E. Haznedaroglu, S. Sozkes*, A.R. Mentes

Department of Pediatric Dentistry, Dentistry Faculty,
Marmara University, Turkey
**Department of Biomaterials, Biomedical Engineering,*
Namik Kemal University, Turkey

e-mail: ehaznedaroglu@marmara.edu.tr

Microhardness evaluation of enamel adjacent to an improved GIC sealant after different enamel pre-treatment procedures

ABSTRACT

Aim *This in vitro study was carried out to evaluate the microhardness of enamel adjacent to a glass ionomer cement (GIC) with high fluoride content used as a sealant (Fuji Triage, GC Corp., Japan) after laser, bur or air abrasion treatment procedures.*
Materials and methods *Study design**:** 200 freshly extracted non-carious human molars were divided into 10 experimental groups according to the enamel pre-treatment method: A air abrasion (Mach 4.1 Kreativ Inc., USA); AP, Air abrasion + conditioning with 20% polyacrylic acid (GC cavity conditioner); L, Er,Cr:YSGG laser application (Waterlase, Biolase Technology, Inc., San Clemente, USA); LP, Er,Cr:YSGG laser application and fissure conditioning; B, ameloplasty carried out with a diamond bur especially designed for preparing fissures (Komet #8833); BP, ameloplasty + fissure conditioning; P, application of 20% polyacrylic acid and all fissures sealed with GIC; C, no fissure treatment, the material was applied directly to the fissures (control); R, application of 37% orthophosphoric acid and fissures sealed with a resin-based sealant (Fissurit; Voco, Germany) (control); N, no treatment (control). Half of each group of teeth were left in artificial saliva for one month and the rest for three months. The teeth were then sectioned and microhardness was measured using a Vickers test apparatus. Kruskal-Wallis, Mann-Whitney U and Dunn's multiple comparison tests were carried out (5% significance).*
Results *After one month results regarding hardness at the base and lateral walls of fissures were significantly higher in groups A, AP, L, LP, B, BP, P and C than in groups R and N (p<0.01), but no difference was seen between the treatment procedures. The results after three months produced similar findings with evenly increased values for all groups.*
Conclusion *The results of this study showed that the tested GIC with a higher fluoride content seemed to improve the enamel hardness of the fissure enamel and could be regarded as an alternative material in cases where resin sealant applications are questionable.*

Keywords *Air abrasion; Fissure sealant; Glass ionomer cement (GIC); Laser; Microhardness.*

Introduction

Caries in children is seen predominantly in occlusal pits and fissures, and these surfaces on permanent teeth are particularly at risk [Oba, et al., 2009]. Sealing occlusal pits and fissures in teeth is a highly effective caries-preventive method [Weintraub, 1989]. Generally, it is accepted that resin-based sealant materials are retained for longer periods than glass ionomer cements [GICs]. However, they are not as sensitive to moisture as resin-based sealants [Oba, et al., 2009; Pereira, et al., 2003]. As a sealant agent, the use of GICs has resulted in a significant reduction in the incidence of caries [Pereira, et al., 2003]. GICs have an anticariogenic potential, which has been related to their fluoride (F) release [Forsten, 1998]. The effect of the released F has been strongly associated with the physical and chemical processess involved in the inhibition of enamel demineralisation and the enhancement of remineralisation [Serra and Cury, 1992].

Studies have shown that retention and microleakage under resin-based materials could be improved after treatment procedures such as conditioning, and/or air abrasion, and/or laser and/or ameloplasty [Hatibovic-Kofman et al., 2001; Hatibovic-Kofman, Wright and Braverman, 1998; Xalabarde et al., 1998]. Similar procedures may apply to GICs but, to the authors' knowledge, there have been no studies directly comparing the retention of GIC sealants with different preparations of pits and fissures. Previous studies have demonstrated that fluoride released by GIC restorations ensures an anticariogenic effect around the enamel and on the adjacent tooth and recently, a high fluoride content GIC has been produced especially for fissure protection [Lobo, et al., 2005]. It has been reported that the major advantage of using this product over other conventional GICs is the level of fluoride released by the sealant, which is considered to be the highest of all GICs [Herle, et al., 2004; Markovic, Petrovic and Peric, 2008].

The aim of this in vitro study was to investigate the effect of a glass ionomer sealant material with a high content of fluoride on the hardness of sound enamel fissures after different enamel pre-treatment procedures and to compare the microhardness of sound enamel after different enamel fissure pre-treatments.

Materials and methods

In this study, 200 extracted, sound third molars were used. The teeth were selected on the basis of laser fluorescence readings lower than 5, as measured on the occlusal surface by a laser diode apparatus (Diagnodent™, Kavo, Biberach, Germany). Subsequently, they were randomly divided into 10 groups of 20 teeth each. The teeth in the 10 groups were prepared as described below (Fig. 1).

- Group (A): the teeth were air abraded using 25-µm α-aluminium oxide particles and a Mach 4.1 machine (Mach 4.1 Kreativ Inc., Albany, USA). The pressure was set at 110 psi and the tip was held at least 10 mm from the tooth and angled toward the lingual side. The surface was examined and the procedure was repeated when necessary. After that the fissure was restored with the GIC studied.
- Group (AP): the teeth were air abraded in the same manner described above and after that, 20% polyacrylic acid (cavity conditioner, GC Corporation, Osaka, Japan) was applied for 10 seconds. The conditioner was removed using an air-water spray and the tooth was gently air dried for 10 sec. GIC sealant application followed.
- Group (L): an Er,Cr:YSGG hydrokinetic dental laser (Waterlase, Biolase Technology, Inc., San Clemente, USA) was used for laser etching. This hard- and soft-tissue laser creates laser-energized, atomised water droplets that act as cutting particles. Laser energy is delivered through a fiberoptic system to a sapphire tip terminal 6 mm long and 600 µm in diameter, bathed in an adjustable air and water vapour. It operates at a wavelength of 2.78 µm and pulse duration of 140 microsec with a repetition rate of 20 Hz. The energy and power densities (5.6 J/cm^2 and 111 W/cm^2 at 2W) were calculated by the manufacturer of the laser unit for the used power adjustment. After laser application, the fissure was sealed with the GIC cement.
- Group (LP): an Er,Cr:YSGG laser was applied and then the fissures were conditioned as described above.
- Group (B): enamel ameloplasty was carried out with a diamond bur especially designed for preparing fissures (Komet No. 8833, Brasseler, Lemgo, Germany).
- Group (BP): after enamel ameloplasty, the fissures were conditioned as described above.
- Group (P): enamel was conditioned as described above.

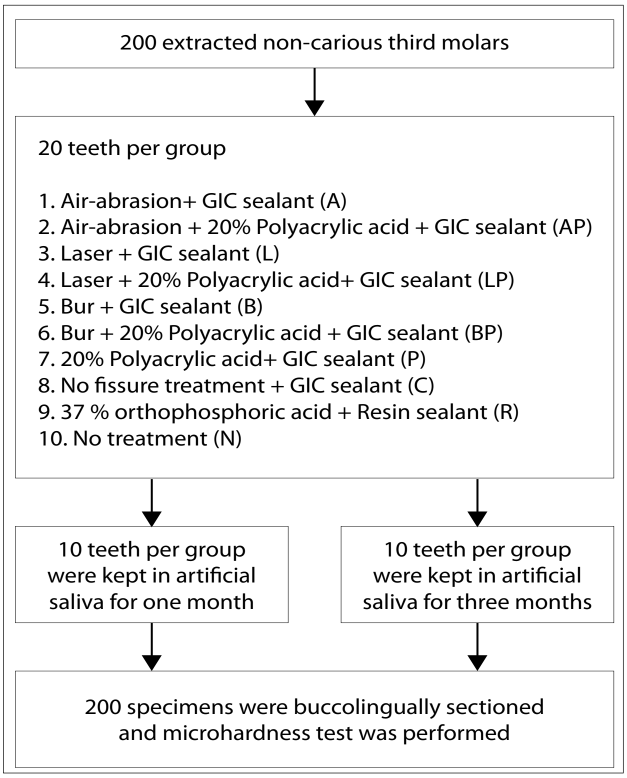

FIG. 1 Illustration of the study design.

- Group (C): no fissure treatment was applied. All the fissures in groups 1 through to 8 were sealed with GIC. Groups 9 and 10 were the control groups.
- Group (R): enamel conditioning was performed with 37% orthophosphoric acid for 15 seconds. After that the tooth surface was rinsed thoroughly with an air-water spray for 15 seconds and then air-dried for approximately 5 sec. The etched enamel was taken a chalky appearance. Then a resin-based sealant (Fissurit; Voco, Cuxhaven, Germany) was used.
- Group (N): no treatment was carried out. Five teeth were kept in artificial saliva for one month while the other five teeth were kept for three months. Fresh solutions of artificial saliva were used each week.

After the experimental period, the samples were sectioned buccolingually by means of diamond cut-off disk mounted on a cutting machine (ISOMET 1000 Precision Saw, Buehler, Lake Bluff, USA). The tooth halves were embedded in epoxy resin with the outer enamel surface perpendicular to the resin surface. The specimens were grinded with Al_2O_3 papers of 400, 600 and 1200 grit (Carborundum, São Paulo, Brazil) and then polished with 1.0 µm of diamond paste (Buehler Metadi, Lake Bluff, USA) using a polishing cloth.

Microhardness measurements were carried out using a Vickers test apparatus under a 200-g load for 10 seconds (Leitz Miniload Model LL, Wilson Mechanical Instrument Hardness Tester, Connecticut, USA). For each tooth, 12 measurements were performed on four different regions of the fissure enamel: closer to

FIG. 1 Scheme of location of microhardness measurements.

the sealant or far from the sealant on the fissure side wall; closer to the sealant or far from the sealant on the fissure base, as seen on Figure 2. Statistical analysis was performed using Kruskal-Wallis, Mann-Whitney U and Dunn's multiple comparison tests (5% significance).

Results

The results showed that the microhardness of all the enamel samples closer to the GIC sealant were higher than that located far from the sealant. Similarly, microhardness at the side walls was higher than the values obtained at the base of the fissure enamel. There were statistically significant differences between the microhardness of the fissure enamel of groups C, L, A, B, P, LP, AP and BP and the control groups (R and N) on both sides closer to the sealant ($p<0.05$) (Tables 1, 2).

Conditioning the enamel with polyacrylic acid also led to significantly increased enamel hardness for all the treatment groups (P>K, AP>A, BP>B) ($p<0.05$) except the LP group, which was not statistically different from the L group. However, the L and LP groups had the hardest values out of all the groups. The results after three months were significantly different in the BP, L, R and N groups.

Groups	Far from the sealant	Close to the sealant	MW	p
AP	339.73±29.94	376.82±38.04	22	0.0034
LP	327.66±9.33	400.85±12.55	0	0.0001
BP	335.63±17.67	412.41±18.22	0	0.0001
P	316.32±17.5	389.24±21.36	0	0.0001
A	295.92±22.62	355.69±26.83	4	0.001
L	317.35±18.95	391.09±21.51	0	0.0001
B	326.41±22.47	386.29±28.48	4	0.001
N	310.65±15.46	318.41±17.46	31	0.151
R	298.39±27.31	311.32±18.44	32	0.174
C	329.29±20.38	374.49±24.95	8	0.001

TABLE 1 Distribution of microhardness scores, mean and standard deviation values for each group at the base and side walls of the fissure (first month) ($p < 0.05$).

Discussion

It is known that the benefits of fluoride-releasing GICs can positively affect the enamel resistance to the carious attack. This was actually shown in 1990 when GICs were tested as liners for amalgam restorations [García-Godoy and Jensen, 1990; Jensen et al, 1990] and on dental structures [Jang et al, 2001; Salar et al, 2007]. Lobo et al. [2005] reported that the fluoride releasing capacity of a resin-modified glass ionomer cement provides cariostatic benefits both in areas adjacent to the sealant or even at a certain distance from it. In our study, the results showed that the hardness of the enamel closer to the sealant was indeed higher than that of enamel far from the sealant. Similarly, the side walls were harder than the base of the fissure enamel. In this study, there were statistically significant differences between the microhardness of the fissure enamel in the GIC groups (C, L, A, B, P, LP, AP and BP) and the control groups (R, N) on both sides closer to the sealant ($p<0.05$). *In vitro* results have shown that fluoride release from conventional GICs are taken up into adjacent enamel resulting in reduced enamel solubility. Also, for these GICs, a large amount of fluoride is released initially, followed by a relatively constant smaller amount over an extended period of time [Forss and Seppa, 1999; Millar, Abiden and Nicholson, 1998]. The results after one month shown in the present study showed that the hardness of the base and lateral walls of the fissures were significantly higher in groups A, AP, L, LP, B, BP, P and C than in groups R and N ($p<0.01$). The results after three months showed similar findings with evenly increased values for all groups. A synergistic cariostatic effect is expected to occur as a function of the integrated retention and fluoride-releasing properties in sealant materials [Lobo, et al.,2005]. Many studies have reported a better efficiency for the resin sealant when the invasive technique is used [Herle, et al., 2004]. According to Herle et al. [2004], with the invasive technique both

Groups	Far from the sealant	Close to the sealant	MW	p
AP	302.95±14.7	381.67±11.54	0	0.0001
LP	327.36±18.7	393.8±22.41	0	0.0001
BP	311.48±32.35	379.73±24.76	6	0.001
P	332.52±14.68	399.52±21.98	1	0.0001
A	311.2±23.68	365.53±24.1	5	0.001
L	309.33±29.71	370.2±19.18	8	0.001
B	312.07±13.66	375.99±26.18	1	0.0001
N	294±32.9	341.23±30.26	16	0.01
R	296.28±13.17	341.06±12.16	0	0.0001
C	284.16±22.27	314.8±25.78	17	0.013

TABLE 2 Distribution of microhardness scores, mean and standard deviation values for each group at the base and side walls of the fissure (first month) ($p < 0.05$).

the GIC and resin-based sealant showed better flow and adaptation to the fissures when compared with the non-invasive technique.

Prior to acid etching and sealant application, it is important to make sure that the occlusal tooth surface and the fissure areas are free from plaque and any debris that might interfere with the etching and sealing procedure. A mechanical preparation consisting of widening the fissures with rotary instrumentation is then suggested to allow a better diagnosis of underlying decalcifications and to improve sealant retention by permitting a deeper sealant penetration and an increase the surface area [De Craene, Martens and Dermaut, 1988; Garcia-Godoy and Araujo, 1994].

The use of lasers as a tool for pretreatment and surface conditioning in pit and fissure sealing has been discussed in a number of recently published papers [Moshonov, et al., 2005; Borsatto et al., 2001]. According to Hossain et al. [2002] Er, Cr:YSGG laser cavity surfaces facilitate good adhesion to the restorative materials. In this study the L and LP groups had the hardest values out of all the groups but no significant differences between the laser and etched bur cavities were found. Regarding air polishing, it has been suggested that it may improve the removal of debris, increase sealant penetration and micromechanical retention of the sealant [Hannig, et al., 2004].

In this study, to increase the retention of the glass ionomer we used different preparation techniques. However, it should be pointed out that conditioning the dental substrate with an acid, even though considered unnecessary with glass ionomers, in fact significantly increases the shear strength of the bond with enamel. In particular, a 20% polyacrylic acid solution has been reported to produce a favourable combination of good shear bond strength and relatively limited enamel erosion surface prior to the placement of GIC [Papacchini, et al., 2005]. In this study conditioning the enamel with polyacrylic acid also significantly increased enamel hardness for all treatment groups (P>K, AP>A, BP>B) ($p<0.05$), except for the LP group, which was not statistically different from the L group. However, the L and LP groups had the hardest values out of all the groups.

In recent years there has been apprehension regarding the leakage of the estrogenic compound BPA from the resin-based sealants [Birkenfeld and Schulman, 1999]. Therefore, the quest to find an alternative to these resin-based sealants is an ongoing challenge. Although GICs have been considered as an alternative, they have always been looked down due to their poor physical properties. Considering the fact that the amount of fluoride released by this high fluoride content product is supposed to be greater than with any other glass ionomers, we may say that it may be promising in terms of its usage as a pit and fissure sealant. Since this was an *in vitro* study, an *in vivo* study should be carried out to ascertain the longevity and cariostatic effect of this new GIC. This is now in progress in our paediatric dentistry department.

Conclusion

The tested GIC with higher fluoride content seemed to improve the enamel hardness of the fissure enamel and could be regarded as an alternative material in cases where resin sealant applications are questionable.

Acknowledgements

This study was supported by GC Corporation and Marmara University Scientific Researches Committee (Project No. SAG-d-300609-0269).

References

- Birkenfeld LH. and Schulman A. , 1999. Enhanced retention of glass ionomer sealant by enamel etching: A microleakage and Scanning electron microscopic study. Quint Int, 30: 712-719.
- Borsatto MC, Corona SA, Dibb RG, Ramos RP, Pecora JD., 2001. Microleakage of a resin sealant after acid etching, Er:YAG laser irradiation and air-abrasion of pits and fissures. J Clin Laser Med Surg, 19: 83–87.
- De Craene GP, Martens C, Dermaut R, 1988. The invasive pit and fissure sealing technique in pediatric dentistry: an SEM study of preventive restoration. J Dent Child, 55: 34–42.
- Forss H, Seppa L., 1999. Prevention of enamel demineralization adjacent to glass-ionomer filling materials. Scand J Dent Res, 98: 173-178.
- Forsten L., 1998. Fluoride release and uptake by glass-ionomers and related materials and its clinical effect. Biomaterials, 19: 503–8.
- García-Godoy F, Jensen ME. Artificial recurrent caries in glass ionomer-lined amalgam restorations. Am J Dent. 1990; 3: 89-93.
- Garcia-Godoy F, Araujo FB., 1994. Enhancement of fissure sealant penetration and adaptation. The enameloplasty technique. J Clin Pediatr Dent, 19: 13–18.
- Hannig M, Gräfe A, Atalay S, Bott B, 2004. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. Journal of Dentistry, 32: 75-81.
- Hatibovic-Kofman S, Butler S. A., Sadek H., 2001. Microleakage of three sealants following conventional, bur, and air-abrasion preparation of pits and fissures. Int J Paed Dent 11: 409–416.
- Hatibovic-Kofman S, Wright GZ, Braverman I., 1998. Microleakage of sealants after conventional, bur, and air abrasion preparation of pits and fissures. Ped Dent, 20: 173–176.
- Herle GP, Joseph T, Varma B, Jayanthi M., 2004. Comparative evaluation of glass ionomer and resin based fissure sealant using noninvasive and invasive techniques-A SEM and microleakage study. J Indian Soc Pedo Prey Dent, 22: 56-62.
- Hossain M, Nakamura Y, Yamada Y, Murakami Y, Matsumoto K., 2002. Microleakage of composite resin restoration in cavities preparerd by Er, Cr: YSGG lazer irradiation and etched bur cavities in primary teeth. J Clin Pediat Dent, 26 (3): 263-268.
- Jang KT, Garcia-Godoy F, Donly KJ, Segura A. Remineralizing effects of glass ionomer restorations on adjacent interproximal caries. ASDC J Dent Child 2001; 68:125–128.
- Jensen M.E., Garcia-Godoy F., Wefel J.S.. Artificial root caries in amalgam restorations: effect of light cured fluoride releasing liners. Am J of Dent 1990; 3: 295–298.
- Lobo MM, Pecharki GD, Tengan C, Silva DD, Tagliaferro PS., 2005. Fluoride-releasing capacity and cariostatic effect provided by sealants. J Oral Sci, 47: 35-41.
- Markovic DL, Petrovic BB, Peric TO., 2008. Fluoride content and recharge ability of five glassionomer dental materials. BMC Oral Health, 8:21.
- Millar BJ, Abiden F, Nicholson JW., 1998. In vitro caries inhibition by polyacid-modified composite resins (‘Compomers’). J Dent, 26, 133-136.
- Moshonov J, Stabholz A, Zyskind D, Sharlin E, Peretz B., 2005. Acid-etched and erbium: yttrium aluminium garnet laser-treated enamel for fissure sealants: a comparison of microleakage. Int J Pediatr Dent, 15: 205–209.
- Oba AA, Dülgergil T, Sönmez IS, Dogan S., 2009. Comparison of caries prevention with glass ionomer and composite resin fissure sealants. J Formos Med Assoc 108: 844–848.
- Papacchini F, Goracci C, Sadek FT, Monticelli F, Garcia-Godoy F, Ferrari M., 2005. Microtensile bond strength to ground enamel by glass-ionomers, resin-modified glass-ionomers, and resin composites used as pit and fissure sealants. Journal of Dentistry, 33: 459-467.
- Pereira AC, Pardi V, Mialhe FL, Meneghim MC, Ambrosano GM., 2003. A 3-year clinical evaluation of glass-ionomer cements used as fissure sealants. Am J Dent, 16: 23–27.
- Salar DV, Garcia-Godoy F, Flaitz CM, Hicks MJ: Potential inhibition of demineralization in vitro by fluoride-releasing sealants. J Am Dent Assoc 2007; 138: 502–506.
- Serra MC, Cury JA., 1992. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quint Int, 23: 143–7.
- Weintraub JA. 1989.The effectiveness of pit and fissure sealants. J Publ Health Dent 49: 317–330.
- Xalabarde A, Garcia-Godoy F, Boj JR, Canalda C., 1998. Microleakage of fissure sealants after occlusal enameloplasty and thermocycling. J Clin Ped Dent, 22: 231–235.