

International Journal of Medical Science and Dental Research

Effect of Invasive and Non-Invasive Techniques on laser fluresence score and retention of Two Pit and Fissure Sealants (in vivo study)

Hatem Abdul Monaem Mahmoud El Bially¹, Khalid Mohamed Noaman², Ahmed Tharwat Al Ammary ³

Abstract:

Objective: This study was conducted to compare clinical effect of invasive and non-invasive techniques on diagnodent pen score and retention of two pit and fissure sealants.

Patients and methods: Total of (84) first permanent molar teeth were sealed in 21 participants aged between 8-12 years enrolled in the study. Baseline Measurements with DIAGNOdent pen were performed on the occlusal surface of each molar tooth. Teeth were randomly assigned into two groups (n=42). A1; Healioseal f. A2; Tetric flow. The sealants were applied by B1, invasive and B2 non-invasive techniques. Participants recalled at 1 week, 1 month and 3 months. Results: Gr_A1 recorded higher fluorescence score mean value than Gr_A2 . The difference was statistically non-significant (p>0.05). Gr_B1 recorded higher fluorescence score mean value than Gr_B2 . The difference was statistically non-significant (p>0.05). The highest fluorescence score mean value recorded after one week storage followed by 1 month storage while the lowest fluorescence score mean value recorded after 3 months storage. The difference was statistically non-significant as proved by Chi square test (p>0.05). Gr_A1 recorded higher success score % value than Gr_A2 group. The difference was statistically significant as revealed by Chi square test (p<0.05), the highest success score % recorded after one week,, while the lowest success score % recorded after 3 months with statistically significant difference as

¹ Assistant lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Assuit-Branch), Al-Azhar University, Egypt.

² Professor and Head of Operative Dentistry Department, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University Egypt.

³ Assistant Professor and Head of Operative Dentistry Department, Faculty of Dental Medicine (Assuit-Branch), Al-Azhar University, Egypt.

¹ Assistant lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Assuit-Branch), Al-Azhar University, Egypt.

² Professor of Operative Dentistry Department, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University Egypt

³ Assistant Professor of Operative Dentistry Department, Faculty of Dental Medicine (Assuit- Branch), Al-Azhar University, Egypt.

proved by Chi square test (p< 0.05). Conclusions: resin based fissure sealant demonstrates effectiveness in caries prevention and retention.

Keywords - Diagnodent pen, Helioseal F, Invasive, Retention, Tetric Flow

I. INTRODUCTION

Dental caries, defined as a carbohydrate-modified local infection that destroys the hard tissues of the teeth, has a multifactorial etiology, in which there is an interaction of various factors, including the host, agent, substrate, and time^[1]. Ninety percent of carious lesions originate in the pits and fissures of the occlusal surfaces of permanent molars^[2]. One preventive option is to seal "imperfections in the enamel", such as pits and fissures ^[3]. However, the presence of sealants influences the performance of conventional methods in detecting occlusal caries and in monitoring its progression. Thus, adjunct methods must be used to improve the monitoring assessments and to increase the diagnostic accuracy^[4].

Some of these methods are based on the fluorescence, which is emitted by the porphyrins present in caries lesions when stimulated at specific excitation wavelengths ^[5, 6]. DIAGNOdent 2190 (LFpen; KaVo, Biberach, Germany) have been currently used as adjunct in caries detection ^[7, 8]. Healthy tooth structure exhibits little or no fluorescence, while carious tooth structure exhibits fluorescence proportional to the amount of decay^[9].

Sealant retention can be improved by cleaning of the occlusal surface prior to sealant placement with mechanical preparation of fissures known as invasive technique^[10].

The applicability of flowable composites as pit and fissure sealants has expanded because of their desirable properties, such as low viscosity, low modulus of elasticity, and ease of handling. It has been proposed that a greater quantity of filler particles may lower the porosity and cause less polymerization shrinkage with better wear resistance, which is particularly important when the material used is of low thickness relative to conventional resin-based pit and fissure sealants^[11, 12].

II. SUBJECTS AND METHODS

- **A. Study design:** Randomized controlled clinical study.
- **B. Study setting:** This study was carried out in Department of Operative Dentistry, Faculty of Dental medicine, Al- Azhar University, Cairo, Boys.
- **C. Study population:** Twenty one participants of both sexes aged 8 to 12years were enrolled for this study according to inclusion and exclusion criteria.

Inclusion criteria:

- 1. Age of the participant was between 8 and 12 years.
- 2. Presence of all four caries-free permanent first molars with clearly visible occlusal surface and deep pits and fissures.
- 3. Evidence of an acceptable home dental cleaning regimen.
- 4. Participant cooperation and acceptance for the treatment.
- 5. No prior dental therapy on respective teeth.
- 6. Possibility to get proper isolation with cotton rolls.
- 7. Approving the participation and filling the consent form.

Exclusion criteria:

- 1. History of any medical disease that might interfere with the study.
- 2. Current participation in other studies.
- 3. History of abnormal para-functional activity.
- 4. Participants undergoing fluoride application regimen.
- 5. Tooth free from any formative and developmental anomalies.
- **D. Ethical considerations:** Approval for this research was obtained from Research Ethics Committee, Faculty of Dentistry, Al Azhar University. The purpose of the present study was explained to the parents and informed consents were obtained according to the guidelines on human research adopted by the Research Ethics Committee, Faculty of Dentistry, Al Azhar University.
- **E. Group assignment:** Eighty four first permanent molars were randomly assigned into two groups: (A) (n=42), according to the type of fissure sealant materials applied as follows; Group A1; sealed with resin fissure sealant (Healioseal F, Ivoclar vivadent). GroupA2; sealed with flowable resin composite (Tetric N flow, Ivoclar vivadent). Each group was divided into two subgroups: (B) (n=21), according to the fissure sealant preparation technique as follows: Subgroup B1; invasive preparation technique. Subgroup B2; non-invasive preparation technique (intact). Each subgroup was further divided according to follow up periods into three classes: (C) (n=7), as follows; Class C1; 1 week, Class C2; 1 month and Class C3; 3 months.
- **D. Participant examination:** Past and present medical histories and dental histories were obtained in the form of a printed health questionnaire which was filled by all parents.
- **E. Selection of teeth:** First permanent molars with deep pits and fissures and fully erupted into the oral cavity were included.
- **F. Teeth preparation:** Before baseline measurements, scaling procedures for each participant was carried out, followed by prophylaxis using a non-fluoridated pumice, rinsed and then dried.
- **G. Preoperative assessment:** DIAGNOdent pen 2190 was used to assess caries lesions before applying fissure sealants in this investigation.
- **H. Intervention:** A split-mouth, single blind clinical trial was performed. One side of the mouth received pit and fissure sealant, while the contralateral side received flowable composite resin. For invasive group: Occlusal fissures and pits of teeth were opened using Fissurotomy Bur (18 SS White, Ivoclar North America, Inc.) in a light sweeping motion. The depth of penetration was confined to enamel. For non-invasive group: Occlusal surfaces of selected teeth were left intact unprepared. Helioseal F and Tetric Flow sealants were applied according to manufacturer's instructions. Participants were recalled at 1week, 1month and 3 months postoperatively and Treated teeth re- examined clinically by the same operator.

Caries assessment using laser fluorescence device: At the end of each follow up periods (1 week, 1month and 3months), Caries assessment was performed with the DIAGNOdent pen to evaluate post sealant application reading values. The data was tabulated and statistically analyzed.

Sealant retention and detachment: After the treatment, at the end of 1 week, 1 month and 3 months. The retention was evaluated using flat mirror and blunt explorer probe by trying to tack off the sealant with an explorer immediately by the same trained examiner. During recall visits the examiner did not know the method used for enamel preparation, therefore the examination is of single-blind type. All partially and non-retained pits and fissure sealants were excluded from the study. Sealed teeth were and categorized as successful or failed.

A-Successful sealant: sealant is completely intact with no signs of partial or complete loss or evidence of occlusal caries.

B-Failed sealant: partialy or completely lost sealant, or detection of occlusal caries^[13]. Ethically, in the event of failure, the fissures were resealed on elimination of the sample.^[14]

I. statistical analysis

Data were presented as percentage. The results were analyzed using Graph Pad Instat (Graph Pad, Inc.) software for windows. Three-way ANOVA was performed to detect effect of each variable (material, technique and time). Chi square test was done to compare in-vivo data. Sample size (n=42/group) was large enough to detect large effect sizes for main effects and pair-wise comparisons, with the satisfactory level of power set at 80% and a 95% confidence level.

III. RESULTS

Regardless to preparation technique or time, totally it was found that, Gr_A1 recorded higher fluorescence score mean value than Gr_A2 . The difference between both groups was statistically non-significant as revealed Chi square test (p=0.8221 > 0.05). Table (1). Gr_B1 recorded higher fluorescence score mean value than Gr_B2 . The difference in the fluorescence scores between both techniques was statistically non-significant as discovered by Chi square test (p> 0.05). Table (1).

Regardless to material or technique, totally it was found the highest fluorescence score mean value recorded after one week storage followed by 1 month storage while the lowest fluorescence score mean value recorded after 3 months storage. The difference in the fluorescence scores between different storage times was statistically non-significant as proved by Chi square test (p=0.9822 > 0.05). Table (1).

Table (1): Statistical analysis using fluorescence score detected by DIAGNOdent pen between both groups as function of preparation technique and evaluation time.

		Gr A1		Gr A2		Statistics
Variable				+		P value
		Gr_A1B1	Gr_A1 B2	Gr_A2B1	Gr_A2B2	
Base - one week		97.43%	79.27%	90.03%	84.47%	0.5586 ns
Base - one month		83.85%	71.39%	69.29%	66.32%	0.6843 ns
Base - 3 months		70%	63.78%	60.89%	57.89%	0.9766 ns
Statistics	P value	0.6196 ns		0.9734 ns		

ns; non-significant (p>0.05)

*; significant (p<0.05)

Regardless to valuation time, totally it was found that, Gr_A1 recorded higher success score % value than Gr_A2 . The difference in the durability between both groups was statistically significant as revealed by Chi square test (p=0.019 < 0.05). For both groups, totally it was found the highest success score % recorded after one week with corresponding least failure score %, intermediate success score % recorded after 1 month with corresponding intermediate failure score % while the lowest success score % recorded after 3 months with corresponding highest failure score %. The difference in the durability between different recall times was statistically significant as proved by Chi square test (p=0.0001 < 0.05).

Table (2) Sealant retention and detachment between both groups as function of evaluation time.

Variable		Gr A1		Gr A2		Chi test
		Successful	Failed	Successful	Failed	P value
Recall time	One week	13 (92.86%)	1 (7.14%)	12 (85.71%)	2 (14.29%)	0.1652 ns
	One month	11 (78.57%)	2 (14.29%)	8 (57.14%)	4 (28.57%)	0.005*
	Three months	8 (57.14%)	3 (21.43%)	3 (21.43%)	5 (35.71%)	<0.0001*
Chi test	P value 0.0013*			<0.0001*		

ns; non-significant (p>0.05)

*; significant (p<0.05)

IV. DISSCUSSION

In recent years, the greatest interest of modern dentistry; it focuses on reducing the risk of caries, preventive practices, and non-invasive conservative techniques where dental structures are protected as much as possible^[15]. Occlusal surfaces have been found to susceptible to caries due to pits and fissures resulting from anatomical structures. The most effective method to prevent caries from these areas is the application of pit and fissure sealants^[16]. Fissure sealants prevent plaque microflora and food-borne debris from accumulation in caries-susceptible pits and fissures ^[18]. Participants in this study aged between 8-12 years. Teeth chosen for sealant application were first permanent molars of all four quadrants in each subject. First permanent molars were chosen as their occlusal surface is most frequently attacked by dental caries^[19].

For the detection and quantification of caries, a laser-based instrument, KaVo DIAGNOdent (DIAGNOdent KaVo, Biberach, Germany). It is a variant of QLF system and was introduced based on research by Hibst and Gal^[23]. DIAGNOdent pen 2190 is the perfect tool to detect fissure and smooth surface caries accurately^[24].

Gr_A1 Helioseal F(white shaded) recorded more pronounced increase in fluorescence score mean value compared to Gr_A2 Tetric Flow(A2) group with statistically non-significant difference this may be explained by Helioseal F(white shaded) intrinsic fluorescence which contain 0.5% titanium oxide . Titanium dioxide (TiO2) is the most common opacifying filler in sealants. This filler facilitates the application of sealant and its visual assessment in recall visits ^[25]. As the TiO2 content increases, fluorescence transmission is attenuated; this indicates that the existing TiO2 in sealants might interfere with the fluorescence transmitted from caries or laser devices. Consequently, they can cause false positive and false negative results. This result agreed with Deery et al^[26]. and Sonmenz et al^[27]. Our results were not supported by findings of Bahrololoomi Z. et al. ^[28], Asksroglou et al^[29].. Krause et al^[30] and Gostanian et al^[25].

With statistically non-significant difference, in this study Gr_B1 (invasive preparation technique) recorded higher fluorescence score mean value than Gr_B2 (non-invasive). Our study result was consistent with the study outcome of Larsen, M.J et al., Krause et al. and Vaarkamp, J et al. Larsen, M.J et al., demonstrated that, Changes in the optical properties of the minerals are caused by an increased pore volume in demineralized enamel^[33]. Thus, LF detects substantially more fluorophores within these pores than within healthy enamel^[34]. Krause et al., claimed that LF values have been reported to be significantly increased in demineralized enamel after the acid-etching procedure^[30]. A study of Vaarkamp, J et al., has shown that hydroxyapatite crystals contribute significantly to scattering^[35]. In contrary to our findings, Diniz et al., concluded that, the thickness of an opaque sealant does not play an important role in the fluorescence readings^[4].

At one week Diagnodent pen value recorded the highest fluorescence followed by 1 month while 3months recorded the lowest values, with statistically non- significant difference.

This non-significant decrease in caries progression from baseline values to 3 months values as detected by (Diagnodent Pen) DP could be related to the constant cycle of the demineralization/ remineralization processes. Bacterial by-products may not be instantly affected by the sealant application. With time, the number of bacteria

and its by-products may decrease. This could be the reason for non-significant decrease in the caries progression as recorded by the DP from 1 week to 3months, these results supported by Nada Jaafar et al^[36]. The results of our study was in contrast to previous in vitro studies have reported that, LF values increased after sealant thermocycling^[4], aging in saline^[30].

In this study Gr_A1 recorded higher success score % value than Gr_A2. The difference in the durability between both groups was statistically significant (p=0.019 < 0.05)meaning that resin based sealant (Helioseal F) showed a higher retention rate than flowable composite (Tetric flow). On the one hand, this may be attributed to low filler content of Helioseal F when compared to Tetric flow resulted in low viscosity. Low viscosity of resin sealant enables it to penetrate deeper in the narrow and irregular anatomy of the fissures. This result is in agreement with other studies $^{[21, 37]}$. On the other hand, Nirwan, et al. $^{[39]}$, agreed with the results of our study.

All tested pit and fissure sealants showed a better performance at 1 week. The retention rate was reduced at each successive time interval from the highest at 1 week to intermediate at 1 month, while the lowest was at 3 months. This result is in agreement with other studies [37, 40-42]. But in our study, the overall observation period was 3 months, while in other studies, the observation period extended for longer periods. This may be explained by that, the challenging media of the oral cavity without a doubt plays a significant role in decreasing the survival rate of all restorative material over an extended period of time. The continuous changes in pH and heat of the oral cavity play a deleterious effect on the restorative material [43]. The outcome of the present study was in disagreement with, Wadhwa, S^[14]., et al., Jafarzadeh et al. [44]., Kasemkhun et al. [45] and Dukic et al [46].

V. CONCLUSION

Although resin based fissure sealant demonstrates effectiveness in caries prevention and retention, both materials could be recommended as materials of choice for pits and fissure sealing procedure.

REFERENCES

- [1] Featherstone, J.D., The science and practice of caries prevention. The Journal of the American dental association, 2000. 131(7): p. 887-899.
- [2] Griffin, S.O., et al., Caries risk in formerly sealed teeth. The Journal of the American Dental Association, 2009. 140(4): p. 415-423.
- [3] Dutra Borges, B.C., et al., Arrest of non-cavitated dentinal occlusal caries by sealing pits and fissures: a 36-month, randomised controlled clinical trial. International dental journal, 2012. 62(5): p. 251-255.
- [4] Diniz, M., et al., The influence of pit and fissure sealants on infrared fluorescence measurements. Caries research, 2008. 42(5): p. 328-333.
- [5] Hibst, R., R. Paulus, and A. Lussi, Detection of occlusal caries by laser fluorescence: basic and clinical investigations. Medical Laser Application, 2001. 16(3): p. 205-213.
- [6] Bader, J.D. and D.A. Shugars, A systematic review of the performance of a laser fluorescence device for detecting caries. The Journal of the American Dental Association, 2004. 135(10): p. 1413-1426.
- [7] Rodrigues, J., et al., Performance of fluorescence methods, radiographic examination and ICDAS II on occlusal surfaces in vitro. Caries research, 2008. 42(4): p. 297-304.
- [8] Diniz, M.B., et al., In vivo evaluation of laser fluorescence performance using different cut-off limits for occlusal caries detection. Lasers in medical science, 2009. 24(3): p. 295-300.
- [9] Hamilton, J.C., W.A. Gregory, and J.B. Valentine, DIAGNOdent measurements and correlation with the depth and volume of minimally invasive cavity preparations. Operative dentistry, 2006. 31(3): p. 291-296.
- [10] Salama, F. and N. Al-Hammad, Marginal seal of sealant and componer materials with and without enameloplasty. International Journal of Paediatric Dentistry, 2002. 12(1): p. 39-46.
- [11] Autio-Gold, J., Clinical evaluation of a medium-filled flowable restorative material as a pit and fissure sealant. Operative dentistry, 2002. 27(4): p. 325.
- [12] Jager, S., et al., Filler content, surface microhardness, and rheological properties of various flowable resin composites. Operative dentistry, 2016. 41(6): p. 655-665.
- [13] Mascarenhas, A.K. and A.M. Moursi, Use of fissure sealant retention as an outcome measure in a dental school setting. Journal of dental education, 2001. 65(9): p. 861-865.

Volume 05, Issue 01 (January-February 2022), PP 90-97

ISSN: 2581-902X

- [14] Wadhwa, S., et al., Comparative Clinical Evaluation of Resin-based Pit and Fissure Sealant and Self-adhering Flowable Composite: An In Vivo Study. International journal of clinical pediatric dentistry, 2018. 11(5): p. 430.
- [15] Newbrun, E., Preventing dental caries: current and prospective strategies. Journal of the American Dental Association (1939), 1992. 123(5): p. 68-73.
- [16] Wyne, A., Caries prevalence, severity, and pattern in preschool children. The journal of contemporary dental practice, 2008. 9(3): p. 24-31.
- [17] Manton, D.J. and L.B. Messer, Pit and fissure sealants: another major cornerstone in preventive dentistry. Australian dental journal, 1995. 40(1): p. 22-29.
- [18] Waggoner, W.F. and M. Siegal, Pit and fissure sealant application: updating the technique. The Journal of the American Dental Association, 1996. 127(3): p. 351-361.
- [19] Macek, M.D., et al., Updated comparison of the caries susceptibility of various morphological types of permanent teeth. Journal of public health dentistry, 2003. 63(3): p. 174-182.
- [20] N., F., Influence of extended light exposure curing times on the degree of conversion of resin-based pit and fissure sealant materials. . Saudi Dent J., 2014(;26:): p. 151–155. .
- [12] Joshi, K., et al., Comparative evaluation of two different pit & fissure sealants and a restorative material to check their microleakage—An In Vitro Study. Journal of International Oral Health: JIOH, 2013. 5(4): p. 35.
- [22] Mickenautsch, S. and V. Yengopal, Validity of sealant retention as surrogate for caries prevention—a systematic review. PLoS one, 2013. 8(10): p. e77103.
- [23] Shi, X.-Q., U. Welander, and B. Angmar-Månsson, Occlusal caries detection with KaVo DIAGNOdent and radiography: an in vitro comparison. Caries research, 2000. 34(2): p. 151-158.
- [24] Kühnisch, J., K. Bücher, and R. Hickel, The intra/inter-examiner reproducibility of the new DIAGNOdent Pen on occlusal sites. Journal of dentistry, 2007. 35(6): p. 509-512.
- [25] Gostanian, H.V., et al., An in vitro evaluation of the effect of sealant characteristics on laser fluorescence for caries detection. Pediatric dentistry, 2006. 28(5): p. 445-450.
- [26] Deery, C., et al., Effect of placing a clear sealant on the validity and reproducibility of occlusal caries detection by a laser fluorescence device: an in vitro study. Caries research, 2006. 40(3): p. 186-193.
- [27] ŞAROĞLU SÖNMEZ, I., et al., Effects of different fissure sealant applications on laser fluorescence measurements. International journal of paediatric dentistry, 2011. 21(1): p. 29-34.
- [28] Bahrololoomi, Z., M. Khodabakhsh, and Y. Khaksar, The effects of opaque and clear pit and fissure sealants on infrared laser fluorescence measurements. Journal of Dentistry, 2014. 15(2): p. 63.
- [29] Askaroglou, E., et al., Effect of sealants on laser fluorescence caries detection in primary teeth. Lasers in medical science, 2011. 26(1): p. 29-34.
- [30] Krause, F., et al., Effects of composite fissure sealants on IR laser fluorescence measurements. Lasers in medical science, 2008. 23(2): p. 133-139.
- [31] Shapira, J., Six-year clinical evaluation of fissure sealants placed after mechanical preparation: a matched pair study. Pediatr. Dent., 1986. 18: p. 204-205.
- [32] Welbury, R., M. Raadal, and N. Lygidakis, EAPD guidelines for the use of pit and fissure sealants. European journal of paediatric dentistry, 2004. 5: p. 179-184.
- [33] LARSEN, M.J. and O. Fejerkov, Chemical and structural challenges in remineralization of dental enamel lesions. European journal of oral sciences, 1989. 97(4): p. 285-296.
- [34] Lussi, A., et al., Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. European journal of oral sciences, 2001. 109(1): p. 14-19.
- [35] Vaarkamp, J., J. Ten Bosch, and E. Verdonschot, Propagation of light through human dental enamel and dentine. Caries Research, 1995. 29(1): p. 8-13.
- [36] Jaafar, N., et al., An in vivo investigation of diagnostic performance of DIAGNOdent pen and the Canary System for assessment and monitoring enamel caries under fissure sealants. Journal of International Society of Preventive & Community Dentistry, 2020. 10(3): p. 246.
- [37] Kumaran, P., Clinical evaluation of the retention of different pit and fissure sealants: a 1-year study. International journal of clinical pediatric dentistry, 2013. 6(3): p. 183.
- [38] Ninawe, N., N.A. Ullal, and V. Khandelwal, A 1-year clinical evaluation of fissure sealants on permanent first molars. Contemporary clinical dentistry, 2012. 3(1): p. 54.
- [39] Nirwan, M., et al., A comparative evaluation of retention of pit and fissure sealant bonded using sixth-, seventh-, and eighth-generation adhesives: An in vivo study. Journal of Indian Society of Pedodontics and Preventive Dentistry, 2017. 35(4): p. 359.
- [40] Reddy, V.R., et al., Retention of resin-based filled and unfilled pit and fissure sealants: A comparative clinical study. Contemporary clinical dentistry, 2015. 6(Suppl 1): p. S18.

- [41] Nogourani, M.K., et al., A 12-month clinical evaluation of pit-and-fissure sealants placed with and without etch-and-rinse and self-etch adhesive systems in newly-erupted teeth. Journal of Applied Oral Science, 2012. 20: p. 352-356.
- [42] Chen, X., et al., Effectiveness of two new types of sealants: retention after 2 years. Clinical oral investigations, 2012. 16(5): p. 1443-1450.
- [43] Versluis, A., W.H. Douglas, and R.L. Sakaguchi, Thermal expansion coefficient of dental composites measured with strain gauges. Dental materials, 1996. 12(5-6): p. 290-294.
- [44] Jafarzadeh, M., et al., Retention of a flowable composite resin in comparison to a conventional resin-based sealant: One-year follow-up. Journal of Dentistry (Tehran, Iran), 2010. 7(1): p. 1.
- [45] ; P.K.S.N.A.P.N.S., The efficacy of dental sealant used with bonding agent on occlusal caries (ICDAS 2-4): A 24-month randomized clinical trial. Int J Paediatr Dent., 2021. 31: p. 760–766.
- [46] Dukic, W. and D. Glavina, Clinical evaluation of three fissure sealants: 24 month follow-up. European archives of paediatric dentistry, 2007. 8(3): p. 163-166.