Enameloplasty effects on microleakage of pit and fissure sealants under load: an in vitro study

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Enameloplasty has been implicated in the successful application of pit and fissure sealants. The present study aimed to evaluate the effect of enameloplasty technique on microleakage of sealants when occlusal force was applied on the teeth. The study also allowed a direct comparison of the effectiveness of enameloplasty, when two different burs were used. Six groups of ten human extracted wisdom teeth were tested. Group A: no load, conventional (no enameloplasty - control); Group B: no load, enameloplasty 1/2 round bur (Brasseler USA); Group C: no load, enameloplasty diamond fissure bur REF/UP 791(Ultradent); Group D: load 500 N, conventional , Group E: load 500 N, 1/2 round bur; Group F: load 500 N, diamond fissure bur. Specimens were thermocycled for 500 cycles at 5˚C and 55˚C with a dwell time of 30 seconds after load application. Microleakage was scored as distance of dye penetration with 0 = no microleakage and 3 = microleakage to underling fissure. The Kruskal-Wallis One Way Anova and Mann -Whitney U test showed group D having the greatest degree of microleakage statistically significant ($p<0.05$) comparing with other groups. Sealants prepared with the conventional technique (A, D) had statistically significant differences in microleakage with those prepared with enameloplasty (B, C, E, F) ($p = 0.01$). Enameloplasty in groups with no load (B, C) did reduce microleakage, but not significantly ($p = 0.3$) However, loaded teeth with enameloplasty appeared to perform superior, as compared to those without ($p=0.005$) Regarding the effect of load, teeth without load (Groups A, B, C) were found to have significantly less microleakage than teeth where mechanical force had been applied (Groups D, E, F), ($p = 0.01$). Microleakage in Group A (conventional technique without load) was significantly less than microleakage in Group D (conventional technique with load), ($p = 0.04$). However, load did not seem to influence microleakage when enameloplasty had been performed. This finding was true for both round bur enameloplasty ($p = 0.29$), and fissure bur enameloplasty ($p = 0.26$). There was no statistically significant difference between Groups B, E (round bur) and C, F (fissure bur) ($p > 0.05$), or between Groups B and C ($p > 0.05$) and between Groups E and F ($p > 0.05$). The results of the study indicated that enameloplasty reduced microleakage of pit and fissure sealants, especially when load was applied to teeth, irrespective of what bur was used to enlarge the fissure, as there was no statistical significant differences between the round and fissured diamond burs. The application of occlusal force to the tooth produces significantly more microleakage, unless enameloplasty is performed.

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INTRODUCTION

Pits and fissures of human teeth are predilection sites for the development of dental caries. Soon after tooth eruption, caries-associated oral bacteria, such as *Steptococcus mutans* and *Lactobacilli*, readily colonize the occlusal fissures due to favorable retention. Therefore, preventive regimens with fissure sealants have been established during the last 30 years.[1]

Although sealants are effective in preventing occlusal caries, their success depends on a meticulous operator technique. Early loss of pit and fissure sealants is considered to be primarily dependent on inadequate isolation of the tooth from salivary contamination during application.[2-5] Gradual additional loss of sealants can be caused by occlusal wear, shearing forces, and marginal failure.[4-7]

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For the adequate retention of the sealant, it is necessary that the tooth has a maximum surface area, deep irregular pits and fissures and that it is clean at the time of sealant placement.[8-13] Enlargment of the occlusal fissures with a bur, in order to increase the surface area of enamel and improve sealant retention, has been recommended.[14-19] Recent studies[18,19] demonstrated that sealants placed over teeth that had no enameloplasty failed to penetrate and adapt into the depth of the fissure, resulting in a thin layer of sealant over the fissures. When enameloplasty was performed, the sealant always penetrated to the depth of the preparation, resulting in excellent adaptation between the sealant and the enamel.[14,19] Adequate resin penetration into the enamel is considered the key to successful bonding of the sealant.[20]

Sealants placed after enameloplasty have demonstrated superior retention as compared to the conventional ones.[21,22] Shapira *et al.* after 6 years of clinical evaluation of pit and fissure sealants, concluded that the retention rate for the mechanically prepared teeth was significantly higher (88%) than for the control group (65%).[23]

Shiota *et al.* and Ripa *et al.* in two separated studies reported that the bases of occlusal grooves or fissures in molars usually have a prismless enamel layer that should be removed to improve retention of sealants.[24,25] In contrast to these findings, Horsted stated that the thin layer has a minimal effect on the retention of the sealant.[26]

Hansen reported that the marginal gap dimension at the restoration/tooth interface is not dependent on the cavity depth, but mostly on the cavity diameter at the occlusal surface.[27]

Various studies on the stress / strain characteristics of human teeth have concentrated mainly to the mechanical properties of the enamel and dentin. Peripheral enamel demonstrates low compressive and high tensile stress, but enamel at the dentin-enamel junction (DEJ) demonstrates a reverse trend.[28] Little is known about the effect of these properties on the dimensional stability of the "cavities" prepared in the teeth and any potential effects on the material – tooth structure interface.

Jorgensen *et al*. studied the deformation of selected types of cavities under load on human teeth and the possible effect of this deformation on the restoration quality of fillings in the cavities.[29] They concluded, that although the magnitude of the biting and chewing forces on teeth under functional conditions are almost unknown, the dimensional instability of empty cavities demonstrated in this study, even at small and moderate loads, indicates a severe risk of percolation by several restoratives and of marginal fracture of occlusal brittle restorations.

As described above, enameloplasty has been implicated in the success or failures of sealants. The present study evaluated the hypothesis that enameloplasty leads to significantly less microleakage, as compared with a conventional technique of sealant application. The experiment allowed for studying enameloplasty when occlusal forces were applied to the teeth, or with no load. It also allowed for a direct comparison of the effectiveness of enameloplasty, when two different burs were used.

MATERIAL AND METHODS

The sealant that was employed in the study was the Ultraseal XT Plus, which is a 60% filled resin, light-cured, radiopaque and fluoride releasing. The material was manipulated in accordance with the directions of the manufacturer.

Sixty human extracted wisdom teeth were collected and stored in saline. The teeth were refrigerated until needed for the study. The samples had also to be free of caries, as detected by a caries detector dye.

The teeth were randomly assigned to one of six groups. Group A: no load (F = 0 N), conventional (no enameloplasty / control); Group B: no load (F = 0 N), enameloplasty with 1/2 round bur Brasseler USA), ((Fig. 1); Group C: no load (F = 0 N), enameloplasty with fissure bur REF/UP 791 (diamond bur by Ultradent), (Fig. 2). Group D: load (F = 500 N), conventional (no enameloplasty); Group E: load (F = 500 N), enameloplasty with 1/2 round bur (Brasseler USA); Group F: (n = 10): load, (F = 500 N) enameloplasty with diamond fissure bur REF/UP 791 (diamond bur by Ultradent).

For those teeth that were randomized to receive enameloplasty, Groups B and E were prepared using 1/2 round bur by Brasseler, in a high speed handpiece (under water jet). Groups C and F were prepared similarly using a diamond fissure bur REF/UP 791 by Ultradent.

The teeth were cleaned by means of a rotary handpiece with a rubber cup using slurry of pumice. The teeth were air-dried and one drop of caries detector dye was applied on the occlusal surface and all pit and fissures. After ten seconds, all teeth were rinsed thoroughly and dried with oil-free air. Teeth that had a blue stain on the surfaces indicating dental caries or demineralization were excluded from the study. The remaining teeth were then brushed and cleaned with pumice, mounted in an acrylic jig and stored in saline solution.

The occlusal surfaces were air-dried and etched with Ultra-etch 35% using blue micro-tip for 15 seconds. Then the teeth were rinsed with air / water spray. Application of Prima Dry, a hydrophilic drying and priming agent, with white mini-brush tip was done for 5 seconds. Teeth were then dried by gently blowing area with moisture-free and oil-free air.

An inspiral brush tip was used to apply Ultra–Seal XT plus to the teeth. Light curing was performed using the 3M Curing Light XT – 1500 / XL 3000 for 20 seconds.

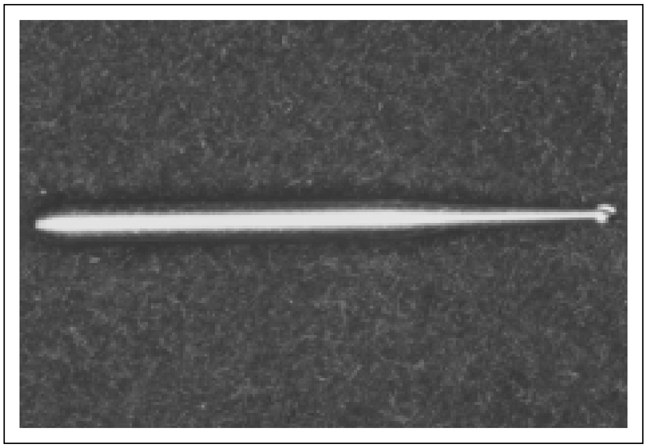

Figure 1. The bur is a 1/2 round bur (Brasseler USA).

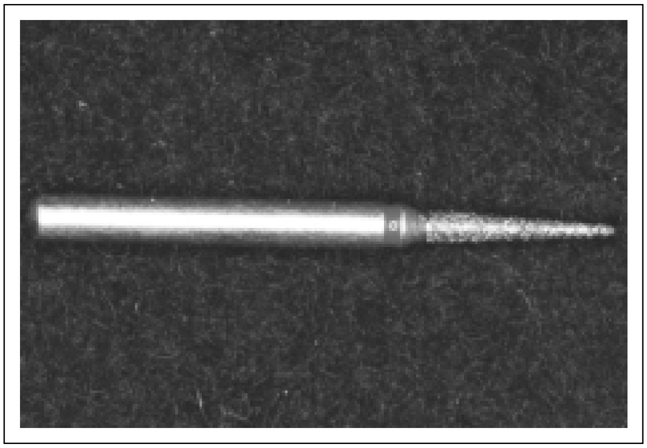

Figure 2. Diamond fissure bur (REF/UP 791 Ultradent).

Samples were stored in saline solution in room temperature prior to application of thermocycling and load. The specimens were thermocycled for 500 cycles at 5°C and 55°C with a dwell of 30 seconds at each temperature prior to load application.

Loading was accomplished using a mechanical testing machine (Instron). In those groups receiving the occlusal load, the force was applied at the peripheral enamel, 2.5mm away from the margin of the tooth, at the level of dentin-enamel junction in cross-section view (Fig. 3). The resin block supporting the tooth was rested on the horizontal cross-head of the testing machine so that the long axis of the tooth was vertical. A metal cylinder with a diameter of 0.9mm was loaded in the longitudinal direction of the tooth to stimulate masticatory forces. Each tooth was subjected to a force of 500 N, at a speed of 1mm/sec. The loading procedure was done at room temperature.

Each specimen was coated with two applications of clear nail polish, except for an area 2.0 mm from sealed occlusal surface. Each apex additionally was sealed with wax. Samples then were immersed in methylene blue dye (1%) for 24hours. After immersion specimens were sectioned with a hard tissue microtome, (Isomet®), in buccolingual direction, along the fissure lines at the point where the load was applied. This was done using a low speed, water-cooled diamond. All specimens were inspected for microleakage with a dissecting optic microscope (Olympus®).

Microleakage, of the methylene blue dye was scored according to the following scale; Score 0: No dye penetration; Score 1: Dye penetration restricted to the outer half of the sealant; Score 2: Dye penetration extending to the inner half of the sealant; Score 3: Dye penetration extending to the underlying fissure.

The non-parametric stastistical techniques Kruskal-Wallis One Way Anova and Mann-Whitney U test were used to test for statistically significant differences between the six groups.

RESULTS

Sixty specimens were originally prepared for this experiment. Three of those had to be excluded from the study, because sealant application was excessive and over the cuspal inclines. Group D (conventional technique under load) showed the greatest degree of microleakage and the difference was statistically significant. Results are shown in Figures 5 to 10.

Regarding the effect of load, teeth without load (Groups A, B, C) were found to have significantly less microleakage than teeth where mechanical force had been applied (Groups D, E, F), ($p = 0.01$). Then the groups were stratified according to their enameloplasty status, and load was evaluated again. Microleakage in Group A (conventional technique without load) was significantly less than microleakage in Group D (conventional technique with load), ($p = 0.04$). However, load did not seem to influence microleakage when enameloplasty had been performed. This finding was true for both round bur enameloplasty ($p = 0.29$), and fissure bur enameloplasty ($p = 0.26$).

Overall, statistically significant differences in microleakage were observed between sealants prepared with the conventional technique (A, D) and those prepared having enameloplasty (B, C, E, F) ($p = 0.01$). Further stratified analyses by load demonstrated that in teeth with no load enameloplasty did reduce microleakage, but not significantly ($p = 0.3$) However, when forces were applied, teeth that had enameloplasty appeared to perform superiorly, as compared to those without ($p = 0.005$)

A statistical analysis was also performed to evaluate if there were any differences in microleakage between the two types of enameloplasty (two different types of burs). There was no statistically significant difference between groups B and E (where round bur was used) and Groups C and F (where fissure diamond bur was used), ($p = 0.26$).

Figure 3. Application of load on the peripheral enamel (black mark).

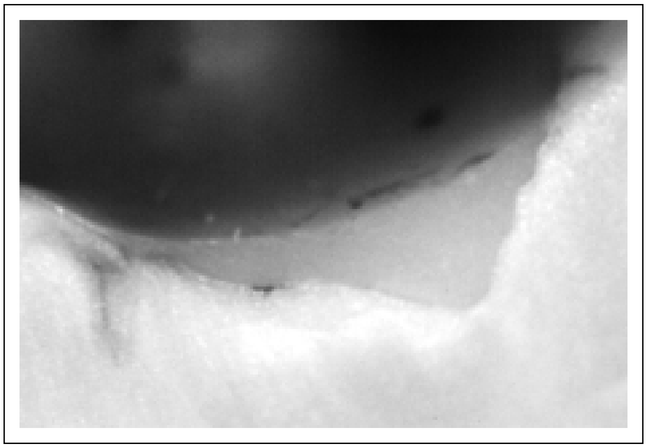

Figure 4. Microleakage of pit and fissure sealant under microscope (conventional technique with load).

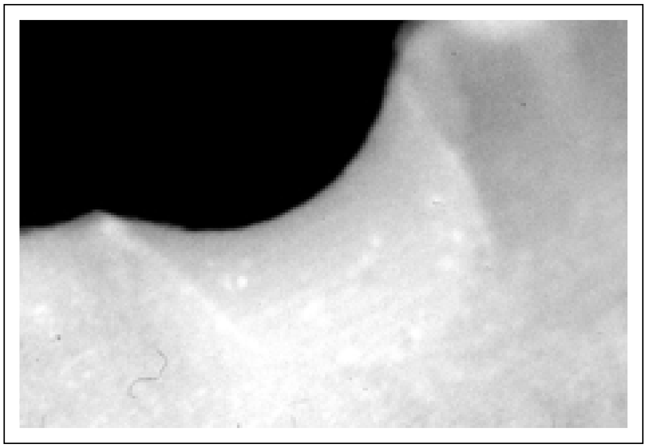

Figure 5. Pit and fissure sealants under microscope (enameloplasty 1/2 round bur).

DISCUSSION

Sealants placed on teeth without enameloplasty mainly bond only to the cuspal incline planes.[20,30] Enameloplasty has been proposed to improve the bond strength, as well as the material penetration, and its adaptation to etched enamel.[11,31]

The present study was able to demonstrate the benefits of enameloplasty against microleakage. Clinical evidence[32,33] tends to support our findings. In a controlled 6-year clinical study, bur-prepared sealants had 88% retention rates, as compared to a 66% retention rate for conventionally sealants.[32] It has been hypothesized that the greater retention can be attributed to the enlargement of the pits and fissures, which produces a greater surface area for bonding, and the use of a thicker layer of sealant which would be more resistant to wear. Also, enameloplasty leads to increased penetration of the material, due to widening and deepening of the pits and fissures and the elimination of organic materials and plaque.[14,17,22,33]

In the present study, the burs that were used for the enameloplasty technique, were 0.5 round carbide and REF / UP 791 fissure diamond. The type of bur did not appear to affect microleakage significantly, although the round bur was slightly better than fissure bur, as evidenced by lowest degree of microleakage.

In this study the effect of enameloplasty on pit and fissure sealants was tested either with or without the application of force. The load that was used (500 N), was approximately the average force that was tested by first molars.[34] In the present study, occlusal load did not seem to reduce the beneficial effects of enameloplasty. In fact load seemed to lead to increased microleakage only when there was no enameloplasty. This result, has obvious clinical implications, as natural chewing and masticatory forces do not seem to influence the pits and fissure sealants adaptation only when enameloplasty is introduced.

Previous studies[29,35,36] have shown that masticatory forces are capable of breaking the bonds created by the acid etch technique between the enamel and composite resin. It has been reported[36] that microleakage of restorations placed *in vivo* in teeth with antagonists was significantly greater than in restorations placed in teeth not subjected to masticatory stress. In the present study, load was applied on the peripheral enamel approximately 2.5 mm from the margin, at the level of dentinoenamel junction. This was based on results of a study[37] that was conducted to evaluate the effect of load on central and peripheral enamel. The result of that study demonstrated increased microleakage on the loaded peripheral sealed enamel, where enamel was not directly supported by dentin.

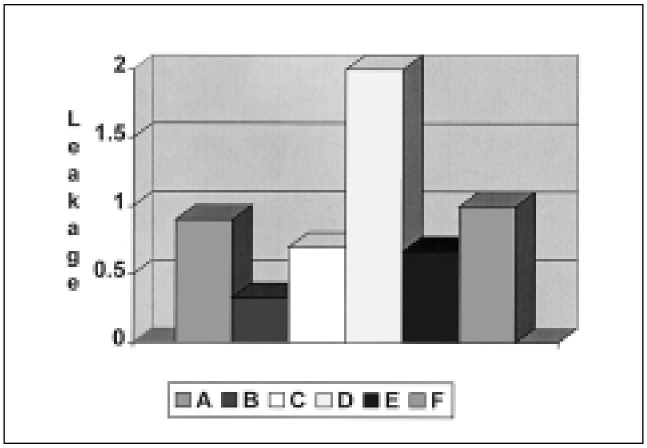

Figure 6. Pit and fissure sealant under microscope (enameloplasty – fissure bur).

A. no load - conventional
B. no load - round carbide bur enameloplasty
C. no load - fissure diamond bur enameloplasty
D. load - conventional
E. load - round bur enameloplasty
F. load - fissure diamond bur enameloplasty

Enameloplasty seems to remove the potential problems associated with placing sealing material on the peripheral enamel. It can be speculated that the benefits of enameloplasty on microleakage may be due to its effect on prismless enamel.

Many reports have stated the existence of prismless layer at the surface of human enamel.[25,26,38] In a study that was conducted by Ripa *et al.*[25] a layer of apparently "prismless" enamel was found on all the deciduous teeth and 70% of the permanent teeth. Kodaka *et al.*[39] recently evaluated the structural and distribution patterns of surface "prismless" enamel in human permanent teeth. They reported that the band-like "prismless" enamel was about 20 - 30μm in thickness and 100 - 300μm in length and was observed in fissure and cervical enamel.

Gwinnett[40-42] found that the presence of prismless layer on the enamel surface might create a reduction in mechanical retention of the sealant. According to this author, this might be due to a difference in topography between the prismless and prismatic enamel. In prismless enamel, no tags of resin representing sealant penetration were seen, but were present where such enamel had been removed. When present, the length of resin projections in association with prismless enamel were significantly shorter than those related to prismatic enamel when viewed by scanning electron microscopy.[20,38,40-42]

This study recommends enameloplasty technique can be considered as a method of fissure preparation prior to sealant placement. Further investigation is needed to determine which is the optimum anatomy of the fissure, to reduce marginal leakage.

	MEAN	S.D
A	0.9	1.1
B	0.33	0.5
C	0.7	1.05
D	2.0	1.05
E	0.67	0.7
F	1.0	0.7

Figure 7. Microleakage results of the study groups.

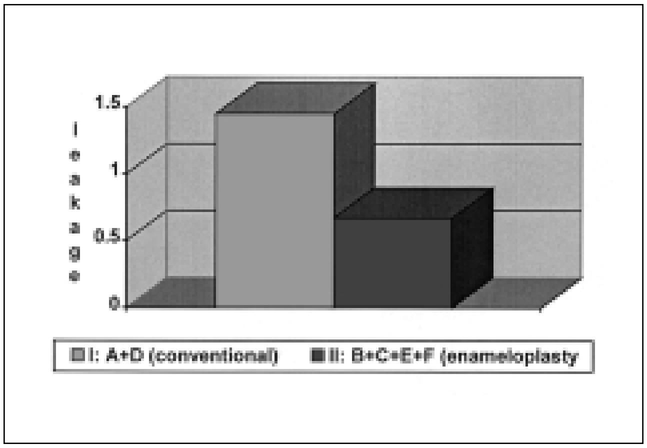

Figure 8. Microleakage vs. Enameloplasty.

A. no load - conventional
B. load - conventional
C. no load - round carbide bur enameloplasty
D. load - fissure diamond bur enameloplasty
E. load - round bur enameloplasty
F. load - fissure diamond bur enameloplasty

	MEAN	SD
I	1.45	1.19
II	0.67	0.78

$z = -2.4207$

$p = 0.0155$

CONCLUSIONS

The results of the study indicate the following:
The introduction of enameloplasty reduced microleakage of pit and fissure sealants, especially when load was applied to teeth.

Enameloplasty remains beneficial, irrespective of what bur was used to enlarge the fissures. There was no statistical significant differences between the round and fissured diamond burs, although the round seemed to be better.

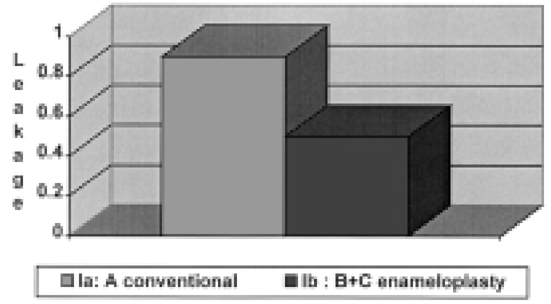

Figure 9. Microleakage vs. Enameloplasty (No Load).

A. no load - conventional
B. no load - 0.5 round carbide bur enameloplasty
C. no load - fissure diamond bur enameloplasty

	MEAN	SD
Ia	1.45	1.19
Ib	0.67	0.78

z = -0.9

p = 0.3

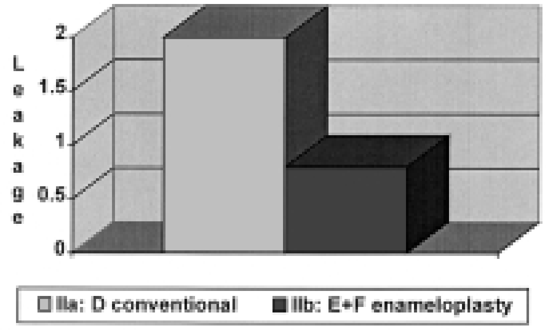

Figure 10. Microleakage vs. Enameloplasty (with Load).

D: load - conventional
E: load - round bur enameloplasty
F: load - fissure diamond bur enameloplasty

	MEAN	SD
IIa	2	1.05
IIb	0.8	0.7

z = -2.8

p = 0.005

	MEAN	SD
a	0.50	0.60
b	0.84	0.90

z = -0.9

p = 0.3

Figure 11. Microleakage vs. type of Bur.

The application of occlusal force to the tooth produces significantly more microleakage, unless enameloplasty is performed.

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