An in vitro study of microleakage of pit and fissure sealants in the presence of occlusal forces

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The purpose of this study was to evaluate the effect of load on microleakage of pit and fissure sealants. In the first phase of the study load of maximal force (F = 880 N) on central and peripheral enamel was applied. Three groups of ten specimens were tested for microleakage. Group A no application of load, Group B application of load on the central pit of the tooth, and Group application of load on the peripheral enamel 2.5 mm away from the margin of the tooth, at the level of dentinoenamel junction. The results of that study showed significant increase of microleakage on the peripheral sealed enamel, as well as enamel microcracks. So, in the phase II sixty wisdom teeth were sealed with Ultra seal XT plus and load of different magnitude was applied on the peripheral enamel. 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. Six groups of ten specimens were tested for microleakage under load. The force was applied at the peripheral enamel, 2.5 mm away from the margin of the tooth, at the level of dentin-enamel junction in cross-section view. Group A: F = 0 N; Group B: F = 300 N; Group C: F = 400 N; Group D: F = 500 N; Group E: F = 600 N; Group F: F = 700 N. Microleakage was scored as distance of dye penetration with 0 = no microleakage and 3 = microleakage to underlying fissure. A linear regression statistics was used with R-squared of 0.31. The groups with load applied (B, C, D, E, F) showed statistically significant results (p<0.05) compared with group A (F = 0 N). Both, the magnitude of the force and tooth morphology may influence the degree of microleakage observed after the placement of sealants.

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INTRODUCTION

A technique termed “occlusal sealing” was introduced in 1965.[1] This is a process of applying (mechanical bonding) a resin material to an acid-etched enamel surface, by sealing existing pits and fissures from the oral environment; thus preventing bacteria from colonizing the pits and fissures, and nutrients from reaching the bacteria already present.[2] Since then, various sealant systems have been developed, including Bis-GMA resins (polymerized either by chemical means or by visible light), polyurethane sealants containing inorganic fluoride compounds, and polyacrylate materials.[1] Inorganic filler particles have been added to sealants to increase the shear bond strength.[3,4] In addition fillers improve the compressive strength, hardness, modulus of elasticity and wear resistance.[5-8]

Sealant longevity is not only influenced by the type of sealant, but also by the position of the tooth in the mouth, the skill of the operator, the age of the child and the eruption stages of the tooth. Thus, the more anterior the tooth and the more skilled the operator, the better the prognosis. Pit and fissure sealants are very technique sensitive materials and special care should be used in their application, to achieve good retention rates.

Adhesion of restorative materials to tooth structure has been, and will always be, of significance in clinical dentistry. When lack of adhesion exists between resin restorations and enamel, then microleakage occurs at the tooth - material interface. This may lead to discoloration, secondary caries and marginal breakdown.[9] To address this problem, Buonocore first introduced the concept of etching enamel with orthophosphoric acid (H_3PO_4).[10] Later studies conducted by Tani and Buonocore, showed that phosphoric acid application practically eliminates all microleakage on etched enamel.[11]

Mechanical loading and the subsequent plastic or elastic deformation of the resin places a much larger force on the bond between the enamel and the resin

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restoration, than the one found with other types of materials. It has been demonstrated that this larger force could result in a breakage of the bond and leakage around a resin restoration even though proper placement procedures have been followed.[12] During chewing, the greatest amount of force is placed on the first molar region.[13] With tougher foods, chewing occur predominantly on the first molars and second premolar areas.[14-16]

Jorgensen *et al*. showed that occlusal loading increased the amount of deformation at the margins of resin composites. They also found that the forces tended to open up the margin between the cavity and the composite filling, even in cases where the cavity had been acid etched prior to the placement of the restorative material, a technique known to provide good sealing.[17] When Rigsby *et al*. evaluated the effect of axial loading and temperature cycling on microleakage of resin restorations, they concluded that microleakage at the enamel acid-etched margins of class V restorations does not occur even if the restored teeth are subjected to thermal and occlusal stresses. The occlusal load in this study was 75 pounds equivalent to 34 MPa.[18]

The marginal adaptation of resin composite restorations placed *in vivo* in acid-etched cavities in teeth with or without antagonists was evaluated by Qvist. It was shown that 71% of the restorations placed in molars with antagonists showed evidence of bacterial penetration, while in teeth without antagonist, only 25% showed bacterial penetration. This was attributed to the plastic or elastic deformation of restored teeth by occlusal forces, which leads to bond failure at the margins of the restoration.[19]

It seems, therefore, that the masticatory forces may be capable of breaking the bonds created by the acid etch technique between the enamel and the composite resin. The question is whether these effects occur with the pit and fissure sealants. Thus, the purpose of this study was to evaluate the effect of load on microleakage of pit and fissure sealants.

MATERIALS AND METHODS

The conventional sealant employed in the study was the Ultraseal XT "plus"™, which is a 60% filled resin, light-cured, radiopaque and fluoride-releasing. This material was selected on the basis of the results of a study that conducted by Bayne *et al*.[20] comparing the properties of flowable composites and concluding that the Ultra-Seal XT Plus demonstrated the highest flow ($534.2 \pm 7.8mm^2$ /30sec/0.5 MPa) and the lowest wear (21 ± 3mm/0.5cycles), properties highly important for a sealant material. Penetration studies on closed capillary tubes, which are somewhat analogous to pits and fissures, have indicated that a sealant will adapt more closely to the enamel surface if it possesses a high coefficient of penetration.[21] Optimal penetration will occur when the sealant has high surface tension and a low viscosity, thus permitting it to flow readily along the enamel surface.

The material was utilized in accordance to 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 were free of caries as were detected by a caries detector dye.

Just prior to sealing the pits and fissures, the teeth were cleaned by means of a rubber cup with slurry of pumice. After air-drying, one drop of caries detector dye was applied on the occlusal surface, for ten seconds. All teeth were then rinsed thoroughly and dried with oil- free air for three seconds. Teeth that had blue stains on the surfaces were not included in the study. The teeth were then brushed and cleaned with pumice, again they were mounted in an acrylic jig and stored in saline solution.

The occlusal surface of teeth was air-dried and then etched with 35% phosphoric acid, using blue micro-tips for 15 seconds and rinsed with air / water spray. An application of Prima Dry, a hydrophilic drying and priming agent was done using a white mini brush for five seconds. Teeth were then dried by gently blowing the surface with moisture-free and oil-free air. Ultra-Seal XT "plus"™ was applied using an inspiral brush tip and light cured for 20 seconds.

The samples were again kept in saline until further use. 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.

The initial phase of the experiment attempted to investigate if there was any statistical or clinical difference among two experimental tooth sites: the central pit and the peripheral enamel. In this first phase, a force of 880 N was applied since Howell *et al*. documented it as the maximal occlusal force. Thus load of 880 N was applied on the central pit of the teeth or at the peripheral enamel 2.5 mm away from the margin of the tooth, at the level of dentin-enamel junction in cross-section view. Fig.1

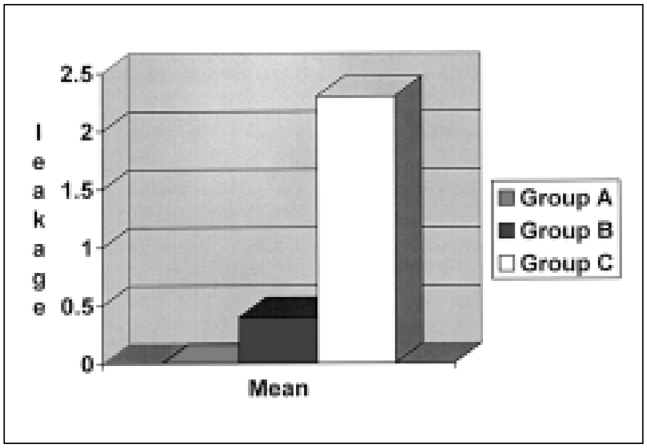
The results of this first phase of the study showed us that there was significant increase in microleakage, especially when load was applied at the periphery. Enamel microcracks and enamel tooth fractures were observed. Fig. 2-5 Based on these results we proceeded to evaluate the effects, if any, of forces of different magnitude on the peripheral enamel of pit and fissure sealants.

Six groups of ten specimens were tested for microleakage under load. As explained earlier, the force was applied at the peripheral enamel, 2.5 mm away from the margin of the tooth, at the level of dentin-enamel junction in cross-section view. The forces that were applied in each group of ten teeth were the following:

Group A: F = 0 N; Group B: F = 300 N; Group C: F = 400 N; Group D: F = 500 N; Group E: F = 600 N; Group F: F = 700 N.

Loading such forces on the enamel was accomplished in a mechanical testing machine (Instron). 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 approximately vertical. A metal cylinder of 0.9mm diameter was loaded in the longitudinal direction

Figure 1. Application of force on the peripheral enamel at dark mark.



Group A: No load
Group B: Load on central pit of occlusal enamel
Group C: Load on peripheral pit of occlusal enamel

	Mean	S.D.
Group A	0.00	0.00
Group B	0.40	0.69
Group C	2.30	0.82

Figure 2. Mean microleakage score in groups loaded in central (B) and peripheral enamel C).

of the tooth to stimulate forces. Each tooth was subjected to a force at a speed of 1.0 mm / second. The loading procedure was done at room temperature.

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

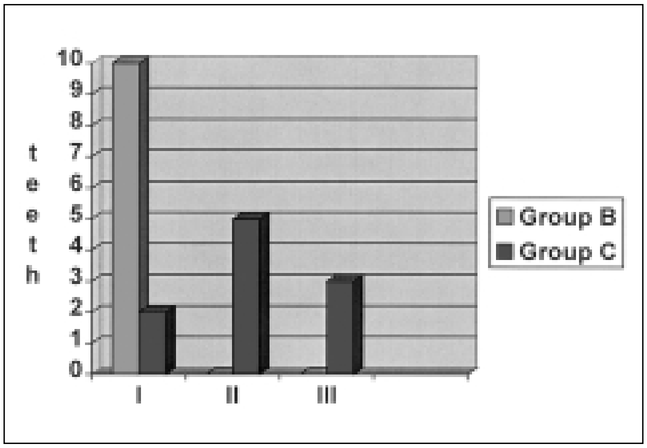


Figure 3. Tooth integrity of the groups in the preliminary study.

Group B: Load on central pit of occlusal enamel
Group C: Load on peripheral pit of occlusal enamel

I: Tooth intact
II: Enamel microcracks
III: Tooth fracture

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.

RESULTS

The results of this study are shown in Figures 6 to 8. Two out of sixty teeth were not included in the study as we were unable to specify the microleakage score as the sealant was over the incline cusp. Linear regression was used to compare the microleakage scores between the different forces; the R-squared was 0.31. Significantly more microleakage was noted for all groups that had force applied ($p<0.05$), as compared to group A, those teeth that received a sealant, but had no forces applied.

DISCUSSION

The integrity of the tooth / sealant material interface dependents upon several factors, such as the mechanical and chemical properties of the material, the anatomy and cleanliness of the fissures, the thermal conditions existing in the oral cavity and the amount and the position of forces that are applied on the tooth.[22]

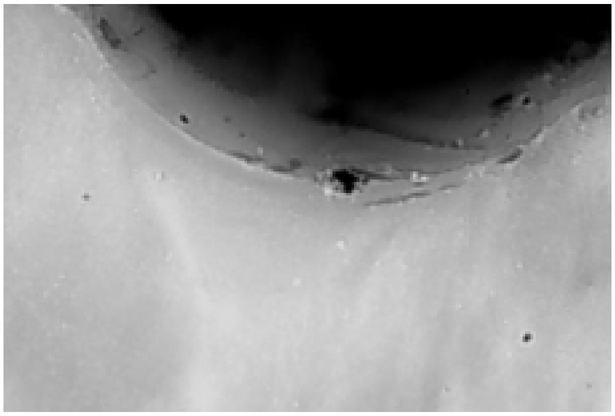


Figure 4. No microleakage on loaded (880 N) central pit of sealants.

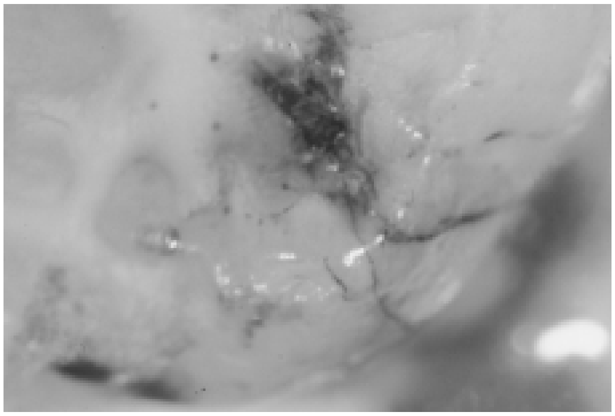


Figure 5. Enamel microcracks on the periphery under 880 N.

Several factors account for the occurrence of microleakage at tooth / restoration interface. Polymerization shrinkage of resin composites induces stresses at the tooth / restoration interface, which disrupt the restoration / tooth bond, resulting in the formation of gaps at the interfaces.[23] Etching of the enamel and proper placement of the composite restorative material enhance the bond strength between enamel and material, which can reach several thousands, pounds per square inch.[24,25] This mechanical bonding to the enamel is necessary to resist the forces of polymer shrinkage during placement, the mismatch in thermal expansion coefficient between enamel and composite and the low elastic modulus of composite material versus enamel.[26-29] Polymer tags that form in direct opposition to irregularities of the enamel surface (created by acid etching) are responsible for the mechanical bond that retains the sealant to enamel.[30]

There are numerous reports in the dental literature, which describe the measurements of biting forces on teeth. Biting forces on adult teeth decrease from the molar region to the incisors with forces on the first molar varying from 390 to 880 N (88-198 lbs.) with an average of 565 N (127 lbs.).[31] Bite force during chewing is not well correlated with maximal strength of the mastication muscles and is probably controlled by other factors such as pain thresholds, sensitivity of the mucosa and periodontal receptors, emotional status, propioceptive reflexes and area of distribution of force. If the force is distributed over a few teeth, the forces tend to be lower.[32,33] Speed, duration and form of the chewing cycle vary with the type of occlusion, kind of food, and presence of dysfunction.[34-36]

As mentioned earlier, during chewing, the greatest amount of force is placed on the first molar region.[13] With tougher foods, chewing occur predominantly on the first molars and second premolar areas.[14-16]

The load that was used in the first phase of the study was the maximal masticatory force that was observed in first molars (198 lbs. / 880 N).[13] As sealants are placed mainly on molars, we chose to look at the maximum force as a first step in our investigation. In this first phase, microleakage was similar for the teeth that had not been subjected to force and those that had received the load in the central pit of the sealed enamel (p=0.108).

It was also demonstrated that this heavy force affects the integrity of the sealant-enamel interface, as well as the integrity of the tooth structure itself, especially when the force was applied on the periphery. A great number of enamel cracks and fractures were seen in this last group. Peripheral enamel demonstrated low compressive strength. Enamel was fractured, but the sealant showed high elasticity, as there were no fractures of the material. A phenomenon of tooth separation from the material, under occlusal load, was observed only when peripheral enamel had no dentinal support.

Enamel has sufficient strength to withstand masticatory pressures and this is in great part due to the cushioning effects of the underlying dentin. The ability of the tooth to withstand great masticatory forces appears to be related to the structural and physical interrelationship between enamel and dentin.[37]

Enamel thickness itself seems to play a role in microleakage. Enamel thickness varies from a maximum of approximately 2.5 mm of working surface to a featheredge at the cervical line. For molars, the minimum enamel thickness is about 0.33 mm.[37]

According to Goel *et al.* the highest compressive stress values in enamel were in the zone immediately adjacent to the mesial DEJ, however, the compressive stresses in the peripheral enamel were low. These were the areas where the enamel was not directly supported by dentin. Areas with dentinal support as in buccal, lingual, mesial, and distal surfaces, distant from DEJ, demonstrated high tensile stress with the lowest compressive stress.[30]

However, studies[15,16,33,35] have shown that chewing forces are controlled by many factors as described earlier, so they tend to be lower than the maximum masticatory force (in a percentage that is not well know). For that reason, we

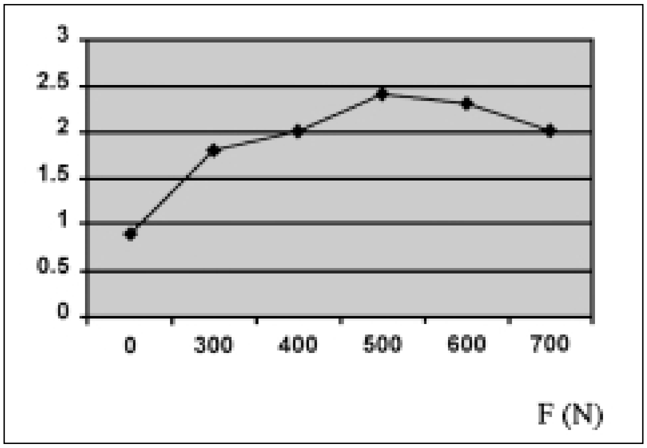


Figure 6. Microleakage scores vs. load application (N).

continued with the second step, which was to test forces of varying lower magnitudes, forces that may better replicate what actually happens in nature. So, in phase II of the experiment we analyzed the effect of such lower forces on sealant microleakage at the peripheral enamel. Our results indicated that the application of load independent of the magnitude (300N - 700N) resulted in statistically significant increase ($p<0.05$) of microleakage of pit and fissure sealants. No tooth fractures or enamel microcracks were seen in any of the groups. Thus, force is an important factor influencing the microleakage of pit and fissure sealants.

In the current literature there is very limited information on the effect of load on microleakage of pit and fissure sealants. More data exist about Class I and V composite restorations. The results of the present study support the findings of Jorgensen *et al.* that occlusal loading increased the amount of deformation at the margins of resin composites.[17] The authors studied the deformation of selected types of cavities in axially loaded human teeth and demonstrated the dimensional instability of unrestored restorations in teeth that were axially loaded at small and moderate load, which indicated a great risk of percolation at the tooth / restoration interface.[17]

Raadal evaluated the microleakage around preventive composite restorations placed in acid etched preparations in enamel only. He subjected these preparations to mechanical loading and thermocycling.[7] He noticed good sealing of occlusal fissures, and that the seal was resistant to thermal and mechanical stresses, which may be experienced *in vivo*. However, the load that was applied in this study (147 N) was small even smaller than the lower force (300 N) that was applied in phase II of our study and might not been enough to replicate natural forces.

Regarding forces of low magnitude, Stewart and Balda conducted a study to evaluate the effect of mechanical loading on composite restorations.[12] The load was applied near the margin of the composite restoration on the composite, with a maximal load of 31 lbs. using 5000 repetitive applications. The fact that no leakage was produced indicated that moderate loading on composite restorations, below the average load for even healthy premolars (50-100 lbs), did not produce sufficient stress or fatigue to break the enamel - composite bond by itself or in conjunction with thermal cycling. Stewart *et al.* evaluated the effect of mechanical loading with and without thermocycling on the marginal integrity of Class V restorations placed on the mid- buccal surfaces of premolars.[12] The results indicated that composite resin restorations placed with the margins entirely in enamel are capable of withstanding repetitive mechanical and thermal stress without significant detrimental effect on the enamel /resin composite bond. In this study the load that was used was 31 lbs., which was too low, even though there were 5000 repetitive applications, trying to mimic the chewing forces.

	Group A 0N	Group B 300N	Group C 400N	Group D 500N	Group E 600N	Group F 700N
Mean	0.9	1.8	2.0	2.4	2.3	2.0
S.D.	1.10	1.18	1.23	0.69	0.62	0.94

Figure 7. Mean microleakage scores in different load application.

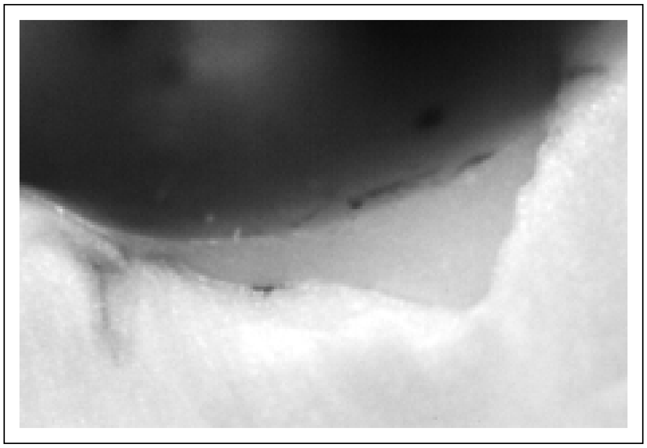


Figure 8. Microleakage in loaded teeth.

CONCLUSIONS

Both, the magnitude of the force and tooth morphology may influence the degree of microleakage observed after the placement of sealants. Clinicians need to pay attention to the placement of sealants especially at the periphery of the tooth.

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