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EFFECTIVENESS OF ULTRASONIC ACTIVATION ASSOCIATED TO GLYCOLIC ACID ON SMEAR LAYER REMOVAL, DENTIN STRUCTURE AND BOND STRENGTH TO ROOT DENTIN

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Abstract

This study evaluated the effects of ultrasonic activation (US) associated to glycolic acid (GA) on smear layer, dentin structure and bond strength (BS) of filling/restorative material to root dentin. The roots were used for antimicrobial activity, dentin structure and BS evaluation, being distributed into seven groups, according to irrigation protocols: G1:DW+US; G2:17% EDTA; G3:QMix; G4:17% GA; G5:17% EDTA+US; G6:QMix+US; G7:17% GA+US. Scanning electronic microscopy, transmission electronic microscopy and push-out were performed, with specific statistical analysis for each evaluation. The highest smear layer removal occured in Groups 6 and 7 ($p<0.05$), and the largest collagen dispersion in Group 7, being similar to Group 2 and 5 ($p>0.05$). The highest BS of filling and restorative material occurred in Groups 6 and 7, and Groups 5, 6 and 7, respectively, being similars between them ($p>0.05$). The use of GA+US promoted effective smear layer removal and dentin structure preservation, improving the BS of filling/restorative material to root dentin.

Keywords: EDTA. Final irrigation. Glycolic acid. QMix. Ultrasonic activation.

1. Introduction

Chemo-mechanical preparation plays an important role in the decontamination of root canals, being essential to achieve successful endodontic treatment (Byström and Sundqvist 1981). However, dentin chips are released during this step, which, associated with organic components, microorganisms and irrigant agents, give rise to the smear layer. It adheres to the dentin surface and promotes obliteration of dentinal tubules (Torabinejad et al. 2002), avoiding the penetration of antimicrobial agents and reducing the bond strength (BS) of filling materials to the root dentin (Shahravan et al. 2007). Thus, the use of final irrigants for smear layer removal is required, cleaning the root canal walls and favoring the adhesion of filling or restorative materials (Perez-Heredia et al. 2008).

Ethylenediaminetetraacetic acid (EDTA) is the final irrigant most widely used in endodontics, with ability to remove the smear layer (Dai et al. 2011). However, it has reduced action on the apical third

(Kuruvilla et al. 2015), is cytotoxic when extravasated to periapical tissues (Botton et al. 2016) and induces significant changes in the dentin structure (Aslantas et al. 2014; Wagner et al. 2017). More recently, an irrigant called QMix, containing 17% EDTA, chlorhexidine and a surfactant agent in its composition, has also been used as a final irrigant, not only contributing to the removal of the smear layer, but also to the decontamination process of the system of root canals (Stojicic et al. 2012). However, the presence of EDTA in its composition can lead to the occurrence of undesirable effects, as previously described. Therefore, some alternatives should be identified, promoting effective smear layer removal and preserving the dentin structure.

Glycolic acid (GA) has organic nature and molecular structure similar to citric acid, being initially used in enamel and dentin conditioning (Cecchin et al. 2018). In endodontics, previous studies have revealed smear layer removal effectiveness and low cytotoxicity (Bello et al. 2019), low surface tension, pH stability and preservation of the structural dentin integrity (Bello et al. 2020). In turn, ultrasonic activation (US) represents a well-established resource aimed at improving the effectiveness of endodontic irrigants (Verhaagen et al. 2014). Souza et al. (2021) showed that the antimicrobial action of GA was increased by using US. However, there are no studies in literature evaluating the effectiveness of GA associated with US on the smear layer removal, as well as its effects on dentin structure and adhesion of filling/restorative materials to the root dentin.

The aim of this study was to evaluate the effectiveness of US associated to GA on smear layer removal and its influence on dentin structure and BS of filling/restorative material to root dentin. The hypotheses were that this association (i) promotes significant smear layer removal, (ii) preserves the dentin structure and (iii) increases the BS of the filling/restorative material to the root dentin.

2. Material and Methods

This study was approved by the Research Ethical Committee of the University of Passo Fundo (protocol 3.110.552).

Smear layer removal evaluation

Seventy single-rooted extracted human teeth without caries cavity were used, which were obtained from the Biobank of the School of Dentistry of University of Passo Fundo (Passo Fundo, RS, Brazil). The teeth were previously sanitized, immersed in distilled water and immediately stored under refrigeration until the beginning of the experiments, in order to preserve their properties. Dental crowns were sectioned with diamond disc so that all roots remained with 15 mm in length. Diamond disc was used to produce two longitudinal grooves in the external root surface without reaching the canal space. The working length (WL) was established by introducing #10 K-file (Dentsply-Sirona, York, PA, USA) into the canal until its tip was visualized at the apical foramen. From this measure, 1 mm was subtracted from the WL. Roots were enlarged to WL using the ProTaper system (Dentsply-Sirona), following the sequence from S1 to F3. Distilled water (DW) (Natupharma, Passo Fundo, RS, Brazil) was used as irrigant solution and renewed at each instrument change.

The 70 samples were irrigated with 5 mL of DW and randomly divided into seven groups (n=10), according to the final irrigation protocol: G1: DW+US; G2: 17% EDTA; G3: QMix; G4: 17% GA; G5: 17% EDTA+US; G6: QMix+US; G7: 17% GA+US. In groups without US, root canals were filled with the tested solution using 5-mL syringe with 19-G needle, until extravasation to the root canal entrance. The solution remained in the root canal for 1 min. In groups with US, after root canal filling with the test solution, US was performed using ultrasonic device (NacPlus, Adiel, Ribeirão Preto, SP, Brazil) and stainless-steel E1-irrisonic endodontic tip (Helse Ultrasonic, Ribeirão Preto, SP, Brazil). The tip was inserted close to WL and activated for 1 minute. Scale power 1 for endodontics (10% power) was used. Finally, irrigation with 5 mL of DW was performed in all groups and the root canals were dried using aspiration cannula.

Roots were split into two halves, obtaining 20 halves per group, which were dehydrated in ethanol, mounted on aluminum stubs, coated with gold palladium and examined using a scanning electronic microscope (SEM) (VEGA LM 3, Tescan, Libušinatř. Kohoutovice, Czech Republic). Images were obtained at

2000× magnification and 20 kV along the coronal (10–12 mm from the apex), middle (6–7 mm from the apex), and apical (1–2 mm from the apex) thirds of each half. Two images were obtained per third, 12 images per root, totaling 40 images for each third and 120 images per group.

The effectiveness of each group was evaluated by scoring system (Prado et al. 2011): 1=no smear layer, all tubules opened; 2=few areas covered by smear layer, most tubules opened; 3=smear layer covering almost all surface, few tubules opened and 4=smear layer covering the entire surface. This evaluation was performed by two previously calibrated and blinded observers. Figure 1 presents an illustration of the scoring system. The Kappa coefficient test showed agreement between researchers (k=0.886). Data were analyzed by Kruskal-Wallis and Mann-Whitney U tests for intergroup comparisons, and the Wilcoxon and Friedman tests for intragroup comparisons (α=5%).

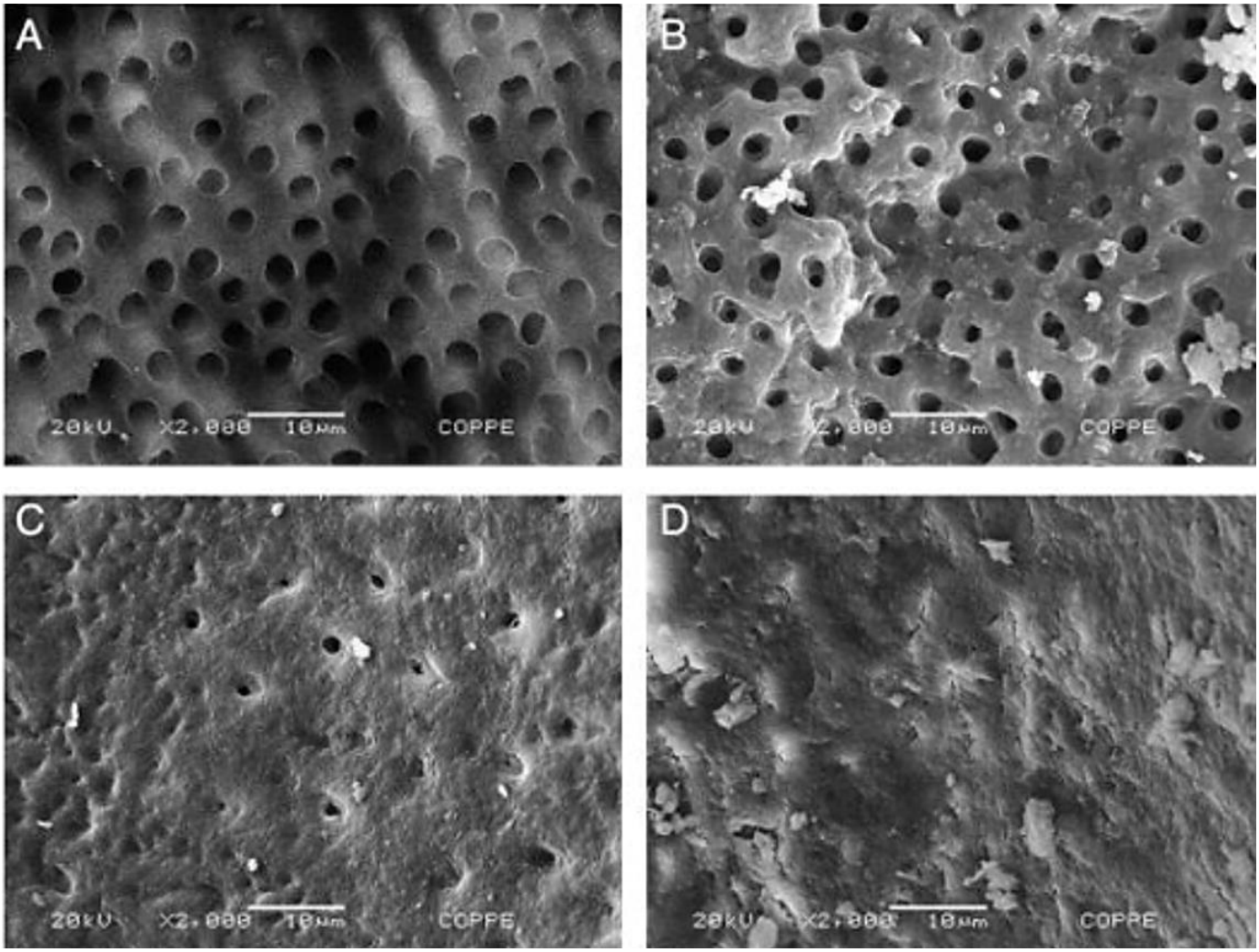

Figure 1. SEM images (x2000) demonstrating the scoring system used to analyse the effectiveness of each group in the smear layer removal.: A - Score 1; B - Score 2; C - Score 3 and D - Score 4.

Dentin structure evaluation

Fourteen bovine incisor teeth were transversely sectioned below the cementum-enamel junction, producing 15-mm-long root segments. Two longitudinal grooves were performed on the buccal and lingual region and two grooves were performed surrounding the root, 5mm and 10mm from the apex, using diamond disc. Instrumentation was performed by hand K-type files (Dentsply-Sirona), using serial instrumentation, enlarging the root canal up to #50 file. Roots were rinsed with 5 mL of DW at every file change. Roots were randomly divided into 7 groups (n=2), similarly to the previous test. Test solutions and irrigation procedures were also the same.

Then, roots were split into two halves, obtaining four samples per group. The surrounding grooves were cleaved, obtaining four samples from the cervical, middle and apical thirds of each root. Samples from the cervical and apical thirds were discarded and samples from the middle third were used for

evaluation by transmission electron microscopy (TEM), as described by Dias et al. (2014). Samples were decalcified, immersed in specific fixative solution and dehydrated, being then included in silicone molds with pure resin and stored in oven at 60ºC for 72 hours. Subsequently, samples were ultramicrotomized in microtome (Leica Microsystems, Wetzlar, Germany). At this stage, sections of 100 nm in thickness were performed in the direction of the long axis of dentinal tubules, deposited in grids and contrast fluid with 2% Uranyl Acetate and Lead Citrate was inserted. Sections were examined by TEM (FEI, Thermofisher Scientific, Hillsboro, Oregon, USA), with power of 200 kV.

Eight images of sections per group were captured at 8,900x magnification. The qualitative analysis of collagen, corresponding to the dentin structure, considered the following classification status: intact(INT), dispersed(DIS) and altered(ALT). This attribution was performed by a senior dentinal ultrastructure examiner, who was previously calibrated and blinded.

For quantitative analysis, the Image J software was used to analyze images captured by TEM. Adjustment for RGB Color was performed, and using the Rotated Rectangle function, 3 areas were selected, in this case, the entrance of dentinal tubules. Subsequently, the image was duplicated, generating a new measurement. It was necessary to make an adjustment of color function to binarize the image, splitting the scale in two colors to analyze the image with collagen and that with no collagen. After selecting the collagen areas, a measurement was generated, and the process was repeated selecting the area free of collagen. The software saves the results of areas, being classified as areas with and without collagen. This process was repeated in 3 different areas of the original image, surrounding the entrance of the dentinal tubule. Subsequently, the results of the three rectangles were exported to the Excel Software, differentiating results of the total area, white area (without collagen) and black area (with collagen), determining the percentage of each area and obtaining the average values of each image.

Regarding the qualitative analysis, data were not submitted to statistical analysis, with description of the dentin collagen appearance. In the quantitative analysis, data were submitted to ANOVA test, followed by the LSD test for multiple comparisons. The percentage of the black (collagen) and white (collagen dispersion) areas of each group was reached (α=5%).

Bond strength evaluation

One hundred and forty single-rooted extracted human teeth were used, which were obtained and prepared as previously described. Seventy roots were used for BS of the filling material and seventy roots were used for BS of the restorative material, being randomly divided into the same seven groups (n=10), as previously described.

In the first evaluation, all roots were filled by lateral condensation using gutta-percha and AH Plus sealer (Dentsply-Maillefer). Excess gutta-percha was removed. In the second evaluation, No. 1 glass fiber posts (GFP) (White Post – FGM, Joinvile, SC, Brazil) were cleaned with 35% phosphoric acid for 30s, rinsed for 30s and gently air-dried. Silane application (3M ESPE) was performed for 1 min, followed by Single-Bond (3M ESPE) adhesive application and light polymerization for 40s with halogen light source and power of 600 mV/cm^2 (Optilux, Demetron Res. Corp, Danbury CT, USA). The root canal dentin was not etched. Subsequently, Rely-X U200 self-adhesive cement was injected with a suitable Centryx syringe and Acudosse needle (DFL, Rio de Janeiro, RJ, Brazil). The GFP was covered with cement and positioned within the root canal at 10 mm level, being held under digital pressure for 20s. Excess cement was removed, being polymerized by 600 mW/cm^2 halogen light source (Optilux) for 30s on each face (buccal, palatal, mesial, distal and occlusal).

All specimens were stored at 37°C and 95% humidity for 21 day for adaptation of the filling/restorative material. Subsequently, roots were transversely sectioned from the root canal entrance into discs of 1 mm in thickness metallographic cutter with diamond disc at at speed of 350 rpm under cooling. The first disc was discarded and the next five discs were selected from each sample, totaling 50 specimens per group in each evaluation. Each disc was subjected to the push-out test in a mechanical testing machine (Emic DL 2000, São José dos Pinhais, PR, Brazil) at speed of 1 mm/min until material displacement. Care was taken to ensure that the contact between the punch tip and the material occurred over the most extended area. Furthermore, the punch tip was centralized in the root canal and positioned

to contact only the material without stressing the surrounding canal walls. The force required to displace the material was recorded in newtons and calculated in megapascal (MPa).

BS calculation and failure patterns were based on previous study (15). BS in MPa was calculated using the formula δ=F/A, where F is the force (N) used by the test machine and A is the area. To calculate the area, the following equation was applied: A=2πr x h, where π is the constant value of 3.14, r is the radius of the intra-radicular space and h is the height (mm). Failure patterns were classified under optical microscopy (Zeiss, São Paulo, Brazil) at 50x magnification, as follows: 1:adhesive, between the dentin and the filling/restorative material, absence of filling/restorative material in the root canal dentine walls; 2:cohesive, failure of the filling/restorative material, presence of filling/restorative material in root canal dentine walls; 3:mixed, both failures (1 and 2) could be observed.

3. Results

The mean and standard deviation values of smear layer removal, collagen area (black area %) and dispersion area (white area %) for each group, are presented in Table 1.

Table 1. Mean and standard deviation of smear layer removal scores for each group; mean and standard deviation of collagen area (black area – %) and dispersion area (white area – %) for each group, at a magnification of 8.900x.

Group	Smear layer Cervical third (Scores)	Smear layer Middle third (Scores)	Smear Layer Apical third (Scores)	Collagen Black area (%)	Dispersion White area (%)
1. DW+US	$3.95\pm 0.41^{A,a}$	$3.96\pm0.14^{A,a}$	$3.99\pm0.26^{A,a}$	63.00 ± 2.08^{A}	22.33 ± 2.40^{A}
2. EDTA	$1.90\pm0.34^{B,a}$	$2.35\pm0.42^{B,a}$	$3.25\pm0.30^{B,b}$	$40.33\pm4.70^{B,C}$	16.33 ± 6.06^{A}
3.QMix	$1.25\pm0.76^{B,a}$	$1.35\pm0.36^{C,a}$	$2.80\pm0.21^{B,b}$	62.66 ± 1.20^{A}	19.33 ± 2.90^{A}
4. GA	$1.95\pm0.46^{B,a}$	$2.05\pm0.29^{B,a}$	$2.75\pm0.38^{B,b}$	48.00 ± 4.50^{B}	36.33 ± 4.37^{B}
5. EDTA+US	$1.50\pm0.21^{B,a}$	$1.45\pm0.26^{C,a}$	$2.50\pm0.31^{B,b}$	$44.33\pm3.92^{B,C}$	$37.00\pm8.00^{B,C}$
6. QMix+US	$1.35\pm0.42^{B,a}$	$1.44\pm0.32^{C,a}$	$1.56\pm0.36^{C,a}$	47.66 ± 1.66^{B}	33.66 ± 5.23^{B}
7. GA+US	$1.45\pm0.38^{B,a}$	$1.52\pm0.44^{C,a}$	$1.58\pm0.22^{C,a}$	32.33 ± 8.41^{C}	50.66 ± 5.84^{C}

* Different capital letters indicate significant differences between groups. Different small letters indicate significant differences between root thirds ($p<0.05$); ** DW=distilled water; US=ultrasonic activation; EDTA=Ethylenediaminetetraacetic acid; GA=glycolic acid.

The intergroup analysis revealed no statistical differences between groups in the cervical third ($p>0.05$). Groups 3 (Qmix), 5 (EDTA+US), 6 (Qmix+US) and 7 (GA+US) were the most effective in the middle third, and groups 6 (QMix+US) and 7 (GA+US) were the most effective in the apical third, with no statistical differences from each other ($p>0.05$). Intragroup analysis revealed no significant differences among groups 1 (DW+US), 6 (Qmix+US) and 7 (GA+US), ($p>0.05$), and the apical third was significantly more deficient in the other groups ($p<0.05$).

The qualitative analysis of TEM revealed INTACT status for control group and DISPERSED status for all other groups. Figure 2 illustrates these statuses. The quantitative analysis showed that group 7 (GA+US) promoted the largest collagen dispersion area, being statistically similar to group 5 (EDTA+US) ($p>0.05$). Furthermore, it was possible to observe that US increased the collagen dispersion ability of test irrigants, being statistically different from the isolated use of these irrigants ($p<0.05$).

The mean and standard deviation of BS values of the filling material and failure pattern percentage for each group are presented in Table 2. The highest BS values were observed for groups 6 (Qmix+US) and 7 (GA+US), being statistically different from all other groups ($p<0.05$).

The mean and standard deviation of BS values of the restorative material failure pattern percentage for each group are presented in Table 3. The highest BS values were observed for groups 5 (EDTA+US), 6 (Qmix+US) and 7 (GA+US), being statistically different when compared to all other groups ($p<0.05$). The chi-square test revealed no statistically significant differences in the failure pattern among groups, with higher predominance of cohesive failure, in both evaluations ($p>0.05$).

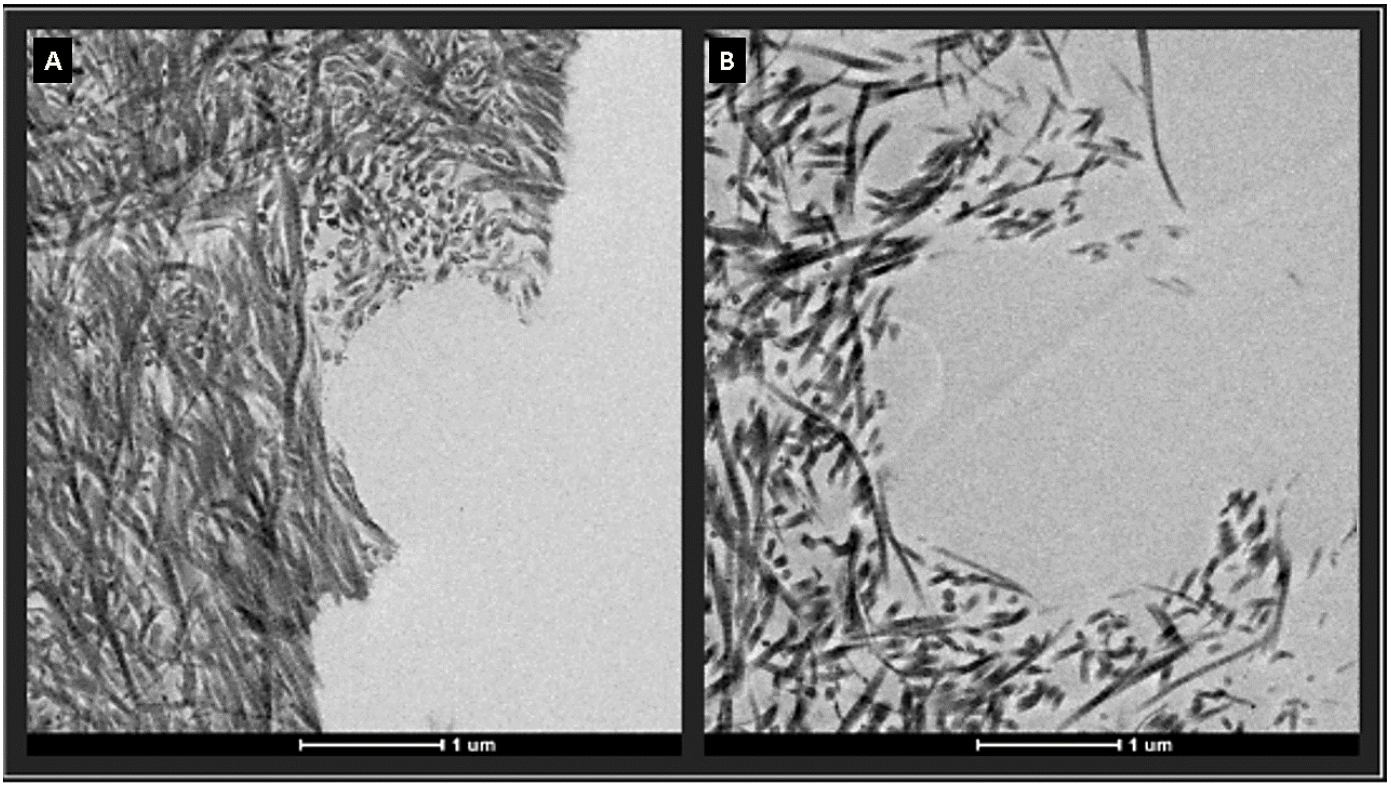

Figure 2. TEM images illustrating: A - intact and B - dispersed collagen.

Table 2. Mean (standard deviation) of bond strength of filling material to root canal dentin (MPa) and percentage of pattern of failure (%) of tested final irrigation protocols.

Group	n	Push Out Bond Strength	Failure mode		
			Adhesive	Mixed	Cohesive
1. DW + US [a]	50	2.20 (1.06)	36.00	8.00	56.00
2. EDTA [b]	50	6.09 (1.56)	34.00	14.00	52.00
3. QMix [c]	50	11.12 (1.89)	30.00	18.00	52.00
4. GA [c]	50	9.10 (1.12)	24.00	22.00	54.00
5. EDTA + US [d]	50	14.92 (1.30)	2.00	30.00	68.00
6. QMix + US [e]	50	20.18 (2.94)	18.00	20.00	62.00
7. GA + US [e]	50	18.10 (1.71)	6.00	24.00	70.00

* Different superscript lowercase letters indicate, in the column, statistically significant differences ($p < 0.05$); ** DW, distilled water; GA, glycolic acid; US, ultrasonic activation.

Table 3. Mean (standard deviation) of bond strength of filling material to root canal dentin (MPa) and percentage of pattern of failure (%) of tested final irrigation protocols.

Group	n	Push Out Bond Strength	Failure mode		
			Adhesive	Mixed	Cohesive
1. DW + US [a]	50	9.91 (0.98)	0.00	0.00	100.00
2. EDTA [b]	50	22.42 (1.07)	4.00	4.00	92.00
3. QMix [c]	50	30.94 (2.67)	0.00	4.00	96.00
4. GA [c]	50	30.73 (2.90)	2.00	0.00	98.00
5. EDTA + US [d]	50	40.17 (2.50)	4.00	0.00	96.00
6. QMix + US [d]	50	42.66 (2.72)	2.00	0.00	98.00
7. GA + US [d]	50	40.33 (2.51)	8.00	0.00	92.00

* Different superscript lowercase letters indicate, in the column, statistically significant differences ($p < 0.05$); ** DW, distilled water; GA, glycolic acid; US, ultrasonic activation.

4. Discussion

During final irrigation, smear layer removal and dentin structure preservation are important aspects for providing conditions for the adequate adhesion of filling materials to the root dentin. In this scenario, the present study evaluated the GA-based final irrigant associated with US, comparing with conventional

irrigants, such as EDTA and QMix. It is known that excessive irrigation time can lead to erosion and formation of cracks in the root dentin. At the same time, the smear layer can be effectively removed, even using short final irrigation period (Çalt and Serper 2002). For these reasons, the final irrigation time of test protocols was set at 1 minute in the present study.

SEM is the method most widely used to quantify the cleaning of root canal walls (Wagner et al. 2017), whereas the score system allows evaluating the smear layer removal, based on the amount of smear layer and opened dentinal tubules (Prado et al. 2011). TEM corresponds to an important tool in studies aimed at assessing the morphology and structural defects of the root dentin, acting in qualitative and quantitative analyses (Wagner et al. 2017). Finally, the push out test represents a consolidated method to evaluate the BS of filling material to the root dentin, being comparable with the clinical reality. Furthermore, it induces effective power for material displacement and less stress at the bond interface (Dias et al. 2014). For these reasons, these tests were adopted to evaluate the variables of the present study.

Smear layer removal was similar among all groups in the cervical third in the present study. This third has greater volume and allows easy access of the irrigant agent, causing effective action, even without US. In the middle third, QMix showed result similar to groups treated with US. This irrigant has a surfactant agent in its composition, reducing surface tension and increasing penetration into the depth of dentinal tubules (Bello et al. 2019), which may have favored these results. Despite the low surface tension presented in a previous study (Bello et al. 2020), the use of GA alone did not promote effective smear layer removal. In the apical third, irrigant agents with low surface tension associated with US (Qmix+US and GA+US) showed the best results. Results reveal difficulty of cleaning this third, requiring not only effective penetration into the root dentin, but also the use of US, which drives the irrigant agent to this area. This result was confirmed by the intragroup findings of the present study, where smear layer removal was lower in the apical third when compared to the other thirds of the root canal.

Middle third fragments were used for TEM evaluation, because central areas of the root canal present more regular and smooth dentin walls (Mai et al. 2010; Qian et al. 2011). Bovine teeth were used in this evaluation because they allowed more standardized collagen pattern (Wagner et al. 2017). According to the present results, US increased the ability to promote collagen dispersion of all test irrigants. US impulses the irrigant solution against the dentinal walls, generating a sequence of shocking waves into the root canal and displacing collagen fibrils from the dentin wall. At the same time, dentin structure degradation was not observed, being a safe procedure in the endodontic therapy. This effect can lead to changes in dentin elastic modulus (John et al. 2011), fracture toughness (Zhang et al. 2010), and bond strength of filling/restorative materials (Mai et al. 2010). Thus, dentin structure preservation is mandatory to adequate root canal sealing and longevity of the endodontic treatment.

The epoxy-resin-based endodontic sealer AH Plus has high flowability and cohesion between its molecules increases the resistance to the filling material displacement, providing effective adhesion to the root dentin (Silva et al. 2016). The results of the present study showed that the use of US over the test final irrigants improved BS, when compared to the isolated use of these chemical substances. Similar findings were observed in a previous study where the association of final irrigant and US resulted in higher BS to the root dentin (Ackay et al. 2015). The hydrodynamic turbulence induced by US associated with the low surface tension of test irrigants, such as QMix and GA, which showed better results, improves irrigant penetration and smear layer removal (Castagna et al. 2013). Then, the dentin structure presents cleaner dentinal wall and more opened dentinal tubules, enabling effective mechanical interlocking of the endodontic sealer. These are related to the adhesion and physicochemical properties, which also help explaining the higher cohesive failure rates in this evaluation.

In the same way, final irrigants associated with US provided higher BS of the restorative material to the root dentin, when compared to final irrigants alone. Similar results were found by Akyus Ekin and Erdemir (2015). The physical properties of test irrigants, as well as the physical principles of US, as previously described, also help to explain the findings of the present study. Resin cements are the most recommended for cementing GFP inside the root canal (Radovic et al. 2008). In the present study, self-adhesive resin cement Rely-X U200 was used, since it provides better adhesion of the GFP to the root dentin and has the purpose of simplifying cementation (Ferracane et al. 2011). The presence of acidic

monomers in its composition demineralizes and infiltrates the root dentin substrate, providing micromechanical retention. In addition, there is a reaction between acid monomers of the cement and hydroxyapatite of the dentin substrate, resulting in additional chemical retention (Pisani-Proenca et al. 2011). This association of mechanical and chemical retention results in effective adhesion to the root dentin, helping to understand the higher incidence of cohesive failure in this evaluation.

The association of GA and US promotes significant smear layer removal in the middle and apical thirds, does not induce dentin structure modification and increases the BS of the filling/restorative material to the root dentin. These findings confirm all hypotheses of the present study. In addition, the use of US improved the results of test final irrigants in all evaluations of the present study. US increases temperature and hydrostatic pressure, enhancing the action of final irrigants inside the root canal (Castagna et al. 2013; Verhaagen et al. 2014; Ackay et al. 2015). As a consequence, cleaner root canal wall is obtained, with dispersed collagen, favoring the adhesion of the filling/restorative material to the root dentin. In this scenario, US is essential to obtain these results.

GA has significant ability to promote smear layer removal (Bello et al. 2019), being the main proposal of a final irrigant agent. The small size of GA molecules and its acidic pH contributes to effective penetration and demineralization of the root dentin (Bello et al. 2020). In addition to this important ability, GA presents low cytotoxicity and does not induce damages to the dentin mechanical properties (Bello et al. 2019). It is an advantage when compared to conventional final irrigants, such as EDTA and QMix. In addition, the results of GA associated to US were satisfactory. Thus, the present study suggests the use of GA + US as alternative for final irrigation protocol, contributing not only for smear layer removal, but also for dentin collagen dispersion and preservation, as well as for the improvement of BS of the filling/restorative material to the root dentin.

5. Conclusions

Under the study limitations, it was possible to conclude that the association of GA and US results in effective smear layer removal, low totoxicity, dentin structure preservation and improvement of BS of the filling/restorative material to the root dentin.

Authors' Contributions: SOUZA, M.A.: conception and design; acquisition of data; analysis and interpretation of data; critical review of important intellectual content; DOS SANTOS, J.P.: acquisition of data; drafting the article; TISSIANI, L.: acquisition of data; drafting the article; MAGALHÃES, U.R.: acquisition of data; drafting the article; BISCHOFF, K.F.: acquisition of data; drafting the article; RICCI, R.: acquisition of data; drafting the article; HOFFMANN, L.T.: acquisition of data; drafting the article; GHIGGI, C.: acquisition of data; drafting the article; DE CARLI, J.P.: acquisition of data; analysis and interpretation of data; critical review of important intellectual content; BERVIAN, J.: acquisition of data; analysis and interpretation of data; critical review of important intellectual content; MOTA, E.G.: acquisition of data; analysis and interpretation of data; critical review of important intellectual content; DE FIGUEIREDO, J.A.P.: acquisition of data; analysis and interpretation of data; critical review of important intellectual content; PALHANO, H.S.: conception and design; acquisition of data; analysis and interpretation of data; critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: This study was approved by the Research Ethical Committee of the University of Passo Fundo (protocol 3.110.552).

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