Effectiveness of fissure sealants on initial caries lesions (ICDAS 1-3) of permanent molars: A 4-year follow-up

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Abstract

Aim The aim of the present prospective study was to evaluate the retention and effectiveness of two types of sealants (clear vs. opaque) on early pit and fissure occlusal non-cavitated ICDAS II #1-3, caries lesions of permanent posterior teeth of children.

Materials and methods Study Design: 6986 pit and fissure occlusal surfaces were randomly sealed with clear or opaque sealants out of which, 5828 sealants were placed on sound and 1158 on questionable surfaces, while 3508 were clear and 3478 opaque sealants. The mean age of the sample at initial sealant placement was 9.5 (±2.9) years and the follow-up time was 12–48 months. The median (IQR, range) follow-up time was 17.9 (8.7–28.6) months. Study inclusion and exclusion criteria applied to the combined database in order to select the study sample. Teethmate™ F-1 natural and opaque sealants (Kuraray, Hattersheim am Main, Germany) were applied following the standard procedure of preparation with moisture control kept by cotton rolls handled Dri-Angles" and a 30 seconds acid-etch with 37% orthophosphoric acid gel was used followed by 10 sec air-water spraying washing and polymerization for 20x2 sec. Sealants were applied on sound tooth surfaces (ICDAS #0) with no visible defects or on surfaces with early caries lesions (ICDAS #1-3), randomly and interchangeably on the upper or lower Jaw. Total retention was considered when all pits and fissures were completely sealed, while partial or complete loss was scored as one code, although was registered separately. Statistics. Separate analyses were performed for each type of failure (loss of sealant or restoration). Cumulative probabilities of failure over time after sealant placement, overall or by specific characteristics, were calculated using the Kaplan-Meier method. Association between these characteristics and the hazard of failure were investigated using appropriate Cox proportional hazard models.

Results Sealed surfaces with ICDAS II # 1-3, showed 100% higher probability of having a restoration and 60% higher probability of sealant loss, with both differences being statistically significant (aHR=2.03, p=0.046), adjusted for age, sex, type of sealant and location of surface. Opaque sealants presented statistically significant (p 0.009) higher re-application rate, while fissures had 70% statistically significant (p<0.001) higher probability for resealing with time compared to pits, with gender not affecting sealant retention while the earlier a sealant was placed on children's teeth, the more probable it was to need resealing or restoration (p <0.012).

Conclusions The therapeutic use of sealants on occlusal surfaces of posterior permanent molars with early carious lesions (ICDAS II 1-3) is inferior compared to sound surfaces, showing higher sealant failures and restorations.

KEYWORDS Fissure sealants, Early caries, Non cavitated lesions, ICDAS II, Permanent molars.

Introduction

The use of sealants as an adequate measure for the primary prevention of caries on sound occlusal surfaces in children and adolescents has been extensively searched and well established in the literature [Deery, 2013; Ahovuo-Saloranta et al., 2017; Colombo and Paglia, 2018]. It is known that a considerable proportion of occlusal surfaces will not be carious, suggesting that sealants under might not be considered cost effective. Trying to overcome this issue Handelman et al. [1972] tried with a preliminary report which was subsequently supported by other authors [Ekman Jensen and Handelman, 1980; Handelman et al., 1982, 1987] to establish the therapeutic use of sealants over carious occlusal surfaces.

Based on this approach several studies were conducted and different guidelines [Beauchamp et al., 2008] and systematic reviews [Griffin et al., 2008; Oong et al., 2008] were published, suggesting the therapeutic use of sealants over carious lesions with the argument that the number of bacteria under sealants decrease and the incipient carious lesions do not progress if the sealants are intact or the surfaces are resealed. However, although there is no doubt of the effectiveness of fissure sealants on sound surfaces [Ahovuo-Saloranta et al., 2017], sealing over active caries lesions as an approach has raised several questions and concerns. One is a philosophical concern suggesting "covering up the disease" and not trying to remineralise or "heal" the surface [Oulis, 1988]. The second is the uncertainty of the retention of sealants on high-risk patients and/or surfaces. If sealant retention over such lesions is compromised the method will lead to multiple resealing, reducing the cost/effectiveness of the sealants. Moreover, the term "risk factor" for surfaces and patients is difficult to be stratified with high validity [Tellez et al., 2011] and is rather loosely used in most studies, explaining to some degree, the contradictory results [Weintraub et al., 1993]. Based on these contradictions, dentists are reluctant to adopt such methods and guidelines in clinical practice [O'Donnell et al., 2013].

In regard to the risk status of children, there are studies showing that sealants are more effective if placed on high rather than low-risk children [Leskinen et al., 2008]. Whereas, other studies have shown, that sealants were only protective in individuals with low or moderate caries activity [Heyduck et al., 2006] or that sealants are not effective when they are applied in a group of high-risk children by general practitioners [Tickle et al., 2007]. In line with the above studies are the results of the study by Bravo et al. [1996]. The authors reported that the higher the dmft values, the higher sealants loss and restorations. The reasoning they suggested was that high-risk children present a higher number of occlusal surfaces with initial carious lesions, which have been inadvertently sealed, resulting in higher sealant failures.

According to Mejare et al. [2003], the contradiction in the literature found on this issue is due to the fact that most studies refer to sound or high-risk children in general terms without specific criteria creating a clear need of studies on children populations categorised as low and high caries risk status and for longer periods of time. Towards this direction, Oulis and Berdouses [2009] applied sealants on children categorised at the time of sealants placement according to their dmft status, as low (dmft=0), moderate (dmft=1-4), high (dmft >4), with almost half of the teeth belonging to the high-risk group and followed them for 3-5 years. Based on the results from that study, it was found that the higher caries risk children showed higher sealant failures and higher occlusal caries prevalence following sealant loss, compared to moderate and low-risk children. However, the uncertainty remains and evidence on the validity of existing systems to predict future caries risk is limited at present [Tellez et al., 2012] or there is no clearly superior method to predict future caries [Twetman, 2016]. On the other hand, there are several guidelines and systematic reviews suggesting that sealing non-cavitated caries lesions is more effective in reducing caries progression, versus unsealing. There is also evidence that sealants are more effective on sound and non-cavitated carious lesions compared to those surfaces without or receiving fluoride varnishes [Wright et al., 2016]. It is interesting though to notice that this systematic review gives strong recommendation to use sealants on sound and non-cavitated occlusal carious lesions combined although based on low quality of studies.

Nevertheless and contrary to these suggestions, 40% of the US dentists do not apply sealants over non-cavitated caries lesions [Tellez et al., 2011] maybe because they are not convinced due to standing beliefs and their difficulty to specify in clinical practise the terms high-risk patients or surfaces [O'Donnell et al., 2013], Other reasons contributing to this choice of the dentists maybe 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] or due to evidence that BPA and some BPA derivatives can pose health risks attributable to their endocrine-disrupting, estrogenic properties [Fleisch et al., 2010]. This uncertainty becomes more complicated from the findings of an *in vivo* study by Hamilton et al. [2001]. In this study it was reported that 44% of the occlusal surfaces initially diagnosed as questionable were found to have caries extending into dentine after they were prepared with air abrasion. Also, the dentists may be not convinced that bacteria under sealants disappear and the lesion does not progress if sealants remain intact since *in vitro* studies have shown that sealants on these surfaces present higher microleakage and lower retention rates [Michalaki et al., 2010]. This finding might be explained from a compromised bonding, due to the histochemical changes of the enamel that take place during the carious process and the lesion development [Michalaki et al., 2016].

Based on the above, the purpose of this prospective study was to evaluate the retention and effectiveness of the therapeutic use of sealants on occlusal surfaces of permanent molars of high-risk children with early carious lesions, ICDAS #1-3 in comparison to sound surfaces, in a clinical practice setting.

Materials and methods

Study design and sampling

The study is an observational prospective multicenter cohort study, based on electronic records which were routinely collected from four private paediatric dental clinics in the area of Athens, Greece. All children who visited one of the collaborating clinics were scheduled for recall appointments every 6 months. A reminding letter was mailed to them one month in advance, and in case they missed that appointment another letter was mailed 6 months later accompanied by a telephone call.

Electronic records of visits in each clinic between March, 2009 and January, 2014 were combined into a single database recording demographic data, dates of visits (multiple visits for each child) and a coded description of operations performed during each visit (in multiple records for separate teeth and surfaces). Study inclusion and exclusion criteria applied to the combined database in order to select the study sample. More specifically, to enter the study, children had to have their first visit before the age of 6 years and have at least one recall visit after sealant placement. Teeth with hypoplastic enamel areas or cavitated enamel lesions extended into dentin (ICDAS II #4-6) were rejected from the study.

Sealants were applied from experienced paediatric dentists and the sample of the study comes from a high-risk population as it is confirmed by a recent pathfinder study where 12-year-old children present DMFS=3.58 [Oulis et al., 2009].

Prior to sealant placement, the five paediatric dentists, were trained and calibrated from the principal investigator (gold standard) on the examination procedure and on caries diagnosis at two levels. The first level included the theoretical part of the process with photographs and extracted teeth covering all the details and asking all the questions. The second level included the clinical examination of first permanent molars in the mouth of children and tested for inter and intra examiner variability according to ICDAS II codes 0, 1-3 combined. Our intention to combine codes 1-3, was based on the fact that in clinical conditions several surfaces with microcavities escape the correct diagnosis and are sealed.

Calibration results after each session were given within 15 minutes and were considered as satisfactory only if Cohen's kappa coefficient of agreement for each dentist vs the gold standard (study's principal investigator) was >0.90, otherwise training was repeated. ICDAS II score repeatability except of the beginning of the study, was taking place in a refreshing course every year thereafter until the end of the study and examiners were assessed through kappa and weighted kappa statistics.

Sealant application

All sealants were applied from the calibrated dentists, helped by trained chair side clinical assistants following the 4-handed sitting Dentistry model. Teethmate™ F-1 natural and opaque fluoride releasing sealant (Kuraray, Hattersheim am Main, Germany) were used as the sealant materials. Full eruption of the tooth was a prerequisite for a sealant to be placed, meaning that the entire occlusal surface was exposed into mouth with no overlying tissue operculum and with the distal gingival tissue lying below the height of the distal marginal ridge.

The application technique included tooth cleaning with pumice without fluoride, moisture control kept by cotton rolls handled Dri-Angles", 30 seconds acid-etch with 37% orthophosphoric acid gel, air-water spraying for 10 sec and polymerisation for 20x2 sec. A Demetron LED Radiometer (Kerr Corporation, Brea, California, USA) was used once a week to measure the performance of the polymerisation devices used in the different offices. The devices minimum irradiance output was at least 800 mW/cm². Sealants were applied on sound tooth surfaces (ICDAS #0) with no visible defects after drying or on surfaces with early caries lesions (ICDAS #1-3), randomly and interchangeably on the upper or lower Jaw.

On every recall visit, all first permanent molars were examined visually for sealants condition or caries development blindly, from an examiner that didn't know the initial categorisation of the surfaces. Total retention was considered when all pits and fissures were completely sealed. Partial or complete loss was scored as one code when part or complete loss of sealants was detected in any pit or fissure of each tooth separately. In teeth with partial or total loss, sealants were reapplied as needed if no sign of any lesion was present.

All sealants were placed by the paediatric dentists while the re-examination of the children at the recall visits and the evaluation of sealant retention or failure were carried out blindly by the assistant of each Dentist who had been trained on assessing the retention of sealants, without knowing the prior history or classification of the tooth surface on which the sealant was applied initially.

Statistical analysis

The main quantity of interest in the current study is the time between sealant application and its failure. Failure was defined in two different ways when at a recall visit it was censored a) restoration of the corresponding surface or b) first reapplication of the sealant or restoration. Follow-up times for surfaces with no failures recorded during the study period were censored at the last visit of each child.

Separate analyses were performed for each type of failure. Cumulative probabilities of failure over time after sealant placement, overall or by specific characteristics, were calculated using the Kaplan-Meier method. Association between these characteristics and the hazard of failure were investigated using appropriate Cox proportional hazard models with adjustment for the potential correlation between surfaces of the same child. Characteristics considered in the aforementioned analyses included gender and child's age at sealant placement, type of surface (i.e. questionable or sound), type of sealant (i.e. clear or opaque) and location of surface (i.e. fissure or pits).

Results

The final sample of the study included 1,743 children contributing data on 6,986 occlusal surfaces, with 1158 sealants on surfaces with early caries lesions (ICDAS # 1-3) and 5828 on sound surfaces (ICDAS #0). The mean age at the initial visit, the gender distribution of the sample along with the type of the sealed surfaces and sealant category are described in Tables 1 and 2.

Median (IQR) time between application of the 1st sealant and last visit of each child within the study period was 17.9 (8.7, 28.6) months and the mean age (SD) of the sample at initial sealant placement was 9.5 (2.9) years, with a prior caries. The proportion of children with more than 1 year gap between their last visit and end of study period was 38.0%. The probability of such gaps was not associated with the type or location of surface (p=0.470 and p=0.143, respectively) nor the gender of the child (p=0.692). However, higher age at 1st sealant placement was associated with higher proportions of such gaps (p=0.005).

In total, from the 6986 sealed surfaces 477 were resealed and 66 were restored at a follow-up visit. Six out of these 66 surfaces were resealed and then were restored at a subsequent follow up visit. Therefore when restoration was considered as failure 66 surfaces were included, when resealing was also considered as failure 537 surfaces were included (477 resealed surfaces and 60 surfaces with restoration without prior resealing).

Time to restoration analysis

Cumulative probabilities of restoration over time since sealant's application are shown in Figure 1. The estimated cumulative % probabilities (95% C.I.) of restoration at 1, 2, 3 and 4 years after sealant's application were 0.2 (0.1, 0.4), 1.1 (0.8, 1.5), 1.9 (1.5, 2.6), 2.4 (1.8, 3.3), respectively.

Figure 2 shows cumulative probabilities of restoration over time according to type of surface, type of sealant, location of surface (only for teeth 16/26) and age at sealant's application. As shown in this figure, type of sealant and location of surface were clearly not associated with the hazard of restoration; p-values from the corresponding univariable Cox models were 0.443 and 0.873 respectively. Similarly, gender was also not associated with the hazard of restoration (p= 0.449, data not shown). Cox models for type of surface and age resulted in lower p-values (0.168 and 0.146,

Gender	N	Mean age at placement Mean (SD)	Follow up time IRQ, range in months
Males	861	9.5 (2.9)	17.8 (8.8, 28.5)
Females	882	9.6 (2.9)	18.0 (8.6, 28.6)
Total	1743	9.5 (2.9)	17.9 (8.7, 28.6)

TABLE 1
Distribution of the sample by gender and duration of follow-up.

	Female	Male	Overall
	N (%)	N (%)	N (%)
Total	3601 (100%)	3385 (100%)	6986 (100%)
Quest-ionable	655 (18.2)	503 (14.9)	1158 (16.6)
Sound	2946 (81.8)	2882 (85.1)	5828 (83.4)
Clear	1894 (52.6)	1614 (47.7)	3508 (50.2)
Opaque	1707 (47.4)	1771 (52.3)	3478 (49.8)
Fissure	2725 (75.7)	2548 (75.3)	5273 (75.5)
Pits	876 (24.3)	837 (24.7)	1713 (24.5)
16	889 (24.7)	863 (25.5)	1752 (25.1)
17	166 (4.6)	165 (4.9)	331 (4.7)
26	871 (24.2)	795 (23.5)	1666 (23.8)
27	165 (4.6)	164 (4.8)	329 (4.7)
36	453 (12.6)	426 (12.6)	879 (12.6)
37	173 (4.8)	170 (5.0)	343 (4.9)
46	460 (12.8)	444 (13.1)	904 (12.9)
47	168 (4.7)	172 (5.1)	340 (4.9)

TABLE 2 Distribution of surfaces and teeth.

respectively) and graphs suggest some discrimination; questionable surfaces and younger age at sealant's application were possibly associated with higher restoration probabilities.

Results from a multivariable Cox model for the hazard of restoration are reported in Table 3, which shows that occlusal surfaces with early carious lesions (ICDAS II #1-3) were significantly associated with approximately double risk of restorations compared to sound surfaces (aHR=2.03, p=0.046), adjusted for age, sex, type of sealant and location of surface. Meaning that, questionable surfaces show 100% higher probability of having a restoration and 60% higher probability of sealant loss. Older age was associated with lower risk of restorations (16% less for one year older, p=0.012). Type of sealant, location of the surface and gender were not significantly associated with the risk of restorations.

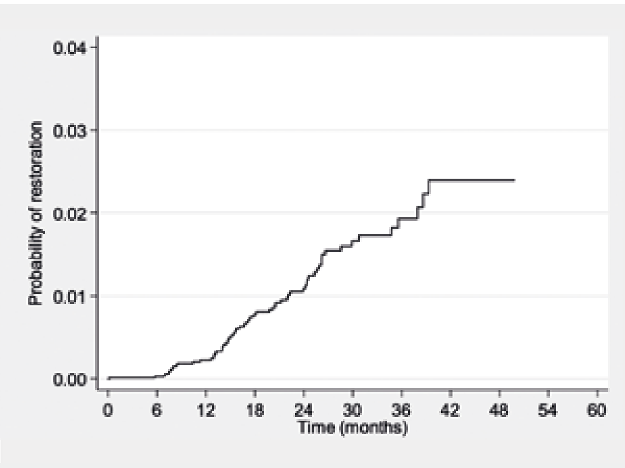

FIG. 1 Cumulative probabilities of restoration over time since sealant's application.

Time to reapplication of sealant analysis

Cumulative probabilities of reapplication over time since first sealant's application are shown in Figure 3. The estimated cumulative % probabilities (95% C.I.) of reapplication at 1, 2, 3 and 4 years after sealant's application were 3.5 (3.0, 4.0), 8.9 (8.1, 9.8), 13.7 (12.4, 15.0), 21.6 (18.0, 25.6), respectively.

Figure 4 shows cumulative probabilities of reapplication over time according to type of surface, type of sealant, location of surface (only for teeth 16/26) and age at sealant's application. As shown in this figure, all parameters were significantly associated with the hazard of reapplication; p-values from the corresponding univariable Cox models were 0.046, 0.009, <0.001 and <0.001, respectively. Gender was still not associated with the hazard of reapplication (p=0.738, data not shown). Occlusal surfaces with early carious lesions, opaque sealants, fissure surfaces and younger age at sealant's application were associated with significantly higher restoration probabilities.

Results from a multivariable Cox model for the hazard of reapplication are shown in Table 3, which shows that questionable surfaces were significantly associated with approximately 70% increased risk of reapplication compared to sound surfaces (aHR=1.69, p=0.001), adjusted for age, sex, type of sealant and location of surface. Older age was associated with lower risk of restorations (13% less for one year older, p<0.001). Opaque sealants were associated with approximately 40% increased risk of reapplication compared to clear ones (aHR=1.40, p=0.011). Pits were associated with 30% lower risk of reapplication compared to fissure (aHR=0.70, p<0.001). Gender was not significantly associated with the risk of restorations (p=0.779).

Discussion

The use of pit and fissure sealants as an effective measure in primary caries prevention of sound surfaces of permanent teeth, compared to no sealants, has been well documented since many years and there is no doubt or controversy on that besides their possible estrogenic effect [Fleisch et al., 2010; Colombo et al., 2018]. Furthermore, it is also well accepted that the therapeutic use of sealants on high-risk children with high-risk surfaces and several non-cavitated caries lesions is a more cost-effective preventive measure if they are regularly monitored and repaired [Welbury et al., 2004; Beauchamp et al., 2008].

The aim of the present study was to evaluate the effectiveness of sealants on non-cavitated carious occlusal surfaces (ICDAS II # 1-3) and to test the null hypothesis that the behaviour of these sealants, in terms of retention and caries development, does not differ from the sealed sound surfaces (ICDAS II # 0).

Based on the findings of this study, the null hypothesis was rejected in both directions. Sealants on surfaces with early carious lesions (ICDAS 1-3) showed 100% higher probability of having a restoration and 69% higher probability of sealant, compared to sound surfaces. In other words, sealants on children with occlusal surfaces having early carious lesions (ICDAS 1-3), were not as protective as we expected.

Our results, as we mentioned in the introduction, verify in some way the controversy with diverging findings in the literature, regarding the effectiveness of sealants in high-risk

	Cox model for the hazard of restoration			Cox model for the hazard of re-application		
Factor	Hazard Ratio	95% C.I.	p-value	Hazard Ratio	95% C.I.	p-value
Type of surface						
Questionable	2.03	1.02, 4.07	0.045	1.69	1.24, 2.30	.001
Sound*	1			1		
Type of sealant						
Clear*	1			1		
Opaque	1.30	0.67, 2.52	0.429	1.40	1.08, 1.82	0.011
Location of surface**						
Fissure*	1			1		
Pits	0.96	0.50, 1.87	0.913	0.70	0.60, 0.82	<0.001
Gender						
Girl	1.26	0.66, 2.41	0.490	1.04	0.80, 1.35	0.779
Boy*	1			1		
Age at 1st application						
per year	0.84	0.74, 0.96	0.012	0.87	0.83, 0.92	<0.001

TABLE 3 A multivariable Cox model for the hazard of restoration and re-application.

children or occlusal surfaces with non cavitated carious lesions. According to our opinion this might be due to several reasons.

First, it is worth noticing that although there are so many studies in the literature testing the effectiveness of sealants in regard to so many parameters (type of sealant material, bonding agent, application technique, comparison with other preventive measures etc), there are also very few studies, testing sealants on high-risk surfaces (cavitated or

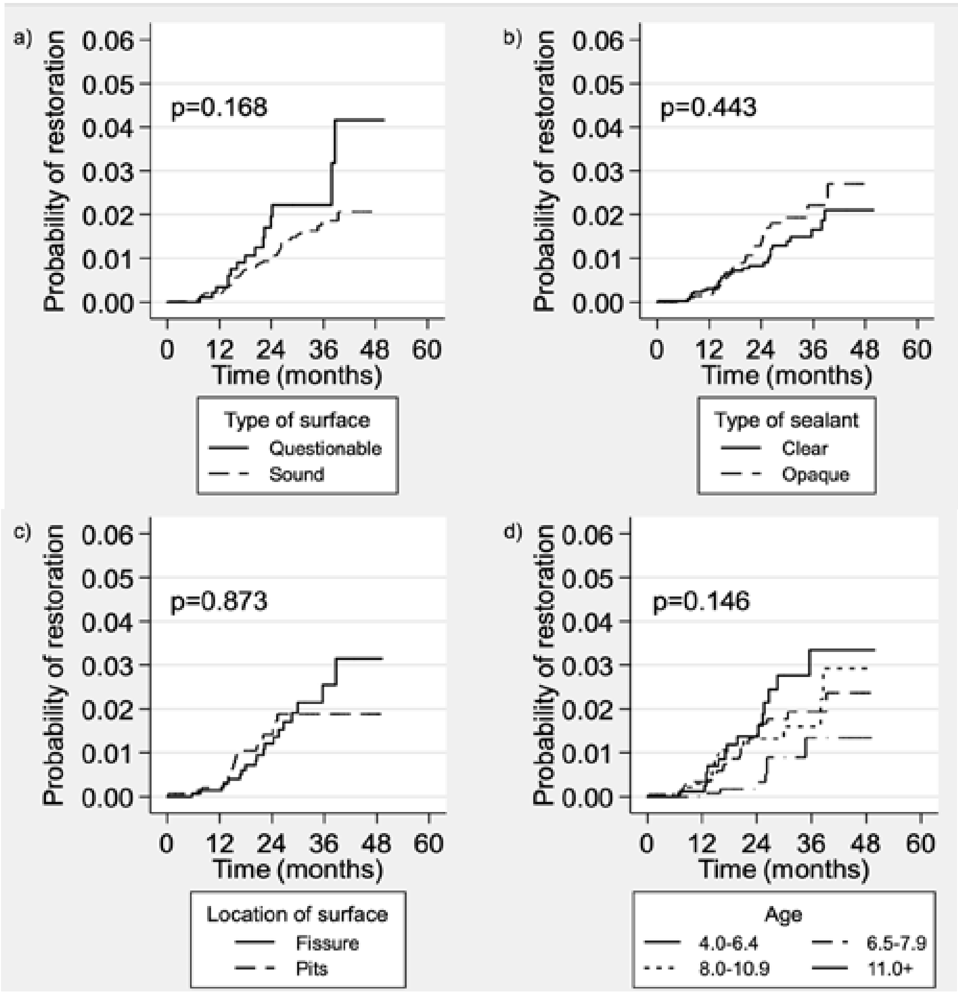

FIG. 2
Cumulative probabilities of restoration over time since sealant's application by a) type of surface, b) type of sealant, c) location of surface (for teeth 16/26) and d) age at sealant's application. P-values from Cox univariable models.

not) for longer periods than 2 or 3 years.

Second, although it is generally accepted that there is a great variability of the morphology (deep or shallow), of the appearance (stains or micro cavities) and the true condition of the occlusal surfaces (ICDAS codes 0 vs 1+2 or 3), most studies refer to their methodology with general terms, without specific criteria. We see for example the terms "sound surfaces" or "high-risk surface" where sealants are tested, irrespectively if the teeth have shallow or deep fissures and compare to surfaces with early, initial, questionable and even non or cavitated carious lesions combined. These "vague" descriptions make comparisons difficult and worsening the confusion due to the different perception of the terms.

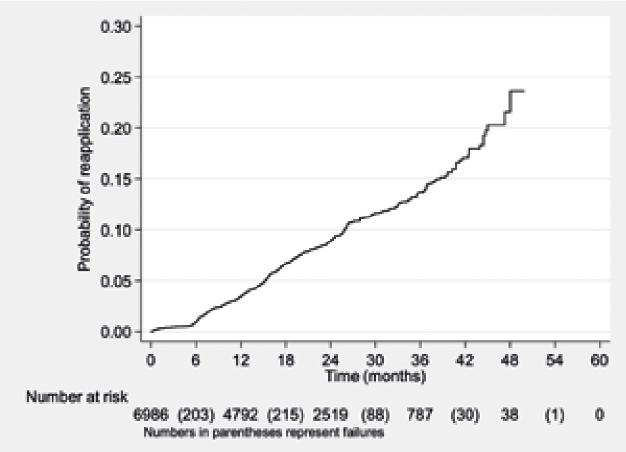

FIG. 3 Cumulative probabilities of reapplication over time since sealant's application.

Moreover, we see that most systematic reviews and guidelines are based mainly on six (6) original studies; most of them are very old, they are using first or second generation resin sealants [Ripa, 1993] and techniques and refer to different population in regard to caries level and for periods mostly up to 2-3 years. The only study using 2nd generation sealants, but still is 25 years old, is the study by Heller et al., [1995]. However, this study is retrospective from patient's records and it is not a study on the field. They also refer to "high-risk" children, although, their dmfs ranged between 2.0-2.2, which means that the children rather belong to at least moderate caries level, but not to high-risk individuals, as it is stated. Another drawback of the study is the condition in the classrooms where the examination was carried out and was not presumably ideal, making the categorization of the surfaces difficult and uncertain with the general term incipient or cavitated. On the contrary, our prospective study was carried out in a standardised clinical setting and sealants were applied from calibrated clinicians on surfaces strictly categorised as ICDAS II #1-3 and followed blindly for up to four years. The children included in this study, belonged to high-risk group with DMFS= 3.52 [Oulis et al., 2009].

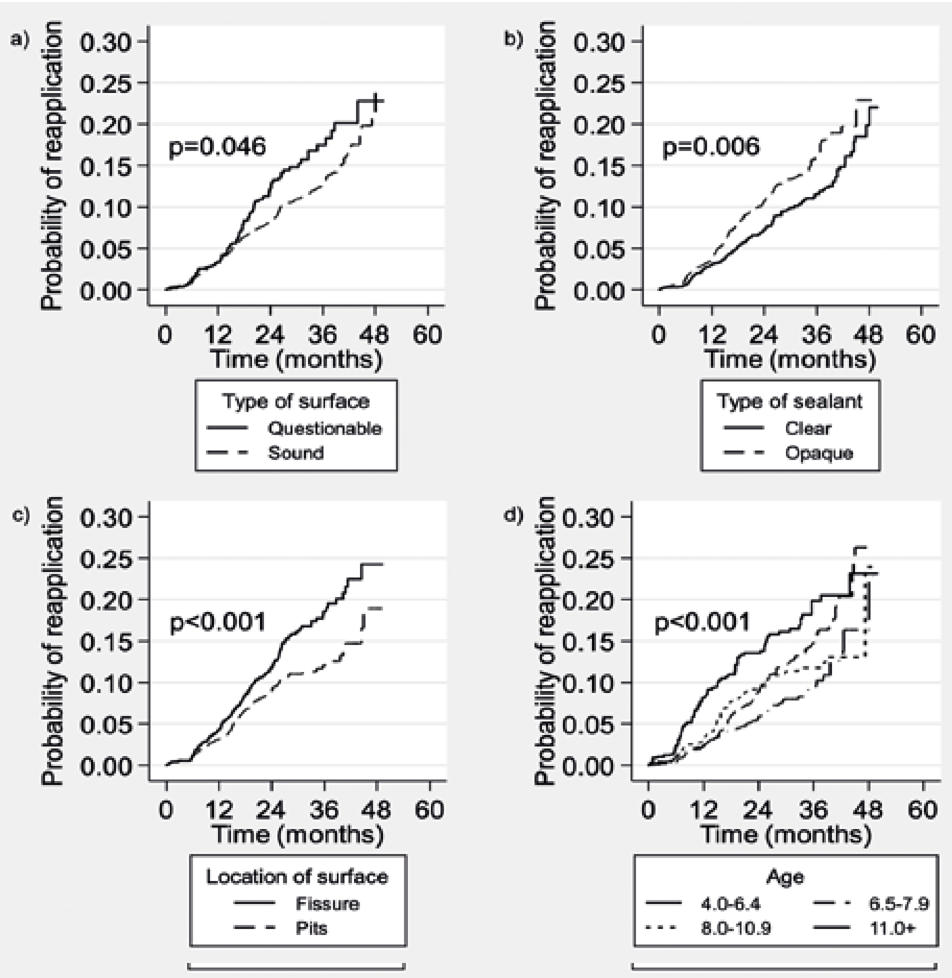

FIG. 4 Cumulative probabilities of reapplication over time since sealant's application by
a) type of surface;
b) type of sealant;
c) location of surface (for teeth 16/26) and;
d) age at sealant's application.
P-values from Cox univariable models.

The explanation of our findings that occlusal surfaces with early carious lesions (ICDAS II# 1-3), showed 100% higher probability of having a restoration and 69% of sealant failure, might be seen from two aspects. The aspect, that high-risks patients, due to higher bacteriologic load present higher cariogenic environment, alone or in combination with the aspect that the enamel of the surfaces on which the sealants are applied is altered, leading to higher sealant failures and restorations.

It is conceivable and well accepted that there are three factors involved in the process influencing the primary and the secondary caries preventive effect of sealants: a) the host (high or low-risk); b) the sealants material used (opaque vs clear) and; c) the condition of the surface *per se* (sound risk vs early non cavitated) on which the sealant is bonded.

In a prior study we have found that sealants applied on teeth of children categorised as high-risk (dmft ≥4) showed higher sealant failures compared to moderate or low-risk. In this study the surfaces were not identified as sound or questionable though [Oulis and Berdouses, 2009]. Therefore, the present study is a continuation of the prior one. The results from this study that sealants on surfaces with early carious lesions resulted to twice as much restorations and approximately 70% more reapplications compared to sound surfaces verify that both situations influence the behaviour and effectiveness of the therapeutic use of sealants in these patients and surfaces. These results are also in agreement with the results from other studies showing that the higher the dft the higher the failure of sealants [Bravo et al., 1996] or that sealants are only protective in individuals with low or moderate caries activity [Heyduck et al., 2006]. The problem though remains that sealants fail on patients and surfaces that need them the most and it is of paramount importance to know why in order to improve their effectiveness even further.

As we know, the carious process leading to carious lesions starts from the imbalance of the risk factors, namely the pathogenic micro-organisms and mainly strep mutants and carbohydrates from the one side, versus the protective factors in the saliva and the preventive measures on the other [Featherstone, 2004]. Studies have shown that there is a statistically significant linear association between the degree of caries activity in children, expressed mainly with D (decay) component and the salivary levels of Streptococcus mutants in the mouth [Ghazal et al., 2018]. Other studies have shown that the salivary enzymes along with oral microorganisms contain esterase at levels capable of hydrolytic-mediated degradation of cured dental resin composites and adhesives [Bourbia et al., 2013]. Esterase has the ability to degrade the polymeric matrix of resin composites and adhesives and promote demineralisation of the tooth surface at the marginal interface. This process compromises the resin-dentin interface, allowing for cariogenic bacterial ingression along the interface, limiting the longevity of resin composite restorations [Kermanshahi et al., 2010]. Therefore, based on this theory we can conclude that the higher amount of strep mutants found in the mouth of higher risk children, the higher caries-activity is found in the mouth, leading to higher probability of the sealants to fail. This conclusion is also supported by a study of Yasaman et al. [2014].

In regard to the second aspect related to the condition of the surface, it is generally accepted that the efficacy of sealants in preventing caries depends on the duration and degree of sealant retention [Droz et al., 2004]. Retention also depends on successful bonding of the sealant to enamel, with the formation of micromechanical tags after an adequate and proper conditioning of the enamel, in order to have mechanical retention of sealants via the direct resin penetration into the porous etched enamel surface [Garcia-Godoy & Gwinnett, 1987]. However, this is an ideal condition and refers to sealants over sound surfaces and not to questionable surfaces or surfaces with early caries lesions. These surfaces, due to the presence of fluoride and the incorporation of organic material in the porous microspaces of the enamel during lesion formation, may have a higher acid resistance compared to sound enamel and reduce resin penetration, compromising adhesion [Davila et al., 1975; Lee et al., 1995; Iijima & Takagi, 2000]. These findings are also in agreement with another *in vitro* study [Osborn et al., 1974], verifying that the enamel of these surfaces is altered and more porous with higher amounts of protein and less amounts of calcium, phosphorus and carbonate. On the other hand, the findings of an *in vitro* study by [Celiberti and Lussi, 2007], have demonstrated that carious fissures cannot be sealed as adequately as sound fissures, questioning the long-term success of sealants placed over occlusal carious lesions. Other studies have shown that these altered occlusal surfaces (ICDAS II 1-3) exhibit significantly more microleakage than sound surfaces [Michalaki et al., 2010], which was more pronounced after thermal and mechanical stressing conditions [Stavridakis et al., 2003]. Meaning that this compromised bonding allows saliva and oral bacteria to infiltrate the spaces between the tooth and the resin and based on the above mentioned mechanism to undermine the sealant and cause the sealant failure [Bourbia and Finer, 2018].

Regarding our finding that older age was statistically influenced by the time of application with lower risk of restorations and reapplications, it might be attributed to the fact that, in younger ages and especially during or immediately after eruption, more carious lesions start developing [Zenkner et al., 2013; Alves et al., 2014], which might have inadvertently been sealed and that this vulnerability is more pronounced in high-risk patients [Manji and Fejerskov, 1994]. Therefore, both the inadequate cleaning and plaque accumulation in connection to the "risk factor" of the patient leads to initial carious lesions or the post eruptive changes of enamel during maturation may cause the inadequate bonding and failure of sealants.

Another finding was that opaque sealants were associated with approximately 40% increased risk of reapplication compared to clear ones (aHR=1.40, $p=0.011$) and pits surfaces were associated with 30% lower risk of reapplication compared to fissure surfaces (aHR=0.70, $p<0.001$). These findings contradict the results of other studies where opaque sealants have shown at least equal or even better retention than clear sealants [Dukic and Glavina, 2007; Nardi et al., 2018]. Another explanation might be that the ability to properly assess retention in opaque sealants is much less error prone than with clear sealants during follow-up [Rock et al., 1989]. On the other hand, studies suggest that the viscosity of the sealant plays an important role in penetrating and forming better micromechanical tags for their retention on the etched surface [Irinoda et al., 2000; Subramaniam et al., 2014] and other studies show that the filled and more viscous sealants, demonstrated similar bond strengths to the unfilled [Wright and Retief, 1984]. All of the above conclude that the critical point for the therapeutic effectiveness of sealants on these surfaces, besides the risk factor of patients, depends on retention which is compromised due to the altered enamel, compared to sound [Kidd and Fejerskov, 2004]. This hypothesis was confirmed from results of a previous *in vitro* study, in which we analysed the enamel mineral composition in terms of Ca and P components of the enamel in early carious lesions (ICDAS II codes 0 and 1-3) and compared them with adjacent areas of sound enamel [Michalaki et al., 2016]. According to the results of that study, the enamel of occlusal enamel of permanent teeth was found to have statistically significant different Ca/P composition rates at the areas of the early carious lesions, compared to sound enamel. Specifically, the EDX analysis indicated changes in the Ca and P contents that were more prominent in ICDAS II code 3 lesions compared to codes 1 and 2 lesions. A condition that resembles the loss of minerals during the development of white and brown spot carious lesions *in vitro*, with a gradient ranging from 74% to 100% from the surface to the bottom of the lesion *in vitro* [Cochrane et al., 10AD; Topoliceanu et al., 2013]. Therefore, occlusal surfaces with early carious lesions classified as ICDAS II codes 1-3, besides the difference in appearance, they differ in terms of mineral contents and tissue quality compared to sound surfaces. This alteration of the enamel starts early, it is more frequent in high-risk children especially during the eruption phase of the posterior teeth. During this phase and due to favourable conditions for plaque accumulation the enamel dissolution begins. Studies have shown that teeth are more caries prone to caries within the first 2–4 years of eruption [Carlos and Gittelsohn, 1965; Manji and Fejerskov, 1994]. During the repeated de- and re-mineralisation cycles, inorganic ions of calcium and phosphates are moving out of the enamel while other inorganic and organic materials (proteins) and stains are entering into the lesions, altering the composition of the tissue. This process depends on the salivary level of mutant's streptococci (low or high) and influences the development of brown discoloured or early carious lesions [Steiner et al., 1998]. The lesion, depending on the local conditions and the host's behaviour and vulnerability, might progress to cavitation or might be delayed and form a questionable or arrested lesion on the occlusal surface. According to some researchers [Noronha et al., 1999; Carvalho, 2014], the repeated cycles of de- and remineralization may lead to lesions formation that are active but very superficial and subclinical in nature (i.e. pre-cavitated) resulting to surface changes. Therefore, from many aspects the enamel of these surfaces is altered and differs in its histochemical composition from sound enamel, which in turn might influence the etching and proper bonding of the sealant to enamel.

Unfortunately, in clinical practice, surfaces with these characteristics, are prepared and sealed as sound with the conventional etching methods, without questioning on whether these surfaces are different or not and probably the usual sealant materials and technique may not be adequate for these surfaces. In the continuation of this clinical research, alternative methods of fissure preparation in terms of different etching times, bonding agents to improve retention [Feigal, 1998] or the use of different materials [Colombo and Beretta, 2018] on such substrates should be tested. In addition, more studies are needed to investigate the hypothesis of a weaker bond of a sealant material to a tooth surface with an established carious lesion. This is the main conclusion of the study after 4 years of clinical observation.

Conclusions

Based on the results of this study it can be concluded that: a) the therapeutic use of sealants on occlusal surfaces of posterior permanent molars with early carious lesions ICDAS II #1-3, resulted in double probability to fail leading to the placement of a restoration and 70% for resealing, after four years of observation; b) Older age was associated with lower risk of restorations (13% less for one year older, $p<0.001$); c) opaque sealants were associated with approximately 40% increased risk of reapplication compared to clear ones; d) sealants on pits were associated with 30% lower risk of reapplication compared to fissure surfaces, and; e) Gender was not significantly associated with the risk of restorations.

These findings might necessitate, besides a closer follow-up for longer period of time and resealing, more research for alternative methods of preparation, in order to improve bonding and retention of sealants on these surfaces for the children that need them the most.

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