caries. J Evid Based Dent Pract 2006;6(1):91-100.

Bravo M, Osorio E, Garcia-Anllo I, Llodra JC, Baca P. The influence of dft index on sealant success: a 48-month survival analysis. J Dent Res 1996;75(2):768-74.

Carvalho JC, Ekstrand KR, Thylstrup A. Dental plaque and caries on occlusal surfaces of first permanent molars in relation to stage of eruption. J Dent Res 1989;68(5):773-79,.

Duangthip D, Lussi A. Microleakage and penetration ability of resin sealant versus bonding system when applied following contamination. Pediatr Dent 2003;25:505-11.

Duangthip D, Lussi A. Effects of application techniques and fissure types on the in vitro performance of two fissure sealants. Am J Dent 2004;17(2):137-42.

Feigal RJ, Musherure P, Gillespie B, et al. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res 2000;79:1850-56.

Foreman FJ. Sealants prevalence and indication in a young military population. J Am Dent Assoc 1994;125:182-86 [Abstract].

Hamilton JC, Dennison JB, Stoffers KW, Welch KB. A clinical evaluation of air-abrasion treatment of questionable carious lesions. A 12-month report. J Am Dent Assoc 2001; 132(6):762-69.

Hatibovic-Kofman S, Wright GZ, Braverman I. Microleakage of sealants after conventional, bur, and air-abrasion preparation of pits and fissures. Pediatr Dent 1998;20(3):173-76.

Heifetz SB, Yaari A, Proskin HM. Retention of a fluoride-releasing sealant compared with its non-fluoride analogue: interim results of a clinical study after an average of eight months. J Clin Dent 2004;15:1-5.

Hicks MJ, Flaitz CM. Caries-like lesion formation around fluoride-releasing sealant and glass ionomer. Am J Dent 1992;5:329-34.

ICDAS: International Caries Detection and Assessment System http://www.dundee.ac.uk/ dhsru/news/icdas.htm.173

Irinoda Y, Matsumura Y, Kito H, et al. Effect of sealants viscosity on the penetration of resin into etched human enamel. Oper Dent 2000 Jul-Aug;25(4):274-82.

Leskinen K, Ekman A, Oulis C, et al. Comparison of the effectiveness of fissure sealants in Finland, Sweden and Greece. Acta Odontol Scand 2008 Apr;66(2):65-72.

Locker D, Jokovic A, Kay EJ. Prevention. Part 8: The use of pit and fissure sealants in preventing caries in the permanent dentition of children. Br Dent J 2003;195:375-78.

Lussi A. Validity of diagnostic and treatment decisions of fissure caries. Caries Res1991; 25(4):296-303.

Makhija SK, Childers NK, Lauten J, et al. Evaluation of initial caries score and caries incidence in a public health sealant program: a retrospective study. Pediatr Dent 2006;Sep-Oct 28(5):420-24.

Marthaler TM. The caries prevention campaign in the Canton of Zurich during1962-1974 along with a statistical result report from the first 8 years. SSO Schweiz Monatsschr Zahndeilkd 1975;85(11):1147-72.

Morphis T, Toumba K. Retention of two fluoride pit and fissure sealants in comparison to a conventional sealant. Int J Paediat Dent 1998;8:203-08.

Oulis CJ, Berdousis E. Sealant retention and caries development after resealing on first permanent molars of low, moderate and high caries risk children. Eur. Archs. Paed. Dentistry 2009:10; pages 211-17

Overdo R, Raadal M. Microleakage in fissures sealed with resin or glass ionomer cement. Scand J Dent Res 1990;98:66-69.

Quinonez RB, Downs SM, Shugars D, Christensen J, Vann WF Jr. Assessing cost-effectiveness of sealant placement in children. J Public Health Dent 2005;65(2):82-89.

Ripa LW. Sealants revised: an update on the effectiveness of pit and fissure sealants. Caries Res 1993;27(Suppl.):77-82.

Semecek JW, Diefenderfer KE, Ahlf RL, Ragain JC. Dental sealant longevity in a cohort of young U.S.A. naval personnel. J Am Dent Assoc 136: 171-78 Feb. 2005.

Stavridakis MM, Favez V, Campos EA, Krejci I. Marginal integrity of pit and fissure sealants. Qualitative and quantitative evaluation of the marginal adaptation before and after in vitro thermal and mechanical stressing. Oper Dent 2003;28(4):403-14.

Thylstrup A, Fegerskov, O. Tooth Surface Interactions and Preventive Dentistry In Rolla G, Songu T, Emderg G., Workshop on Tooth Surface Interactions and Preventive Dentistry (1980 Oslo, Norway) Edit.1981, IRL Press (London, Washington, D.C)

Tickle M, Yeung CA, Milsom KM, Blinkhorm AS. The prescription and outcomes of fissure sealants applied to a group of high caries risk children by general dental practitioners working in the North West England. Community Dent Health 2007; 24(3):135-39.