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ORIGINAL RESEARCH

In vitro Evaluation of the Efficacy of 2% Carbonic Acid and 2% Acetic Acid on Retrieval of Mineral Trioxide Aggregate and their Effect on Microhardness of Dentin

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ABSTRACT

Introduction: In this *in vitro* study, the efficacy of 2% carbonic acid and 2% acetic acid on the surface, microhardness of white mineral trioxide aggregate (MTA) and dentin after 1 day of setting and 21 days of setting of MTA is measured.

Materials and methods: Tooth molds were made using 60 single-rooted premolars by slicing them to 4 mm in the mid-root region. White MTA (Angelus) was mixed and packed in the molds. Three experimental groups were formed and exposed to 2% carbonic acid, 2% acetic acid, and saline for 10 minutes on 1 and 21 days of setting respectively. Vickers hardness test of white MTA and dentin was done before and after exposure. Data were subjected to analysis of variance (ANOVA) and *post hoc* tests.

Results: The results show that 2% acetic acid was significantly effective in reducing the microhardness of white MTA compared to 2% carbonic acid and saline on exposure for 10 minutes.

Conclusion: The results of the present study indicate that 2% acetic acid has maximum efficacy in reducing the surface microhardness of partial and completely set MTA, followed by 2% carbonic acid.

Clinical significance: The following study will help find an adjunct for retrieval of MTA, which was found difficult with the existing methods.

Keywords: Acetic acid, Dentin, Microhardness, Mineral trioxide aggregate.

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INTRODUCTION

The aim of endodontic therapy is to treat apical periodontitis.[1] Most endodontic failures occur due to the improperly sealed root canal fillings.[2-5] An ideal orthograde or retrograde root canal filling material should prevent the chances of contamination of the root canal system by the surrounding tissues.[6]

Mineral trioxide aggregate (MTA) has been an effective material in endodontic treatment procedures[7,8] from the day of its introduction by Torabinejad et al in 1993. Mineral trioxide aggregate has been used in various procedures, such as pulp capping, pulpotomy, repair of perforations, retrograde filling, root canal filling, and apexogenesis.[9,10] Studies have reported that the compressive strength of MTA in the presence of moisture after 24 hours is 40 MPa and after 21 days, it is 67.3 MPa.[11] Despite all the benefits, MTA possesses some disadvantages, such as potential for tooth discoloration, high cost, long setting time, difficult handling, and difficulty in retrieval after setting.[12,13]Although root canal therapies have a high degree of success, failures can also occur after treatment.[14] In the previous studies, rotary and ultrasonic instruments are found to be inefficient in the removal of MTA from the canal.[15,16] Thus, there is a need for having a solvent for MTA.[17,18] Mineral trioxide aggregate is a mixture of Portland cement, bismuth oxide, and gypsum.[19] Because of the chemical similarity between MTA and Portland cement, some investigators suggested it to be a substitute for MTA.[20] The surface hardness of Portland cement is reported to be reduced by organic acids[21-23] like carbonic acid, citric acid, tartaric acid, and acetic acid. When the organic acids come in contact with the cement, the reactions with the hydrates of the portlandite, calcium silicate (C-S-H), and aluminate produce calcium and aluminum salts mainly, whose solubility varies in water (from high to very high). Eventually, the porosity of the material increases and the mechanical strength of the structure decreases. The chemical compositions of MTA and Portland cement are found to be similar in nature; MTA may also interact similarly with the weak organic acids aiding in its retrieval from the root canals.

Dentin is formed by organic and inorganic components. Calcium (Ca) and phosphorus (P) are the main inorganic components of the hard tissue. Change in the original proportion of organic and inorganic components may reduce the microhardness and lead to increase in the permeability and solubility of the canal dentin, inhibiting the resistance to bacterial ingress and resulting in leakage.[24] During the use of these solvents, dentin is also exposed to them which may alter the dentin microhardness. Thus, it becomes important to investigate the effect of these solvents on the radicular dentin of the root canal. The purpose of this study is to compare and evaluate the efficacy of two organic acids on the dissolution of MTA (after partial and complete set) and the effect of the same on microhardness of dentin. Thus, we hypothesized that the tested solvents may alter the surface microhardness of white MTA (WMTA) and dentin.

OBJECTIVES

The aim of this *in vitro* study was to evaluate the efficacy of 2% carbonic acid and 2% acetic acid on the surface microhardness of WMTA and dentin after 1 day of setting and 21 days of setting of MTA.

MATERIALS AND METHODS

Sixty molds were made using single-rooted premolars by slicing them to 4 mm in the mid-root region having the thickest diameter. The root canals were prepared to a 6 number Gates Glidden drill. White MTA (Angelus, Londrina, PR, Brazil) was mixed with the powder: Liquid ratio of 3:1, to a thick putty consistency and incrementally packed in the canals with a stainless steel condenser (Hu-Friedy, Chicago, IL) (Fig. 1A). Moist cotton pellets were placed on each sample condensed with WMTA and was stored in 100% humidity. All the specimens were examined and confirmed under the optical microscope (LABOMED CX RII). Specimens with cracks, defects, or gaps were excluded.

Thirty specimens were tested for hardness after 24 hours of set MTA using Vickers microhardness testing machine (Leica VMHT Auto, version 10, UK). Indentations were made with a square-based pyramid-shaped diamond indenter at three widely similar positioned locations at a level of 0.5 mm, using 100 gm weight for 5 seconds. To calculate the microhardness value in each sample (Vickers hardness number [VHN]) the average length of the two diagonals of the indentation was used (Fig. 2). Vickers microhardness value was calculated and displayed on the digital readout of the tester. The specimens were then randomly divided into three groups (Fig. 1B) with 20 teeth in each group and exposed to experimental solutions in the following manner: Group 1: 2% carbonic acid (CAG); group 2: 2% acetic acid (AAG); group 3: Normal saline (CG).

The first 30 samples were continuously in contact of the solutions for 10 minutes (Fig. 1C) followed by microhardness testing of WMTA and dentin. Similar procedures were repeated for the rest of the 30 samples after 21 days of setting of WMTA. From the obtained data, the mean microhardness values of dentin and WMTA before and after the exposure to the chemical solutions for all groups were calculated. Comparison of mean change in the microhardness values of WMTA and the dentin within the groups was done with the repeated analysis of variance (ANOVA) test and the intergroup comparison was done with *post hoc* test.

RESULTS

Table 1 describes the comparison of mean baseline values of microhardness of dentin and MTA by Vickers microhardness test at 100 gm for 5 seconds before exposure to acids, done after 24 hours of MTA placement and after 21 days of MTA placement. By applying Student's unpaired t-test, there is no significant difference between the mean values of microhardness of dentin calculated

Figs 1A to C: (A) Tooth slices; (B) groups; and (C) samples in contact with the test solutions

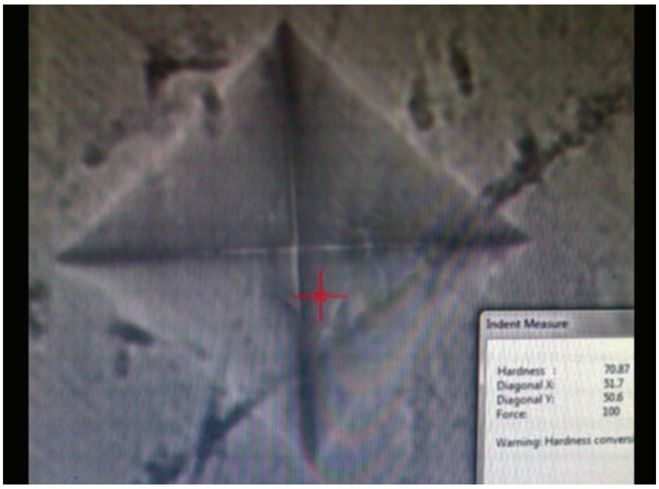


Fig. 2: Indentation of Vicker's Hardness needle

after 24 hours and after 21 days of MTA placement ($p > 0.05$) and highly significant difference was seen for mean values of MTA after 24 hours and after 21 days of MTA placement during comparison ($p < 0.0001$). Graph 1 explains the graphical representation of the same.

Table 2 describes intergroup comparison of microhardness of dentin by Vicker's microhardness test at 100 gm weight for 5 seconds before exposure to acids, after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups. By applying Student's unpaired t-test, there is no significant difference between mean values of microhardness dentin after 24 hours and after 21 days of MTA placement comparison in all three groups ($p > 0.05$). Graph 2 explains the graphical representation of the same.

Table 3 describes intergroup comparison of MTA by Vickers microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement. By applying Student's unpaired t-test, highly significant difference between mean values of MTA after 24 hours and after 21 days of MTA placement comparison in all three groups was found ($p < 0.0001$). Graph 3 explains the graphical representation of the same.

Table 4 describes intergroup comparison of microhardness of dentin by Vickers microhardness test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups. By applying one-way ANOVA test, there is significant difference between all the three groups ($p < 0.05$ – significant). Graph 4 explains the graphical representation of the same.

Table 5 shows intergroup comparison of MTA microhardness by Vickers test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups. By applying one-way ANOVA test, there is highly significant difference between 24 hours and 21 days of placement of MTA. Graph 5 explains the graphical representation of the same.

Table 1: Comparison of mean baseline values of microhardness of dentin and MTA by Vickers microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement

n = 30	*After 24 hours of MTA placement Mean ± SD*	*After 21 days of MTA placement Mean ± SD*	*Student's unpaired t-test value*	*Significance*
Microhardness of dentin	65.57 ± 2.37	65.77 ± 2.31	0.33	p > 0.05 NS
MTA	48.00 ± 1.68	60.53 ± 2.78	21.12	p < 0.01 NS

NS: Not significant; HS: Highly significant

Table 2: Intergroup comparison of microhardness dentin by Vickers microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups

Groups	*After 24 hours of MTA placement Mean ± SD*	*After 21 days of MTA placement Mean ± SD*	*Student's unpaired t-test value*	*Significance*
2% carbonic acid group (n = 10)	64.20 ± 1.75	64.70 ± 1.42	0.70	p > 0.05 NS
2% acetic acid group (n = 10)	63.00 ± 1.94	63.30 ± 1.77	0.12	p > 0.05 NS
Saline (control) group (n = 10)	65.00 ± 1.41	65.00 ± 1.41	0	p > 0.05 NS

NS: Not significant

Table 3: Intergroup comparison of MTA by Vickers microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement

Groups	*After 24 hours of MTA placement Mean ± SD*	*After 21 days of MTA placement Mean ± SD*	*Student's unpaired t-test value*	*Significance*
2% carbonic acid group (n = 10)	46.00 ± 2.21	56.00 ± 1.63	11.52	p < 0.01 HS
2% acetic acid group (n = 10)	43.00 ± 2.16	51.40 ± 1.26	10.6	p < 0.01 HS
Saline (control) group (n = 10)	47.00 ± 1.70	61.00 ± 1.70	11.97	p < 0.01 HS

HS: Highly significant

Table 4: Intergroup comparison of microhardness dentin by Vickers microhardness test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups

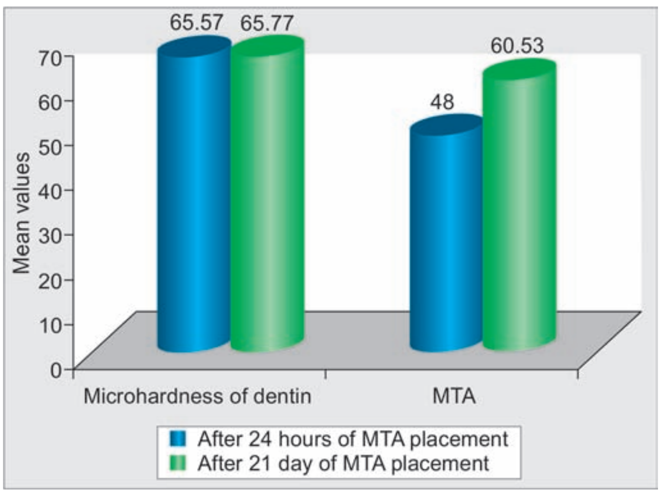
Groups	*2% carbonic acid group (n=10)*	*2% acetic acid group (n=10)*	*Saline (control) group (n=10)*	*p value*†	*2% carbonic acid group vs 2% acetic acid group*	*2% acetic acid group vs saline group*	*2% carbonic acid group vs saline group*
After 24 hours of MTA placement Mean (SD)	64.20±1.75	63.00±1.94	65.00±1.41	0.03*	0.15	0.017*	0.271
After 21 days of MTA placement Mean (SD)	64.70±1.42	63.30±1.77	65.00±1.41	0.043*	0.10	0.029*	0.50

†Repeated measures of ANOVA, followed by Tukey's *post hoc* analysis; *$p<0.05$ – significant

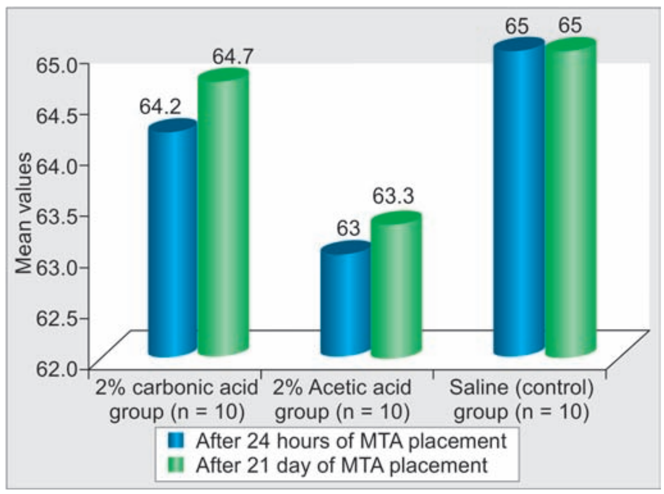
Table 5: Intergroup comparison of MTA microhardness by Vickers test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups

Groups	*2% carbonic acid group (n=10)*	*2% acetic acid group (n=10)*	*Saline (control) group (n=10)*	*p value*†	*2% carbonic acid group vs 2% acetic acid group*	*2% acetic acid group vs saline group*	*2% carbonic acid group vs saline group*
After 24 hours of MTA placement Mean (SD)	46.00±2.21	43.00±2.16	47.00±1.70	< 0.001**	0.005*	< 0.001**	0.401
After 21 days of MTA placement Mean (SD)	56.00±1.63	51.40±1.26	61.00±1.70	< 0.001**	< 0.001**	< 0.001**	< 0.001**

†Repeated measures of ANOVA, followed by Tukey's *post hoc* analysis; *$p<0.05$ – significant; **$p<0.001$ – highly significant



Graph 1: Comparison of mean baseline values of microhardness of dentin and MTA by Vicker's microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement

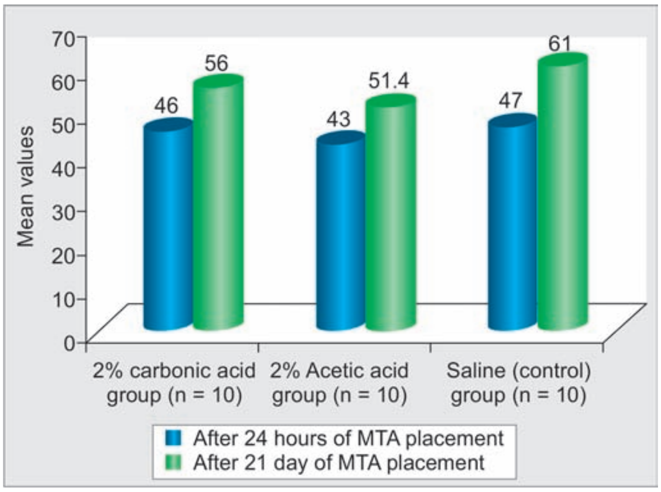


Graph 2: Comparison of microhardness dentin by Vicker's microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups

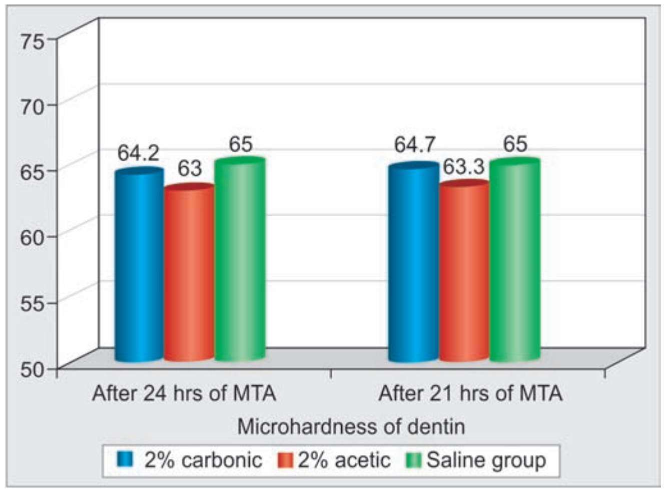
DISCUSSION

This study was aimed to evaluate the effect of 2% carbonic acid and 2% acetic acid on the surface properties of WMTA by microhardness determination, which indirectly provided the evidence of alterations in the mineral content of the dental hard tissues. Mineral trioxide aggregate is a type of hydraulic cement, which sets and is stable under water.[25-27] It has been observed that hydration reactions and maturation of MTA continues within the 1st week of setting.[28] Calcium hydroxide (CH) and C-S-H will be the main hydration products in the reaction of tricalcium silicate (C3S) and dicalcium silicate (C2S) of MTA with water.[19,26] Studies have concluded that the carbonation results in a decrease in Portland cement's tensile strength and resiliency.[20] pH of 5.48 of carbonic acid makes it a weak organic acid. In Portland cement-like materials, such as MTA, carbonation will result in the conversion of portlandite, calcium hydroxide to calcite, $CaCO_3$. Long-term contact of MTA with carbonic acid decomposes the CSH gel into calcium carbonate, acid-insoluble silica gel, and water. As the reaction of acid and base leads to formation of salt and water, complete carbonation eventually results in decalcification of the CSH gel that leads to decrease in strength.

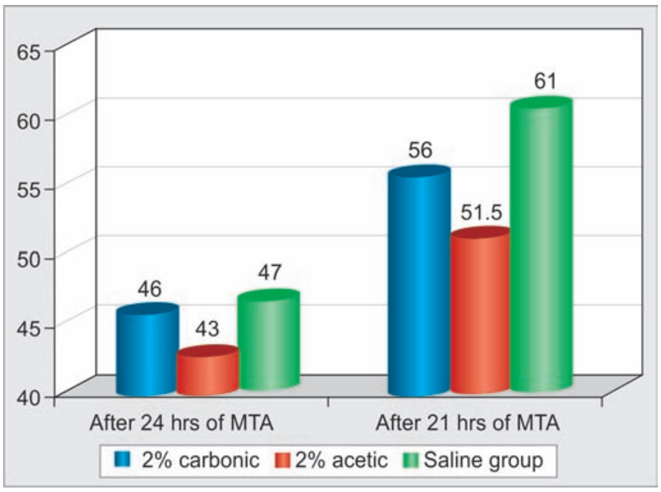
The results of the Vickers microhardness test showed that the carbonic acid specimens had microhardness of partial and completely set MTA significantly higher than that of acetic acid and saline groups; this may be due to the poorly crystallized C-S-H in the previous



Graph 3: Comparison of microhardness dentin by vicker's microhardness test at 100 gm weight for 5 seconds before exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups



Graph 4: Intergroup comparison of microhardness dentin by Vickers microhardness test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups



Graph 5: Intergroup comparison of MTA microhardness by Vickers test at 100 gm weight for 5 seconds on exposure to acids after 24 hours of MTA placement and after 21 days of MTA placement in 2% carbonic acid, 2% acetic acid, and saline groups

groups. The calcium-depleting nature of this acid may have the potential to decrease the strength of MTA by interrupting the crystallization of C-S-H as reported by Smith et al.[16] Rai et al[21] studied the effect of 20 wt% of tartaric acid during the process of hydration of Portland cement in which he proved that tartaric acid is a solution which strongly retards Portland cement's hydration. The organic acid interacts chemically with the cement's mineral phases, leading to the formation of calcium tartarate hydrate with the evolution of heat and thereby leads to decrease in amounts of C3S, C2S, and C3A phases. Due to these chemical interactions, it might be possible to use these organic acids that may dissolve the obturated calcium silicate-based material in the procedure of retreatment as acetic acid is a weak organic acid. The results of the present study indicate that acetic acid and carbonic acid cause significant decrease of surface microhardness of partial set MTA. Thus, the use of these irrigants within 24 hours of placement of WMTA is not recommended, but can be an adjunct for the retrieval of partial set WMTA whenever indicated. The other part of the study evaluated the effect of these solutions on the microhardness of dentin. In the present study, Vickers hardness values for dentin were measured with similar indentations as on the MTA surface at the level of 0.5 mm from the root canal walls and with a load of 100 gm for time period of 5 seconds. The decalcifying efficacy of acids and chelating agents depends mostly on the application time, the pH, the concentration of the solution, and the hardness of dentin.[29-32] The results of the present study suggest that all the solutions decreased microhardness of root canal dentin significantly ($p < 0.05$). In the present study, acetic acid reduces the microhardness of the dentin slightly more as compared to carbonic acid. Studies have shown that NaOCl caused maximum reduction in the microhardness of dentin followed by carbonic, citric, and tartaric acids.[32,33] As per annals, carbonic and citric acid solutions have greater effect on reducing dentin microhardness due to its chelating property.[28,33-36]

The chemical analysis and X-ray diffraction have proved that 18.8% of the WMTA is insoluble in water and its crystallinity is close to 80%[37-39] due to which, there are nonsignificant results of the control group.

In dentin, calcium is available in the form of a complex within the hydroxyapatite crystals, which impedes complete reaction of dentin with the acid. The other organic acids like carbonic and acetic acids, thus, also have some calcium depleting action and the carboxyl groups present in them possess efficiency of reducing the microhardness of dentin.

CONCLUSION

The results of the present study suggest that 2% acetic acid has highest efficacy in reducing the surface microhardness of partial and completely set MTA, followed by 2% carbonic acid.

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