ORIGINAL RESEARCH

Influence of Filling Material Remnants on the Diffusion of Hydroxyl Ions in Endodontically Retreated Teeth: An *Ex Vivo* Study

Natanael Henrique Ribeiro Mattos[1], Camila Paiva Perin[2], Liliane Roskamp[3], Cristiano Miranda de Araújo[4], Marilisa Carneiro Leão Gabardo[5], Vânia Portela Ditzel Westphalen[6], Luiz Fernando Fariniuk[7], Flares Baratto–Filho[8]

Abstract

Aim: To assess the influence of remnants of filling material on hydroxyl ion diffusion from calcium hydroxide (CH) paste, measured by the pH value, in retreated teeth.

Materials and methods: A total of 120 single-rooted extracted teeth were prepared up to a size 35 hand file and filled. For retreatment, the specimens were divided into four groups ($n = 20$): ProTaper Universal Retreatment (PUR), PUR with additional instrumentation (PURA), Mtwo Retreatment (MTWR), and MTWR with additional instrumentation (MTWRA). Negative (NEG) and positive (POS) control groups were composed by 20 specimens each one. The specimens, except NEG, were filled with CH paste. The retreated groups were scanned using cone-beam computed tomography (CBCT) for analysis of filling remnants. The pH assessment was performed at the baseline, after seven, 21, 45, and 60 days of immersion in saline. Data were analyzed using Shapiro–Wilk and Levene's test, followed by a two-way analysis of variance (ANOVA) and Tukey's test.

Results: Additional instrumentation (PURA and MTWRA) were superior regarding removal of the filling material (p <0.05); however, without significant difference (p >0.05). The mean pH value in all groups increased (p <0.05). After 60 days, no statistical difference was observed among POS and PURA; and MTWR and MTWRA. There was less diffusion of hydroxyl ions when the amount of remnants was greater than 59%.

Conclusion: Additional instrumentation improved the ability to remove filling material in both systems. All groups presented increasing pH; however, the higher the amount of remnants, the lower the diffusion of hydroxyl ions.

Clinical significance: The amount of remnants allows less diffusion of calcium hydroxyl ions. Thus, additional instrumentation improves the ability to remove these materials.

Keywords: Calcium hydroxide, Endodontics, Gutta–percha removal, Root canal retreatment, Rotary instruments.

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Introduction

The goals of root canal treatment are cleaning and shaping the root canal system and sealing it in three dimensions to provide aseptic conditions in order to promote repair and/or maintain periapical health with the tooth in function.[1] However, several conditions may demand endodontic retreatments, such as persistent and progressive periapical infection and obturation failure.[2]

Root canal retreatment is one of the greatest technical difficulties faced by endodontists, due to the filling materials representing a mechanical barrier that often requires considerable time and effort to be removed.[3] The maintenance of remnants in the root canal can compromise the success of retreatment because the material adhered to the dentin walls potentially shelters microorganisms and necrotic remains. Thus, the development of intraradicular infection can interfere with the adhesion of new filling materials to the root canal walls.[4]

Root canal anomalies can be challenging due to the difficulty of accessing or removing residual root filling.[5,6] In this context, the effective removal of the obturation in endodontic retreatments is considered essential, but generally not completely achieved.[7] Various techniques have been proposed with this aim, including ultrasonic, heat-carrying instruments, chemical products, endodontic hand files, and engine-driven rotary files.[8,9] Studies also reveal the inability to remove all filling material when comparing the use of rotary systems for retreatments, such as PUR and MTWR.[10,11]

[1–4]Department of Dentistry, Universidade Tuiuti do Paraná, Rua Sydnei Antonio Rangel Santos, Curitiba, Paraná, Brazil

[5]School of Health Sciences, Universidade Positivo, Rua Professor Pedro Viriato Parigot de Souza, Curitiba, Paraná, Brazil

[6,7]School of Life Sciences, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição, Curitiba, Paraná, Brazil

[8]Department of Dentistry, Universidade da Região de Joinville, Rua Paulo Malschitzki, Joinville, Santa Catarina, Brazil

Corresponding Author: Marilisa Carneiro Leão Gabardo, School of Health Sciences, Universidade Positivo, Rua Professor Pedro Viriato Parigot de Souza, Curitiba, Paraná, Brazil, Phone: +55 41 3526-5121, e-mail: marilisagabardo@gmail.com

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Additionally, to reduce or eliminate microorganisms and their by-products, intracanal medication has been recommended in a complementary manner.[12,13] Calcium hydroxide is one of the most effective intracanal medicaments used in endodontics due to its bactericidal properties,[14] and is widely used to act as a physical barrier in the root canal preventing reinfection, interrupting the nutrient supply to the remaining bacteria, and thus delaying recontamination.[15] This material produces a highly alkaline environment (pH 12.5–12.8), which is unfavorable for the survival of microorganisms.[13,16] Results suggest that hydroxyl ions (OH^-) released by CH through the dentinal tubules are responsible for the alkaline environment.[17] Thus, higher pH values indicate a greater release of OH^-. Previous results have demonstrated that CH pastes, when placed into the root canal system, release the OH^-, which then diffuses through the root dentine and cementum, reaching the periradicular tissues. An important aspect of this diffusion process is to deliver the ions throughout the entire root canal system – that is, to all the dentine tubules, isthmus, lateral canals, and accessory canals – since bacteria may be present in any or all of these sites. Increasing the pH in the dentine can therefore inhibit and eliminate bacteria that remain following the mechanical procedures (instrumentation) and irrigation of the root canal during treatment,[18] which justifies investigations regarding the pH values. These values can also be influenced by two important factors: the vehicle aggregated to the powder during CH paste manipulation, once the aqueous vehicles promote higher ionic dissociation speed compared to the viscous,[12,19,20] and the anatomy of the dentinal tubules.[21]

In this sense, research was conducted to evaluate the diffusion capacity of CH[19,21,22] since the knowledge is limited when retreatment is involved in the analysis. However, knowing that remnants of filling material can act as a physical barrier, avoiding ionic diffusion of CH paste, this *ex vivo* study aimed to compare pH changes over time in teeth submitted to four different retreatment techniques.

The null hypothesis was that there would be no significant difference in pH values when comparing the different retreatment techniques.

Materials and Methods

This *ex vivo* laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) guidelines[23] (Flowchart 1).

Ethical Aspects

This research was approved by the Institutional Research Ethics Committee (Registration No. 48588).

Sample Size Calculation and Teeth Selection

Post hoc power analysis (1-β) of the sample size for this study was performed using G*Power software (version 3.1.9), with a 95% confidence interval for the analyzed variables. The test power was determined to be 0.99 for the number of samples included ($n = 120$).

One hundred and twenty single-rooted straight teeth were included, with completely formed apices, without calcifications or internal resorption, and extracted for periodontal reasons were selected from the Dental School's teeth bank and stored for 15 days in 10% formalin solution. All teeth were cleaned and examined, and if craze lines, cracks, and surface defects were detected, the teeth should be excluded. The teeth selected were stored in 0.9% saline solution (Eurofarma, São Paulo, Brazil) at 37 ± 1°C till use.

Flowchart 1: Explaining the study

In endodontic retreatment, the amount of remnants allows less diffusion of calcium hydroxyl (CH) ions.

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To assess the influence of remnants of filling material on hydroxyl ion diffusion from CH paste, measured by the pH value, in retreated teeth.

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This research was approved by the Institutional Research Ethics Committee, with registration no. 48588.

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120 single-rooted extracted teeth

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Group I (*n* = 20): ProTaper Universal Retreatment (PUR)
Group II (*n* = 20): PUR with additional instrumentation (PURA)
Group III (*n* = 20): Mtwo Retreatment (MTWR),
Group IV (*n* = 20): MTWR with additional instrumentation (MTWRA)
Negative group (*n* = 20): Empty root canals
Positive group (*n* = 20): Canals irrigated with EDTA 17% for 3 minutes and filled with CH paste

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Residual filling material on the canal walls and pH evaluation

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CBCT images acquisition post-retreatment for analysis of residual filling material, and digital pH meter for pH analysis

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Additional instrumentation (PURA and MTWRA) were superior regarding removal of the filling material. The mean pH value in all groups increased. After 60 days, no statistical difference was observed among positive and PURA and MTWR and MTWRA. There was less diffusion of hydroxyl ions when the amount of remnants was greater than 59%.

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Additional instrumentation improved the ability to remove filling material in both systems. All groups presented increasing pH; however, the higher the amount of remnants, the lower was the diffusion of hydroxyl ions.

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No funding

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No conflict of interest

Root Canal Preparation and Filling

All procedures were performed by a single operator.

A coronal access cavity was prepared using diamonds burs 1013 and 3080 (KG Sorensen, São Paulo, Brazil). The root canals were negotiated using a size of 10 K-files (Dentsply Sirona, Ballaigues, Switzerland), and the working length (WL) was established at 1 mm from the major apical foramen. Root canal preparation was performed by step-back technique to the size 35 Flexofile (Dentsply Sirona, Ballaigues, Switzerland). Irrigation was accomplished using 2 mL of 2.5% sodium hypochlorite (NaOCl) at each instrument change (Miyako do Brasil Industria e Comercio Ltda., São Paulo, Brazil).

Eighty specimens were dried with paper points (Dentsply Ind. Com. Pirassununga, São Paulo, Brazil). For the obturation (lateral condensation technique), size 35 gutta–percha cones and accessory cones (Dentsply Indústria e Comércio Ltda., Pirassununga, Brazil), with AH Plus sealer (Dentsply Sirona GmbH, Konstanz, Germany) were used. Zinc oxide/zinc sulfate cement (Citodur®; DoriDent, Wien, Austria) was placed over the filling material as a temporary restorative material.

Negative control was composed of empty root canals just after preparation (n = 20), and POS of canals irrigated with EDTA 17% (Biodinâmica, Ibiporã, Brazil) for 3 minutes and filled with CH paste just after preparation. These specimens were not obturated, retreated, or submitted to CBCT.

CBCT Images Acquisition before Retreatment

The specimens were scanned with CBCT (Kodak 9000C 3D, Carestream Health Inc., New York, USA) with an isotropic voxel size of 76 µm, tube potential 60 kV, and tube current 5 mA for 10.8 s. The teeth were identified in a wax device according to Zancan et al.[20] that allowed the same position for the next examination. After that, the specimens were stored in 0.9% saline solution at 37 ± 1°C for further procedures.

Retreatment

After 3 months, the 80 specimens were randomly divided into four groups (n = 20) according to the technique used in the retreatment.

Group I

ProTaper® Universal Retreatment (Dentsply Sirona, Ballaigues, Switzerland): D1 (30/0.09) in cervical, D2 (25/0.08) in middle, and D3 (20/0.07 taper) in apical third until the WL. F1 (20/0.07) was used for retreatment. According to the manufacturer's instructions, the instruments were driven by X-Smart electric motor (Dentsply Sirona, Ballaigues, Switzerland).

Group II

ProTaper® Universal Retreatment with additional instrumentation: PUR plus ProTaper® instruments F2 (40/0.06), F3 (40/0.06), and F4 (40/0.06) (Dentsply Sirona, Ballaigues, Switzerland).

Group III

Mtwo® Retreatment: R25/.05 and R15/.05 (VDW, Munich, Germany). Retreatment with 10.04 (20/0.06), 15.05 (20/0.06), and 20.06 (20/0.06) instruments driven by VDW Silver motor (VDW, Munich, Germany) according to the manufacturer's instructions.

Group IV

MTWR with additional instrumentation: MTWR plus Mtwo® 10/0.04, 15/0.05, 25/0.06, 30/0.05, 35/0.04, and 40/0.04 (VDW, Munich, Germany).

During the shaping, irrigation was performed with 5 mL of 2.5% NaOCl using a syringe and 27-G needle NaviTip (Ultradent Products, South Jordan, USA). Subsequently, the canals were irrigated with EDTA 17% (Biodinâmica) for 3 minutes to remove the smear layer. The final irrigation was performed with 5 mL of 2.5% NaOCl. The canals were dried with paper points (Dentsply India Pvt Ltd.). Then, the root canals were filled UltraCal XS® (Ultradent).

Then, all-access cavities were sealed using composite resin (Z350®; 3M ESPE, St. Paul, USA), and the external surface of the crown and root apex were protected with cyanoacrylate adhesive (Loctite Super Bonder®; Henckel, São Paulo, Brazil) and epoxy resin (Araldite®; Maxepoxi Ind. Com. Ltd., São Paulo, Brazil). All samples were stored separately in deionized water at 37 ± 1°C for 24 h.

CBCT Images Acquisition Post-retreatment and Analysis

After retreatment of the four groups, CBCT images were again obtained similarly to the first run.

The images were viewed in different planes (sagittal, coronal, and axial) of 0.5 mm thickness. Navigation in axial slices of 0.5 mm/0.5 mm involved the coronal to the apical direction (and the apical to coronal direction). The area of residual filling on all root canal surfaces (total area) was measured. The percentage of residual filling material in the root canal walls was calculated using the following equation: (area of the remnant/area of the root canal)*100. Image-Pro Plus 3.0 (Media Cybernetics, Silver Spring, USA) was used to determine the area of the root canal wall with filling remnants. The mean total surface area covered by residual remnants was statistically evaluated for all groups.

The pH Assessment

The pH assessment was performed at baseline, after 7, 21, 45, and 60 days of immersion. The pH was measured using a digital pH meter (Quimis, Diadema, Brazil) previously calibrated with pH 7.0 and 4.0 buffer solutions.

Statistical Analysis

Shapiro–Wilk normality test and Levene's test for homogeneity of variance across the groups were performed. Two-way ANOVA and test Tukey's test were used for multiple comparisons. The software used in the analysis was SPSS version 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The significant level adopted was p <0.05.

Results

Residual Filling Material on the Canal Walls

When the total surface area of canals was compared in both orientations (vertical and horizontal), PURA and MTWRA were superior when compared to other groups regarding removal of the filling material (p <0.05); however, no significant difference occurred between both (p >0.05) (Table 1).

The pH Evaluation

The results are shown in Table 2, Appendix 1. Statistical comparison of pH mean values at baseline and day 60 showed that all groups

Table 1: Percentage of remnants of filling material on root canal walls according to the orientation (vertical or horizontal) performed

Variable	*n*	*Mean (± SD)*
Vertical sections		
PUR	20	59.1 (17.7)
PURA	20	29.9 (17.1)
MTWR	20	57.7 (15.5)
MTWRA	20	22.5 (13.3)
Total	80	42.3 (22.7)
Horizontal sections		
PUR	20	60.3 (16.6)
PURA	20	29.4 (17.2)
MTWR	20	58.1 (13.1)
MTWRA	20	23.5 (17.1)
Total	80	42.8 (22.9)

SD, standard deviation; PUR, ProTaper Universal Retreatment; PURA, PUR with additional instrumentation; MTWR, Mtwo Retreatment; MTWRA, MTWR with additional instrumentation

Table 2: Comparison of pH in relation to group vs day of evaluation (mean ± SD)

Day of evaluation	*PUR*	*PURA*	*MTWR*	*MTWRA*	*NEG*	*POS*
Baseline	6.39 ± 0.24^{Aa}	6.33 ± 0.13^{Aa}	6.39 ± 0.22^{Aa}	6.33 ± 0.16^{Aa}	6.33 ± 0.15^{Aa}	6.31 ± 0.13^{Aa}
7	7.25 ± 0.42^{Ab}	6.61 ± 0.26^{BCb}	6.90 ± 0.23^{Bb}	6.66 ± 0.22^{BCb}	6.44 ± 0.19^{Ca}	6.65 ± 0.18^{BCb}
21	7.41 ± 0.25^{ADb}	7.30 ± 0.29^{ADb}	6.95 ± 0.29^{Bb}	7.55 ± 0.26^{ADc}	6.64 ± 0.24^{Cab}	7.58 ± 0.22^{ADc}
45	8.39 ± 0.33^{Ac}	8.57 ± 0.31^{Ac}	7.63 ± 0.36^{Bc}	8.61 ± 0.31^{Ad}	6.84 ± 0.20^{Cb}	8.08 ± 0.17^{Dd}
60	8.45 ± 0.32^{Ac}	8.68 ± 0.36^{ABCc}	8.75 ± 0.33^{ACd}	8.89 ± 0.34^{BCd}	7.27 ± 0.19^{Ec}	8.80 ± 0.23^{Ce}

SD, standard deviation; PUR, ProTaper Universal Retreatment; PURA, PUR with additional instrumentation; MTWR, Mtwo Retreatment; MTWRA, MTWR with additional instrumentation; NEG, negative; POS, positive. Two-way ANOVA test and Tukey's test (p <0.05). Different lowercase letters in the same column indicate statistically significant differences for comparisons within the same group. Different capital letters in the same line indicate statistically significant differences for comparisons among groups

presented increasing values (p <0.05). In the NEG group, this increase occurred more slowly, demonstrating no significant difference between baseline and day 7 (p >0.05).

On day 7, PUR presented a higher and statistically significant pH mean value (p <0.05). On day 21, PUR, PURA, MTWRA, and POS differed from the other groups, with higher mean values (p <0.05). On day 45, PUR, PURA, and MTWRA, the same occurred, with these groups showing higher mean values, differing from the others (p <0.05). Finally, on day 60, all groups presented higher mean values, highlighting MTWRA (8.89 ± 0.34) (p <0.05).

Discussion

Different types of intracanal medicaments, including CH, phenols, aldehydes, halides, steroids, antibiotics, and a mixture of medications are used for root canal disinfection.

Calcium hydroxide favorably increases the root surface pH value, maintaining it high, with the release of calcium (Ca^{2+}) and OH^-, to exert antimicrobial activity and induce dentinogenesis. Hydrated CH with a molecular weight of 74.08 is commonly used in dentistry. In presence of water, ionic dissociation occurs. Thus, when applied as an intracanal medicament, these ions penetrate into the dentinal tubules. When the OH^- ions diffuse, the pH will be raised, causing the destruction of bacteria, reduction of osteoclastic activity, inactivation of bacterial enzymes, and activation of alkaline phosphatase which is involved in mineralization.[17,18] This justifies the need for investigation regarding pH values.

In this study, the null hypothesis was rejected once differences occurred among groups on different evaluation days. Also, additional instrumentation improved the ability to remove filling material, and lower CH ion diffusion occurred when the amount of remnants was greater than 59%.

Non-surgical endodontic retreatment requires the removal of previous root canal filling materials to enable access to contaminated canal space for cleaning. Then, the effective removal of the obturation generally is not achieved.[3,7] When comparing the use of rotary systems for retreatments, such as PUR and MTWR, many authors concluded that the complete removal of filling does not occur.[10,11]

Rossi–Fedele and Ahmed[24] conducted a systematic review to discuss the effectiveness of different instrumentation procedures in removing root canal filling materials. According to the authors, reciprocating and rotary systems exhibited similar abilities in removing root filling material, corroborating the present findings. However, it is important to highlight that in PURA and MTWR, additional instruments were used to theoretically remove more material, which resulted in significant differences, in agreement with the results of a previous study.[11] The additional instrumentation used in PURA and MTWRA aims to refine the root canal preparation, particularly in the apical third, as the diameter of the apical retreatment instrument is normally in a clinical situation smaller than that of the file used for the instrumentation.[9]

In this sense, the literature is scarce when dealing with diffusion through retreatments. Only a few authors have evaluated the dissolution capacity of CH in cases of root canal retreatment.[25] During retreatment, the dentinal tubule openings may be obstructed with gutta–percha and the remaining sealer. This can impact on the penetration and diffusion of OH^- through dentinal tubules. The permeability of dentin is directed mostly by dentin tubule anatomy, diameter, density, and length as well as features of the solute, such as charge and size. Dentin is a substrate, whereas CH is a material. The size of the dentin tubules correlates with the size of the CH particles.

The results revealed that all groups presented increasing pH from baseline to day 60, with a statistical difference. Kazemipoor et al.[26] demonstrated that after CH pastes were placed into the root canals, pH in cavities prepared on the surfaces of the non-retreated teeth, increased as opposed to the retreated samples, suggesting that the obstruction of dentinal tubules by the remaining gutta–percha and sealer can reduce diffusion of OH^- of the material. After 60 days, the findings of this study support that residual root filling material influences the pH value and diffusion capacity of CH paste in a root canal filled with paste, once PURA and MTWRA (fewer remnants) showed the higher mean values, highlighting the last one that differed from the other groups significantly.

Intracanal medication with CH paste aims to expand the spectrum of action against microorganisms due to ionic dissociation through the dentinal tubules, which raises the pH of the medium.[13,14,17] It is known that some vehicles are more favorable to inducing this ion release from CH pastes.[16,27,28] According to Mori et al.,[16] distilled water or 0.2% chlorhexidine present a better diffusion capacity than propylene glycol or 2% chlorhexidine. Here, Ultracal (Ultradent-UltraCal XS, South Jordan, USA) was used, which contains an aqueous vehicle in its composition, which shows a progressive increase in alkalinity, according to some results.[29,30] Studies indicate that aqueous vehicles diffuse a greater amount of ions, or this occurs more quickly.[13,19] Even in analyzing ionic diffusion through dentin in retreated teeth, CH paste prepared with saline solution had high pH around the roots after seven and ten days.[25]

Other variables may influence dentin permeability. Among the intrinsic factors is cited the anatomy of the dentinal tubules[21] and the patients' age, with dye penetration areas systematically decreasing with increasing age, and from coronal to apical regions.[31] Among the extrinsic factors that can also influence dentin permeability are the presence of a smear layer and, probably, the presence of materials adhered to the dentinal walls that obliterate the entrance of the dentinal tubules and prevent the diffusion of substances,[32] however, according to dos Santos et al.,[33] Also, OH^- are able to diffuse through dentinal tubules, so it is possible to re-establish the permeability of dentine to ionic diffusion after retreatment. On the other hand, cleaner walls can mean greater permeability, allowing, in cases of endodontic retreatment, better penetration of intracanal medication and, consequently, increasing the chances of post-intervention success.

The addition of materials such as chitosan, a cationic polymer derived from the exoskeleton of crustaceans (such as crabs), is a promising vehicle for CH to maintain an alkaline pH and allow sustained release of calcium ions in the root canal system.[28]

The limitation of this study is related to the experimental model because it is difficult to control all the variables, reducing confounding. Also, the lack of accuracy is not properly addressed, which can be a disadvantage compared to other methods.

As for the methods adopted here, single-rooted teeth were used to promote the best filling of the root canal and maximum ions diffusion. In relation to the analyzes carried out, it can be argued that the assessment of the material removal capacity includes the measurement of the area of remnants after the endodontic intervention, and this procedure became more accurate with the use of CBCT,[34] a technique of choice in this research. In contrast, the use of software for measuring areas of waste provides greater data fidelity when compared to visual assessments and has been used in studies involving retreatment.[35,36] Regarding the determination of diffusion of OH^- release, the electric device (pH meter) to measure hydrogen-ion activity (acidity or alkalinity) was used by other authors[19,22] and is well established in the literature. Future studies with other methods can potentially identify results that increase what was found here.

Conclusion

Additional instrumentation improved the ability to remove filling material for both systems. Hydroxyl ions were able to diffuse through dentinal tubules, and all groups presented increasing pH; however, the higher the amount of remnants, the lower the diffusion of these ions.

Clinical Significance

The amount of remnants allows less diffusion of calcium hydroxyl ions. Thus, additional instrumentation improves the ability to remove these materials.

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Appendix 1: Multiple comparions of pH in relation to groups vs day of evaluation

Group	*p-value*
PUR	
PURA	0.068
MTWR	0.653
MTWRA	**0.001**
NEG	**<0.001**
POS	**0.004**
PURA	
PUR	0.068
MTWR	0.805
MTWRA	0.726
NEG	**<0.001**
POS	0.937
MTWR	
PUR	0.652
PURA	0.805
MTWRA	0.091
NEG	**<0.001**
POS	0.248
MTWRA	
PUR	**0.001**
PURA	0.726
MTWR	0.091
NEG	**<0.001**
POS	0.997
NEG	
PUR	**<0.001**
PURA	**<0.001**
MTWR	**<0.001**
MTWRA	**<0.001**
POS	**<0.001**
POS	
PUR	**0.004**
PURA	0.937
MTWR	0.248
MTWRA	0.997
NEG	**<0.001**

Bold values are statistically significant. PUR, ProTaper Universal Retreatment; PURA, PUR with additional instrumentation; MTWR, Mtwo Retreatment; MTWRA, MTWR with additional instrumentation; NEG, negative; POS, positive. Two-way ANOVA and Tukey's test ($p < 0.05$).