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Clinical and antibacterial effectiveness of three different sealant materials

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Purpose. The aim of this work is to study and compare the retention rate, caries-preventing and antibacterial effects of resin-modified glass ionomer and flowable composite in comparison to conventional fissure sealant.

Methods. Forty-five children aged 7-10 years with newly erupted lower first permanent molars were randomly divided into three equal treatment groups. Group I: sealed by a conventional resin sealant; Group II: sealed by resin modified glass ionomer (RMGI); and Group III: sealed by flowable composite. Retention and caries status of the sealed teeth were recorded after 1 month, 6 months, year and 2 years. In addition, Streptococcus mutans counts were assessed at baseline, 1 day, 1 month, 6 months, 1 year and 2 years after sealant application. Data were analyzed by Fisher exact, chi-square and ANOVA tests.

Results. Group III and Group I showed significantly higher retention rates than Group II fissure sealant ($p<0.05$). There were no differences of the caries-preventive effects between the tested sealant materials throughout the duration of the study. Streptococcus mutans counts were significantly lower in group II compared to group I or group III up to 6 months of the study ($p<0.05$). After 1 year of the study the differences of Streptococcus mutans counts were not significant ($p>0.05$).

Conclusion. This study indicated a lower retention of RMGI compared to flowable composite and resin sealant without significant difference in caries prevention or long-term bacterial inhibition.

Keywords: sealant, retention, caries, bacteria

Introduction and literature review

By the end of the 20th century, the global distribution of dental caries among school children showed wide variations between developed and developing countries[1]. However, it has been reported that the relative contribution of pit and fissure caries to overall caries level in 12-year-old children is about 80%.[2,3,4] In this context, use of pit and fissure sealants as an adjunct to oral health care strategies and fluoride therapy in preventing dental caries would be worthwhile.

Nevertheless, the capacity of a sealant to prevent dental decay relies directly upon the ability of the sealant material to thoroughly fill pits, fissures, and/or morphological defects and remain completely intact and bonded to enamel surfaces for a lifetime.[5] Additionally, studies demonstrated that incipient carious lesions may inadvertently be sealed with dental sealants and the fate of bacteria is of significance.[6] Therefore, additional antibacterial protection provided by sealants, especially against Streptococcus mutans, would be of value to prevent subsequent deterioration at the sealant-tooth interface and caries initiation.[7]

Nowadays, the most widely used fissure sealants are based on bisphenyglycedyl dismethacrylate (BisGMA) resins or urethane-based products. Since the introduction of resin sealant, various materials and techniques have been developed and/or proposed to improve the sealing quality of pits and fissures and to enhance sealant longevity.[8,9] Glass ionomer materials have been successfully employed for a number of applications and recently a growing interest in their use as fissure sealants has aroused. [10,11] However, conventional, chemically cured glass ionomer cements tried as pit and fissure sealants generally exhibited a poor retention rate and are too viscous to penetrate deeply into narrow fissures.[12] Many investigators found the retention rate of conventional glass ionomer to be lower than that of resin sealants, but without significant differences in caries prevention. [13,14,15] However, other research reports, did not support this view. [16,17]

On the other hand, the visible-light-polymerized resin-modified glass ionomer (RMGI) exhibited a significantly better compressive strength than conventional auto-cured glass ionomer cements.[18] Furthermore, RMGI has higher fracture and wear resistances than does self-cured glass ionomer without hindering the rate of fluoride release.[19] These improvements of glass ionomer materials are expected to increase the effectiveness of RMGI sealant.

In the past several years, the use of flowable composites as pit and fissure sealants has been widely suggested because of their beneficial properties, such as low viscosity, low modulus of elasticity, and ease of handling.[20,21] It has been reported that higher amount of filler particles in flowable composites provide lesser porosity and better wear resistance than conventional resin-based pit and fissure sealants.[22] Autio-Gold reported an equivocal retention rate and cariostasis of flowable composite and conventional sealants.[23]

Improving sealant materials is important since it may dramatically change the calculations on cost-benefit. Although there is a growing interest in the use of resin-modified glass ionomer and flowable composite as pit and fissure sealing materials, the evidence is still limited relative to evaluating and comparing their clinical effectiveness. Moreover, the antibacterial activities of composite and glass ionomer have only been examined in number of in-vitro studies.[24,25,26] No in-vivo studies have been conducted to clarify this issue. These studies reported different levels of short-term antibacterial properties of diverse materials against cariogenic bacteria. The authors of this study were not aware of any published data comparing the long-term effectiveness of these materials in lowering cariogenic bacteria when used as sealants.

Aim of the work

The aim of this study was to:

1. Clinically assess the retention rates and caries-preventive effects of resin-modified glass ionomer and flowable composite in comparison to conventional fissure sealant;
2. Compare the long-term inhibitory effects of the tested materials on salivary streptococcus mutans counts.

Materials and methods

Study design

This study was carried out as a controlled experimental clinical trial to test and compare the clinical and antibacterial effectiveness of the tested materials.

Subject selection

Forty-five caries-free children aged 7-10 years were randomly selected from Pedodontic Clinic, Faculty of Dentistry, Tanta University. Each child had 2 lower first permanent molars with at least two-thirds of occluso-cervical length erupted. The eruption time of these molars did not exceed 3 years. Teeth were selected based on the existence of deep narrow central fissures and supplemental grooves with no evidence of cavitation, pre-cavitation, or probe catching with stained fissures.[27] Children with chronic systemic diseases or children with physically or mentally handicaps were excluded from the study. Also, those taking antibiotic therapy in the last 3 months before the start of the study were not included.

The children were randomly divided into 3 equal groups composed of 15 children each according to the type of sealant used.

Group I:

The fissures of the 2 newly erupted lower first permanent molars were sealed by a conventional-light-cured bisGMA resin fissure sealant (***Helioseal F****, Viva Dent Benderstrasse.Schaan, Liechtenstein, Austria*). This group was considered a positive control group.

Group II:

The fissures of the 2 newly erupted lower first permanent molars were sealed by visible light-cured resin modified glass ionomer (RMGI) (***FujiII LC****, GC, Tokyo, Japan*).

Group III

The fissures of the two newly erupted lower first permanent molars were sealed by flowable composite (***Tetric Flow****, Viva Dent Benderstrasse.Schaan, Liechtenstein, Austria*).

The teeth were cleaned with a dry pointed bristle brush in a low-speed hand piece and isolated with a rubber dam. Occlusal surfaces of Groups I and III were etched with 37% phosphoric acid gel (*Ultra Etch, Ultradent Products Inc., USA*) for 30 seconds and the RMGI group participants were scrapped with *GC Dentine Conditioner* (GC, Tokyo, Japan) for 20 seconds according to the manufacturers, instructions.

The surfaces were rinsed for 10 seconds with an air/water spray and occlusal fissures were dried with oil-free compressed air for 15 seconds.

The sealants were applied to etched surfaces of the treatment groups according to manufactures' instructions using a syringe needle tip included with each material. All sealants were then photo-cured for 40 seconds. The occlusion was examined after sealant application and the high spots were adjusted.

Sealant retention and dental caries status of all teeth were evaluated at 1 month, 6 months, 1 year and 2 years after sealant application under normal clinical conditions with a dental operating light, mouth mirror, and sharp dental explorer. The retention of the sealant was scored as:

1. Totally present: no crevice detected by explorer
2. Partially lost: partial exposure of fissures
3. Totally lost: complete loss of sealant (28)

Caries presence was scored without radiographs, according to World Health Organization dental caries criteria.[27] Diagnosis was primarily visual; probing was used only to confirm diagnosis.

Saliva sample collection and microbiological procedures

Saliva samples were taken before sealant application to obtain base-line streptococcus mutans count for each participant. Another sample was taken 1 day after sealant application from all groups. Subsequently, saliva samples were taken at 6 months, 1 year and 2 years.

Saliva samples were taken in the morning before breakfast and the participants were requested not to perform tooth cleaning on the sampling days. The samples were collected with the children sitting, swallowing, and allowing saliva to pool in the mouth for 2 minutes. All saliva samples were taken from underneath the tongue by means of a sterile plastic pipette.[29] Subjects gave approximately 2 mL samples of unstimulated whole saliva collected into sterile test tubes.

Samples were immediately transported to the laboratory and processed within 30 minutes. Aliquots of 0.5 ml of saliva were diluted in 10-fold solution of sterile, phosphate-buffered saline and 20 mL was plated on Mitis-Salivarius agar, which is supplemented with bacitracin (0.2units/ ml) and 10% sucrose. The plates were incubated in 5% carbon dioxide environment at 37 0C for 48 hours.[30]

The bacteria were counted blindly of study groups with the help of a coordinator. Mutans streptocci identification was based on its distinct colony morphology which appear as hard, coherent, dark blue, and berry-like with raised colonies varying in size from 0.5 to 1 mm in diameter.[31] (Figure 1)

Figure 1. Streptococcus mutans colonies cultured on mitis salivarius bacitracin medium

Patients' rights

Informed consents were obtained from the parents of children after the nature and aim of the trial were outlined and it was explained that some of their teeth would be fissure sealed and that participation is voluntary. Children received oral hygiene instructions and they continued their usual oral hygiene practices including the use of fluoridated toothpaste throughout the study.

Examiner reliability

All sealants placements and dental examinations were conducted by one examiner. Intra-examiner calibration was assessed before the start of the study by re-examination of 10 children with sealed lower permanent molars with 1 week interval between examinations (kappa = 0.8 for both caries detection and sealant retention).

Statistical analysis

Data were collected, presented, and statistically analyzed using the SPSS statistical package system.[32] Fisher exact test, chi square test, ANOVA, and LSD post hoc tests were used according to the type of data. Partial and complete sealant losses were summed to facilitate statistical analysis. The level of significance used was 5%.

Results

The clinical evaluation of retention of the 3 sealants in the tested groups during the study period is shown in Table I and Figure 2. As determined by the Fisher exact test, there was no statistically significant difference between the 3 groups after 1 month (p = 0.36). However, chi-square test revealed that there were statistically significant differences between the 3 groups after 6 months, 1 year and 1 years (p = 0.02, 0.003, 0.00, respectively). Tetric flow showed the highest retention rate followed by Helioseal F and lastly FujiII LC fissure sealant. At the end of the study, the sample attrition in Groups I, II, and III were 2, 3 and 1 children respectively.

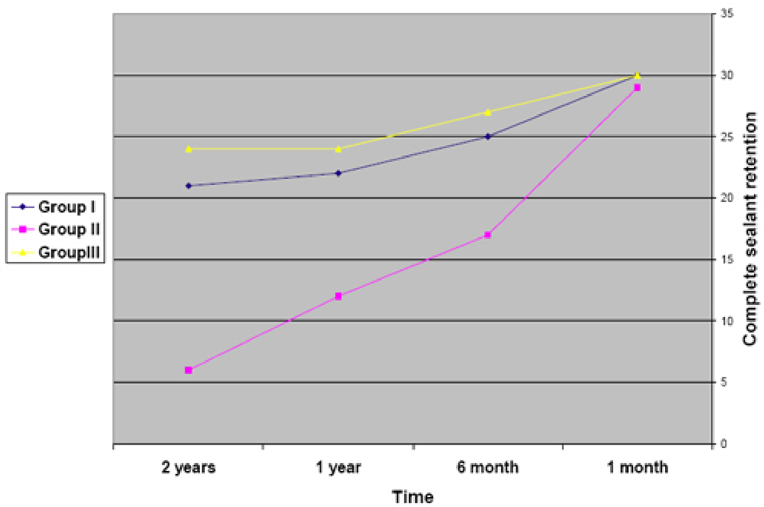

Figure 2.: Retention rate of the study groups during follow up periods

Table I. Comparison of retention rate between tested groups during study period.

Time	1 month			6 months			One year			Two years		
Group	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII
No sealant loss	30	29	30	25	17	27	22	12	24	21	6	24
Partial sealant loss	0	1	0	3	5	3	3	7	4	2	5	3
Complete sealant loss	0	0	0	2	6	0	3	7	0	3	13	1
P-value	0.36			0.02*			0.003*			0.00*		

* Significant at 5% level

As shown in Table II, no caries were found among the study groups either at 1 month or 6 months follows up. Similarly, 96% of the sealed teeth in all groups were sound after 1 year and 2 years from the start of the study.

Table II. Caries status of the sealed teeth among study groups during different follow up periods

Time	1 month			6 months			One year			Two years		
Group	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII
Sound	30	30	30	30	28	30	27 96.4%	25 96.1%	27 96.4%	25 96.1%	23 96%	27 96.4%
Carious	0	0	0	0	0	0	1 3.6%	1 3.9%	1 3.6%	1 3.9%	1 4%	1 3.6%

A comparison between salivary Streptococcus mutans colony forming units (CFU) in the 3 groups during the study is shown in Table III. The mean number of Streptococcus mutans colony forming units was 231 x 10^3, 223 x 10^3, and 227 x 10^3, respectively at the start of the study. The differences between all groups were not statistically significant (F = 0.27, p = 0.77). After one day of sealant application until 6 months follow up, the mean Streptococcus mutans count was highest in Group III, followed by Group I and lastly Group II. ANOVA and LSD tests revealed that the differences between all groups were statistically significant (F =46.8, 90.6, 75.6, respectively, p = 0.00). After one year until the end of the study, the differences of the mean streptococcus mutans count between study groups were not statistically significant (F = 0.47, 0.72, p = 0.64, 0.52, respectively).

Table III. Salivary streptococcus mutans count in study groups (CFU/ml)

Time	Group I	Group II	Group III	F	LSD
	$\overline{X}$ ± *SD*	$\overline{X}$ ± *SD*	$\overline{X}$ ± *SD*		
Baseline	231×10^3 13×10^3	223×10^3 11×10^3	227×10^3 12×10^3	0.27 P= 0.77	
One day	213×10^2 7×10^2	174×10^2 6.5×10^2	231×10^2 1×10^3	46.8* P= 0.00	*Group I vs group II** *Group I vs group III** *Group II vs group III**
One month	242×10^2 9.6×10^2	203×10^2 605×10^2	284×10^2 5.5×10^2	90.6* P= 0.00	*Group I vs group II** *Group I vs group III** *Group II vs group III**
Six months	284×10^2 8.5×10^2	234×10^2 1.5×10^3	341×10^2 6×10^2	75.6* P= 0.00	*Group I vs group II** *Group I vs group III** *Group II vs group III**
One year	237×10^3 6.4×10^3	238×10^3 4×10^3	235×10^3 13×10^3	0.47 P= 0.64	
Two years	243×10^3 5.1×10^3	234×10^3 6.5×10^3	249×10^3 8.6×10^3	0.72 P= 0.52	

*Significant at 5% level

Discussion

In this study, sealant application was performed during the posteruptive maturation phase of first permanent molars when they are at risk of developing dental caries. This may highlight the differences in caries preventive effects between the tested materials. [10] Diagnosis of fissure caries, especially under defective sealants presented more difficulties without radiographs.[13] However, caries diagnosis was performed primarily visually to avoid exposing the children to unnecessary radiations.

The retention rates of resin sealant and resin modified glass ionomer observed at the end of this study were congruent to those of recent trials using the same materials.[13,21,33] Furthermore, the considerably lower retention rates obtained with resin modified glass ionomer (Group II) compared with resin sealant (Group I) in all follow up periods were in agreement with previous investigations.[15,34] This could be attributed to the deep narrow fissure systems selected for this trial which might result in an entrapment of air voids under relatively thick RMGI, hence reducing the strength of adhesive joints.[35] This, also, confirmed the early assumption that glass ionomer sealant should only be used for fissures more than 100 µm in width.[36] Additionally, glass ionomer sealants are more technique-sensitive than resin-based sealants and a minute saliva exposure would predispose surface degradation and early loss of sealant.[35]

In the present work, the significantly higher retention of flowable composite (Group III) in comparison to resin-modified glass ionomer (Group II) paralleled that of Pardi et al.[21] This could be due to a poorer bond of resin-modified glass ionomer to tooth structure than flowable composite.[37] The better retention of flowable composite than the conventional resin-based fissure sealant observed in this study was in accordance with Corona et al (5) but was not in agreement with Autio-Gold.[23] These discrepancies in results might be attributed to the differences in the follow up periods between these studies. It is to be noted that, while flowable composite sealant material used in this trial had an optimal performance throughout the duration of the study, these results might not be applied for all diverse flowable composite materials with different compositions.

In this study, the rate of sealant loss as a function of time seemed to be different between the tested materials. There was an initial high rate of resin-modified glass ionomer sealant loss; while for resin-based and flowable composite sealants, the rate of loss seemed to be fairly constant. The initial high loss of glass ionomer sealant has been reported by other researchers[37,38] and is thought to depend mainly on unfavorable fissure morphology.

Nevertheless, the question of cariostasis remains the main issue to address. After two years of this clinical trial, 4% and 3.6% of resin-modified glass ionomer (Group II) and flowable composite (Group III) sealed teeth became carious which was in a complete agreement with Pardi et al.[21] Also, the 3.9% carious teeth detected in Group I at the end of this work was in accordance with the 2 year study of Fross et al.[13]

The nearly similar caries increment detected in the present work among the study groups, despite of a clear difference in the retention rate, are analogous to those seen in previous studies employing comparable materials and give credence to the concept of a possible benefit of glass ionomer sealant.[34,39] The fact that the glass ionomer remains in the deeper recesses of the occlusal fissures may explain why no caries was recorded in Group II teeth despite macroscopic sealant loss. Moreover, glass ionomer acts as a mean of sustained fluoride release to the adjacent tooth structure and to the oral environment.[40] On the other hand, the results of the present study contradicted that of Poulsen et al who reported that the glass ionomer sealant had a less caries-protective effect than resin-based sealant.[10] These differences in results might be due to differences in selection criteria, of the sealed teeth, different caries diagnostic criteria and the use of chemical-cured glass ionomer sealant in the former study instead of light-cured resin-modified glass ionomer tested in this work.

With the recent advances in materials sciences, the mechanical properties of dental materials have been extensively studied. However, little efforts have been made to revise their biological properties. Thus, in this study, examination of the antibacterial properties of the sealants against streptococcus mutans was conducted. In this context, long-term in vivo studies are preferred than in vitro studies as they can predict if the antibacterial activities will last for extended periods of times. Moreover, the in vivo situations allow the tested microorganisms to interact at their full viability with the oral flora.[41,42]

In this study, culturing of Streptococcus mutans was made by use of mitis salivarius bacitracin (M SB) agar medium. Although some investigators have reported that trypticase yeast-extract cystine sucrose bacitracin (TYCSB) medium yields a significantly higher amount of mutans Streptococcus from oral samples than does MSB medium. However, non-mutans Streptococcus are more abundant on TYCSB than on MSB medium and, in some instances, so numerous that they make detection of the mutans streptococci is difficult.[43] Therefore, MSB medium was preferred in the present study.

In this work, the immediate reductions of salivary streptococcus mutans counts noted in all groups after one day of sealant application were in a complete agreement with Going, who reported that, the acid etching procedure itself reduces the number of cultivable microorganism by approximately 95%.[44] Additionally, teeth prophylaxis before sealant application may contribute to the observed diminution of streptococcus mutans count.

The results of the present work clearly demonstrate that Streptococcus mutans counts were significantly lower in Group II compared to Group I or Group III up to 6 months of the study. This reconfirms and extends the knowledge base gathered from previous investigations on the antibacterial property of glass ionomer materials.[25,26] The etiology of the observed reduction of streptococcus mutans counts in group II children is speculative at this time and may be related to fluoride released by ionomeric materials.[42] Additionally, a significant amount of aluminum release from glass ionomer that has been previously reported may play a role in this bacterial inhibition.[45] Aluminum has an inhibitory effect on ATPase enzyme of streptococcus mutans which plays an important role in the maintenance of the bacterial metabolism and intracellular pH.[46,47]

Similarly, the significantly lower counts of streptococcus mutans, observed in this study in Group I compared to Group III for 6 months follow up, emphasizes the antibacterial effects of fluoride-releasing resin sealants observed in previous studies.[7,48] Also, the significantly higher streptococcus mutans counts observed in Group III compared to Group I and Group II until the end of 6 months of this study come in a line with some in vitro studies.[41,49] This could be explained by the fact that resin composite extracts and unpolymerized ethylene glycol monomers, released from composite resins, have growth-promoting effects on cariogenic bacteria.[50,51]

After one year of the clinical trial until the end of this study, there was no statistically significant difference of streptococcus mutans count between all groups which was in accordance with previous studies.[43,52] The recovery of bacteria throughout the study may stem from the potential streptococcus mutans reservoirs on soft tissues that remain unaffected by sealant application.[53] Moreover, the release of fluoride from glass ionomer was found to be the greatest in the first few months, after which it decreases to a constant level over a prolonged period of time.[54] This might explicate the recolonozation of streptococcus mutans in Group II after one year until the end of the study.

Finally, due to the relatively small sample size of this work, this study could be considered a pilot clinical trial which recommends further studies involving larger sample sizes.

Conclusion

The results of this study indicated a lower retention of RMGI compared to flowable composite and resin sealants without significant difference in caries prevention or long term bacterial inhibition.

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Notes

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References

1. Whelton H. Overview of the impact of changing global patterns of dental caries experience on caries clinical trials. J Dent Res. 2004;83(spec iss): 29-34.
2. Mejàre I, Kallestal C, Stenlund H, Johansson H. Caries development from 11 to 22 years of age: a prospective radiographic study. Prevalence and distribution. Caries Res. 1998;32: 10-16.
3. Ripa L, Leske G, Varma G. Longitudinal study of the caries susceptibility of occlusal and proximal surfaces of first permanent molars. J Public Health Dent. 1998;48: 8-13.
4. Whelton H, O'Mullane D, Cronin M. Survey of Oral Health of Children and Adolescents 1997-a report for the Mid-Western Health Board. Limerick (Ireland): Mid Western Health Board; 1998. three-letter_month_if_anyday_if_any.

5. Corona M, Borsatto C, Garcia L. Randomized controlled trial comparing the retention of flowable composite system with a conventional fissure sealant: one year follow up. Int J Ped Dent. 2005;15: 44-50.
6. Takahashi Y, Imazato S, Russel R, Noiri Y, Ebisu S. Influence of resin monomers on growth of oral streptococci. J Dent Res. 2004;83(4): 302-306.
7. Preetha V, Shashikiran N, Reddy V. Comparison of antibacterial properties of two fluoride-releasing and a non-fluoride-releasing pit and fissure sealants. Ind Soc of Ped and Prev Dent. 2007;25: 133-136.
8. Brown J, Barkmeie W. A comparison of six enamel treatment procedures for sealant bonding. Pediatr Dent. 1996;18: 29-31.
9. Kersten S, Lutz F, Schabpach P. Fissure sealing: optimization of sealant penetration and sealing properties. Am J Dent. 2001;14: 127-131.
10. Poulsen S, Beiruti N, Sadat N. A comparison of retention and the effect on caries of fissure sealing with a glass ionomer and resin-based sealant. Community Dent Oral Epidemiol. 2001;29: 289-301.
11. Beiruti N, Frencken J, Helderman W. Caries-preventive effect of a one-time application of a composite resin and glass ionomer sealants after 5 years. Caries Res. 2006;40(1): 52-59.
12. Croll T. Glass ionomer for infants, children and adolescents. JADA. 1990;120(1): 65-68.
13. Fross H, Saarni U, Seppa L. Comparison of glass ionomer and resin-based fissure sealants: a2-year clinical trial. Community Dent Oral Epedemiol. 1994;22(1): 21-34.
14. Songpaisan Y, Bratthal D, Phantumvanit. Effects of glass ionomer cements, resin-based fissure sealants and HF application on occlusal caries in developing country field trial. Community Dent Oral Epidemiol. 1995;23(1): 25-29.
15. Raadal M, Utkilen AB, Nilsen OL. Fissure sealing with a light-cured resin-reinforced glass ionomer cement (Vitrebond) compared with a resin sealant. Int J Pediatr Dent. 1996;6(4): 235-239.
16. Mckenna E, Grundy G. Glass ionomer cement fissure sealants applied to operative dental auxiliaries--retention rate after one year. Aust Dent J. 1987;32(3): 200-203.
17. Ganesh M, Tandon S. Clinical evaluation of FUJI VII sealant material. J Clin Pediatr Dent. 2006;31(1): 52-57.
18. Mallmann A, Ataíde JC, Amoedo R, Rocha PV, Jacques LB. Compressive strength of glass ionomer cements using different specimen dimensions. Braz Oral Res. 2007;21(3): 204-8.
19. Mitra SP. Adhesion to dentine and physical proberties of a light-cured glass ionomer liner\base. J Dent Res. 1991;70: 72-74.
20. Gungor H, Altay N, Alpar R. Clinical evaluation of a poly-modified resin composite- based fissure sealant: two year results. Oper Dent. 2004;29(3): 254-260.
21. Pardi V, Periera AC, Meneghem C. Clinical evaluation of three different materials used as pit and fissure sealants : 24 month results. J Clin Pediatr Dent. 2005;29(2): 133-137.
22. Czemer A, Weiller M, Ebert J. Wear resistance of flowable composites as pit and fissure sealants. J Dent Res. 2000;79: 279-(abstract 1087).
23. Autio-Gold J. Clinical evaluation of medium-filled flowable restorative materials as a pit and fissure sealant. Oper Dent. 2002;27(4): 325-329.
24. Matalon S, Slutzky H, Weiss E. Surface antibacterial properties of packable resin composite: part I. Quintes Int. 2004;35(3): 189-193.
25. Slutsky H, Weiss E, Liwinstein I, Slutzky S, Matalon S. Surface antibacterial properties of resin and resin-modefied dental cements. Quintes Int. 2007;38(1): 55-61.
26. Menon T, Kumar C, Dinesh K. Antibacterial activity of glassionomer restorative cements and polyacid modified composite resin against cariogenic bacteria. Ind J Med Micr. 2006;24(2): 150-151.
27. Word Health Organization. WHO Basic Oral Health Survey, 4th edition. Geneva: WHO; 1997.
28. Boksman L, Carson B. Two-year retention and caries rate of Ultraseal XT and Fluoroshield light-cured pit and fissure sealant. Gen Dent. 1998;46: 184-187.
29. Ogaard B, Arends J, Helseth J. Fluoride level in saliva after bonding orthodontic brackets with a fluoride containing adhesive. Am Orthod Detofac Orthoped. 1997;58: 206-213.
30. Gold O, Jordan H, Van haut J. Selective medium for streptococcus mutans. Arch Oral Biol. 1973;18: 1357-1364.
31. Notle W. Streptococci: in :Oral microbiology with basic microbiology and immunology. (edition4thed). St. Louis (MO): C.V Mobsy Company; 1982. 28- 326.
32. Norusis MJ. SPSS\PC+ statistics 7.5 for the IBM PC\XT\AT and PS\2. Chicago (IL): SPSS Incorporated; 1996.
33. Periera AC, Pardi V, Mialhe FL. A 3-year clinical evaluation of glass ionomer cements used as fissure sealants. Am J Dent. 2003;16(1): 23-27.
34. Smales R, Wrong KC. 2-year clinical performance of a resin-modefied glass ionomer sealant. Am J Dent. 1999;12(2): 59-61.
35. Mejare A, Major I. Glass ionomer and resin-based fissure sealans: a clinical study. Scand J Dent Res. 1990;98: 345-350.
36. Mclean J, Wilson A. Fissure sealing and filling with an adhesive glass ionomer cement. Br Dent J. 1974;136: 269-276.
37. Klipatrick M, Murray J, McCbet F. A clinical comparison of a light cured glass ionomer sealant restorstion with a composite sealant restoration. J Dent. 1996;24: 399-405.

38. Fross H, Halme E. Retention of a glass ionomer cement and resi-based sealant and effect on carious outcome after 7 years. Community Dent Oral Epidemiol. 1998;26: 21-25.
39. Hicks HJ, Flaitz CM. Occlusal caries formation in vitro: Comparison of rein-modefied glass ionomer with fluoride-releasing sealant. J Clin Pediatr Dent. 2000;24(4): 309-314.
40. Mark M, Edward J, Jeffery A. Using rein-modefied glass ionomer as an occlusal sealant:A on-year clinical study. JADA. 1996;127: 1508-1514.
41. Hansel C, Leyhausen C, Mai U, Geurtsen W. Effects of various resin composite (co) monomers and extracts on two caries-associated microorganisms in vitro. J Dent Res. 1998;77(1): 60-67.
42. Karanika-Kouma A, Dionysopoulos P, Koliniotou-Koubia E. Antibacterial p[roperties of dentin bonding systems, polyacid-modefied composite resins and composite resins. J Rehab. 2001;28: 157-160.
43. Svanberg M, Mjor I, Qrstavik D. Mutans streptococci in plaque from margins of amalgam, composite and glass ionomer restorations. J Dent Res. 1990;69(3): 861-864.
44. Going R. Sealant effect on incipient caries enamel maturation and future caries susceptibility. J Dent Educ. 1984;48: 35-41.
45. Savarino L, Cervellati M, Stea S, Cavedagna D, Donati M. In vitro investigation of aluminum and fluoride release from compomers, conventional and resin modified glassionomer cements: a standardized approach. J Biomater Sci Polym. 2000;11: 289-300.
46. Sturr M, Marquis R. Inhibition of proton-trans and aluminum locating ATPases of Streptococcus mutans and Lactobacillus casei by fluoride. Arch Microbiol. 1990;155: 22-27.
47. Hayacibara M, Rosa O, Koo H. Effects of fluoride and aluminum from ionomeric materials on S. mutans Biofilm. J Dent Res. 2003;82(4): 267-271.
48. Loyola-Rodrigues J, Garcia-Godoy F. Antibacterial activity of fluoride releasing sealant on mutans streptococci. J Clin Pediatr Dent. 1996;20: 109-111.
49. Friedle K, Schmalz G, Hiller K. Liquid culture tests of the effect of dental materials on bacterial growth. Dtsch Zahnartztl. 1992;47: 826-831.
50. Imazato S, McCabe J, Tarumi H, Ehara A, Ebisu S. Degree of conversion of composites measured by DTA and FTIR.. Dent Mater. 2001;17: 178-183.
51. Takahashi Y, Imazato S, Russel R, Noiri Y, Ebisu S. Influence of resin monomers on growth of oral streptococci.. J Dent Res. 2004;83(4): 302-306.
52. Van Dijkin J, Persson S, Sjostrom S. Presence of streptococcus mutans and lactobacilli in saliva and on enamel, glass ionomer cements, and composite resin surfaces. Scand J Dent Res. 1991;99: 13-19.
53. Van Haute J. Mechanisms and implications. Microbiol Abstr. 1976;1: 2-32.
54. Thornton J, Retief D, Bradley E. Fluoride release from and tensile bond strength of Ketac-Fil and Ketak-Silver to enamel and dentine. Dent Mater. 1986;2: 241-245.