Influence of Different Enamel Substrates on Microtensile Bond Strength of Sealants After Cariogenic Challenge

Kamila Rosamilia Kantovitz[a]/Fernanda Miori Pascon[b]/Marcelo Correa Alves[c]/
Francisco Humberto Nociti, Jr[d]/Cinthia P. Machado Tabchoury[e]/Regina Maria Puppin-Rontani[f]

Purpose: To evaluate the microtensile bond strength (μTBS) of resin sealer on enamel substrates after cariogenic challenge.

Materials and Methods: Enamel blocks were obtained from human third molars and randomly divided into 6 groups (n = 10) according to enamel substrates (S: sound, CL: caries-like lesion, or CLTF: caries-like lesion + topical fluoride application) and sealant material (F: FluroShield, or H: Helioseal Clear Chroma). Sealants were placed on enamel surfaces, stored in 100% humidity (24 h, 37°C), and longitudinally sectioned into hourglass shapes. According to the groups, pH cycling was applied and the μTBS test was performed. The fracture patterns were assessed by SEM.

Results: Regarding substrates, the highest μTBS values in MPa were observed for CLTF enamel (26.0 ± 7.6), followed by S (22.0 ± 7.4) and CL (15.5 ± 4.9). A significant interaction was found between material and pH cycling (p = 0.0395). F (23.9 ± 7.6) showed higher μTBS values than H (18.3 ± 7.5) when submitted to pH cycling. The majority of samples presented mixed failure.

Conclusions: Enamel substrate significantly affected μTBS, with the highest values for remineralized caries-like enamel lesions. Furthermore, μTBS values were dependent on both materials and pH cycling.

Keywords: preventive therapy, microtensile bond strength, fissure sealing, enamel, caries-like lesion, cariogenic challenge model.

J Adhes Dent 2011; 13: 131-137.
doi: 10.3290/j.jad.a19230
Submitted for publication: 09.02.09; accepted for publication: 16.11.09.

Initial enamel caries lesions are usually not treated operatively to avoid the sacrifice of sound hard tissues.[11] Thus, preventive action at an early stage is important to prevent caries development. The maintenance of oral hygiene in conjunction with dietary advice, fluoride therapy, and prudent use of pit-and-fissure sealants has been shown to be a reliable preventive strategy in these populations.[29] Noninvasive pit-and-fissure sealing has proven to be effective for caries prevention in several studies.[25] In order to obtain long-term clinical success, the sealant retention and the integrity of sealant-enamel bond are important criteria for successful prevention of leakage of bacteria and oral fluids that initiate pit-and-fissure caries.[25]

Some factors that can affect the adhesion of sealants to enamel are the structure and organization of dental enamel. With regard to the condition of the substrates, etching sound enamel with phosphoric acid may form porosities on the surface, allowing the resinous sealants to penetrate into microdepressions created by the acid and yield high enamel bond strengths.[5,19] White spot lesions are characterized by a loss of mineral in the bulk of enamel, whereas the surface of the lesion remains relatively intact.[27] The tiny pores within the lesion body may act as diffusion pathways for acids and minerals.[27] In this case, the aim of the sealing regimen is to occlude these pores with light-curing resins by

[a] *Affiliate Professor, Affiliate Assistant Professor, Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

[b] *Assistant Professor, Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

[c] *PhD Student, Department of Anatomy, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

[d] *Professor, Department of Prosthodontics and Periodontics, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

[e] *Associate Professor, Department of Physiologic Sciences, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

[f] *Professor, Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.*

Correspondence: *Professor Regina Maria Puppin-Rontani, Pediatric Dentistry Division, Department of Pediatric Dentistry, Piracicaba Dental School, UNICAMP, Av. Limeira, 901, Piracicaba, SP, Brazil 13414-900. Tel: +55-19-21065286, Fax: +55-19-21065218. e-mail: rmpuppin@fop.unicamp.br*

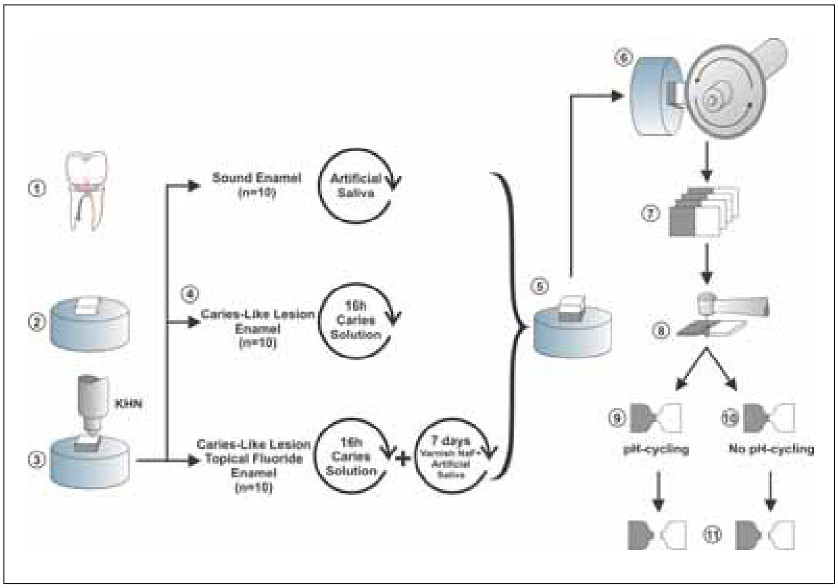

Fig 1 Representative scheme of methodology and experimental design: (1) Root section 1 mm below the cementoenamel junction; (2) Enamel block preparation (4x4 mm); (3) Surface microhardness determination (KHN); (4) Experimental group distribution; (5) Sealant application (4 mm height) (FluroShield or Helioseal Clear Chroma); (6) Longitudinal cutting of the sample into a series of 1-mm-thick slices; (7) Slice preparation (four slices per enamel block); (8) Hourglass preparation (ca 1 mm^2 cross-sectional area); (9,10) Submission or not to pH cycling; (11) Microtensile bond strength test (μTBS).

penetration into the lesion body, preventing the dissolution of enamel at the advancing front of the lesion.[8,17] Moreover, after curing the material, a mechanical support of the fragile enamel framework in the lesion might be achieved.[17] In the remineralized enamel, its crystalline structure is stabilized by the acquisition of fluoride, which competes with and displaces the hydroxyl groups of the hydroxyapatite molecule to form fluoridated hydroxypatite.[6] Moreover, the formation of calcium fluoride has been reported to reduce the bond strength of resin to enamel.[14]

Considering the structure of the different enamel substrates, such as caries-like lesions or remineralized caries-like lesions, no study has hitherto focused on sealant application on different enamel substrates in an attempt to prevent the development of the initial lesion. In addition, such treated substrates are constantly submitted to cariogenic challenge, mainly in high caries-risk children. A dynamic chemical model, termed pH cycling, has been used in order to simulate the oral conditions of high caries-risk children in in vitro studies.[2]

As such, the aim of this in vitro study was to evaluate the microtensile bond strength (μTBS) of resinous sealant materials on different enamel substrates after a cariogenic challenge (pH cycling). The null hypothesis was that there are no statistically significant differences in the bond strength of different resinous sealant materials on different enamel substrates when submitted to pH cycling or not.

MATERIAL AND METHODS

This study was conducted after approval from the Ethics Committee of the Piracicaba Dental School, University of Campinas (protocol #046/2006).

Experimental Design

The factors under study were: enamel conditions (sound, caries-like lesion, and caries-like lesion + topical fluoride application) and sealant materials (FluroShield and Helioseal Clear Chroma). The experimental samples consisted of 60 enamel blocks, which were randomly assigned to six treatment groups (n = 10). The response variables were bond strength values and type of failure pattern, as assessed by scanning electron microscopy (SEM).

Preparation of Enamel Blocks

Ninety-seven impacted human third molars were selected that had been extracted for clinical and orthodontic reasons and were free from apparent caries on the buccal, palatal, or lingual surfaces. All teeth were examined under 20X magnification (Leica MZ6; Wetzlar, Germany) to exclude those with any enamel defect. The teeth were cleaned and stored in 0.5% chloramine T solution for up to 2 months after extraction. Their roots were sectioned off 1 mm below the cementoenamel junction using a double-face diamond saw and discarded (KG Sorensen; São Paulo, SP, Brazil). Each tooth was longitudinally sectioned along the fissure orientation (Isomet, Buehler; Lake Bluff, IL, USA) in order to obtain 194 buccal, palatal or lingual enamel surfaces. The enamel surfaces were flattened (4x4 mm) on a water-cooled mechanical grinding machine, using 400-, 600-, and 1200-grit Al_2O_3 abrasive paper (Arotec; São Paulo, Brazil), and cloth polished with 1.0-μm diamond paste (Buehler Metadi II) (Fig 1). Care was taken not to expose the underlying dentin. For block selection, surface microhardness (SMH) determination was accomplished using a Future-Tech FM-ARS microhardness tester (Future-Tech; Tokyo, Japan) with a Knoop diamond under a 50-g load for 5 s.[7] Five indentations were made at the center of the enamel surface (Fig 1). One hundred twenty-

Table 1 Brand, composition, manufacturers, and batch number of the sealants

Material	Composition	Manufacturer and batch #
FluroShield	Urethane modified bis-GMA dimethacrylate; barium aluminoborosilicate glass (30%), polymerizable dimethacrylate resin, bis-GMA, sodium fluoride, dipentaerythritol pentaacrylate phosphate, titanium dioxide, amorphous silica.	Dentsply DeTrey; Konstanz, Germany # 317131
Helioseal Clear Chroma	Bis-GMA, triethylene glycol dimethacrylate (>99 wt%). Additional contents are stabilizers, catalyts and pigments (<1 wt%).	Ivoclar Vivadent; Schaan, # F54463 Liechtenstein

three enamel blocks with 341.6 ± 18.0 Knoop Hardness Number units (KNH) were selected for this study. The rejected blocks (71) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the means. Twenty sound enamel blocks were kept in a humid environment until the experiment (10 enamel blocks were used in the FluroShield group and 10 in the Helioseal Clear Chroma group), and 103 blocks were used for caries-like lesion induction.

Artificial Caries-like Lesion Formation

One hundred-three enamel block surfaces were isolated with double coats of acid-resistant nail varnish (Colorama; São Paulo, Brazil), except for the polished enamel area (4x4 mm) (Fig 1). Artificial caries-like lesions were produced by suspending each enamel block in 32 ml of a solution containing 0.05 M acetate buffer 50% saturated with enamel, pH 5.0, for 16 h at 37°C. To prepare this solution, enamel powder (particles of 74 to 105 µm) was agitated in 0.05 sodium acetate buffer, pH 5.0, for 96 h at 37°C (0.50 g/l).[18] The solution was used at a proportion of 2.0 ml per mm^2 of exposed enamel area. After caries-like lesion induction, 63 blocks with known enamel SMH (81.9 ± 22.8 KHN) were selected. The rejected blocks (60) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the mean. Twenty enamel blocks with artificial caries-like lesions were then kept in a humid environment until the experiment (10 enamel blocks were used in the FluroShield group and 10 in the Helioseal Clear Chroma group) and 43 blocks were used for topical fluoride application on caries-like lesions using 5% NaF varnish, simulating a remineralization procedure.

Artificial Caries-like Lesion Remineralization

Following caries-like lesion formation, 43 enamel blocks were submitted to topical fluoride application. The enamel surfaces of these blocks were coated with 5% NaF varnish (Duraphat, Colgate-Palmolive; S. Bernardo do Campo, SP, Brazil), using a microbrush. The varnished blocks were individually immersed in 20 ml of artificial saliva (1.5 mM calcium, 0.9 mM phosphate, 150 mM KCl in 0.1 M Tris buffer, 0.05 µg F/mL, pH 7.0) at 37°C for 1 week. The solution was applied at a proportion of 1.25 ml per mm^2 of exposed enamel area.[9] The varnished blocks were then removed from the artificial saliva and rinsed with distilled deionized water (pH 6.0). After topical fluoride application, twenty blocks with known enamel SMH (140.4 ± 36.5 KHN units) were selected. Ten enamel blocks were used in the FluroShield group and ten in the Helioseal Clear Chroma.The rejected blocks (23) were those that did not fit into the mean and standard deviation range, which was considered as 10% above or below the mean.

Experimental Groups

Enamel blocks were then randomly divided into six groups (n = 10) according to enamel substrates and sealants: Sound enamel + FluroShield (SF); caries-like lesion + FluroShield (CF); caries-like lesion + topical fluoride application + FluroShield (CFF); sound enamel + Helioseal Clear Chroma (SH); caries-like lesion + Helioseal Clear Chroma (CH); caries-like lesion + topical fluoride application + Helioseal Clear Chroma (CFH) (Fig 1).

Sample Preparation

After enamel substrate preparation, the polished enamel surface of the blocks was etched using 37% phosphoric acid gel for 30 s, rinsed for 10 s with water, and dried. The sealants were then applied to a flat surface to build up a bonding surface block of about 4 mm in height, with 2-mm-thick increments; samples were then light cured for 40 s (FluroShield) or 20 s (Helioseal Clear Chroma) (Fig 1). The light curing was carried out using the Elipar Tri-light unit (3M ESPE; Seefeld, Germany) at 800 mW/cm^2 light intensity. The sealed blocks were stored for 24 h at 37°C and 100% humidity. The brand names, composition, manufacturers, and batch numbers of the sealants are listed in Table 1.

Afterwards, each sample was longitudinally cut into a series of 1-mm-thick slices by means of a water-cooled diamond blade (Isomet, Buehler) (Fig 1). Four slices were obtained per block and trimmed to an hourglass shape using a cylindrical diamond bur (FG 3097, KG Sorensen) mounted in a high-speed handpiece (Fig 1). Each hourglass was isolated with double coats of acid-resistant nail varnish (Colorama), except for the bonding area.

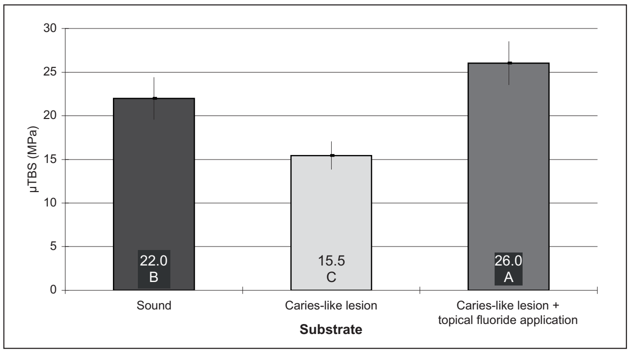

Fig 2 µTBS mean (MPa) and 95% confidence intervals for different enamel substrates. Different letters represent statistically significant difference by Tukey test ($p<0.05$). The data of sealant groups were combined.

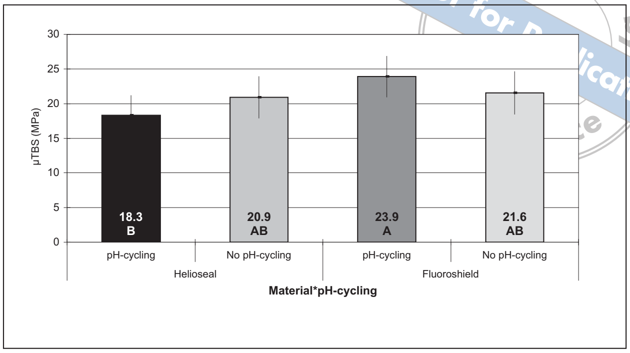

Fig 3 Means (MPa) and 95% confidence intervals of the µTBS measured. Different letters represent statistically significant difference by Tukey test ($p<0.05$). The data of enamel substrate groups were combined.

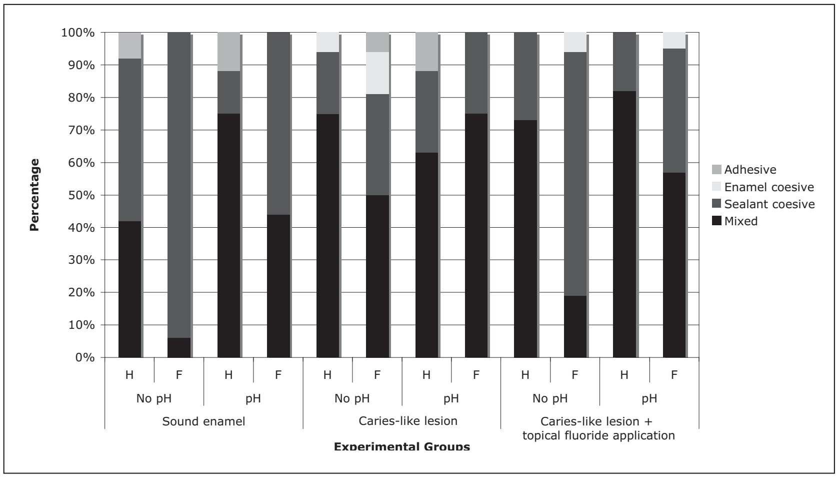

Fig 4 Percentage of failure patterns in the experimental groups after µTBS test. H – Helioseal Clear Chroma; F-FluroShield; No pH – No pH cycling; pH – pH cycling.

pH Cycling Model

The hourglass specimens were then submitted to pH cycling or not (Fig 1). Two specimens from each sample were subjected to 7-day pH cycling, simulating a cariogenic challenge.[2,9] Each cycle consisted of a 3-hour immersion in demineralizing solution followed by a 21-hour immersion in remineralizing solution. Hourglass specimens were individually immersed in 40 ml of a demineralizing solution (2 mM calcium, 2 mM phosphate in 0.075 M acetate buffer, 0.03 µg F/ml, pH 4.3, 37°C) used at a proportion of 2.5 ml per mm^2 of exposed bonding area. Specimens were then washed in deionized water for 30 s, dried with absorbent paper and individually immersed in 20 ml of a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM KCl in 0.1 M tris buffer, 0.05 µg fluoride/ml, pH 7.0, 37°C) applied at a proportion of 1.25 ml per mm^2.[9] Both solutions contained thymol crystals to prevent microbial growth.

Microtensile Bond Strength Test (µTBS)

The hourglass-shaped specimens were individually mounted using cyanoacrylate glue (Super Bonder; São Paulo, Brazil) in a metallic port that was attached to the universal testing machine (4411 Instron; Canton, MA, USA). The µTBS test was performed at a crosshead speed of 0.5 mm/min. The cross-sectional area at the site of fracture was measured with a digital caliper (Mitutoyo; Suzano, Brazil) with an accuracy of 0.01 mm. The load (in Kgf) and the bonding surface area of each specimen was recorded on a worksheet. The microtensile bond strengths were calculated in MPa, using the formula: R=F (Kgf)/A (cm). Pretest failures were not included in the statistical analysis.

Determination of Failure Pattern

The failure sites were gold-sputter coated (Bal-Tec SCD 050 Sputter Coater, Bal-Tec; Balzers, Liechtenstein) and observed with SEM (JEOL JSM 5600LV; Tokyo, Japan) at an accelerating voltage of 15 kV, a working distance of 20 mm, and a magnification of 100X. For each specimen, the failure pattern was defined into four types: mixed failure; adhesive failure; cohesive failure in enamel; or cohesive failure in sealant. A blind calibrated examiner (K.R.K.) evaluated the failure pattern. The intra-examiner coincidence level of failure pattern was analyzed with Spearman's correlation test and was 95%.

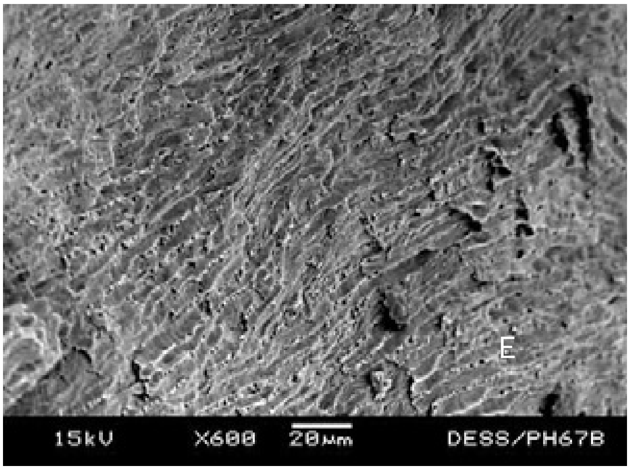

Fig 5A SEM photomicrograph illustrating enamel cohesive failure (600X). E: enamel.

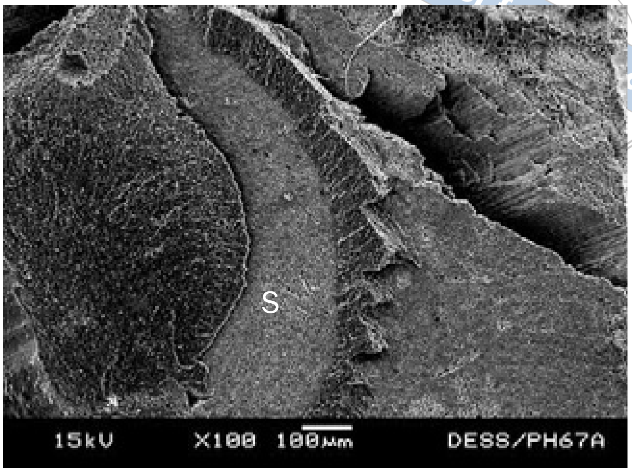

Fig 5B SEM photomicrograph illustrating sealant cohesive failure (100X). S: sealant.

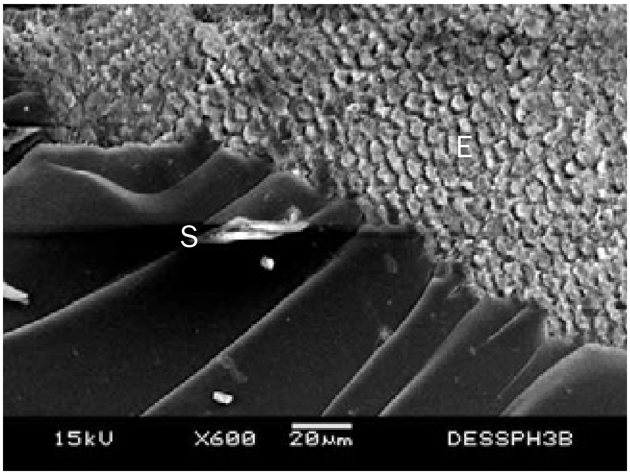

Fig 5C SEM photomicrograph illustrating mixed failure (600X). E: enamel; S: sealant.

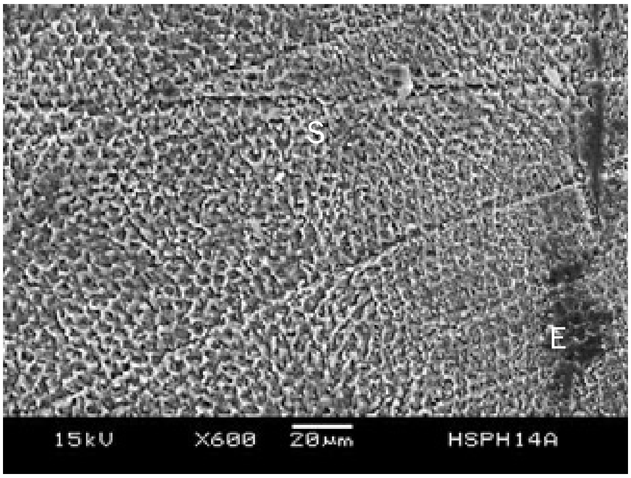

Fig 5D SEM photomicrograph illustrating adhesive failure. S: sealant, E: enamel.

Statistical Analysis

Original data from μTBS test were transformed ($x^{0.5}$) before applying 3-way ANOVA and Tukey's test, because variances were not homogeneous. A multi-factor ANOVA was applied to the μTBS data to analyze the interactions among the factors (enamel substrates, materials and pH cycling). In order to assess significant differences within these factors, Tukey's test was applied. The SAS system software (version 8.02, SAS Institute; Cary, NC, USA) was used and the significance level was set at 5% ($p < 0.05$).

RESULTS

According to 3-way ANOVA statistical analysis, there were no interactions among: enamel substrates and materials ($p = 0.1347$); enamel substrates and pH cycling ($p = 0.8126$); and enamel substrates, materials and pH cycling ($p = 0.0949$). A significant difference was found among the enamel substrate factors examined ($p < 0.001$). The mean values for enamel substrates in the μTBS test (MPa values) and their 95% confidence intervals are shown in Fig 2. The data of sealant groups were combined. Significant differences were observed between the materials and an effect of pH cycling when Tukey's test was applied ($p = 0.0395$). The data of substrate enamel groups were combined (Fig 3). FluroShield (23.9 ± 7.6) presented higher μTBS values than Helioseal Clear Chroma (18.3 ± 7.5), when the materials were submitted to pH cycling. Sealant materials showed no difference in μTBS when not submitted to pH cycling, as shown in Fig 3.

The percentage of failure pattern for all groups is presented in Fig 4. Mixed failure (cohesive in the sealant and cohesive in the enamel, Fig 5) was the most frequently observed failure type for all groups, with the exceptions of SF and CFF when not submitted to pH cycling.

DISCUSSION

Fissure sealants are currently one of the most effective tools available for protection against caries development on the occlusal surfaces of high caries-risk children. An important parameter in the evaluation of the clinical success of sealing procedures is the ability of the material to adhere to the enamel surface.[1] The bond strength is an indicator of this ability and the μTBS test was chosen due to its more accurate assessment of the interfacial bond strength of material and dental substrates, since it provides uniform stress distribution over small-sized specimens.[21] Another aspect to be considered is the condition of the enamel, which is of great importance for sealant performance and efficacy.

The null hypothesis that there are no differences in the bond strength of the different enamel substrates and resinous sealant materials was rejected. In this study, differences were noted between the μTBS of sound enamel, enamel with caries-like lesions, and enamel bearing a caries-like lesion but treated with topical fluoride. The fluoride application of 5% NaF varnish on caries-like lesions of enamel increased the μTBS values, compared to the values of sound enamel and caries-like lesions. This could be explained by the structural,[15,16] chemical, and physical properties of the different substrates, and suggests that the mineral status of the underlying dental hard tissue could influence bond strength.

Little is known about the μTBS of fissure sealants when applied to different dental substrates. When analyzing remineralized enamel, a study[13] found clinically acceptable tensile bond strength values for sealed, pretreated enamel surfaces, in accordance with data from the current study. Conversely, other studies have indicated that topical fluoride application fills the interprismatic spaces occupied by $Ca_5(PO)_3$ and CaF_2 and reduces the bonding capacity of adhesives.[15,16] On the other hand, studies have shown that tensile[28] or shear[10,12] bond strength is not significantly different in groups with and without fluoride pretreatment. In these studies, researchers saw globular structures only on the prism cores of ground enamel surfaces etched with H_3PO_4 containing higher fluoride concentrations; they did not observe adverse effects on the bond strength of bonding resin to etched enamel.

Experimental studies have shown that varnishes supply fluoride more effectively than do other topical agents.[4,24] The use of a varnish as a vehicle for topical fluoride application was chosen in this study due to its prolonged period of contact with the enamel surface, allowing greater uptake of fluoride ions into the enamel and making it more resistant to demineralization.[3,22] However, in the present in vitro study, the remineralization procedure used does not reproduce the dynamics observed in the in vivo remineralization events.

For sound enamel substrates, results demonstrated intermediate μTBS values (22.0 MPa) when compared with topical fluoride application and enamel with caries-like lesions. Similar results were found in a report that evaluated the μTBS of different types of materials used as pit-and-fissure sealants for sound ground enamel.[20]

In the caries-like lesions on enamel, the surface topographies appear relatively smooth and intact with a slightly larger pore volume than sound enamel.[26] With regard to acid etching of these substrates, it was reported that the surface morphologies of lesions that were acid etched for 30 s were rough with loss of prisms; this may provide a more reactive surface for fluoride treatment and perhaps enhance the rate of remineralization.[26] In this context, it may be appropriate to perform the topical fluoride application on the caries-like lesions before sealant application, instead of applying sealant directly on the early caries lesions, which presented the lowest μTBS values in the present study.

In this study, a caries risk situation was simulated by a pH cycling model. Regardless of the substrate, pH cycling did not influence the μTBS values for either of the materials. However, pH cycling resulted in higher μTBS values for Fluroshield than Helioseal. Under this condition, Helioseal demonstrated a higher percentage of adhesive failures, showing a weakening of bonding interface. This finding was not observed for Fluroshield, possibly due to its composition. The difference between the sealants tested may occur as a consequence of the presence of fluoride and fillers (30% barium aluminoborosilicate) in the Fluroshield composition, since both materials are resin based. The presence of fillers in FluoroShield may increase the mechanical resistance of the material, and the bond strength of resin-based materials may depend on the length, shape, and mechanical properties of resin tags.[23] In addition, the fluoride content of Fluroshield seems to protect the bonding interface from cariogenic challenge, when compared with Helioseal under the same conditions, showing a higher percentage of mixed and sealant cohesive failures. Especially the interface below the infiltrated zone, between demineralized, maybe poorly infiltrated enamel and the sealant seem to be the weakest link in bonding.

The microtensile bond strength may represent an indicator of the sealant's clinical retention ability. Thus, the results of this in vitro examination may provide information additional to that gained in clinical practice. However, these findings should not be directly extrapolated to clinical conditions. Further in vitro and in situ studies and clinical evaluations are required to assess the long-term bonding performance of these sealants and, thus, predict the quality of the adhesion obtained as well as the sealant degradation in a high caries-risk situation, indicating their failures.

CONCLUSION

It can be concluded that the μTBS was significantly influenced by different enamel substrates, where remineralized caries-like enamel lesion substrates demonstrated the highest μTBS values. The μTBS values are dependent on both materials and pH cycling.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Pediatric Dentistry and Cariology Division (University of Campinas), Mr. Marcelo Correa Maistro and Mr. Waldomiro Vieira for their cooperation and assistance. This research was supported by FAPESP – São Paulo Research Support Foundation (grant number 05/60595-1).

REFERENCES

1. Ahovuo-Saloranta A, Hiiri A, Nordblad A, Worthington H, Mäkelä M. Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2004;3:CD001830. Update in: Cochrane Database Syst Rev. 2008;4:CD001830.
2. Argenta RM, Tabchoury CP, Cury JA. A modified pH cycling model to evaluate fluoride effect on enamel demineralization. Braz Oral Res 2003;17: 241-246.
3. Beltrán-Aguilar ED, Goldstein JW, Lockwood SA. Fluoride varnishes. A review of their clinical use, cariostatic mechanism, efficacy and safety. J Am Dent Assoc 2000;131:589-596.
4. Bravo M, Garcia-Anllo I, Baca P, Llodra JC. A 48-month survival analysis comparing sealant (Delton) with fluoride varnish (Duraphat) in 6- to 8-year-old children. Community Dent Oral Epidemiol 1997;25:247-250.
5. Buonocore M. Adhesive sealing of pits and fissures for caries prevention, with use of ultraviolet light. J Am Dent Assoc 1970;80:324-330.
6. Cartwrigth HV, Lindahl RL, Bawden JW. Clinical findings on the effectiveness of stannous fluoride and acid phosphate-fluoride as caries reducing agents in children. ASDC J Dent Child 1968;3:36-40.
7. Cury JA, Rebelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CP. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 2000;34:491-497.
8. Davila JM, Buonocore M, Greeley CB, Provenza V. Adhesive penetration in human artificial and natural white spots. J Dent Res 1975;54:999-1008.
9. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S. Enhancement of remineralization in vitro and in vivo. In: Leach S.A (ed). Factors relating to demineralization and remineralization of the teeth. Proceedings of a Workshop 1985. Washington DC: Oxford, 1986:23-34.
10. Garcia-Godoy F. Shear bond strength of a resin composite to enamel treated with an APF gel. Pediatr Dent 1993;15:272-274.
11. Kidd EAM, Van Amerongen JP. The role of operative treatment. In: Fejerskov O, Kidd E (eds). Dental caries. The disease and its clinical management. Ames, Iowa, USA: Blackwell Munksgaard, 2003:245-250.
12. Kimura T, Dunn WJ, Taloumis LJ. Effect of fluoride varnish on the in vitro bond strength of orthodontic brackets using a self-etching primer system. J Orthod Dentofacial Orthop 2004;125:351-356.
13. Koh SH, Chan JT, You C. Effects of topical fluoride treatment on tensile bond strength of pit and fissure sealants. Gen Dent 1998;46:278-280.
14. Larsen MJ, Fejerskov O. Structural studies on calcium fluoride formation and uptake of fluoride in surface enamel in vitro. Scand J Dent Res 1978;86:337-345.
15. Lee H, Stoffey D, Orlowski J, Swartz ML, Ocumpaugh D, Neville K. Sealing of developmental pits and fissures. Effects of fluoride on adhesion of rigid and flexible sealers. J Dent Res 1972;51:151-152.
16. Low T, von Fraunhofer JA, Winter GB. The bonding of a polymeric fissure sealant to topical fluoride-treated teeth. J Oral Rehabil 1975;2;303-307.
17. Meyer-Lueckel H, Paris S, Mueller J, Colfen H, Kielbassa AM. Influence of the application time on the penetration of different dental adhesives and a fissure sealant into artificial subsurface lesion in bovine enamel. Dent Mater 2006;22:22-28.
18. Paes Leme AF, Tabchoury CPM, Zero DT, Cury JA. Effect of fluoridated dentifrice and acidulated phosphate fluoride application on early artificial carious lesions. Am J Dent 2003;16:91-95.
19. Papacchini F, Cury AH, Goracci C, Chieffi N, Tay FR, Polimeni A, Ferrari M. Noninvasive pit and fissure sealing: microtensile bond strength to intact bovine enamel of different pit and fissure sealants in a simplified fissure model. J Adhes Dent 2006;8:375-380.
20. Papacchini F, Goracci C, Sadek FT, Monticelli F, Garcia-Godoy F, Ferrari M. Microtensile bond strength to ground enamel by glass-ionomers, resin-modified glass-ionomers, and resin composites used as pit and fissure sealants. J Dent 2005;33:459-467.
21. PashLey DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono T, Fernandes CA, Tay F. The microtensile bond test: a review. J Adhes Dent 1999;1:299-309.
22. Petersson LG, Arthursson L, Ostberg C, Jönsson G, Gleerup A. Caries-inhibiting effects of different modes of Duraphat varnish reapplication: a 3-year radiographic study. Caries Res 1991;25:70-73.
23. Sakakai T, Fukushima T, Kawai S, Matsumoto M. Effect of physical properties of direct bonding adhesives on bonding to etched enamel. J Prosthet Dent 1994;71:522-529.
24. Seppä L, Leppänen T, Hausen H. Fluoride varnish versus acidulated phosphate fluoride gel: a 3-year clinical trial. Caries Res 1995;29:327-330.
25. Simonsen RJ. Pit and fissure sealant: review of the literature. Pediatr Dent 2002;24:393-414.
26. Tandon S, Mathew TA. Effect of acid-etching on fluoride-treated caries-like lesions on enamel: a SEM study. ASDC J Dent Child 1997;64:344-348.
27. ten Cate JM, Larsen MJ, Pearce EIF, Fejerskov O. Chemical interactions between the tooth and oral fluids. In Fejerskov O, Kidd E (edd). Dental varies. The disease and its clinical management. Ames: Blackwell Munksgaard, 2003:49-68.
28. Thornton JB, Retief DH, Bradley EL Jr, Denys FR. The effect of fluoride in phosphoric acid on enamel fluoride uptake and the tensile bond strength of an orthodontic bonding resin. Am J Orthod Dentofacial Orthop 1986; 90:91-101.
29. Vinnet D, Tandon S. Comparative evaluation of marginal integrity of two new fissure sealants using invasive and non-invasive techniques: a SEM study. J Clin Pediatr Dent 2000;24:291-298.

Clinical relevance: The success of sealants is dependent upon the material's retention, and the in vitro microtensile bond strength may be an indicator of this clinical ability.