



• U

C • FMUC

FACULDADE
DE MEDICINA
UNIVERSIDADE
DE COIMBRA

Integrated Master in Dentistry

^{99m}Tc in the evaluation of microleakage of composite resin restorations with Optibond™ XTR. *In vitro* study.

Ana Sofia Avidago Laranjo*

Advisor: Professor Eunice Virgínia Valdez Palmeirão Carrilho*

Co-advisor: Professor Manuel Marques Ferreira*

*Faculty of Medicine, University of Coimbra

Address: Av. Bissaya Barreto, Bloco de Celas, 3000-075 Coimbra, Portugal

Coimbra, June 2013

ABSTRACT

Introduction: OptiBond XTR™ recently introduced in the market is a new mild self-etch adhesive bonding agent which is claimed to feature an enhanced etching ability of the primer in order to the decrease of the pH.

Aim: Evaluate the microleakage of dental restorations using Optibond™ XTR. The null hypothesis was that the type adhesive system doesn't have influence in what concerns to microleakage.

Materials and methods: Thirty noncarious extracted human molars were selected and cut in two equal halves occlusogingivally. Class V cavities (4mm/3mm/3mm) were prepared on the buccal or lingual surfaces of each tooth with gingival margin walls in enamel. The specimens were randomly divided into 4 groups: 1- Optibond™ XTR was applied; 2 –Clearfill™ SE BOND was applied; 3 – the cavities weren't restored; 4 – Optibond™ XTR was applied. In groups 1, 2 and 4 the enamel was conditioned (37% orthophosphoric acid) before the adhesive application and restored with SonicFill™. The specimens were stored in distilled water (37°C, 7 days) and then went through thermocycling (500 cycles, 5°C and 55°C, dwell time 30"). Two coats of nail polish were applied to the external surface around of each restoration except to the negative control group, where the crowns were completely sealed. The specimens were submersed in a solution of ^{99m}Tc-Perchnetate. The radioactivity was counted 3 hours later. The nonparametric Kruskal-Wallis test, with Bonferroni correction at a significance level of 5%, was used for statistical analyses.

Results: Results showed that there weren't statistically significant differences ($p>0.05$) among the groups restored with Clearfill™ SE and Optibond™ XTR.

Conclusion: Based on the results of this study, the Optibond™ XTR doesn't reduce microleakage compared to Clearfill™ SE BOND.

Key-words: dental adhesion; self-etch adhesive; composite restorations; microleakage; polymerization shrinkage; thermocycling

INTRODUCTION

Resin composites were introduced in 1962 as a class of dental restorative materials¹ which are largely used in Dentistry. Despite their excellent aesthetics, composite restorations are subject to shrinkage which occurs during polymerization of all resin composites, affecting the physical properties of the composites and the marginal integrity of the restorations.² Several studies demonstrated that microleakage remains the major cause for composite restorations' failures implying postoperative sensitivity, margin colorations, secondary decay or pulpal inflammation.³⁻⁸ Microleakage could be defined as the passage of bacteria, fluids, molecules or ions between the cavity wall and the restorative material applied to it.^{9,10} Adhesives are necessary to prevent leakage on resin composite restorations while dental composites are not able to bond to dental tissues.¹⁰

Adhesion to tooth substrate is based on an exchange process involving replacement of minerals removed from the hard tissue by resin monomers that upon setting become micromechanically interlocked in the created porosities.^{3,11} This process involves two phases: one phase consists on the removal of calcium phosphates, by which microporosities are exposed in both enamel and dentine surfaces; the other, the so-called hybridization phase, involves infiltration and subsequent *in situ* polymerization of resin within these microporosities. The result is a micromechanical interlocking of the resin with the tooth structure.^{12,13}

In modern Dentistry the interaction of adhesives with the dental substrate is based on two different strategies, commonly described as an etch-and-rinse and a self-etch approach.

The majority of currently popular adhesive systems were developed under total etch technique.¹⁰ This approach, also known as etch-and-rinse and proposed by *Fusayama et al.* in 1972¹⁴, implies an acid-etching to enamel and dentine in order to remove the smear layer, open the dentinal tubules, and increase dentinal permeability.¹⁵ The following step consists of the application of a primer containing specific monomers with hydrophilic properties, such as 2-Hydroxyethyl methacrylate (HEMA), dissolved in organic solvents like acetone, ethanol or water.¹⁶ In a third step, a hydrophobic resin is applied and penetrates the collagen network exposed by the acid-etching procedure.¹⁶

Differently from their etch-and-rinse counterparts, self-etch adhesives do not require a separate etching step, as they contain acidic monomers that simultaneously etch and prime the dental substrate.¹⁶ Due to these acidic characteristics, self-etch adhesives are able to dissolve the smear layer and demineralize the underlying dentine/enamel.¹⁷

OptiBond XTR™ (Kerr, Orange, CA, USA) was recently introduced in the market. This is a light-cured, mild, self-etch adhesive bonding agent which is claimed to feature an enhanced etching ability of the primer. Such ability results from the rapid evaporation of acetone that concentrates water and GPDM monomers, thus lowering the pH from the initial value of 2.4 to 1.6.¹⁷

There are several methods by which microleakage can be studied, such as the use of radioactive isotopes. Technetium is an artificial element obtained by the radioactive decay of molybdenum. ^{99m}Tc decays with a half-life of 6 hours by isometric transition and emission of 140.5 keV of gamma radiation.¹⁸

The aim of the present study is to evaluate the microleakage of dental restorations using Optibond™ XTR. The null hypothesis was that the type adhesive system doesn't have influence in what concerns to microleakage.

METHOD AND MATERIALS

Thirty non-carious extracted human molars were collected and stored in normal saline solution 0,9% (B. Braun, 11496403, Queluz de Baixo, Barcarena) at 5°C no more than 6 months. After this, all teeth were sectioned in occluso-cervical direction with a saw of the Exakt System 300 (Exakt System, 22851 Norderstedt, Germany) in two equal halves. Class V cavities were prepared on the buccal or lingual surfaces of each specimen with gingival margin walls in enamel and with standard dimensions. A transparent resin mold was performed to design the cavities with these dimensions: 4 mm mesiodistally and 3 mm occlusogingivally. The preparations were done with burs (Proclinic, 51/09, Nyon, Swiss) maintaining an internal angle of 90 degrees in order to create margins walls of approximately 2 mm. All of the margins were stated in enamel.(Figure 1)

The sixty specimens were divided into four groups: twenty for each study group (group 1 and 2) and ten for each control group (group 3 and 4).

Group 1: In this study group the cavities were restored with adhesive system Optibond™ XTR (Kerr, 35122, Orange, CA, USA) (Figure 5). Only the enamel was conditioned during 30 seconds with 37% orthophosphoric acid gel Octacid Jumbo (Clarben, T012RD, Lindigo, Sweden) (Figure 2) and after this period was washed with an air/water jet per 30 seconds, too. After this, OptiBond™ XTR primer was applied to enamel/dentin using scrubbing motion during 20 seconds and dried with medium air pressure for 5 seconds. The OptiBond™ XTR adhesive was applied (Figure 3) with a microbrush to enamel/dentine surface using light brushing motion during 15 seconds, dried with medium air pressure and then strong air for at least 5 seconds, and finally light cured during 10 seconds using light cure BluePhase™ G2 (Ivoclar Vivadent, 5VDC, Liechtenstein, Austria) (Figure 4). A SonicFill™ System (Kerr/Kavo, 3691651, Bismarckring, Biberach) was activated and the Sonic Fill™ composite shade (A2) was placed in one bulk increment followed by shaping the buccal surface and light cured for 20 seconds using light cure BluePhase™ G2 (Ivoclar Vivadent, 5VDC, Liechtenstein, Austria). Restorations were polished using Sof-Lex Disk System. (Brown/Orange/Light Orange/Yellow, 3M ESPE, N301289, St. Paul, MN, USA)

Group 2: In this study group the cavities were restored with adhesive system Clearfil™ SE BOND (Kuraray, 041872, Okayama, Japan)(Figure 6). Only the enamel was conditioned during 30 seconds with 37% orthophosphoric acid gel (Figure 2) and after this period was washed with an air/water jet per 30 seconds, too. After this Clearfil™ SE BOND primer was applied to enamel/dentin using scrubbing motion and left for 20

seconds, and dried thoroughly with mild air flow. Clearfil™ SE BOND bond was applied to enamel/dentin surface using light brushing motion (Figure 3), dried with air flow gently and light cured during 10 seconds using light cure BluePhase™ G2 (Figure 4). A SonicFill™ System was activated and the Sonic Fill™ composite shade (A2) was placed in one bulk increment followed by shaping the buccal surface and light cured for 20 seconds using light cure BluePhase™ G2. Restorations were polished using Sof-Lex Disk System.

Group 3: In this control group (positive control) the class V cavities of 10 specimens weren't restored.

Group 4: In this control group (negative control) the cavities were restored with adhesive system OptiBond™ XTR. Only the enamel was conditioned during 30 seconds with 37% orthophosphoric acid gel (Figure 2) and after this period was washed with an air/water jet per 30 seconds, too. After this, OptiBond™ XTR primer was applied to enamel/dentin using scrubbing motion during 20 seconds and dried with medium air pressure for 5 seconds. The OptiBond™ XTR adhesive was applied with a microbrush to enamel/dentine surface using light brushing motion during 15 seconds (Figure 3), dried with medium air pressure and then strong air for at least 5 seconds, and finally light cured during 10 seconds using light cure BluePhase™ G2. A SonicFill™ System was activated and the Sonic Fill™ composite shade (A2) was placed in one bulk increment followed by shaping the buccal surface and light cured for 20 seconds using light cure BluePhase™ G2 (Figure 4). Restorations were polished using Sof-Lex Disk System.

Table I. Materials used in the restorations

Material	Lot no.	Manufacture
OptiBond™ XTR	35122	Kerr
Clearfil SE Bond	41872	Kuraray
SonicFill™	3691651	Kerr/Kavo



Figure 1. Class V cavity on the buccal surface



Figure 2. Enamel conditioning with 37% orthophosphoric acid gel



Figure 3. Application of a self-etch adhesive system



Figure 4. Polymerization of the adhesive



Figure 5. The self-etch adhesive OptiBond™ XTR



Figure 6. The self-etch adhesive Clearfil™ SE Bond

All restorative procedures were performed by the same operator and all the specimens were stored in distilled water at 37°C for a period of 7 days. Afterwards they went through thermocycling 500 cycles between 5°C and 55°C with a dwell time of 30 seconds. Two coats of red nail polish (Resist and Shine L'Oréal, 16G901, Paris, France) were applied to the external surface around of each restoration of groups 1, 2 and 3 with 2 mm of margins (Figures 7, 8 and 9). The negative control group (group 4) was completely sealed (Figure 10). The specimens were submersed in a solution of ^{99m}Tc-Perchnetate for 3 hours (Figure 11). After this period all varnish was removed with a bisturi (Figure 12) and the radioactivity was counted by a gamma camera.



Figure 7. Application of two coats of red nail polish around restorations of group 1



Figure 8. Application of two coats of red nail polish around restorations of group 2



Figure 9. Application of two coats of red nail polish around restorations of group 3



Figure 10. Application of two coats of red nail polish covering all the crowns of group 4

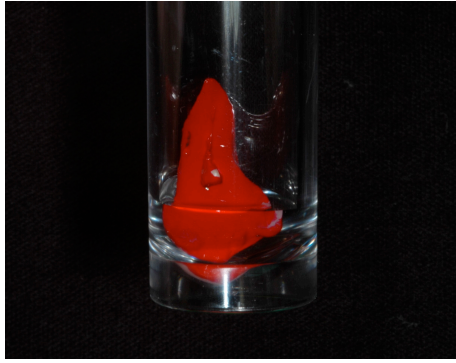


Figure 11. Immersion in a solution of ^{99m}Tc -Pertechnetate for 3 hours

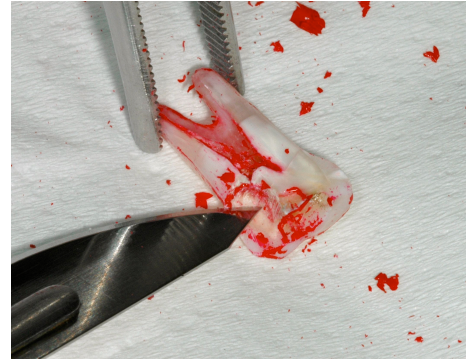


Figure 12. Preparation of the specimens for further evaluation in the gamma camera

The statistical analysis was made by using the program SPSS 20 and comparisons were done by nonparametric Kruskal-Wallis test with Bonferroni correction at a significance level of 5%.

RESULTS

In the present study 60 specimen were used, assigned to group 1 (n = 20), group 2 (n = 20), positive control group 3 (n = 10) and negative group 4 (n = 10). Four different values were found: average counts, mid counts, maximum counts and pixels. After acquiring these values the comparison between groups was made by using the mid counts of each tooth (Table II and Figure 13).

Table II. Statistical analysis of mid counts

Groups	Median	Interquartile range	Minimum	Maximum
Group 1 (n=20)	0,066	0,0202	0,0635	0,030
Group 2 (n=20)	0,077	0,0156	0,077	0,017
Group 3 (n=10)	0,313	0,0933	0,349	0,131
Group 4 (n=10)	0,035	0,0077	0,0355	0,014

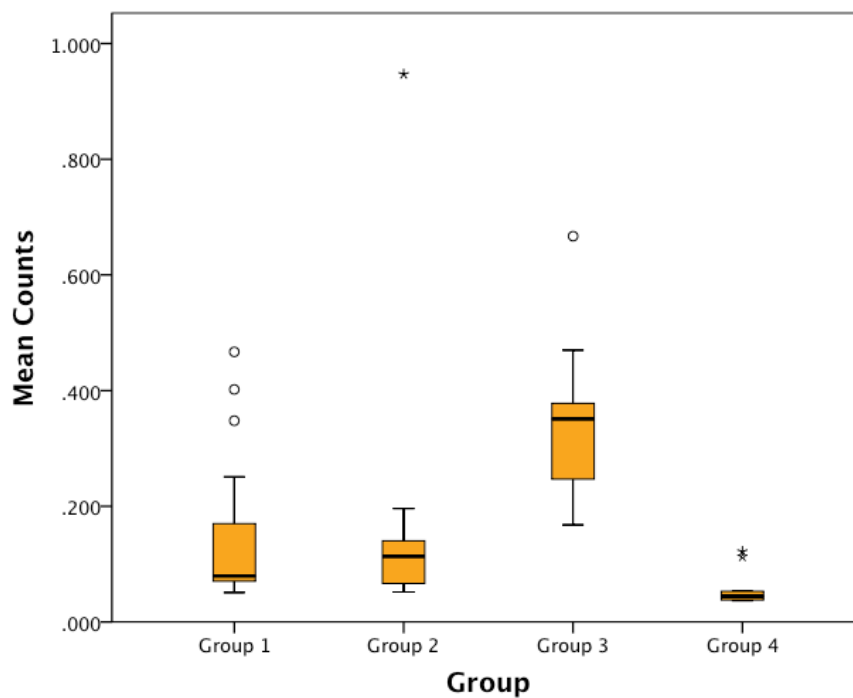


Figure 13. Statistical analysis of microleakage of each group

First of all a test of the normal distribution of the values and of the homogeneity of their variances was made for this multiple group comparison in order to choose the statistical test. As there was no normality between the values a nonparametric test was selected and comparisons were done by nonparametric Kruskal-Wallis test with Bonferroni correction at a significance level of 5%. We established the level of statistical significance at $p < 0.05$. If $p < 0.05$ it indicates significant difference and $p < 0.001$ indicates highly significant difference among groups.

Data analysis showed that there were statistically significant differences between the experimental groups (G1 and G2) and the control groups (G3 and G4) (Table III). Regarding the comparison of scores obtained by experimental groups (G1 and G2) results showed that there were no statistically significant differences ($P > 0.05$) among the experimental groups restored with Clearfill™ SE BOND and Optibond™ XTR with respect to microleakage scores (Table III). However, after meticulous analysis, it was found that Optibond™ XTR exhibited less microleakage compared to Clearfill™ SE BOND (Table II). Apart from these differences, highly significant difference was observed between negative and positive control groups (G3 and G4) which presented a p-value less than 0.001 (Table III).

Table III. Comparisons between groups done by nonparametric Kruskal-Wallis test with Bonferroni correction at a significance level of 5%

Groups	p-value
G1 vs. G2	1,000
G1 vs. G3	0,008
G1 vs. G4	0,025
G2 vs. G3	0,006
G2 vs. G4	0,033
G3 vs. G4	<0,001

DISCUSSION

“Minimally-invasive Dentistry” is a modern approach focus on the achievement of a more conservative cavity design, but providing sufficient access for the complete removal of the carious tissue.¹⁵ The restorative procedure is based on the bonding effectiveness of adhesive materials with no need to remove dental structure for additional mechanical retention.¹⁵ The bond strength and the clinical longevity of these adhesive restorations depend on: the heterogeneity of tooth structure and composition, the hydrophilicity of the exposed dentine surface, the features of the dental substrate after cavity preparation¹⁹⁻²¹ and the characteristics of the adhesive itself as to its physicochemical properties and its strategy of interaction with dental tissues.^{22,23}

The fundamental mechanism of bonding to enamel and dentine is essentially based on an exchange process where the minerals removed from the dental hard tissues are replaced by resin monomers that upon polymerization become micro-mechanically interlocked in the created porosities.^{19,24}

On enamel, acid-etching can significantly enhance bonding of restorations to this tissue.^{17,25} This technique selectively dissolves enamel crystals in the prism structure, which results in a roughened surface that will allow monomer diffusion by capillary attraction^{15,26} producing micro-mechanical interlock within this etched structure.^{27,28} The etching approach still provides the best achievable bond to the dental substrate which not only seals the restoration margins in long term, but also protects the more vulnerable bond to dentine against degradation.²⁹

While adhesion to enamel is effectively achieved with this hybrid interlocking, adhesion to dentine was not reliable.¹² The main hindrance is the heterogeneous nature of this substrate, with hydroxyapatite deposited on a mesh of collagen fibers.²⁴ In addition, dentine is intimately connected with pulpal tissue by means of numerous fluid-filled tubules which render this exposed surface naturally moist and thus intrinsically hydrophilic.^{30,31} This hydrophilicity definitely represents one of the major challenges for the interaction of modern adhesives with dentine. The presence of cutting debris on instrumented dental surfaces in the form of smear layer and smear plugs that obstruct the dentine tubules is also a primary co-factor that may not be underestimated.^{31,32}

One of the strategies of bonding dental substrate is the self-etch approach which is able to dissolve the smear layer and demineralize the underlying dentine or/and enamel.³³ There are currently 4 different types of SE adhesives based on its acidity or etching aggressiveness: the ultra-mild SE (pH about 2.5), the mild SE (pH about 2), the intermediary strong SE (pH about 1.5), and the strong SE (pH < 1).^(8, 34-36) The strong SE

produces a deep demineralization similar to etch-and-rinse approach but the products originated are not rinsed away.¹⁵ On the other hand, mild and ultra-mild self-etch adhesives only partially demineralize the dentine, leaving a substantial amount of hydroxyapatite crystals around the collagen fibrils.¹⁵ That remains available within the submicronscale hybrid layers^{8,37,38} for possible additional chemical interaction³⁹ which may contribute significantly to the stabilization of the adhesive interface over time.⁸ This chemical bonding is due to the presence of specific functional monomers in the composition of this adhesives, such as 10-MDP, 4-META and phenyl-P.^{8,37} These monomers contain carboxylic and phosphate groups are able to ionically link to calcium in hydroxyapatite.⁴⁰ Some studies showed that 10-MDP is more effective and also more stable in an aqueous environment than 4-MET and phenyl-P.^{37,41}

Self-etch adhesives have the advantage of demineralizing and infiltrating the tooth surface simultaneously to the same depth, ensuring complete penetration of the adhesives. In order to this propriety, this approach has been claimed to be more user-friendly and less technique-sensitive resulting in a clinically reliable performance.⁴² In this study we used two different self-etch adhesives: Clearfill™ SE BOND and Optibond™ XTR. Clearfil SE Bond is a mild, two-step self-etch adhesive system with a pH of 2.0 and its bonding ability has been validated by several *in-vitro* studies^{8,15,38,43}. In particular, its improved ability to bond to dentine *in vitro* has been related to the presence of 10-MDP, which is able to react with residual hydroxyapatite within the hybrid layer.^{8,37} Optibond™ XTR is a new self-etch adhesive introduced in April 2011 which is claimed to feature an enhanced etching ability of the primer.¹⁷ According to the manufacturer, the pH of OptiBond™ XTR primer is 2.4 until it is dispensed. Acetone rapidly evaporates from the material, increasing the concentration of glycerol phosphate dimethacrylate and thereby reducing pH to 1.6.⁴⁴ Few previous studies have investigated the performance of this adhesive. *Walter et al.* reported that the mean shear bond strength of Optibond XTR™ to bovine dentine at 24 hours was similar to that of Clearfil™ SE Bond.³⁸

Although adhesive restorations tend to fulfill the main requirements of a more conservative and aesthetic treatment, their clinical longevity is still a topical issue, mainly due to the degradation of the adhesive interface over time.¹² The main cause of failure of composite fillings is related to the occurrence of marginal leakage, which eventually leads to marginal discoloration, secondary caries, and subsequent loss of retention.^{25,45}

The polymerization shrinkage of resin-based restorative materials produces stress at the adhesive interface, which could lead to bonding failure with gap formation. The stress generated could reach up to 10 MPA, leading to a marginal breakdown.⁴⁶

Microleakage evaluation is the most common method of assessing the sealing efficiency of a restorative material.⁴⁷ There are several methods by which microleakage can be

studied such as the use of dyes, chemical tracers, radioactive isotopes, artificial caries, scanning electron microscopy, neutron activation analysis, and electrical conductivity.⁴⁸

In this study we used radioactive isotopes which permits detection of minute amount of leakage and also have the advantage over dyes as tracers, for their presence can be readily detected in very small concentrations.^{49,50} The use of ^{99m}Tc radionuclide is a qualitative and nondestructive method, enables to measure the microleakage from the same specimen at intervals over extended periods, without destroying the sample.⁵¹

The aim of this study is the evaluation of microleakage of dental restorations with SonicFill™ using Optibond™ XTR compared to the universal self-etch adhesive Clearfill™ SE BOND. Thirty non-carious extracted human molars were selected and sectioned in two equal halves occlusogingivally. Class V cavities were prepared on the buccal or lingual surfaces of each specimen with gingival margin walls in enamel and with standard dimensions. This type of preparation was done in order to involve two dental hard structures, enamel and dentine, and to create very similar cavities where the variety of anatomy is not a relevant problem, as in the occlusal surface of the molars. This patronization allowed a more reliable comparison between the groups and made this microleakage study of adhesive systems more relevant.

Restorative procedures were performed by the use of two self-etch adhesives with no acid conditioning of the dentin. However the enamel was pre conditioned with 37% orthophosphoric acid gel with the use of both systems. The bonding capacity of self-etch systems to enamel still remains critical and some authors suggest that self-etch adhesives have poor adhesion to the enamel tissue.^{36,42,52-55} Typically a separate phosphoric acid-etching of unground enamel surfaces is recommended.^{12,56} 30–37.5% phosphoric acid is now apparently preferred because of its known advantage in efficiently etching this dental substrate.²⁵ According to Cardoso *et al.* selective etching of enamel followed by the application of the self-etch adhesive to both (etched) enamel and (non-etched) dentine may represent the best option to effectively and durably bond to the dental substrate.¹⁵ Although a preliminary phosphoric acid etching of the enamel was found to be beneficial⁵⁷, Van Meerbeek *et al.* recommend that phosphoric acid should not be extended to dentine, as self-etch adhesives bond suboptimally to deeply demineralized dentine.⁸

The greatest problem of direct resin composite restorations is the polymerization shrinkage of the resin materials⁵⁸ which may result in a marginal breakdown. According to Idriss *et al.* and Ben Amar *et al.* the choice of material and placement technique are important determining factors in microleakage.^{59,60} Since difficulties imposed by the cavity configuration (C-factor) play an important role in stress development, many researchers have suggested the use of “incremental layering techniques” for resin-composite

restorations to reduce the polymerization shrinkage stress and cuspal deflection.⁶¹⁻⁶⁵ Park *et al.* concluded that the bulk filling technique yielded significantly more cuspal deflection than the incremental filling techniques and suggested that the cuspal deflection resulting from polymerization shrinkage can be reduced by incremental filling techniques to obtain optimal outcomes in clinical situations.⁶³ Moreover Lee *et al.* observed that cuspal deflection increased with increasing cavity dimension and C-factor, defending the use of incremental filling technique. However the literature is not conclusive about the advantage of the incremental layering technique over the polymerization shrinkage.⁶⁵ According to Loguercio *et al.*, some evaluated effects of polymerization shrinkage such as gap width, adhesive bond, strength and the cohesive strength of the resin composite were not reduced by the filling technique under the different C-factor cavities.⁶⁶ In addition, Versluis *et al.* concluded that the incremental filling technique increased the deformation of the restored tooth and could produce higher polymerization stresses at the restoration interface compared to bulk filling.⁶⁷

Another important determining factor is the type of resin composite used in adhesive restorations. All the restorative procedures of groups 1,2 and 4 are done with the resource of a new flowable composite SonicFill™ introduced in the dental market in 2010.⁶⁸ SonicFill™ incorporates a nano-hybrid resin with special modifiers that react to sonic energy. This sonic energy is applied with a particular handpiece and induces a decrease of 87% of the viscosity. This decrease of the viscosity of the composite enables the quick placement and precise adaptation to the cavity walls. When the sonic energy is stopped, the composite returns to a more viscous, non-slumping state that is perfect for carving and contouring.⁶⁸ According to Behle and Bayne *et al.*, one important property of a flowable resin composite is related to its elastic modulus, which is significantly lower than that of traditional hybrid composites (30 to 50%).^{69,70} A lower elastic modulus decreases the polymerization shrinkage of restorative composite resins, preserving the hybrid layer and avoiding marginal gap formation.⁷¹

Conversely, some studies have shown higher shrinkage for flowable composites,^{72,73} indicating a potential for higher interfacial stresses with the same behavior of hybrid restorative composite resins.⁷³

Another advantage of this composite is the rapid placement through a single increment up to 5mm due to reduced polymerization shrinkage, thereby reducing working time.⁶⁸ According to Frankenberger, SonicFill showed comparable results to conventional resin composites when placed in a first increment of up to 5 mm.⁷⁴ Thompson in a study concluded that the volumetric shrinkage of SonicFill is significantly less than most traditional composite materials.⁷⁵

In this study the cavity preparation and all restorative procedures were done by the same operator as the tests of each sample group in order to minimize clinician influence as some authors recommended.^{76,77} After that all the specimens were stored in distilled water at 37°C for a period of 7 days to simulate the corporal temperature. Then they went through thermocycling 500 cycles between 5°C and 55°C with a dwell time of 30 seconds. It is important that *in vitro* testing of adhesive restorations tries to simulate the oral environment and tests that involve accelerated aging through water storage, thermocycling and cyclic loading have been developed.⁷⁸⁻⁸¹

Thermocycling is a stress test comprising cyclic thermal fluctuations. Basically it is *the in vitro* process of subjecting the restoration on the tooth to temperature extremes compatible with the oral cavity. This simulates the introduction of hot and cold extremes in the oral cavity and shows the relationship between the coefficients of thermal expansion of tooth and restorative material.⁸² Due to differences in the thermal expansion coefficients of composite and tooth substrate, thermal stress is the highest at the adhesive interface.⁷⁶ The thermocycling regimens vary between studies with respect to the number of cycles, temperature and dwell time. The number of cycles used in the present study was 500 (5°C to 55°C with a dwell time of 30 seconds. Radovic I, Vulicevic ZR, Godoy GF also used 500 cycles as it was based on the current ISO standard.⁷⁶ However some authors reported that that number of cycles is probably too low to achieve a realistic ageing effect as Gale and Darvell (1999).⁸³ Recent studies have used various number of thermocycles: approximately 1500 cycles between 10°C and 50°C after 3 months of storage (Trites *et al.*, 2004), 500 cycles between 5 and 55°C (Bishara *et al.*, 2007), and 6000 cycles between 5 and 55°C (Faltermeier *et al.*, 2007).⁸³

In order to prevent the infiltration of the isotope, two coats of red nail polish were applied to the external surface around each restoration with 2 mm of margins, except to the negative control group where all the surfaces were sealed. The specimens were immersed in a solution of ^{99m}Tc-Perchnetate for 3 hours. After this period the specimen were washed with water and dried on absorbent paper. Then, the varnish was removed with a bisturi which influenced loss of tooth structure upon removal. Finally a gamma camera counted the radioactivity. The radioisotope infiltrated between the gaps created at the adhesive interface as a result of the polymerization shrinkage. That was measured by the gamma camera based on the radioactivity of the ^{99m}Tc-Perchnetate.

Based on the results of this *in vitro* study both adhesive systems showed microleakage. There were no statistically significant differences ($p > 0.05$) among the experimental groups restored with Clearfill™ SE BOND and Optibond™ XTR. Also in a study performed by Marchesi *et al.* they concluded that Optibond™ XTR and Clearfil™ SE Bond yielded similar

results.³⁸ However, after meticulous analysis, it was found that Optibond™ XTR exhibited less microleakage compared to Clearfill™ SE BOND.

There were significant differences between the study groups and the control groups, which mean that both adhesives were effective. We only found highly significant differences between control groups ($p < 0.001$). The negative control group showed significantly lower values of infiltration, demonstrating that the two varnish layers were effective in sealing the surfaces, thus preventing side microleakage. As expected, more microleakage occurred in the positive control group where the cavities weren't restored.

In the future more studies must be done to evaluate the microleakage of restorations using Optibond™ XTR as adhesive system. Whereby the use of ^{99m}Tc radionuclide is a nondestructive method of assess microleakage without destruction of the sample, the specimen could be taken over another microleakage such as scanning electron microscopy. This would be an interesting comparison between both methods. Another suggestion could be eventually an increase of the number of thermocycles as some authors have already suggested. In addition another test group with no acid conditioning of enamel should be added to the study in order to evaluate how this procedure can be dismissed when Optibond™ XTR is used.

CONCLUSION

Based on this microleakage study, it can be concluded that Optibond™ XTR does not reduce microleakage when compared to the universal self-etch adhesive, Clearfil™ SE BOND. The performance of OptiBond™ XTR should be further evaluated with long-term studies.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Eunice Virgínia Valdez Palmeirão Carrilho, my advisor, for her professional guidance and valuable support, encouragement and for making this project possible and to my co-advisor, Professor Manuel Marques Ferreira for his kindness and constructive suggestions during the planning and development of this research work. Thank you very much!

I must acknowledge Dr. Ana Margarida Coelho Abrantes and Cláudia Brites for their availability and help with the laboratory process. In addition, I would like to thank Dr. João Eduardo Lopes Casalta for his help in doing the statistical analysis.

My grateful thanks are also extended to Kerr, Meodontal and 3M for offering me the materials for my research project. Without the support of these companies this in vitro study would not be possible.

A very special thanks goes to Dr. Helga Sousa for her availability and promptness demonstrated in the language correction of this study.

REFERENCES

1. Zimmerli B, Strub M, Jeger F, Stadler O, Lussi A. Composite materials: Composition, properties and clinical applications. *Schweiz Monatsschr Zahnmed.* 2010; 120:972-979
2. Caldwell R, Kulkarni G, Titley K. Does Single Versus Stepped Curing of Composite Resins Affect Their Shear Bond Strength? *J Can Dent Assoc* 2001; 67(10):588-592
3. Geerts S, Bolette A, Seidel L, Guéders A. An In Vitro Evaluation of Leakage of Two Etch and Rinse and Two Self-Etch Adhesives after Thermocycling. *International Journal of Dentistry.* 2012; 852841.
4. Siso HS, Kustarci A, Goktolga EG. Microleakage in resin composite restorations after antimicrobial pre-treatments: effect of KTP laser, chlorhexidine gluconate and clearfil protect bond. *Operative Dentistry.* 2009; 34:321-327.
5. Perdigao J, Swift EJ Jr. Fundamental concepts of enamel and dentin adhesion. In: Roberson TM, Heymann HO, Swift EJ Jr, editors. *Sturdevant's Art & Science of Operative Dentistry.* 5th ed. St. Louis: Mosby; 2006. p. 262–264.
6. Duarte S Jr, Dinelli W, Carmona Da Silva MH. Influence of resin composite insertion technique in preparations with a high C-factor. *Quintessence International.* 2007; 38 (10): 829–835.
7. Ansari ZJ, Sadr A, Moezizadeh M *et al.* Effects of one-year storage in water on bond strength of self-etching adhesives to enamel and dentin. *Dental Materials Journal* 2008; 27(2): 266–272.
8. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A. State of the art of self-etch adhesives. *Dental Materials.* 2011; 27: 17–28.
9. Waldman GL, Vaidyanathan TK, Vaidyanathan J. Microleakage and Resin-to-Dentin Interface Morphology of Pre-Etching versus Self-Etching Adhesive Systems. *The Open Dentistry Journal*, 2008; 2: 120-125.
10. Fusayma T, Nakamura M, Kurosaki N, Iwaku M. Non-pressure adhesion of a new adhesive restorative resin. *J Dent Res* 1979; 58:1364-1370.
11. Van der Vyver PJ, Wet FA. The current stage of dentine bonding systems a review of materials and techniques. *SADJ* 2000; 55(9): 457-85.
12. Ozer F, Blatz MB. Self-Etch and Etch-and-Rinse Adhesive Systems in Clinical Dentistry. *Compend Contin Educ Dent.* 2013 Jan;34(1):12-14, 16, 18.
13. Summit JB, Robbins JW, Hilton TJ, Schwarz RS. *Fundamentals of Operative Dentistry.* 3rd ed. Hanover Park, IL: Quintessence Publishing; 2006. p. 183-242.
14. Arias VG, Campos IT, Pimenta LAF. Microleakage Study of Three Adhesive Systems. *Ann Stomatol (Roma).* 2012; 3(1): 19–23.

15. Cardoso MV, Almeida Neves A, Mine A, Coutinho EV, Van Landuyt K, De Munck J, Van Meerbeek B. Current aspects on bonding effectiveness and stability in adhesive dentistry. *Australian Dental Journal* 2011; 56(1): 31–44.
16. Tay FR, Sano H, Carvalho R, Pashley EL, Pashley DH. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. *J Adhes Dent* 2000; 2:83–98.
17. Rengo C, Goracci C, Juloski J, Chieff N, Giovannetti A, Vichi A, Ferrari M. Influence of phosphoric acid etching on microleakage of a self-etch adhesive and a self-adhering composite. *Australian Dental Journal* 2012; 57: 220–226.
18. Mazzi U. Technetium in Medicine. In: Zolle I, editor. *Technetium-99m pharmaceuticals: preparation and quality control in nuclear medicine*. 1st ed. Berlin: Springer; 2007: p.95.
19. Van Meerbeek B, De Munck J, Mattar D, Van Landuyt K, Lambrechts P. Microtensile bond strengths of an etch and rinse and self-etch adhesive to enamel and dentin as a function of surface treatment. *Oper Dent* 2003; 28: 647–660.
20. Cardoso MV, Coutinho E, Ermis RB, et al. Influence of dentin cavity surface finishing on micro-tensile bond strength of adhesives. *Dent Mater* 2008; 24: 492–501.
21. Neves AA, Coutinho E, Cardoso MV, Lambrechts P, Van Meerbeek B. Current concepts and techniques for caries excavation and adhesion to residual dentin. *J Adhes Dent* 2011; 3(1):7-22.
22. De Munck J, Van Landuyt K, Peumans M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. *J Dent Res* 2005; 84: 118–132.
23. Van Landuyt KL, Snauwaert J, De Munck J, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials* 2007; 28:3757–3785.
24. Van Meerbeek B, Yoshida Y, Van Landuyt K, et al. In: Summitt JB, Robbins JW, Hilton TJ, Schwartz RS, eds. *Fundamentals of Operative Dentistry. A Contemporary Approach*. 3rd ed. Chicago: Quintessence Publishing, 2006:183–260.
25. Vaidyanathan TK, Vaidyanathan J. Recent Advances in the Theory and Mechanism of Adhesive Resin Bonding to Dentin: A Critical Review. *Journal of Biomedical Materials Research Part B: Applied Biomaterial* 2008; 88(2): 558–578.
26. Gwinnett AJ, Matsui A. A study of enamel adhesives. The physical relationship between enamel and adhesive. *Arch Oral Biol* 1967; 12:1615–1620.
27. Miyasaki M, Hirohata N. Influence of self-etching primer drying team on enamel bond strength of resin composites. *J Dent* 1999; 27: 203-207.
28. Borges MAP, Matos IC, Dias KRHC. Influence of Two Self-Etching Primer Systems on Enamel Adhesion. *Braz Dent J* 2007; 18(2): 113-118.

29. De Munck J, Van Meerbeek B, Yoshida Y, et al. Four-year water degradation of total-etch adhesives bonded to dentin. *J Dent Res* 2003; 82: 136–140.
30. Cardoso MV, Moretto SG, Carvalho RCR, Russo EMA. Influence of intrapulpal pressure simulation on the bond strength of adhesive systems to dentin. *Braz Oral Res* 2008; 22: 170–175.
31. Pashley DH. Smear layer: an overview of structure and function. *Proc Finn Dent Soc* 1992; 88: 215–224.
32. Ermis RB, De Munck J, Cardoso MV, et al. Bond strength of self-etch adhesives to dentin prepared with three different diamond burs. *Dent Mater* 2008; 24: 978–985.
33. Tay FR, Sano H, Carvalho R, Pashley EL, Pashley DH. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. *J Adhes Dent* 2000; 2: 83–98.
34. Van Meerbeek B, Vargas M, Inoue S et al. Adhesives and cements to promote preservation dentistry. *Operative Dentistry* 2001; 6: 119-144.
35. Van Meerbeek B, Munck J, Yoshida Y et al. Buonocore Memorial Lecture-adhesion to enamel and dentin: current status and future challenges. *Operative Dentistry* 2003; 28 (3): 215–235.
36. Van Meerbeek B, Peumans M, Poitevin A et al. Relationship between bond-strength tests and clinical outcomes. *Dental Materials* 2010; 26(2): 100–121.
37. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Oka- Zaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van MeerBeek B. Comparative study on adhesive performance of functional monomers. *J Dent Res* 2004; 83: 454–458.
38. Marchesi G, Frassetto A, Visintini E, Diolosa M, Turco G, Salgarello S, Di Lenarda R, Cadenaro M, Breschi L. Influence of ageing on self-etch adhesives: one-step vs. two-step systems. *Eur J Oral Sci* 2013; 121: 43–49.
39. De Munck J, Van Meerbeek B, Vargas M, et al. One day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. *Oper Dent* 2005;30: 39–49.
40. Yoshida Y, Van Meerbeek B, Nakayama Y, et al. Evidence of chemical bonding at biomaterial-hard tissue interfaces. *J Dent Res* 2000; 79: 709–714.
41. Sano H, Yoshikawa T, Pereira PN, et al. Long-term durability of dentin bonds made with a self-etching primer, in vivo. *J Dent Res* 1999; 78: 906–911.
42. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. *Dent Mater* 2005; 21: 864–881.
43. Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. *Dent Mater* 2010; 26: 78–93.

44. Walter R, Swift EJ, Boushell LW, Braswell K. Enamel and dentin bond strengths of a new self-etch adhesive system. *J Esthet Restor Dent* 2011; 23: 390–398.
45. Hegde MN, Vyapaka P, Shetty S. A comparative evaluation of microleakage of three different newer direct composite resins using a self etching primer in class V cavities: An in vitro study. *J Conserv Dent*. 2009; 12(4): 160–163.
46. Duquia C, Osinaga PW, Demarco FF, de V Habekost L, Conceição EN. Cervical microleakage in MOD restorations: In vitro comparison of indirect and direct composite. *Oper Dent*. 2006; 31: 682–7.
47. Yamazaki PC, Bedran-Russo AK, Pereira PN, Swift EJ Jr. Microleakage evaluation of a new low-shrinkage composite restorative material. *Oper Dent*. 2006; 31-6:670–676.
48. Gogna R, Jagadis S, Shashikal K. A comparative in vitro study of microleakage by a radioactive isotope and compressive strength of three nanofilled composite resin restorations. *J Conserv Dent*. 2011; 1.4(2) :128-131.
49. Alain AH, Toh CG. Detection of microleakage around dental restorations: a review. *Oper Dent* 1997; 22:173-85.
50. Prasad KBS, Sudhakaran S. Radioactive isotope evaluation of coronal leakage after endodontic treatment in teeth restored with three different intracoronar restorative materials: an in vitro study. *World J Dentistry* 2011; 2(1): 35-38.
51. Ferreira MM, Abrantes M, Carrilho EV, Botelho MF. Quantitative scintigraphic analysis of the apical seal in Thermanfil/Topseal and RealSeal /Realseal filled root canals. *World J Stomatol* 2013; 2(2): 30-34.
52. Apap M. Adhesifs auto-mordancants: un bon plan? *L'Information Dentaire* 2007; 43: 2804–2816.
53. Degrange M. Les systèmes adhésifs amélo-dentinaires. *Réalités Cliniques* 2005; 16 (4): 327-348.
54. Perdigao J, Monteiro P, Gomes G. In vitro enamel sealing of self-etch adhesives. *Quintessence International* 2009; 40(3): 225–233.
55. Van Landuyt KL, Kanumilli P, Munck J, Peumans M, Lambrechts P, Van Meerbeek B. Bond strength of a mild self-etch adhesive with and without prior acid-etching. *Journal of Dentistry* 2006; 34(1): 77-85.
56. Manuja N, Nagpai R, Pandit IK. Dental adhesion: mechanism, techniques, and durability. *J Clin Pediatr Dent* 2012; 36(3): 223-234.
57. Taschner M, Nato F, Mazzoni A, et al. Role of preliminary etching for one-step self-etch adhesives. *Eur J Oral Sci* 2010;118: 517–524.
58. Mousavinasab SM, Khosravi K, Tayebghasemi N. Microleakage Assessment of Class V Composite Restorations Rebonded with Three Different Methods. *Dent Res J* 2008; 5 (1):21-26.

59. Idriss S, Abduljabbar T, Habib C, Omar R. Factors associated with microleakage in Class II resin composite restorations. *Oper Dent* 2007; 32(1): 60-6.
60. Ben Amar A, Slutzky H, Matalon S. The influence of 2 condensation techniques on the marginal seal of packable resin composite restorations. *Quintessence Int* 2007; 38(5): 423-8.
61. Davidson CL, Feilzer AJ. Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. *Journal of Dentistry* 1997; 25(6): 435-440.
62. Lutz E, Krejci I, Oldenburg TR. Elimination of polymerization stresses at the margins of posterior composite resin restorations: a new restorative technique. *Quintessence International* 1986; 17(12): 777-784.
63. Park J, Chang J, Ferracane J, Lee IB. How should composite be layered to reduce shrinkage stress: incremental or bulk filling? *Dental Materials* 2008; 24(11): 1501-1505.
64. Suliman AA, Boyer DB, Lakes RS. Cusp movement in premolars resulting from composite polymerization shrinkage. *Dental Materials* 1993; 9(1): 6-10.
65. Lee MR, Cho BH, Son HH, Um CM, Lee IB. Influence of cavity dimension and restoration methods on the cusp deflection of premolars in composite restoration. *Dental Materials* 2007; 23(3): 288-295.
66. Loguercio AD, Reis A, Ballester RY. Polymerization shrinkage: effects of constraint and filling technique in composite restorations. *Dental Materials* 2004; 20(3): 236-243.
67. Versluis A, Douglas WH, Cross M, Sakaguchi RL. Does an incremental filling technique reduce polymerization shrinkage stresses? *Journal of Dental Research* 1996; 75(3): 871-878.
68. Sybron Dental Specialties Inc. SonicFill™ System [Internet]. Kerr; 2011. [access 2012 jan 20]. Available from: www.sonicfill.eu/
69. Behle C. Flowable composites: properties and applications. *Pract Period Aesthetic Dent* 1998; 10: 347-351.
70. Bayne SC, Thompson JY, Swift Jr EJ, Stamadiades P, Wilkerson JA. A characterization of first-generation flowable composites. *J Am Dent Assoc* 1998; 129: 567-577.
71. De Goes MF, Giannini M, Di Hipólito V, Carrilho MRO, Daronch M, Rueggeberg FA. Microtensile Bond Strength of Adhesive Systems to Dentin with or without Application of an Intermediate Flowable Resin Layer. *Braz Dent J* 2008; 19(1): 51-56.
72. Labella R, Lambrechts P, Van Meerbeek B, Vanherle G. Polymerization shrinkage and elasticity of flowable composites and filled adhesives. *Dent Mater* 1999; 15: 128-137.
73. Braga RR, Hilton TJ, Ferracane JL. Contraction stress of flowable composite materials and their efficacy as stress-relieving layers. *J Am Dent Assoc*. 2003; 134: 721-728.

74. Frankenberger R. Marginal Quality and Associated Cusp Displacement of SonicFill™ Restorations. SonicFill Portfolio of Scientific Research [Internet]. Kerr Corporation; 2012. Available from: www.kerrdental.com/sonicfill.
75. Thompson JY. SonicFill™ Volumetric Shrinkage. SonicFill Portfolio of Scientific Research [Internet]. Kerr Corporation; 2012. Available from: www.kerrdental.com/sonicfill.
76. Salz U, Bock T. Testing Adhesion of Direct Restoratives to Dental Hard Tissue – A Review. *J Adhes Dent* 2010; 12: 343-371.
77. Nalcacl A, Ulusoy N. Effect of thermocycling on microleakage of resin composites polymerized with LED curing techniques. *Quintessence International*. 2007; 38(7): 433-439.
78. Bedran-De Castro AK, Pereira PNR, Pimenta LAF, Thompson JY. Effect of thermal and mechanical load cycling on nanoleakage of class II restorations. *J Adhes Dent* 2004; 6: 221-226.
79. Cavalcanti AN, Mitsui FHO, Silva F, Peris AR, Bedran-Russo A, Marchi GM. Effect of cyclic loading on the bond strength of class II restorations with different composite materials. *Oper Dent* 2006; 33: 163-168.
80. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: Methods and Results. *J Dent Res* 2005; 84: 118-132.
81. Bravis T, Pilecki P, Wilson RF, Fenlon M, Timothy F, Watson TF, Foxton RM. Effect of loading on the microtensile bond strength and microleakage of a self-etching and etch-and-rinse adhesive in direct class II MOD composite restorations in vitro. *Dental Materials Journal* 2012; 31(6): 924–932.
82. Singla R, Bogra P, Singal B. Comparative evaluation of traditional and self-priming hydrophilic resin. *J Conserv Dent*. 2012; 15(3): 233–236.
83. Yuasa T, Iijima M, Ito S, Muguruma T, Saito T, Mizoguchi I. Effects of long-term storage and thermocycling on bond strength of two self-etching primer adhesive systems. *European Journal of Orthodontics* 2010; 32: 285–290.