Effect of Conditioning Protocols and Ultrasonic Application of an Infiltrant Resin in White Spot Lesions

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The aim of this study was to evaluate different conditioning protocols and sonic/ultrasonic application of an infiltrant resin (IR) in artificial white spot lesions (AWSL). the V/L surfaces of 48 molars were induced to an AWSL and divided in 6 groups, according to the conditioning protocols and application technique: 15% hydrochloric acid (HA) + manual application of the IR; HA + 37% phosphoric acid (PA) + manual application of the IR; HA + ultrasonic application (U) of the IR; HA + sonic application (S) of the IR; PA+HA+S; and PA+HA+U. For the Penetration Depth (PD), the crowns were etched with HA for 120s. The IR Icon® (DMG) was applied according to the manufacturer`s instructions. The crowns were dye penetrated (0.1% red fluorophore rhodamine B isothiocyanate for 12h) and bleached with 30% hydrogen peroxide for 12 h. The discs were immersed in a 50% ethanol solution, containing 100 µM of sodium fluorescein. The PD (in µm) was measured using confocal laser scanning microscopy (20x). The bond strength (BS) was performed by michoshear test (0.5 mm/min). Data were submitted to 2-way ANOVA and Tukey (α=0.05). For BS, the interaction was not significant ($p>0.05$). For PD, the main factors were significant (application - $p<0.001$; conditioning technique - $p=0.003$). The ultrasonic application showed the highest PD values. PA+HA presented higher results than HA. The sonic/ultrasonic applications and the use of phosphoric acid prior to hydrochloric acid improved PD of the infiltrant resin. Conditioning protocols or application techniques did not influence BS values.

Key Words: dental enamel, ultrasonics, hydrochloric acid, phosphoric acid.

Introduction

One of the challenges of the esthetic restorative dentistry is to provide non-invasive treatment alternatives for the surface enamel lesions and the White Stain lesions, diagnosed according to the ICDAS classification system (International System for the Detection and Diagnosis of Cavities) as 2 N (1). The white stains are signs of a demineralization process that could evolve into a dentin cavity lesion. These stains occur when the pathogenic bacteria penetrate the base of the enamel and produce organic acids, eliminating calcium and phosphate ions from the enamel, which may be remineralized or not (2).

A conservative approach to treat these lesions is the microabrasion technique, firstly described by Croll in 1998, which consists in a selective removal with the association of an erosive agent (mainly hydrochloric and phosphate acids) and an abrasive agent (pumice stone or silicon carbide), applied with low-speed rotation or spatula; nevertheless, this technique may produce increased surface roughness (3). A less invasive alternative, with promising in vivo results (4), is the infiltration of low-molecular weight resin that penetrates the demineralized tissue, without the necessity to create a cavity, with previous use of 15% hydrochloric acid gel (HCl), which creates smooth porosity allowing the resin infiltration.

Studies have investigated the mechanism of "cause and reaction" and the effects that can be obtained by the infiltration of resins to the affected dental structure. Paris et al. (5) evaluated the penetration factor by modifying the viscosity of these resins in order to obtain a higher penetration depth and observed that the experimental resins that did not contain Bis-GMA presented better performance. An infiltration technique, with a low-viscosity photoactivated resin (Icon®, DMG) was studied. Subramaniam et al. (6) evaluated the fabricants protocol with excellent clinical results; however, Shivanna, Shivakumar (7) concluded that a modification in the treatment time may be necessary to achieve acceptable results.

Once bond strength of resin composite is decreased in hypomineralised enamel, Chay et al. (8) evaluated the bond strength values of the composite resin in demineralized and sound enamel to improve the performance of previous treatments with different techniques and strategies with infiltrating resins. The authors concluded that the pre-treatment of hypomineralised enamel with infiltrant resin may be beneficial to the adhesion of resin composite.

To obtain effective impregnation of the infiltrant resins within the enamel surface, enamel etching should be performed using 15% hydrochloric acid for 2 min (9). Without the conditioning step, it is difficult to occur the resin infiltration; otherwise, increased application times may promote an eroded surface with impurities over the porosities (5,10). Also, the application method of the infiltrant system may directly influence the penetration quality. Active manual application could increase the bond strength values to enamel, due to the increased solvent evaporation and higher penetration of the infiltrating resin in the enamel substrate, once the application following the manufacturer`s instructions showed a limited permeation into the demineralized enamel (11).

The confocal laser microscopy (CSLM), a microscopic observation technique that presents excellent results in different scientific fields like medicine, biology, materials, geology, and more, is a useful tool to provide information about the penetration depth of infiltrant resin in demineralized enamel (2,5,10,12,13). Its success is due to the advantages it has over the traditional optical technology (like image sharpness and contrast, better resolution, and others) (12,13). It allows a 3-D analysis of an object and different "sampling optical sections".

With the intention to increase BS values between dental substrates and adhesive/resin, recent studies have demonstrated the use of a sonic device with a frequency of 170 Hz, which may improve BS between dentin and adhesive systems (14,15). The sonic devices have already been used in different stages of the endodontic treatment (16). By the other way, the ultrasonic devices produce frequencies between 24000 and 30000 Hz, through a transducer, using ceramic components to transmit a mechanical vibration in the same frequency. When these ultrasonic waves are applied over an element in its liquid state, they influenced on bubble formation and further implosion (17).

In the contemporary literature, there are no studies regarding the application of a low-molecular weight infiltrant resin in enamel using sonic or ultrasonic devices. Thus, this study evaluated the penetration depth into dental enamel submitted to an artificial white spot lesion, using the low-molecular weight infiltrant resin, modifying the application strategies (incorporating sonic and ultrasonic application) and conditioning protocols, and the influence in the bond strength values between the composite resin and enamel. The hypothesis was: the application strategies or the conditioning protocols would influence the penetration depth and the bond strength values in enamel.

Material and Methods

Tooth Selection

This research project was approved by the Research Ethics Committee of our Institution. Forty-eight permanent third human molars were used for the experiment. Teeth were cleaned with periodontal scalers (Duflex, SS White, Rio de Janeiro, RJ, Brazil) and stored in distilled water at room temperature. The teeth selected followed the inclusion criteria: absence of cavity lesions, white stains, cracks or structural alterations and restorations.

Development of the Artificial White Spot Lesion

In each tooth, an area of 4 mm x 4 mm was delimited at the buccal and lingual surfaces with adhesive tape and measured with a caliper rule, resulting in 96 specimens. The remaining area of the tooth was protected with nail polish. Following, an initial periapical digital radiography image was taken (Imax Mural model 70 Kv 180CM with an exposition time of 0.5 s) to observe the sound enamel before the immersion in the demineralizing gel. Teeth were cut at the cementoenamel junction (CEJ) using a diamond saw (ISOMET 1000, Buehler, Lake Bluff, IL, USA), constantly refrigerated with water, in a mesiodistal position to obtain the buccal and lingual surfaces.

Demineralizing acid gel was produced with 14 g/L of hydroxyethylcellulose (HEC) – pH=5, adjusted with 0.05 M of lactic acid, and hydrolyzed by agitation during 30 min. The specimens were incubated for 24 h at 37 °C $\pm$ 1°C and were kept hydrated in a second container at 37 °C for 4 weeks. After this period, a periapical digital radiography image was taken with the same technical specifications already mentioned, to observe the presence or absence of any kind of cavity lesions, resulted by the exposition to the demineralizing gel (18).

Experimental Groups

Subsequently, the buccal specimens (n=48) were randomly divided in 6 groups for analysis of the penetration depth of resin infiltration by microscopy confocal analysis. These six groups resulted from the combination of the main factors application techniques (manual, sonic and ultrasonic application) and conditioning protocols (15% hydrochloric acid [HA] and HA + 37% phosphoric acid [PA]): 1) manual application of HA; manual application of HA + PA; HA + ultrasonic application (U); HA + sonic application (S); PA+HA+S; and PA+HA+U. Groups and application protocol are described in Table 1. Table 2 presents the materials used in this study. Each group was composed of eight hemi-teeth.

The same randomization protocol (simple systematic randomization, Biostat 5.0 software, Tefé, AM, Brazil) was performed in the lingual specimens (n=48) that were assigned to micro-shear bond strength test.

Bond strength (BS) Evaluation: Microshear Test

After the infiltration protocols, the lingual specimens

Table 1. Experimental groups and application protocol

Experimental group	Application protocol
Manual application of HA	Etching the marked area of the tooth with 15% hydrochloric acid (HA) for 2 min. Washing and drying for 30 s. Application of ethanol for 30 s. Manual application of the infiltrant resin for 3 min. Photopolymerization - 40 s with LED 1200 mW/cm² (Radii Plus, SDI, Victoria, Australia)
Manual application of PA+HA	Etching the marked area of the tooth with 37% phosphoric acid (PA) for 15 s. Washing and drying for 30 s. Application of HA for 2 min. Washing and drying for 30 s. Application of ethanol for 30 s. Manual application of the infiltrant resin for 3 min. Photopolymerization - 40 s with LED 1200 mW/cm² (Radii Plus, SDI, Victoria, Australia).
HA + Ultrasonic application	Etching the marked area of the tooth with HA for 2 min. Washing and drying for 30 s. Application of ethanol during 30 s. Application of the infiltrant resin with the ultrasonic device for 3 min. Photopolymerization - 40 s with LED 1200mW/cm² (Radii Plus, SDI, Victoria, Australia)
HA + Sonic application	Etching the marked area of the tooth with HA for 2 min. Washing and drying for 30 s. Application of ethanol during 30 s. Application of the infiltrant resin with the sonic device for 3 min. Photopolymerization - 40 s with LED 1200mW/cm² (Radii Plus, SDI, Victoria, Australia)
PA + HA + Ultrasonic application	Etching the marked area of the tooth with PA for 15 s. Washing and drying for 30 s. Application of HA for 2 min. Washing and drying for 30 s. Application of ethanol for 30 s. Application of the infiltrant resin with the ultrasonic device for 3 min. Photopolymerization - 40 s with LED 1200 mW/cm² (Radii Plus, SDI, Victoria, Australia)
PA + HA + Sonic application	Etching the marked area of the tooth with PA for 15 s. Washing and drying for 30 s. Application of HA for 2 min. Washing and drying for 30 s. Application of ethanol for 30 s. Application of the infiltrant resin with the sonic device for 3 min. Photopolymerization - 40 s with LED 1200 mW/cm² (Radii Plus, SDI, Victoria, Australia)

Table 2. Materials used for the study, composition, manufacturer and batch number

Material	Composition*	Manufacturer	Batch number
Icon® - Infiltrant resin	TEGDMA, initiators – additives	DMG Brazil, Piratuba, SP, Brazil	#632178
Icon® - Etch	15% hydrochloric acid, (salicylic and pyrogenic) surface reaction substance	DMG Brazil, Piratuba, SP, Brazil	#632178
Icon® - Dry	99% ethanol	DMG Brazil, Piratuba, SP, Brazil	#632178
Condac 37% - phosphoric acid	37% H_3PO_4 (phosphoric acid) thickener, coloring, deionized water	FGM, Joinville, SC, Brazil	#080814
Adper™ Single Bond 2 - adhesive system	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, copolymer dimethacrylates, poliacrylic acid and acetic acid	3M ESPE, St. Paul, MN, USA	#512002
Filtek™ Z350 XT -composite resin	Bis-GMA, Bis-EMA, UDMA, TEGDMA	3M ESPE, St. Paul, MN, USA	#455416

were randomly selected to evaluate the bond strength by microshear test. The specimens were mounted in PVC tubes (Tigre, São Paulo, SP, Brazil) using acrylic resin (Acrylic Resin VIPI Flash, VIPI, Pirassununga, SP, Brazil). The dental enamel surfaces were conditioned with 37% phosphoric acid for 30 s, cleaned and dried for 30 s. Afterwards, the adhesive system was applied (Adper™ Single Bond 2, 3M ESPE, St. Paul, MN, USA) according to the manufacturer`s instructions. Tygon tubes with 1 mm height and 0.7 mm diameter (Tygon® Medical Tubing, Saint Gobain, Akron, OH, USA) were filled with composite resin (FilteK™ Z350 XT, 3M ESPE, St. Paul, MN, USA), over a glass plate. After observing the absence of bubbles, they were put on the enamel surface and photopolymerized for 40 s with a LED device (1200 mW/cm², RadiiCal, SDI, Victoria, Australia), periodically monitored by a radiometer (Led - Kondortech, Kondortech Equipamentos Odontológicos LTDA, São Carlos, SP, Brazil). Four tygons were positioned on each specimen; and a mean of these 4 bond strength values were obtained for each specimen, which was considered for statistical purposes. The test was performed in a Universal Testing Machine (Kratos Dinamômetros, São Paulo, SP, Brazil) at a cross-head speed of 0.5 mm/min, using an orthodontic wire (0.3 in diameter) positioned as closest as possible to the adhesive interface. The bond strength values were expressed in Megapascal (MPa).

After the microshear test, a stereoscopic magnifier (STEMI 2000-C; Carl Zeiss, Germany) was used to classify the fracture modes (50× magnification): Type 1: Adhesive failure at the enamel-adhesive interface; Type 2: Cohesive failure at the enamel if the failure happens exclusively at the enamel; Type 3: Mixed failure (both fracture modes).

Sample Preparation for Penetration Depth (PD) Evaluation: Confocal Laser Scanning Microscopy

After obtaining the artificial white spot lesions before submitted the specimens to the 6 experimental groups (infiltration protocols), the specimens were conditioned with 15% hydrochloric acid for 2 min, according to the manufacturer`s instructions (Icon®, DMG), and cleaned for 60 s. The hemi-teeth were colored with the first contrast agent (0.1% red fluorophore rhodamine B isothiocyanate, Sigma Aldrich, Steinheim, Germany), immersed during 12 h at 37 °C (17).

At this time, the 48 buccal hemi-teeth were submitted to the 6 experimental groups, as described above. To evaluate the penetration depth of the infiltrant resin, the hemi-teeth were longitudinally cut in a lingo-buccal direction (ISOMET 1000), to obtain discs of 1 mm±0.1 mm thick. The discs were stored in a 30% hydrogen peroxide solution for 12 h at 37ºC, in order to remove all the fluorophore that did not penetrate. Then, the samples were thoroughly washed with deionized water and polished with abrasive paper # 1200, 2400, 4000, with the intention to obtain clean cuts for the confocal laser microscopy observation (CLSM) (NikonA1RSiMP, NIKON, Tokyo, Japan). To visualize the structures or the pores on the non-infiltrated regions of the artificial lesions, the discs were immersed in a 50% ethanol solution, containing 100 µM of sodium fluorescein (Sigma Aldrich, St. Louis, MO, USA) during 3 min. Then, the discs were washed with deionized water for one minute, according to a previous protocol (19).

The discs were visualized in confocal laser scanning microscopy using 20X magnification, in oil immersion (aperture of 0.75). For GFP (Bioluminiscent and fluorescent proteins), a 488nm laser was used for stimulation and bandpass of 500-550 nm. For DAPI (Bioluminiscent and fluorescent proteins), a 405nm laser was used for stimulation and bandpass of 425-475 nm. The software Imaging Software NisElements 4.20 (NIKON, Tokyo, Japan) was used to visualize the images and create 3D animation with the use of Z slices (18). To measure the penetration depth, the images were sent to the image software J 1.47 for Macbook, performing a pixel calibration to turn them into micrometers (µm) of the infiltrant resin, analyzing the three most representative sites. The demineralized enamel lesions were represented by green color, and the infiltration depth by a red color (Fig. 1). The ratio between the demineralized region and the infiltrated region was calculated in percentages.

Statistical Analysis

Data obtained by bond strength (MPa) and penetration depth (%) were analyzed by 2-way ANOVA (application technique *vs.* conditioning protocols) and Tukey post-test (p=0.05). The Sigma Plot 11 software (Systat Software, San Jose, California, USA) was used for statistical analysis. Data from the fracture modes were qualitatively analyzed.

Results
Strength Test

The mean values and standard deviation of the bond strength (in MPa) for the different experimental groups are illustrated in Table 3. Two-way analysis of variance detected that the cross-product interaction, as the main factors (application technique and conditioning protocols), were not significant (p >0.05).

Table 4 showed the failure modes in each experimental group. The most predominant failure observed was cohesive failures, in all experimental groups.

Penetration Depth by Confocal Laser Scanning Microscopy

The obtained images from confocal laser scanning

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microscopy, suggestive from each experimental group, are illustrated in Figure 2. The lesion depth (μm) and the penetration depth (μm) of the infiltrant resin for all experimental groups may be visualized in Figure 3.

The mean penetration depth values and standard deviation for the different experimental conditions are demonstrated in Table 5. Two-way analysis of variance demonstrated that the cross-product interaction (application techniques vs. conditioning protocols) was also not significant (p=0.429), but the main factors were (application technique, p< 0.001 and conditioning protocols, p=0.003). The manual application presented statistically lower results compared to the sonic application (p<0.001). The sonic application presented statistically lower results compared to the ultrasonic application (p=0.01). In relation to the conditioning protocols, the use of PA + HA showed statistically higher results than the isolated application of HA (p=0.003).

Discussion

Resin infiltration is a predictable approach that penetrates enamel porosities in natural white spot lesions, impairing lesion progression and prevent cavitation (20). The recent investigations are focused on new protocols to facilitate the infiltrant resin impregnation into the demineralized enamel (or white spots), in faster periods, to facilitate the inhibition of the lesion progression rapidly.

The use of ultrasonic application may be an interesting approach, as it could be noted the higher penetration depth values when it was used, compared to sonic and manual application. The explanation for the better results when using an ultrasonic device, compared to a sonic device, may be due to the surface vibration, which increased penetration speed, facilitates the infiltrant resin impregnation and destroys the microscopic bubbles formed within the resin (14). Moreover, the lower frequencies produced by the sonic device promote less bubbles and release more energy, opposed to higher frequencies (ultrasonic activation), which produce more bubbles with lower size and lower energy release (17).

The infiltration protocol with a sonic or ultrasonic device was evaluated with both conditioning techniques: 15% hydrochloric acid and 37% phosphoric acid in order to investigate differences between the application protocols of the infiltrating resin Icon®. The lowest values, in terms of penetration depth, were found with the manual application of the infiltrant resin. Several researches have evaluated the bond strength values of different adhesive systems with manual active application on dentin/enamel surface, compared to manual passive application. The results showed higher values for the active application (21,22). In this study, the infiltrant resin was just left in the surface, without vigorous application. Maybe, if the resin was gently agitated with manual pressure, the results would be similar
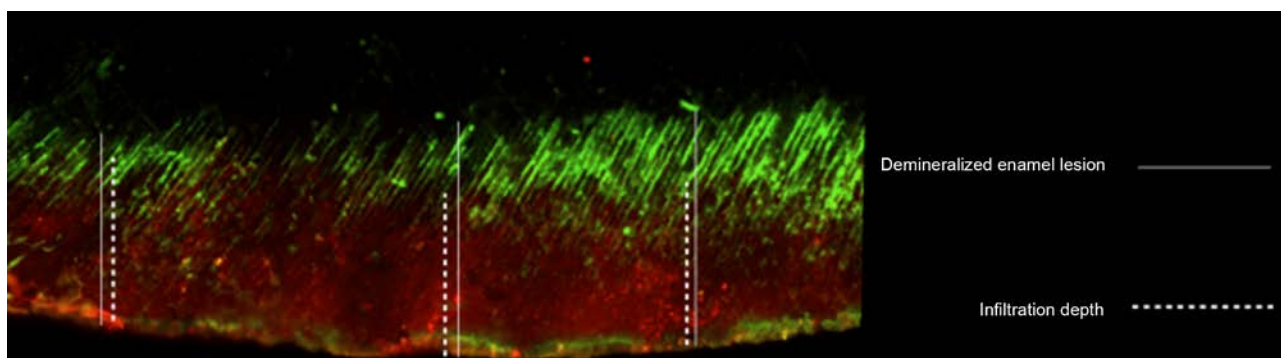

Figure 1. Mensuration of the penetration depth (in red) of the infiltrant resin and the artificial enamel lesion (in green).

Table 3. Mean bond strength values and standard deviation, in MPa, for the application techniques and the conditioning protocols*

Application techniques	Conditioning protocols	
	HA	HA + PA
Manual	22.6 ± 6.2 a	25.1 ± 8.1 a
Sonic	21.6 ± 6.4 a	19.6 ± 7.5 a
Ultrasonic	23.4 ± 6.3 a	24.3 ± 5.8 a

*Same letters indicate no statistically significant differences (p> 0.05)

Table 4. Mean percentage (%) of the failure modes* found in each experimental group

| Application techniques	Conditioning protocols	
	HA	HA + PA
Manual	16.6/50.0/33.4	33.4/50/16.6
Sonic	0/83.4/16.6	16.6/50/33.4
Ultrasonic	16.6/66.8/16.6	16.6/50/33.4

*adhesive/ cohesive/ mixed

to those found by the above-mentioned authors.

Colorants like rhodamine and sodium fluorescein allow a selective visualization due to the stimulation and wave length compression of the TEGDMA resin emission. These colorants were used to evaluate the penetration depth of the infiltrant resin. This technique showed positive

correlation and reproductability (19,23). The reference method to measure the colorants is the lesion depth and the infiltrated depth, defined from the surface until the deepest region of the lesion (colored in green and red fluorescein, respectively) (19,23) as showed at the present study.

As mentioned in previous studies, in 70% of cases, 90%
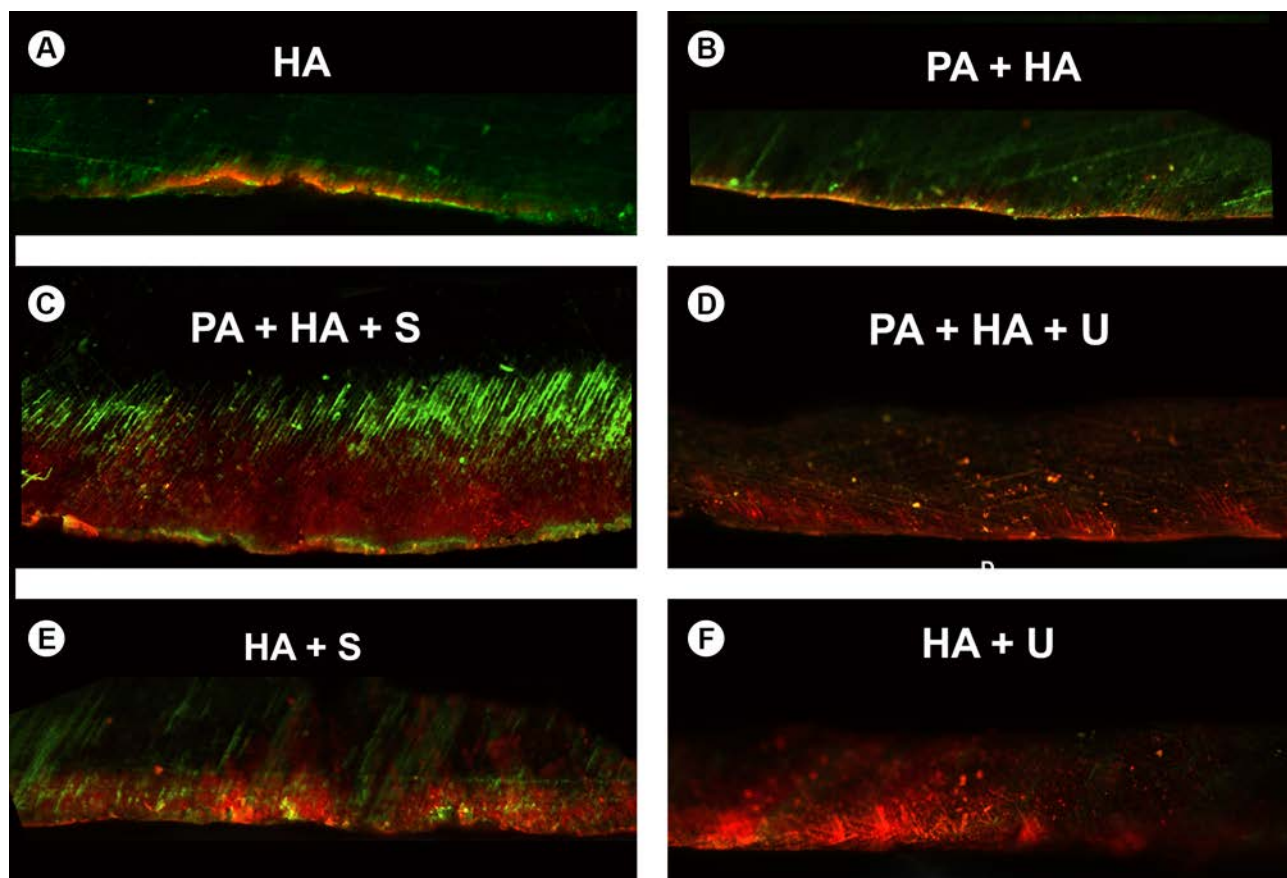
Figure 2. Illustrative images obtained by confocal laser scanning microscopy, according to each experimental group, as follows: A: 15% hydrochloric acid (HA) + manual application of the infiltrant resin; B: HA + 37% phosphoric acid (PA + HA) + manual application of the infiltrant resin; C: PA + HA + sonic application (S) of the infiltrant resin; D: PA + HA + ultrasonic application (U) of the infiltrant resin; E: HA + sonic application (S) of the infiltrant resin; F: HA + ultrasonic application of the infiltrant resin.
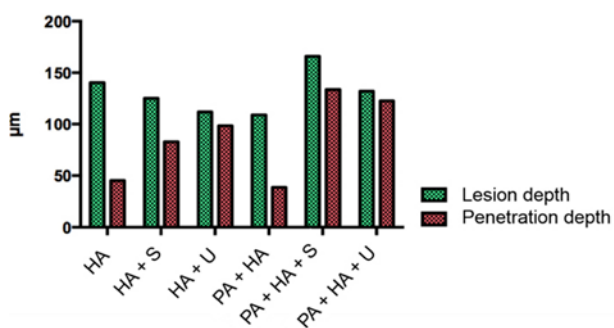

Figure 3. Lesion depth and penetration depth (in μm) of the infiltrant resin in the groups, where HA: 15% hydrochloric acid; PA: 37% phosphoric acid; U: ultrasonic application; S: sonic application.

Table 5. Penetration depth values (%) and standard deviation for the different experimental groups*

Application techniques	Conditioning protocols		Main Factor
	HA	HA + PA	
Manual	28.0 ± 9.3	34.5 ± 7.7	31.3 ± 8.7 a
Sonic	66.8 ± 13.4	84.2 ± 8.9	75.5 ± 14.1 b
Ultrasonic	84.1 ± 10.1	94.1 ± 5.3	89.1 ± 9.2 c
Main Factor	59.6 ± 26.3 A	71.1 ± 27.8 B	

* Different letters indicate statistically significant difference ($p<0.05$). HA: 15% hydrochloric acid; PA: 37% phosphoric acid;

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of the base of the eroded surface may present impurities accumulation due to the increased acid application time (5,9,10). Therefore, as an effort to preserve the dental surface, being less invasive regarding the conditioning protocols (specifically to avoid the longer contact time of HA with enamel), the combination of PA + HA with the sonic and ultrasonic application was studied. The association of both acid agents showed higher penetration depth values compared to single application of HA. PA is extensively known as the gold standard acid agent used to etch the enamel surface prior to adhesive procedures (24,25). One may be speculated that the hypomineralised base could be eliminated, and PA acts as a booster agent of HA over the demineralization process.

The bond strength values obtained from microshear test did not present significant statistical differences between the groups, being not possible to correlate to the penetration depth evaluation. It was suspected that the higher penetration depth of the infiltrant resin would result in higher bond strength values for the composite resin; however, it did not occur. Likewise, this observation was noted in previous studies, which did not correlate the penetration depth and the bond strength values (24,25).

It is worth to mention that, as an in vitro study, there are limitations regarding the artificially developed white spot lesions. Studies have reported that artificial enamel lesions are more permeable and show less organic content on their surface (25). In situ or in vivo studies, where natural saliva and the acquired pellicle are presented, may demonstrated different results. In this way, more in vitro and long-term studies are required to confirm the findings, and also to validate the application strategies and protocols that may have clinical promising results.

Finally, the results of this study showed that neither sonic/ ultrasonic application of the infiltrant resin or the conditioning protocols, influenced the bond strength values between the infiltrant resin and composite resin. However, the sonic/ultrasonic applications and the combination of 37% phosphoric acid prior to 15% hydrochloric acid application increased the penetration depth values of the infiltrant resin.

Resumo:

O objetivo deste estudo foi avaliar diferentes protocolos de condicionamento e a aplicação sônica/ultrassônica de uma resina infiltrante (RI) em lesões de mancha branca produzidas artificialmente (LMBA). As superfícies vestibulares/linguais de 48 molares foram induzidas à formação de LMBA e divididas em 6 grupos, de acordo com os protocolos de condicionamento e técnica de aplicação da resina infiltrante: ácido hidroclorídrico 15% (AH) + aplicação manual da RI; AH + ácido fosfórico 37% (AF) + aplicação manual da RI; AH + aplicação ultrassônica da RI (U); AH + aplicação sônica da RI (S); AF + AH + S; e AF + AH + U. para o grau de penetração (GP), condicionou-se as coroas com AH por 120 s. A RI Icon® (DMG) foi aplicada de acordo com as instruções do fabricante. As coroas foram coradas (rodamina B 0,1% por 12 h) e clareadas com peróxido de hidrogênio 30% por 12 h. os discos foram imersos em solução de etanol 50%, contendo 100 μM de fluoresceína sódica. O GP (em μm) foi mensurado por meio de microscopia confocal a laser (20×). A resistência de união (RU) foi calculada pelo teste de microcisalhamento (0,5 mm/min). Os dados foram submetidos ao teste ANOVA 2 fatores e Tukey (α=0,05). Para RU, a interação não foi significante (p>0,05). Para GP, os fatores principais foram significantes (técnica de aplicação - p<0,001; protocolos de condicionamento - p=0,003). A aplicação U mostrou os maiores valores de GP. AF+AH demonstrou resultados superiores ao grupo AH. As aplicações sônica/ultrassônica e o uso do ácido fosfórico antes do ácido hidroclorídrico aumentaram o GP da resina infiltrante. Os protocolos de condicionamento ou as técnicas de aplicação não influenciaram os valores de RU.

References

1. Pitts N. "ICDAS"--an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Commun Dent Health 2004; 21:193-198.
2. Askar H, Schwendicke F, Lausch J, Meyer-Lueckel H, Paris S. Modified resin infiltration of non-, micro- and cavitated proximal caries lesions in vitro. J Dent 2018;74:56-60.
3. Paic M, Sener B, Schug J, Schmidlin PR. Effects of microabrasion on substance loss, surface roughness, and colorimetric changes on enamel in vitro. Quintessence Int 2008;39:517-522.
4. Ammari MM, Jorge RC, Souza IPR, Soviero VM. Efficacy of resin infiltration of proximal caries in primary molars: 1-year follow-up of a split-mouth randomized controlled clinical trial. Clin Oral Investig 2018;22:1355-1362.
5. Pini NIP, Lima DANL, Sundfeld RH, Ambrosano GMB, Aguiar FHB, Lovadino JR. Tooth enamel properties and morphology after microabrasion: an in situ study. J Investig Clin Dent 2017;8(2).
6. Subramaniam P, Girish Babu KL, Lakhotia D. Evaluation of penetration depth of a commercially available resin infiltrate into artificially created enamel lesions: An in vitro study. J Conserv Dent 2014;17:146-149.
7. Shivanna V, Shivakumar B. Novel treatment of white spot lesions: A report of two cases. J Conserv Dent 2011;14:423-426.
8. Chay PL, Manton DJ, Palamara JE. The effect of resin infiltration and oxidative pre-treatment on microshear bond strength of resin composite to hypomineralised enamel. Int J Paediatr Dent 2014;24:252-267.
9. Yim HK, Kwon HK, Kim BI. Modification of surface pre-treatment for resin infiltration to mask natural white spot lesions. J Dent 2014;42:588-594.
10. Meyer-Lueckel H, Paris S. Improved resin infiltration of natural caries lesions. J Dent Res 2008;87:1112-1116.
11. Hashimoto M, Ohno H, Kaga M, Sano H, Tay FR, Oguchi H, et al. Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems. J Dent 2002;30:99-105.
12. Paris S, Bitter K, Renz H, Hopfenmuller W, Meyer-Lueckel H. Validation of two dual fluorescence techniques for confocal microscopic visualization of resin penetration into enamel caries lesions. Microsc Res Tech 2009;72:489-494.
13. Askar H, Lausch J, Dorfer CE, Meyer-Lueckel H, Paris S. Penetration of micro-filled infiltrant resins into artificial caries lesions. J Dent 2015; 43:832-828.
14. Cuadros-Sanchez J, Szesz A, Hass V, Patzlaff RT, Reis A, Loguercio AD. Effects of sonic application of adhesive systems on bonding fiber posts to root canals. J Endod 2014;40: 1201-1205.
15. Mena-Serrano A, Garcia EJ, Loguercio AD, Reis A. Effect of sonic application mode on the resin-dentin bond strength and nanoleakage of simplified self-etch adhesive. Clin Oral Investig 2014;18:729-736.
16. Herrera DR, Santos ZT, Tay LY, Silva EJ, Loguercio AD, Gomes BP. Efficacy of different final irrigant activation protocols on smear layer removal

by EDTA and citric acid. Microsc Res Tech 2013;76:364-369.

17. Albu S, Joyce E, Paniwnyk L, Lorimer JP, Mason TJ. Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinus officinalis for the food and pharmaceutical industry. Ultrason Sonochem 2004;11:261-265.

18. Gelani R, Zandona A, Lippert F, Kamocka M, Eckert G. In Vitro Progression of Artificial White Spot Lesions Sealed With an Infiltrant Resin. Oper Dent 2014;39:481-488.

19. Paris S, Schwendicke F, Seddig S, Müller WD, Dörfer C, Meyer-Lueckel H. Micro-hardness and mineral loss of enamel lesions after infiltration with various resins: influence of infiltrant composition and application frequency in vitro. J Dent 2013;41:543-548.

20. Swamy DF, Barretto ES, Mallikarjun SB, Dessai SSR. In vitro Evaluation of Resin Infiltrant Penetration into White Spot Lesions of Deciduous Molars. J Clin Diagn Res 2017;11:ZC71-ZC74.

21. Higashi C, Michel MD, Reis A, Loguercio AD, Gomes OM, Gomes JC. Impact of adhesive application and moisture on the mechanical properties of the adhesive interface determined by the nano-indentation technique. Oper Dent 2009;34:51-57.

22. Zander-Grande C, Ferreira SQ, da Costa TR, Loguercio AD, Reis A. Application of etch-and-rinse adhesives on dry and rewet dentin under rubbing action: a 24-month clinical evaluation. J Am Dent Assoc 2011;142:828-835.

23. Meyer-Lueckel H, Paris S. Progression of artificial enamel caries lesions after infiltration with experimental light curing resins. Caries Res 2008; 42:117-124.

24. Ozer F, Blatz MB. Self-etch and etch-and-rinse adhesive systems in clinical dentistry. Compend Contin Educ Dent 2013;34:12-14.

25. Lausch J, Paris S, Selje T, Dorfer CE, Meyer-Lueckel H. Resin infiltration of fissure caries with various techniques of pretreatment in vitro. Caries Res 2015;49:50-55.

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