OBSERVATION OF TAGS AND HYBRID LAYER OF A SINGLE BOTTLE CONVENTIONAL ADHESIVE SYSTEM AND A SELF-ETCHING ADHESIVE SYSTEM, ON SOUND DENTIN

L.S. Machado[1], R.H. Sundfeld[1], J.D. Cardoso[1],
F.G. Oliveira[1], A.P.B.R. da Silva[1],
G. Delicio[1], R.S. de Alexandre[1],
M.L.M.M. Sundefeld[2]

[1] Department of Restorative Dentistry.
[2] Discipline of Biostatistics.
Araçatuba Dental School – UNESP,
São Paulo, Brazil.

ABSTRACT

This experimental light microscopy study investigated the formation of a hybrid layer and resin tags on sound dentin, after utilization of conventional and self-etching adhesive systems. After restorative procedures, the specimens were decalcified in a formic acid and sodium citrate solution, embedded in paraffin, sectioned at 6-µm thickness and stained by the Brown & Brenn method for analysis and measurement by light microscopy (AXIOPHOT) (400x). The results were statistically analyzed by analysis of variance, at a significance level of 5%. Based on the results, it could be concluded that the conventional adhesive allowed the formation of a thicker hybrid layer than the self-etching adhesive, with similar penetration into the dentinal tubules (resin tags).

Key words: sound dentin; conventional adhesive; self-etching adhesive; hybrid layer; resin tags.

AVALIAÇÕES EM MICROSCOPIA ÓPTICA COMUM DA CAMADA HIBRIDA DE ADESÃO E DOS PROLONGAMENTOS RESINOSOS (TAGS) EM TECIDO DENTINÁRIO HÍGIDO. EFEITOS DE UM SISTEMA ADESIVO RESINOSO FRASCO ÚNICO E DE UM AUTOCONDICIONANTE

RESUMO

Analisamos nesta pesquisa laboratorial/microscópica, o poder de formação da camada híbrida de adesão e dos prolongamentos resinosos (tags) em tecido dentinário hígido, empregando, para tanto, nesse substrato dental, um sistema adesivo convencional e outro autocondicionante. Após a realização dos procedimentos restauradores; os espécimes foram descalcificados em solução de ácido fórmico/citrato de sódio, incluídos em parafina, cortados a uma espessura de 6 micrometros, corados pelo método Brown & Brenn, para posteriormente, serem analisados e mensurados em microscopia óptica comum (AXIOPHOT) (400X). Após a coleta dos resultados, estes foram submetidos à análise estatística ANOVA, ao nível de 5%. De acordo com os resultados obtidos pudemos concluir que o adesivo convencional possibilitou a formação de uma camada híbrida de adesão mais espessa do que o adesivo autocondicionante e um mesmo nível penetração no interior do túbulo dentinário (Tags).

Palavras chave: dentina hígida, adesivo convencional, adesivo autocondicio-nante, camada híbrida e tags.

INTRODUCTION

The increasing search for esthetic treatments, combined with the considerable advances in preventive dentistry, changed the dental practice in the last decades. The conventional concepts of cavity preparation at the beginning of the last century were replaced by a more conservative philosophy, thanks due to the adhesion of new restorative materials to the dental hard tissues.

Adhesive restorations are currently among the most important and investigated subjects in Dentistry, because the advent and evolution of adhesive systems has widened the treatment possibilities, by utilization of adhesive restorations or direct or indirect techniques, as well as by the combination of restorative materials and prosthetic rehabilitation.

It is classically known that the process of material adhesion to the enamel structure submitted to acid etching has a micromechanical nature[1], because the etching agent allows the penetration of adhesives by capillarity, thereby forming the resin tags, which promote a highly effective mechanical interlocking between the resin material and this surface[1-4].

The indisputable results achieved by the adhesion of resin materials to the enamel structure[1,3,5-8] were not comparably effective in the dentin or cementum tissues[9-12].

Nakabayashi in 1989[9] reported that the process of bonding of adhesive materials to the tooth structure submitted to acid etching is mainly micromechanical in nature, because the acid etchants promote complete removal of the smear layer and smear plug, followed by surface dentin demineralization, yielding a collagen network with low mineral content. Therefore, the primer of the adhesive system may penetrate into the etched dentinal tubules, the collagen network of the surface intertubular and peritubular dentin and the peritubular dentin of the dentinal tubule walls, forming the hybrid layer.

In the following year, Fusayama[10] suggested an adhesive technique called “total etching”, which proposes acid etching of enamel and dentin and the use of hydrophilic adhesive monomers.

Nevertheless, new materials and techniques have been proposed for dental etching, including the single-bottle adhesive systems[6] and later the self-etching adhesive systems[3,4,11], for both enamel and dentin. These systems do not require the previous utilization of an etchant, thus simplifying the clinical steps of the technique and reducing the chair time[12].

The latter do not require previous acid etching, simultaneously providing surface dentin demineralization and infiltration of resin monomers. Therefore, from a theoretical standpoint, there is no difference between the degree of demineralization and the degree of penetration of the adhesive system[12-16]. However, the most recent self-etching adhesive systems are considered more aggressive, insensitive to the surface characteristics of the substrate and able to form a similar hybrid layer as that achieved by conventional and total-etch adhesives[15-17]. However, these data were obtained from scanning electron microscopy studies, which generate images of very small interface regions. Little consistent information is available on the performance of these systems on large interface areas of the enamel and dentin[6,11]. Thus, this study investigated, by light microscopy analysis, the ability of a conventional and a self-etching adhesive system to form the hybrid layer and resin tags on sound dentin.

MATERIAL AND METHODS

The study was conducted on 20 sound posterior teeth obtained from patients aged 20 to 40 years. After extraction, the teeth were cleaned and stored in distilled water until utilization. The study was revised and approved by the Institutional Review Board (Araçatuba - UNESP).

Prophylaxis was performed with pumice and water and a Robinson brush, followed by rinsing and drying; wide Class I cavities were manually performed, involving nearly the entire occlusal surface, using a diamond bur n. 1092 (KG Sorensen Ind & Com, Alphaville, São Paulo, Brazil), at high speed under air-water cooling.

The cavities were medium to deep. Depth was standardized by the length of the diamond bur employed and cavities had divergent walls in pulpal-occlusal direction, plain pulpal wall and occlusal margin in enamel. Final cleaning of the cavities was performed with water spray and light air drying; the teeth were then divided in 2 study groups with 10 specimens each.

Only the specimens in Group I were submitted to acid etching of enamel and dentin, by applying 37% phosphoric acid gel on the enamel for 1 minute and for 20 seconds on the dentin.

The cavities were then carefully rinsed with water for 15 seconds and dried. After drying, the enamel surface should be completely dry and the dentin should appear moist. To achieve this clinical effect the dentin tissue was protected with a small cotton pellet while the etched cavities were dried.

With the aid of a bristle brush adapted to an inspiral brush tip, one coat of the adhesive agent PQ1 (Ultradent Products, Inc., South Jordan, USA) (Table 1) was uniformly applied under moderate attrition on the entire surface of etched enamel and dentin. The adhesive agent was then lightly air-dried for 5 seconds and light-cured with halogen light (Ultralux – Dabi Atlante Dabi Atlante, Ribeirão Preto, SP, Brazil) for 20 seconds. After

Table 1: Composition of adhesive materials employed.

ADHESIVE MATERIALS Adhesive system	CLASSIFICATION	COMPOSITION	Solvent	Number of steps
PQ1 (Ultradent)	Light-cured adhesive system	2-hydroxyethyl methacrylate (Hema), camphorquinone, Bis-GMA	Ethyl alcohol Water	2 1
		liquid A (green cap) 2-hydroxyethyl methacrylate (Hema), purified water, ethanol, toluene hydroxybutyrate (THB), amorphous silica		
Xeno III (Dentsply)	Self-etching, light-cured adhesive system	**liquid B (yellow cap)** Functional methacrylate with phosphoric acid (Pyro-EMA), mono fluoro phosphazene modified polymethacrylate resin (PEM-F), urethane dimethacrylate, toluene hydroxybutyrate (THB), camphorquinone, ethyl-4-dymethylaminobenzoate		

application of the adhesive system, the cavities were restored with composite resin Vitalescence (Ultradent Products, Inc., South Jordan, USA) by the incremental technique. Each increment was obliquely applied; the first increment of resin material was applied on the buccal wall, followed by the lingual wall and finally by the last increment, until the entire cavity was filled. Each increment was light-cured for 40 seconds with a light-curing unit Ultralux (Dabi Atlante, Ribeirão Preto, SP, Brazil) directed to the wall corresponding to the application of composite resin.

The specimens in group II received the self-etching adhesive system Xeno III (Dentsply - De Trey Gmbh, Konstanz, Germany) (Table 1). Equal doses of the liquids A and B of the self-etching adhesive system were mixed for nearly 5 seconds, with the aid of an application tip supplied by the manufacturer. The adhesive agent was applied under moderate pressure, with the aid of the application tip, on all cavity surfaces. After 20 seconds, the adhesive was uniformly diffused by gentle air drying for nearly 2 seconds, followed by light curing with halogen light (Ultralux – Dabi Atlante Dabi Atlante, Ribeirão Preto, SP, Brazil) for 10 seconds. Thereafter, the cavities were restored with composite resin QuixFil (Dentsply - De Trey Gmbh, Konstanz, Germany), which was carefully applied in the prepared cavity with the aid of a Centrix syringe, in layers of up to 4-mm thickness. Each increment was light cured with a light curing unit Ultralux (Dabi Atlante, Ribeirão Preto, SP, Brazil) for 20 seconds. Additionally, the restoration was light-cured through the buccal or lingual enamel walls.

The teeth were then decalcified in a solution containing equal proportions of 50% formic acid and 20% sodium citrate[11]. The acidic solution was changed every five days. The complete decalcification of each specimen was radiographically checked[11]. This process promotes complete removal of dental enamel, leaving only the decalcified dentin tissue, which was, in effect, the subject of this study. After decalcification, the restorations were removed and the specimens were embedded in paraffin.

The specimens were serially sectioned in longitudinal direction at 6-μm thickness and sequentially mounted on glass slabs. Fifteen slabs for each specimen containing nearly six sections each were selected by systematic sampling, at an interval proportional to the number of sections obtained for each specimen[11]. The selected specimens were then

Table 2: Analysis of variance of mean thickness of hybrid layer in µm, for conventional (PQ1) and self-etching (XENO III) adhesive systems.

Source of variation	SS	df	MS	F	p value
Between groups	18.68889	1	18.68889	36.02379693	0.0000112*
Within groups	9.338272	18	0.518793		
Total	**28.02716**	**19**			

* significant at the level of 5%

Table 3: Analysis of variance of mean length of resin tags in µm, for conventional (PQ1) and self-etching (XENO III) adhesive systems.

Source of variation	SS	df	MS	F	p value
Between groups	23.07668	1	23.07668	3.377924431	0.082639
Within groups	122.9691	18	6.831615		
Total	**146.0458**	**19**			

* significant at the level of 5%

stained by the Brown & Brenn method[18]. The best histological sections on each slab were then submitted to analysis on a light microscope Axiophot (ZEISS DSM-940 A, Oberkochen, Germany) at 400x, with a micrometric ocular 40/075.

Measurement of the hybrid layer and resin tags was carefully performed over the whole extension of each histological section by a single examiner. Three measurements were obtained for each section, for each end-point. Consequently, for each selected slab, the thickness of the hybrid layer and the length of resin tags corresponded to the mean of the three measurements.

Therefore, fifteen means of the three measurements were obtained for each specimen, for both end-points, i.e. hybrid layer and resin tags. The means of each end-point corresponding to each specimen were submitted to analysis of variance at a significance level of 5%.

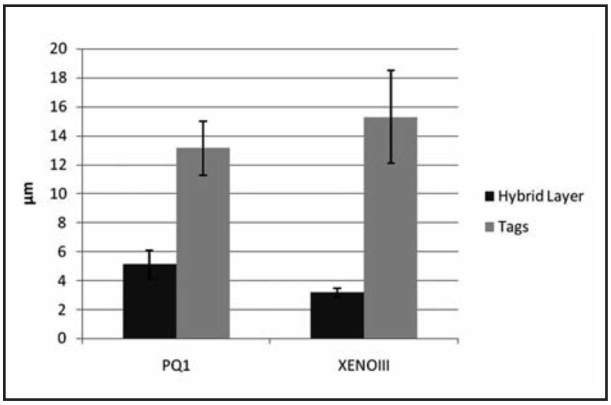

Fig. 1: Graph of mean thickness of hybrid layer (µm) and resin tags of the groups treated with conventional (PQ1) and self-etching (XENO III) adhesive systems.

RESULTS AND STATISTICAL ANALYSIS

Light microscopy analysis revealed a zone of altered staining in dentin tissue, following the use of both adhesive systems (Figures 2 and 3). The hybrid layer stained strongly and was detected as a uniform surface layer that could be distinguished from the underlying unaltered dentin (HL); the resin tags within the dentin tissue usually stained the color of the Hybrid Layer (T); and of the underlying unaltered dentin (D). The present study revealed that the resin tags presented a funnel shaped appearance, conceivably the result of widening of the tubules by removal of the mineralized peritubular dentin.

The means of specimens for the parameters hybrid layer and resin tags, for both study groups, were statistically analyzed by analysis of variance at a significance level of 5% (Table 2, 3 and 4). A statistically significant difference was observed for the parameter hybrid layer; the conventional adhesive PQ1 exhibited a significantly greater thickness of the hybrid layer (Table 2, Figure 1 and 2). No significant difference was observed in the length of resin tags between the conventional adhesive system PQ1 and the self-etching adhesive system Xeno III (Table 3, Figure 1 and 3).

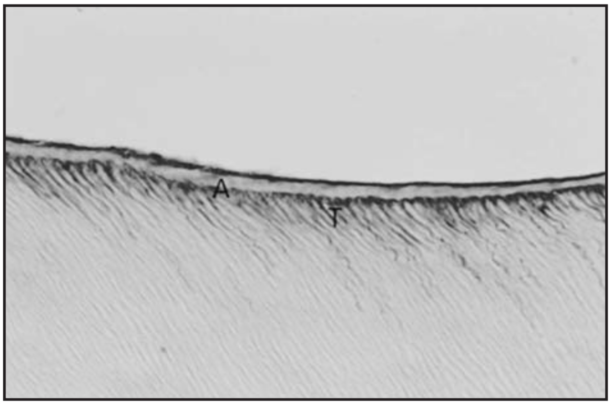

Fig. 2: Histological section of conventional adhesive material PQ1, analyzed by light microscopy – 400X. Hybrid layer (A) and resin tags (T).

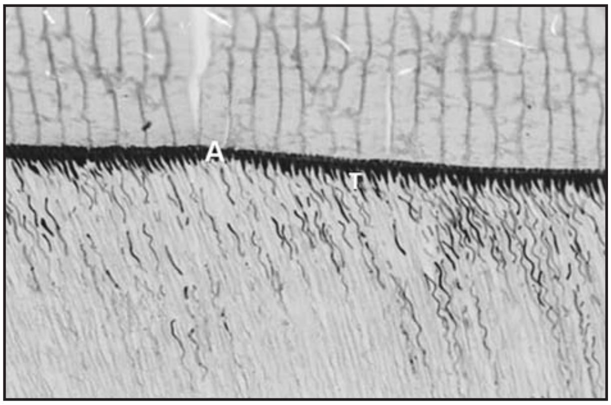

Fig. 3: Histological section of self-etching adhesive material Xeno III, analyzed by light microscopy – 400X. Hybrid layer (A) and resin tags (T).

DISCUSSION

One of the main concerns of contemporary dentistry has been to enhance the sealing of restorations. Within this context, the evolution of adhesive dentistry has been considerably important, in view of the several studies conducted on dentin bonding[11-17]. Within this context, the aim of this study was to evaluate and compare the penetration of a conventional and a self-etching adhesive system on sound dentin. Light microscopy analysis of histological sections of human dental tissue was performed as suggested by Tay et. al. in 1995[19] and Sundfeld et. al. in 2005[11], to evaluate the hybrid layer and resin tags in the dentinal tubules. Brown & Brenn's staining technique stains the resin structures intensely, allowing for good microscopic observation of the structures investigated[18].

It should also be highlighted, as mentioned by Sundfeld et al. in 2005[11], that even when the histological section of the specimen obtained on the soft tissue microtome was not adequate for good observation of the resin tags, it was always adequate for observation of the hybrid layer.

Observation of the hybrid layer revealed a significantly greater thickness for the conventional adhesive system compared to the self-etching adhesive system. This could be due to the complete removal of the smear layer and smear plug and consequent dentin demineralization promoted by the 37% phosphoric acid. Since the conventional adhesive system exhibits a more acidic pH and a higher pKa value than the self-etching adhesive system, it would increase the depth of dentin mineralization with greater exposure of collagen fibers[14].

Table 4: Mean thickness (µm) of hybrid layer and of resin tags for both conventional (PQ1) and self-etching (XENO III) adhesive systems.

	PQ1	Xeno III
Hybrid Layer ±SD	5.10 ± 0.97	3.17±0.30
Resin Tags ±SD	13.13±1.84	15.28±3.20

SD – Standard Deviation

Moreover, with application of the self-etching adhesive, the byproducts of dental demineralization are not eliminated. This may limit the demineralizing action because these byproducts may act as a buffering agent in the tooth structure. Coupled to the lack of complete removal of the smear layer present in the structure, this would lead to a more superficial penetration of the adhesive system in the dentin, thus reducing the thickness of the hybrid layer compared to the conventional adhesive system[15,16,20].

The self-etching adhesive Xeno III contains acidic monomers which promote dissolution of the smear layer. The resulting product reacts with the acidic monomer, neutralizing the acidity of the adhesive[16,17]. In addition to the acidic and hydrophilic monomers, these systems also contain water, which is necessary to promote the ionization of the acidic monomer[21]. Single-bottle self-etching adhesives contain more water than two-bottle conventional adhesives. Thus, the single-bottle adhesive contains a higher percentage of monomers with hydrophilic and acidic characteristics. Moreover, these low molecular weight monomers enhance the penetration in demineralized dentin. The fact that these adhesives contain a larger proportion of water, which is necessary for the ionization process, also implies that they contain a smaller proportion of hydrophobic monomers[22]. This explains their low viscosity and high hydrophilicity, and accounts for the formation of a thinner coat of adhesive[22,23].

According to some authors, the quantity of water in self-etching adhesives may impair their durability, because this water is not completely evaporated and gives rise to small lacunae at the adhesive interface[21-23]. This water in the adhesive interface might lead to incomplete curing of the adhesive, conferring inferior mechanical properties on the hybrid layer, leaving the collagen fibers unprotected and increasing the susceptibility to degradation[23].

The resin tags are formed by capillary infiltration of adhesive in the dentinal tubules[9]. The length of resin tags in the present study was not significantly different between specimens treated with the conventional and self-etching systems.

According to Nakabayashi et al. in 1989[9] and Van Meerbeek et al. in 2003[24], these resin tags may or not be associated with the formation of the hybrid layer in the tubule, which would enhance the bond strength. Studies evaluating the potential correlation between the length of resin tags and the bond strength revealed that they are not correlated with the bond strength[25].

As previously reported, self-etching adhesives maintain the smear plug, which might act as a barrier against the formation of resin tags. However, this study revealed similar penetration for both systems. Two possible explanations for this phenomenon are suggested. The first is related with the high concentration of low molecular weight monomers and water in their composition, which, combined with the lack of pulp pressure, may contribute to the penetration of the self-etching adhesive system. The second possibility is the presence of fillers in the conventional adhesive (PQ1). The addition of fillers increases the viscosity of the adhesive[25], conceivably reducing the penetration of the resin material in the dentinal tubules. Moreover, Can Say et al. in 2006[25] demonstrated that there is a higher concentration of fillers in the openings of dentinal tubules. Few particles are able to penetrate into the hybrid layer, also due to the reduced interfibrillar space (20nm)[23] that may also reduce the penetration.

According to the present results, both materials presented the ability to form a hybrid layer and resin tags. Clinical studies and laboratory investigations on the bond strength should be conducted to investigate their real effectiveness.

CONCLUSION

According to the present results, it was concluded that the conventional adhesive PQ1 allowed the formation of a thicker hybrid layer than the self-etching adhesive Xeno III; however, they exhibited a similar level of penetration into the dentinal tubule (resin tags).

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CORRESPONDENCE

Renato Herman Sundfeld, Discipline of Restorative Dentistry – Araçatuba Dental School – UNESP. Rua José Bonifácio 1193, São Paulo, Brazil. CEP16015-050.
e-mail: sundfeld@foa.unesp.br

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