Evaluation of the dentin-resin modified glass-ionomer bonding interface after different cavity preparations and dentin treatments.

REBECA LIMA AFONSO[1], RENATO HERMAN SUNDFELD[2], MARIA LÚCIA MARÇAL MAZZA SUNDEFELD[3], LUIZ ANDRÉ FREIRE PIMENTA[4], ANDRÉ LUIZ FRAGA BRISO[5]

Abstract

Objectives: To evaluate the bonding interface of the dentin-resin modified glass-ionomer Fuji II LC (RMGIC), using two cavity preparation techniques and two different dentin treatments.

Methods and Materials: Primary molars were selected and randomly divided into 4 groups. The cavities were made by conventional technique (CT) – low speed (# 4 round burs) or by Atraumatic Restorative Treatment (ART) - using excavators. Two dentin treatments were performed: 36% phosphoric acid (P.A), and 12.5% polyacrylic acid (PAA). The restorative procedures were: (G-I) ART+PA; (G-II) CT+PA; (G-III) ART+PAA; (G-IV) CT+PAA. The teeth were subsequently stored for 24h in water, after which they were decalcified, embedded in paraffin and then sectioned (6µm thick) and sequentially mounted on glass slides for microscopic investigations. These sections were stained with Brown & Brenn staining for analysis on a light microscope AXIOPHOT (ZEISS, Oberkochen, Germany) at 400x magnification, with a micrometric ocular 40/075. The hybrid layer thickness and resin tag length were measured and submitted for statistical analysis using ANOVA and Tukey's test.

Results: At the dentin-RMGIC interface, a hybrid layer was found in all groups. Only the specimens submitted to CT + PAA did not reveal the presence of resin tags in dentin. The groups treated with the ART technique presented an irregular cavity surface, and several bacterial colonies throughout the cavities were detected.

Conclusions: It was concluded that CT or ART, in conjunction with dentin etching, both resulted in considerable micromechanical interaction between RMGIC and primary dentinal tissue.

Clinical Significance: It is known that the bonding process to the tooth structure, particularly dentinal tissue, is one of the factors related directly to the success of the restoration and that adhesion of materials might be optimized using different surface treatments.

Due to good acceptance by patients, the modified ART technique has been increasingly performed in Pediatric Dentistry. The presence of a hybrid layer and the formation of tags in dentin after the application of this technique, is an important factor for its indication.

Keywords: bonding interface, primary tooth; atraumatic restorative treatment, resin-tags, hybrid layer, resin modified glass ionomer.

Short Title: Analysis of the dentin-RMGIC interface.

Introduction

The bonding process to the tooth structure, especially dentinal tissue, is one of the factors directly related to the sealing ability of the material, which is considerably important for the occurrence of postoperative sensitivity and development of secondary caries lesions.[1] Thus, it can be stated that one of the most important objectives of adhesive systems is to promote good bonding between resin-based materials and the tooth structure, especially dentin.[2]

Because adhesive materials are technique-sensitive, the application technique requires specific conditions to obtain successful results. In Pediatric Dentistry, particularly, the use of such materials is a matter of concern, since patient behavior is often uncooperative.[3]

Initially suggested as an option for treatment in areas where cavity preparation and conventional restorative procedures are not possible, the Atraumatic Restorative Treatment (ART)[4] has also been presented as an option for the treatment of non-compliant children.[5] This technique is different from the conventional technique because it removes the carious lesion with manual instruments only, and restorations are performed with glass ionomer cements (GIC).[5,6] However, the simplicity and success of this technique has led to its use in private practice as well.[3,5] For those cases where more resources are available, the modified ART technique using different materials and instruments might optimize the quality of the restorations

[1] *PhD student in Pediatric Dentistry, School of Dentistry at Araçatuba, UNESP, Sao Paulo, Brazil*
[2] *Associate Professor of Restorative Dentistry, School of Dentistry at Araçatuba, UNESP, Sao Paulo, Brazil*
[3] *Assistant Professor of Biostatistics, School of Dentistry at Araçatuba, UNESP, Sao Paulo, Brazil*
[4] *Clinical Professor, Department of Dental Ecology, School of Dentistry at Chapel Hill, UNC*
[5] *Assistant Professor of Restorative Dentistry, School of Dentistry at Araçatuba, UNESP, Sao Paulo, Brazil*

Correspondence to:
Prof. Dr. André Luiz Fraga Briso, School of Dentistry at Araçatuba – UNESP, Department of Operative Dentistry, Rua José Bonifácio 1193, CEP: 16015 050, Araçatuba, São Paulo, Brazil. Fax: +55 (018) 36363251; email: alfbriso@foa.unesp.br

Table 1. Tukey's test comparing the hybrid layer thickness for the different dentin treatments.

Group	n	Mean (µm)	SD	p < 0.05
1	9	3.57	0.65	ab
2	10	4.38	0.78	a
3	10	3.98	1.02	a
4	10	2.70	0.31	b

Different letters mean significant statistical differences between groups ($p < 0.05$)

and, consequently, their long term performance. Thus, it has been suggested the use not only of conventional GIC, but also Resin Modified Glass Ionomer (RMGIC), resin composites, or even the association of both.[3]

It has also been demonstrated that adhesion of these materials to the dental enamel might be optimized using different surface treatments.[2] Nonetheless, there is a lack of studies evaluating the effect of these treatments on dentin,[7] especially when the modified ART technique is employed.

The null hypothesis tested was that neither the preparation technique nor the dentin treatment would interfere with the morphological characteristics of the RMGIC-dentin interface.

The purpose of this study, therefore, was to investigate the effect of two preparation techniques and two different dentin treatments on the morphological interface between a RMGIC and the dentinal tissue in primary teeth, as well as to measure the thickness of the hybrid layer and the length of the resin tags .

Methods and Materials

Thirty nine carious primary molars presenting wide dentinal cavities were selected for this study. All had been extracted for reasons unrelated to this study, and the "Free and Informed Consent Form" was signed by parents or responsible person. Before commencement of the study, the research protocol was submitted to and evaluated by the Institutional Review Board in order to obtain approval.

The teeth were cleaned and stored in distilled water for 15d until their preparation.[6] They were then randomly divided into 4 groups, according to the restorative procedures.

For groups 1 and 3, the operative procedures began with the removal of enamel using round diamond burs # 1092 (KG Sorensen, Sao Paulo, SP, Brazil) at high speed. Excavators were then used to remove the softened dentin until resistance was found, even if the dentin presented with staining.

The specimens in groups 2 and 4 were subjected to conventional cavity preparation using rotary instruments. The enamel was removed using diamond round burs # 1092 (KG Sorensen) at high-speed under water cooling, followed by carious tissue removal using excavators and # 4 round stainless burs at low-speed. The carious tissue removal was checked by evaluating the consistency of the remaining dentin with an explorer.

After cavity preparation, the teeth were rinsed for 10s and air-dried for 3s.

The teeth in groups 1 and 2 were etched with 36% phosphoric acid gel (Dentsply Ind. Com., Rio de Janeiro, RJ, Brazil) for 15s, and the cavities rinsed for 15s and blotted dry.

For the teeth in groups 3 and 4, the dentin was treated with 12.5% polyacrylic acid (GC Corporation, Tokyo, Japan), applied for 20s by active form. The teeth were then rinsed with water for 15s and also blotted dry.

Immediately thereafter, the cavities were restored with RMGIC FUJI II LC (GC Corporation, Tokyo, Japan), following the manufacturer's instructions.. The material was light-cured with a halogen light unit, Ultralux (Dabi Atlante, Ribeirao Preto, SP, Brazil) for 40s. The light density was checked throughout the experiment (400mW/cm^2).

The specimens were then decalcified in 17% EDTA solution, changed every 7d. Complete decalcification of each specimen was checked using radiographs and penetration of a needle through the tooth[7].

After decalcification, the restorations were carefully removed, and the specimens embedded in paraffin. The specimens were then serially sectioned longitudinally through the crown (6µm thick) and sequentially mounted on glass

Table 2. Mean and Standard Deviation of the resin tag length.

Group	n	Mean	SD
1	9	9.81	2.26
2	10	10.65	2.11
3	10	9.11	3.46

slides. Fifteen sections of each specimen were selected by systematic sampling, with an interval proportional to the number of sections achieved for each specimen. These sections were stained with Brown & Brenn staining for analysis on a light microscope AXIOPHOT (ZEISS, Oberkochen, Germany) at 400x magnification, with a micrometric ocular 40/075.

Measurements of the thickness of the hybrid layer and the length of the resin tags of each section were taken by careful analysis of the entire extension of the ground section using a single calibrated examiner. Three measurements were collected for each section. Consequently, for each section analyzed, the length of the resin tags corresponded to the mean of the three measurements performed. Thus, three means were obtained for each specimen. The means of tag length and hybrid layer thickness corresponding to each specimen were submitted to statistical analysis using ANOVA and Tukey's test to compare the groups ($p < 0.05$).

Results

Hybrid layer thickness and resin tags length

With regard to the thickness of the hybrid layer, there was a significant statistical difference ($p < 0.0001$). The Tukey's test demonstrated that Group 4 (conventional technique + PAA + Fuji II LC), which presented the lowest thickness, was significantly different statistically to Group 2 (conventional technique + PA + Fuji II LC), and Group 3 (ART technique + PAA + Fuji II LC). However, Group 1 (ART technique + PA + Fuji II LC) did not present any significant statistical difference when compared to any Group (Table 1)

With regard to the length of the resin tags, Group 4 (conventional technique + PAA + Fuji II LC) was not included for statistical analysis, since there were no detected resin tags in dentin when this treatment was performed. Thus, ANOVA was used to analyze data from Groups 1 – 3 demonstrating that there is no significant statistical difference among groups ($p=0.5186$). The mean of the resin tag length and the standard deviation for each group are presented in Table 2.

Morphological analysis

Microscopic evaluation revealed an area of altered staining in the dentin, corresponding to the interaction between the RMGIC and the dentinal tissue.

For the qualitative morphological analysis of the sections, the hybrid layer and resin tag formation in dentin, as well as the presence of bacteria in the remaining dentinal tissue and regularity of the preparation was considered.

In general, the RMGIC-dentin interface was observed in all groups (Figure 1). The outer layer can be identified (stained in light red), suggesting the presence of the resin copolymer from Fuji II LC (GC Corporation), which did not penetrate into demineralized dentin and was retained after the removal of the restoration (A); the inner layer (hybrid layer) which was stained in violet and could be observed as a uniform layer on the surface demarcated from the underlying unaltered dentin (HL); the resin tags within the dentinal tissue usually stained in violet (T), the underlying unaltered dentin (D).

Regarding the specimens in Group 1 (ART technique + PA +

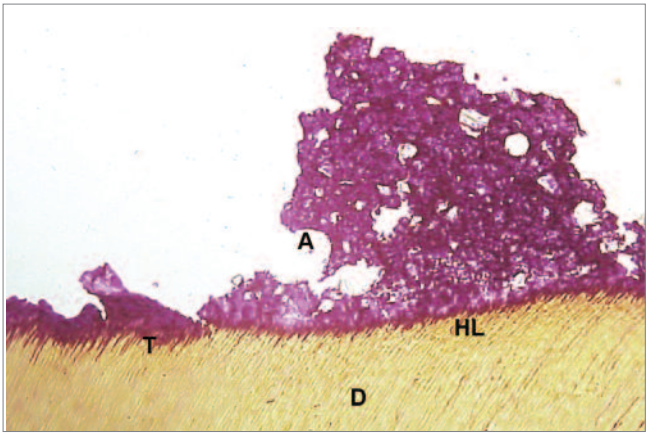

Figure 1 – General view of the RMGIC-dentin interface. (A) - restoration, (HL) - hybrid layer, (T) – resin tags in dentin, (D) - unaltered dentin (400x).

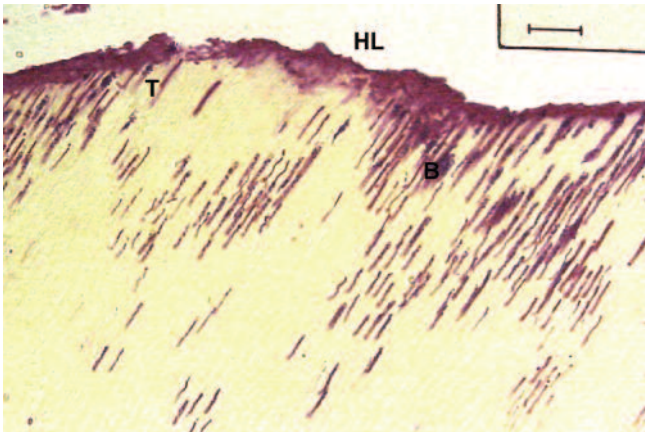

Figure 2 – Histological section of group 1, revealing irregularities on the surface, area of RMGIC-dentin interaction – hybrid layer (HL), resin tags in dentin (T) and bacterial colonies (B) (400x).

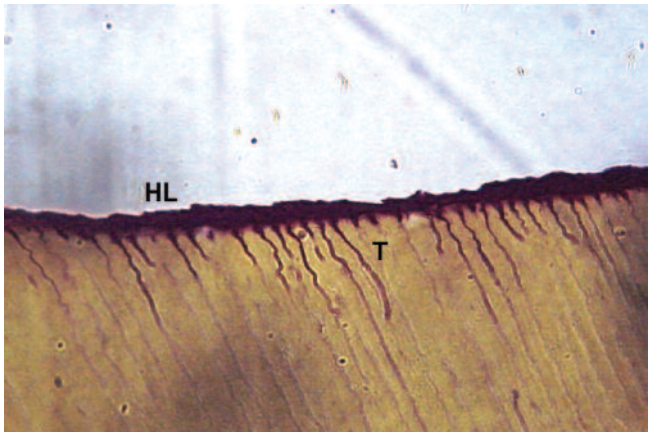

Figure 3 – Histological section of group 2, revealing area of RMGIC-dentin interaction – hybrid layer (HL) and clear and continuous resin tags in dentin (T) (400x).

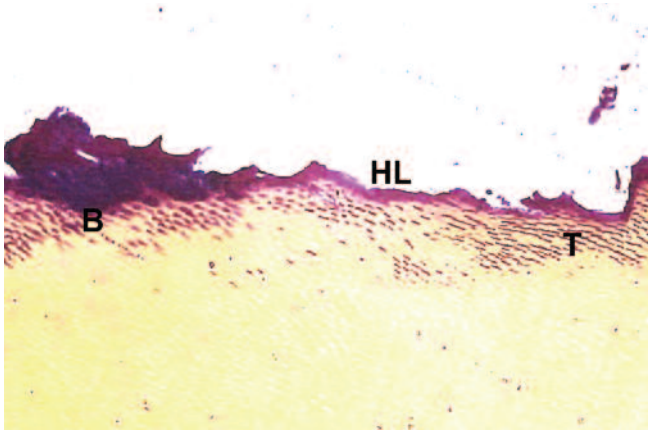

Figure 4 – Histological section of group 3, revealing irregularities on dentin surface, hybrid layer (HL), resin tags in dentin (T) and presence of bacterial colonies (B) (400x).

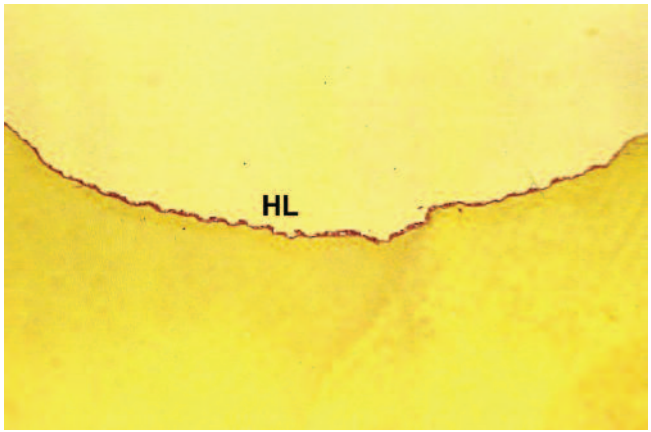

Figure 5 – Histological section of group IV, revealing thin area of interaction – hybrid layer (HL), and absence of resin tags in dentin (100x).

Fuji II LC), irregularities on the dentin surface of cavity preparations, as well as a large amount of bacterial colonies can be observed (Figure 2). The interaction between the restorative material and the remaining dentinal tissue revealed variable thicknesses in all sections, and also the presence of several well-defined tags. In the case of the specimens in Group 2 (conventional technique + phosphoric acid + Fuji II LC), the formation of a clear area of interaction between the restorative material and the remaining dentinal tissue, with mild variation in thickness and throughout the cavity extension, was noted (Figure 3). Several well-defined and uniform tags in all cavities were also observed.

As with the specimens in Group 1, the specimens in Group 3 (ART technique + PAA + Fuji II LC) revealed irregularities on the dentin surface and the presence of bacterial colonies within the dentinal tubules, as well as within the RMGIC-dentin interface. An evident area of interaction between the RMGIC and the dentinal tissue was also observed. However, in some areas, in which disorganized dentinal tissue was present, this interaction was not as evident (Figure 4). Nevertheless, several well-defined tags in almost all sections were detected.

With regard to the specimens in Group 4 (conventional technique + PAA + Fuji II LC), the RMGIC-dentin interface exhibited an area of continuous interaction. However, it was thinner than those observed for other groups. The cavities displayed a regular surface with an absence of bacteria (Figure 5).

Discussion

The ART technique was initially indicated to be used in areas with poor infra-structure, using excavators for caries removal, and conventional glass ionomer cement as restorative material.[4,5]

However, many studies have stated that ART is well indicated in Pediatric Dentistry, since it is well accepted by patients, and also contributes to the establishment of a relationship of trust between patients, parents and the dentist.[8,9] These aspects have also been highlighted in another study,[10] in which it was observed that 95% of children treated by the ART technique were cooperative for subsequent new procedures.

Considering the limitations of conventional glass ionomer cements as a definitive restorative material, the present study

used a modified ART technique,[11] restoring the cavities with a RMGIC.

The use of RMGIC in association with acid etching is of interest, since this technique could be beneficial to marginal sealing.[1,11-14]

It is known that the objective of etching the tooth structure is to create a more reactive surface, which is able to promote chemical and/or micromechanical bonding between the restorative materials and the tooth.[2, 7, 14, 18]

It has also been assumed that bonding to the tooth structure depends on several factors.[15,16] However, a close contact between the restorative material and the tooth structure is fundamental to obtain successful bonding.[14] Consequently, two different acids were evaluated in this study as conditioners - a 36% phosphoric acid gel and a 12.5% polyacrylic acid. The results of the present study demonstrated that both etching agents allowed the formation of an area of interaction between dentin and the restorative material, since the hybrid layer formation was observed in all the specimens investigated, as reported in other studies.[17,18]

In the present study, when those areas of interaction were thicker and better defined, a clear hybrid layer could be observed, suggesting that not only microretention had occurred, but also chemical bonding. Thus, for the groups submitted to phosphoric acid etching in dentin, the presence of a well defined hybrid layer was observed. In these cases, the larger thickness of the hybrid layer could be related to the demineralization of the intertubular and peritubular dentin promoted by the phosphoric acid, followed by the penetration of the resin copolymer in this demineralized area which is rich in exposed collagen fibrils, resulting in the formation of a resin-ionomer hybrid layer.

The measurement of hybrid layer thickness demonstrated that there was no significant statistical difference among Groups 1, 2 and 3. However, in Group 4, apart from the presence of a clear and continuous hybrid layer, it was significantly thinner when compared to the other groups (Table 1 and Figure 5).

The results of this study also revealed that the RMGIC was able to penetrate into the dentinal tubules, especially the resinous component (HEMA). Despite the lack of reaction between the ionomeric part of the material and the resin, the presence of resin tags was observed . Furthermore, it is emphasized that conventional glass-ionomer cement has particles with dimensions ranging from 20 to 50µm. Even though the manufacturer does not give information of the precise size of the particles of Fuji II LC, it is assumed that they may be larger than 2.5µm, which would not allow the penetration of such components into the dentinal tubules. In this study the formation of tags in dentin with similar lengths, in both types of preparation was also observed. However, these structures were not detected in the group in which the carious tissue was removed with round burs and etched with 12.5% polyacrylic acid.

This fact might be related to preparation using burs might result in a thicker smear layer and smear plugs in dentin, which would not be totally removed by the organic acid and, consequently, would avoid the contact with the restorative material. Based on that, we could affirm that the hybrid layer could be primarily influenced by the type of instrument employed and the area of dentin where it is formed.[19] Thus, the presence of a smear layer and especially a smear plug would probably prevent the formation of tags in this group. Therefore, it might be assumed that the smear produced by using round burs possibly caused the obliteration of the dentinal tubules, and that the polyacrylic acid promoted only a superficial cleaning of the dentin, and did not remove the smear plug, resulting in non-penetration of the resin in the tubules. This fact was not observed in cavities prepared with excavators, where their action did not obliterate the tubules, thus allowing penetration of the resin material inside the dentin tubules.

It is important to mention that the morphologic aspect of the cavities observed in microscopy was different in the groups where burs were used and in those that employed excavators for caries removal. When using burs, the blades cut the dentin regularly, which was different from those the specimens prepared with excavators.

Moreover, the groups where excavators were used revealed a large amount of bacterial colonies within the dentin, which could be cause for concern.[20-22] Such bacterial colonies could only be observed due to the staining employed (Brown & Brenn), which has affinity to gram-positive and gram-negative bacteria, as well as to the organic component of restorative materials.

Therefore, it is important to improve the quality of restorative materials to seal the cavity effectively. The sealing ability of Fuji II LC was evaluated in restorations performed in non-carious dentin and in caries-affected dentin, revealing that marginal leakage at the enamel-glass ionomer interface was considerably controlled only when the tooth surface was etched with phosphoric acid[1]. It has also been reported that sealed carious lesions exhibited little or no increase in depth when compared to lesions kept open, which presented a significant increase in depth after one year of evaluation.[20]

In addition, the modified ART technique has been increasingly applied in Pediatric Dentistry, due to the good acceptance by patients. The presence of a hybrid layer, and the formation of resin tags in dentin, after the application of this technique, is an important factor for its indication.

According to the morphological characteristics observed at

the RMGIC-dentin interface in this study, it is possible to affirm that the use of excavators or rotary cutting instruments, in conjunciton with phosphoric acid etching in dentin, resulted in formation of effective micromechanical interaction with the dentin in primary teeth, with the formation of a hybrid layer and resin tags within the dentinal tissue. Moreover, conventional cavity preparation followed by the application of 12.5% polyacrylic acid did not result in the formation of tags. This result rejects the null hypothesis, that neither the preparation technique nor the dentin treatment would interfere in the morphological characteristics of the RMGIC-dentin interface.

The success achieved by the original ART technique performed in poor communities could now also be achieved by the modified ART technique, especially in the treatment of noncompliant children. Moreover, the findings of the present study indicate that the modified ART might be also successfully employed in private practice.

Conclusion

- The use of excavators or rotary cutting instruments, in conjunciton with phosphoric acid etching in dentin, resulted in the formation of a hybrid layer and resin tags within the dentinal tissue.
- Conventional cavity preparation followed by the application of 12.5% polyacrylic acid did not result in the formation of resin tags.
- In the groups prepared with excavators (ART), there was a noticeable presence of bacteria and irregularities on the dentin surface.

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