Spectrophotometric analysis of the color stability of white mineral trioxide aggregate in contact with four different irrigating solutions - An *in vitro* study

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**Abstract**

**Aims:** This study aims to compare the color stability of white mineral trioxide aggregate (wMTA) in contact with four irrigating solutions.

**Settings and Design:** Original research study.

**Subjects and Methods:** Fifty cylindrical discs of wMTA, 10 mm in diameter and 2 mm in height were prepared using a mold. Samples were incubated at a temperature of 37°C and at 100% humidity for the material to reach its optimal mechanical properties. The samples were divided into 6 groups: Group A: dry (*n* = 5); Group B: distilled water (DW) (*n* = 5); Group C: 5% sodium hypochlorite (NaOCl) (*n* = 10); Group D: 2% chlorhexidine gluconate (CHX) (*n* = 10); Group E: 17% aqueous ethylene diamine tetra-acetic acid (EDTA) (*n* = 10); Group F: 0.2% Chitosan (*n* = 10) Each disc was immersed into the irrigating solution for a period of 24 h. All the specimens were photographed using a digital camera before and after immersion. The assessment of color change of each disc of wMTA was conducted by a spectrophotometer. The Commission Internationale de l’Eclairage system was used to calculate the differences in color.

**Statistical Analysis Used:** Statistical Package for the Social Sciences version 16.0 (Chicago, IL, USA) at a significance level of *P* < 0.05 was used.

**Results:** All groups except group A exhibited discoloration of wMTA. The mean values for change in color was highest with Group D, followed by Group C, F, E, B, and group A. Only Group B when compared to group A did not show any statistically significant difference (*P* = 0.948) whereas all the other four groups showed a highly statistically significant difference (*P* < 0.001).

**Conclusions:** 2% CHX causes maximum discoloration of wMTA followed by 5% NaOCl, 0.2% Chitosan and least discoloration with 17% aqueous EDTA and DW.

**Keywords:** 0.2% chitosan; discoloration; irrigants; white mineral trioxide aggregate

**INTRODUCTION**

In the last two decades, mineral trioxide aggregate (MTA) has become one of the most widely studied Endodontic material.[1] MTA materials are obtained from a Portland cement principal compound. The trioxide aggregate in MTA is composed of calcium, aluminum and selenium. MTA has several beneficial properties such as biocompatibility, bioactivity, hydrophilicity, radiopacity, sealing ability, and low solubility. It was initially introduced as a root-end filling material and for root perforation repair.[2] However,

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it is presently used for a variety of applications such as pulp capping, apexification procedures (immature apex and reimplanted teeth), in pulpotomies, in root resorption, for revascularization of immature necrotic permanent teeth and many other endodontic procedures.\textsuperscript{[2]}

The earliest MTA products were grey and most of the initial research was done on this formulation. Due to staining concerns due to grey MTA (GMTA) residues in the clinical crown, the white version of MTA was launched in 2002.\textsuperscript{[3]} Nevertheless, tooth discoloration was reported with both GMTA and white MTA (wMTA) variant. In fact, both types of MTA induced significantly decreased 3 parametric values of the Commission International de l’Eclairage’s (CIE) \textit{L*a*b} color system, with the change in color being greater with GMTA. GMTA led to clinically appreciable crown discoloration after 1 month, whereas the total color change caused by wMTA exceeded the discernible threshold for the human eye after 3 months. This suggests that the application of GMTA in the aesthetic zone should be circumvented, whereas wMTA should be used with caution when placed in pulp chambers.\textsuperscript{[4]}

The goal of endodontic therapy is to prevent and treat apical periodontitis. However, the aesthetic result is equally important, especially in the anterior region. Pulp therapy procedures such as direct pulp capping, stepwise excavation, Cvek’s pulpotomy and regenerative endodontics involve the placement of materials in the coronal third of the tooth, which may have caused discoloration.\textsuperscript{[5]} The resultant damage to the quality of treatment presented dissatisfaction in 31.6\%-57\% of the patients.\textsuperscript{[6]} Subsequent color change caused by the interaction of calcium silicate-based materials (CSBM) and irrigation solutions will have a notable impact on both treatment planning and clinical outcomes.

Irrigating solutions like sodium hypochlorite (NaOCl), Chlorhexidine (CHX), ethylene diamine tetra-acetic acid (EDTA) and Chitosan are routinely used in chemical cleaning and shaping of the canals. In frequently performed endodontic procedures, those which require placement of MTA, there is always contact with a certain residual irrigant or chelating agent which could potentially affect its optical properties.

No original research study has yet aimed at comparing Chitosan with routinely used irrigants. Therefore, the purpose of this study was to evaluate color stability of wMTA after immersion in four different irrigating solutions such as NaOCl, CHX, aqueous EDTA, Chitosan and distilled water (DW). The null hypothesis of this study was that none of the irrigating solutions will cause a change in the color stability of wMTA.

**SUBJECTS AND METHODS**

**Sample preparation**

wMTA (Angelus Solucoes Odontologicas, Londrina, Brazil) was mixed according to the manufacturers’ instructions using a stainless-steel spatula (#982, GDC) and a glass slab. It was condensed with a stainless-steel condenser into 10 mm diameter and 2 mm high stainless-steel cylindrical mold. The sample discs were stored in an incubator at 37\(^\circ\)C and 100\% humidity during the setting to attain their optimal mechanical properties. After 24 h, the discs were retrieved and a pretreatment colored photograph of all the 50 samples was captured by a digital camera (Canon EOS REBEL T6i/750 D, Tokyo, Japan).

All the 50 specimens were randomly divided into six groups: Group A (\(n = 5\)): Dry; Group B (\(n = 5\)): DW; Group C (\(n = 10\)): 5\% NaOCl (Prime Dental Products Private Limited, Thane, Maharashtra, India); Group D (\(n = 10\)): 2\% CHX Gluconate (ASEP-RC, ANABOND STEDMAN PHARMA RESEARCH (P) LTD, Chennai, Tamil Nadu, India); Group E (\(n = 10\)): 17\% Aqueous EDTA (Dent Wash, Prime Dental Products Private Limited, Thane, Maharashtra, India); Group F (\(n = 10\)): 0.2\% Chitosan (Chitogrant, Everest Biotech, Bengaluru, Karnataka, India). These groups were divided on the basis of the irrigating solution in which the samples were immersed for a period of 24 h. After 24 h, these samples were removed, dried and a posttreatment colored photograph was captured with a digital camera [Figure 1].

**Spectrophotometric analysis**

A SS 5100H Spectrophotometer (Premier Colorsan Pvt Ltd, Navi Mumbai, Maharashtra, India) was used to analyze the color changes in wMTA. The spectrophotometer was calibrated with the measurement of a pure white standard (100\% reflection) in the wavelength spectrum of visible light (380 nm–740 nm) every time. All measurements were performed thrice and averaged. Measurements were carried out by the same operator prior to and after immersion of the wMTA discs in the irrigating solutions.

![Figure 1: Postimmersion and dried white mineral trioxide aggregate discs placed on white mixingpad; dry, distilled water, 5% sodium hypochlorite, 2% chlorhexidine gluconate, 17% aqueous ethylene diamine tetra-acetic acid and 0.2% chitosan (from left to right)](image-url)
The spectrophotometer was equipped with a black circular Bakelite sample assembly 10 mm in diameter. The specimen was placed in the circular opening to ensure the accurate disc position. Standard D65 illumination was selected. The CIE system was used to calculate the difference in color. The value of the luminance (L) and the chromatic components (a and b) were measured before and after immersion in different solutions. Total change in color (ΔE) was calculated according to the following equation:

\[ \Delta E = (\Delta a^2 + \Delta b^2 + \Delta \lambda^2)^{1/2} \]

L* values stand for lightness, on a continuous scale from black (0) to white (100), a* values represent red (+80a*) to green (−80a*) and b* values represent yellow (+80b*) to blue (−80b*) color variations. Where in ΔE values that were ≥3.7 were accepted as clinically perceptible change in color.

**Method of data analysis**

Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Statistical analysis was done by using descriptive statistics such as Mean, and standard deviation (SD) for quantitative data. One-way ANOVA test was applied to compare inter-group color change of wMTA. Tukey post hoc test was used to perform multiple comparison tests. \( P < 0.05, \text{considered as significant as alpha error set at 5% with confidence interval of 95% set in the study. Power of the study was set at 80% with beta error set at 20%.} \)

**RESULTS**

On the assessment of color stability, samples from Group A and B did not show any discernible changes, whereas samples from Group C, D and F displayed a stark difference in color among each other and also in comparison with Group A and B. Group C appeared visibly dark greyish-brown and samples of Group D appeared dark greyish-blue and brownish in parts. Samples from Group E on the other hand appeared whitish-blue and brighter than group A. Samples in group F appeared glossy, textured and light-yellowish-brown [Figure 1].

All the values obtained by spectrophotometric analysis were then subjected to statistical analysis. The results of mean, SD and standard error are presented in Table 1 and Graph 1. The highest mean values for color change (ΔE) of group were presented by Group D, followed by Group C, Group F and Group E, Group B and least by Group A. Collective comparison of color change of wMTA when in contact irrigating solutions, when subjected to ANOVA F test are presented in Table 2. When comparing all the mean values of color change (ΔE) of all the six groups it was seen that the value of \( F = 397.99 \) and the results were statistically highly significant (\( P < 0.001 \)).

**DISCUSSION**

wMTA was mainly developed to control the discoloration potential of GMTA in an attempt to impart good aesthetics. It has been observed clinically that many lightly colored dental products when in contact with customarily used endodontic materials undergo discoloration. The difference between the GMTA and the wMTA has been reported to be in the lack of iron in the white version. In addition to the color change in the biomaterial, changes may occur in the color of the tooth; this limits its use in anterior teeth.

| Groups                          | Mean   | Standard deviation | Standard error |
|---------------------------------|--------|--------------------|----------------|
| Group A (dry)                   | 3.43   | 0.15               | 0.067          |
| Group B (distilled water)       | 3.98   | 0.164              | 0.073          |
| Group C (5% sodium hypochlorite)| 17.65  | 0.76               | 0.23           |
| Group D (2% chlorhexidine)      | 19.91  | 1.53               | 0.48           |
| Group E (17% EDTA)              | 6.87   | 0.83               | 0.26           |
| Group F (0.2% chitosan)         | 10.86  | 1.025              | 0.32           |

EDTA: Ethylene diamine tetra-acetic acid

On multiple comparisons using Tukey's post hoc test only Group B when compared with Group A did not show any statistically significant difference (\( P = 0.948 \)) whereas all the other 4 groups showed a highly statistically significant difference (\( P < 0.001 \)) in color. Group B was individually compared to all groups, a highly statistically significant mean difference in ΔE of wMTA was seen. When Group C was compared with Group D, Group E Group F to assess the ΔE, the mean difference was highly statistically significant with \( P < 0.001 \). The mean difference between group D and group E as well as group D and group F to assess ΔE, was statistically highly significant with \( P < 0.001 \). Group E and group F when assessed for ΔE presented with a highly statistically significant mean difference with \( P < 0.001 \) [Table 3 and Graph 2]. Hence, all the five groups except group A presented with a ΔE value ≥ 3.7.
A probable mechanism of tooth discoloration by wMTA is due to oxidation of the iron content of set material, which is accredited to the calcium aluminoferrite phase of the powder. Kang et al. stated that the degree of discoloration that occurs depends on the content of metal components such as bismuth, iron, aluminum, and magnesium oxides. Bismuth oxide (BO) on interaction with collagen, gets converted to a black precipitate. Also, on oxidation of BO, its oxygen becomes unstable, reacts with carbon dioxide in the air and produces bismuth carbonate, which causes discoloration. When exposed to high temperatures or light irradiation in an oxygen-free environment it undergoes dissociation and produces metallic bismuth and oxygen. MTA discolouration may be possibly due to absorption of haem from hemoglobin. Lenherr et al. suggested that the porosities of the material may absorb blood components and may be responsible for the perceivable discoloration. Namazikhah et al. demonstrated that the material microstructure shows pH-dependent porosities which may uptake blood components. This is of clinical relevance because Portland cement-based materials are usually placed in direct proximity to vital, vascularized tissue.

Another reason for wMTA discoloration is the combination of the oxygen-free environment with light found in clinical scenario. When exposed to light in an oxygen-free environment, BO, which is supposedly the dominant cause of MTA discoloration, dissociates into dark-colored crystals of metallic bismuth and oxygen. BO is activated by both visible and ultraviolet (UV) light in akin to heating. The UV-visible diffuse spectrum for nanocrystallite BO spans from wavelengths shorter than 300–500 nm, with a

Table 2: Comparison of colour change (ΔE) of white mineral trioxide aggregate when in contact with different media and solutions using a spectrophotometer using ANOVA F-test

| Groups                  | Mean   | Standard deviation | Standard error | ANOVA F-test | P, significance |
|-------------------------|--------|--------------------|----------------|--------------|----------------|
| Group A (dry)           | 3.43   | 0.15               | 0.067          | F= 397.99    | P<0.001, highly significant |
| Group B (distilled water)| 3.98   | 0.164              | 0.073          |              |                |
| Group C (5% sodium hypochlorite) | 17.65  | 0.76               | 0.23           |              |                |
| Group D (2% chlorhexidine)     | 19.91  | 1.53               | 0.48           |              |                |
| Group E (17% EDTA)       | 6.87   | 0.83               | 0.26           |              |                |
| Group F (0.2% chitosan)  | 10.86  | 1.025              | 0.32           |              |                |

P>0.05 (not significant), EDTA: Ethylene diamine tetra-acetic acid

Table 3: Inter group pair–wise comparison of colour change (ΔE) of white mineral trioxide aggregate when in contact with different irrigating solutions using a Spectrophotometer using Tukey’s post hoc test

| Group                  | Comparison group | Mean difference | P, significance |
|------------------------|------------------|-----------------|----------------|
| Group A (dry)          | Group B (distilled water) | 0.54            | P=0.948        |
| Group C (5% sodium hypochlorite) | Group D (2% chlorhexidine) | 14.21           | P<0.001, highly significant |
| Group D (2% chlorhexidine) | Group E (17% EDTA) | 16.47           | P<0.001, highly significant |
| Group E (17% EDTA)     | Group F (0.2% chitosan) | 7.42            | P<0.001, highly significant |
| Group D (2% chlorhexidine) | Group C (5% sodium hypochlorite) | 13.67           | P<0.001, highly significant |
| Group E (17% EDTA)     | Group F (0.2% chitosan) | 15.92           | P<0.001, highly significant |
| Group F (0.2% chitosan) | Group C (5% sodium hypochlorite) | 10.78           | P<0.001, highly significant |
| Group B (distilled water) | Group E (17% EDTA) | 6.87            | P<0.001, highly significant |
| Group C (5% sodium hypochlorite) | Group F (0.2% chitosan) | 6.97            | P<0.001, highly significant |
| Group D (2% chlorhexidine) | Group E (17% EDTA) | 9.05            | P<0.001, highly significant |
| Group E (17% EDTA)     | Group F (0.2% chitosan) | 3.99            | P<0.001, highly significant |

P>0.05 (not significant), EDTA: Ethylene diamine tetra-acetic acid

Graph 2: Inter group individual pair wise comparison of ΔE white mineral trioxide aggregate after contact with irrigation solutions
Mehta, et al.: Discoloration of white MTA and chitosan reported that this residue [2,13] may be due to the reduction in NaOCl to sodium chloride in the mean ∆E value of 18.4; which was similar to the results in our study, i.e., 19.91 for Group D (2% CHX). The results of their study determining the mean ∆E value of NaOCl was 15.2 which also corroborated with finding of this study, where in the mean ∆E of Group C (5% NaOCl) was 17.65 [Table 1 and Graph 1].

Sobhnamayan et al.[24] assessed that the effect of different irrigation solutions on the color stability of three CSBM corroborated with the present study. A clinically perceptible change in color was seen with all the testing materials when in contact with CHX and NaOCl. Angelus wMTA showed the highest discoloration with CHX where the precipitate called para-chloroaniline. Since, NaOCl and CHX both seem to induce a discoloration in wMTA and interact with each other, there should be a thorough flushing out of the these irrigants before placement.[23]

In a study by Keskin et al.[8] color stabilities of CSBM in contact with different irrigating solutions has been assessed. In their study both NaOCl and CHX exhibited clinically perceptible change in color (∆E). Comparing with the results of the current study, he stated that in contact with Angelus wMTA, NaOCl showed a statistically more significant difference as compared to CHX (P < 0.05) and vice versa for ProRoot MTA. Greater interaction with ProRoot MTA than Angelus wMTA was due to a higher content of BO. In the present study, Group C when compared with Group D, resulted in a mean difference of 2.25 and a highly statistically significant difference in ∆E, P < 0.001 [Table 3].

NaOCl is one of the most routinely used solutions for root canal irrigation.[19,20] One of the early studies has shown that residuum NaOCl tend to crystallize and to occlude dentinal tubules. Zou et al.[23] reported that this residue could penetrate into dentin to a depth of 77–300 µm and would be difficult to excavate from the root canals.[21] It has been documented that NaOCl has a very noteworthy impact on pigmentation caused by CSBM. However, this effect was less prominent than discoloration observed when NaOCl was used alone.[22] The 5% NaOCl solution that was used in our study caused a dark greyish-brown change in colour of wMTA [Figure 1]. The reason for this may be due to the reduction in NaOCl to sodium chloride by BO; thus, NaOCl causes BO oxidation, which renders it unstable, resulting in a reaction with CO₂ present in the air that creates bismuth carbonate, which is light sensitive thereby causing MTA's discoloration. Contact of bismuth-containing substances with NaOCl lead to a dark brown, nearly black discoloration.[2,13]

CHX is a synthetic cationic biguanide containing two symmetric 4-chlorophenyl rings and two biguanide groups, joined by a central hexamethylene chain.[23] CHX as well as tetracyclines have a unique feature of antimicrobial substantivity. The positively charged ions released by CHX are adsorbed into dentine and prevent microbial colonization beyond the actual period of time of application.[23] Therefore, CHX is a frequently used irrigating solution in cases with open apices, periapical lesions and perforations. In a clinical scenario the chances of residual CHX molecules are very high and hence chances of interaction with retrograde filling material like wMTA also increases.[23]

Its optimal antimicrobial activity is achieved within a pH range of 5.5–7.0. Hence, it is likely that alkalinizing the pH by adding Ca (OH)₂ released from wMTA to CHX will lead to its precipitation thereby decreases its effectiveness. This could also be a reason for discoloration of MTA when in contact with CHX.[23] It is also probable that CHX can undergo a reaction with BO which could have Lead to the discoloration. The interaction between CHX and NaOCl results in the formation of a neutral and insoluble orange precipitate. In a study by Lee et al.[25] it has been proven the physical properties of MTA are affected even after 7 days. The current study also evaluated the effect of 17% aqueous EDTA on the color stability of wMTA. EDTA is used for removal of the mineralized portion of smear layer. Recently, EDTA was introduced in regenerative endodontic procedures as the only irrigant in the second visit due to its ability to release growth factors from the dentin and induce cell attachment and differentiation.[24] On visual examination, based on the pre and the post immersion digital images clinically perceptible change in the sample discs of group E can be appreciated. Only one study in literature by Sobhnamayan et al.[24] has compared the effect of NaOCl, CHX and EDTA on colour stability of wMTA, where in maximum
discoloration was seen when in contact with CHX and least but statistically significant $P < 0.05$ with EDTA. In our study, the Group E (EDTA) had a mean $\Delta E$ value 6.87 whereas as in the study by Sobhnamayan et al.[24] the mean $\Delta E$ value for EDTA was 10.9 [Table 1 and Graph 1]. On inter-group pair wise comparison of $\Delta E$ of wMTA using Tukey’s post hoc test, the mean $\Delta E$ difference between Group E and Group C was 10.78; with group D being 13.04 and with Group F being 3.99 were highly statistically significant, $P < 0.001$ [Table 3]. The MTA samples in the current study were in contact with aqueous 17% EDTA for 24 h. As it chelates the calcium ions in the setting MTA it is safe to say that it can also be a causative factor for discoloration in wMTA discs which on visual assessment appeared lighter, brighter, whitish-blue [Figure 1] and brittle.

The color stability of wMTA has been assessed in our study when in contact with 0.2% Chitosan (Group F). Chitosan is a natural polysaccharide, which is biocompatible, biodegradable, bio-adhesive and lacks toxicity.[26] Adsorption, ionic exchange, and chelation are probably responsible for formation of complexes between Chitosan and metal ions. At an acidic pH, Chitosan presents remarkable chelating ability. It is specifically directed to the chelation of calcium ions resulting in smear layer removal from root canal walls similar to EDTA and citric acid, but without resulting in root dentin erosion.[26]

Since it is a biocompatible chelating agent and it is extremely well tolerated in periapical tissues. When Chitosan comes in contact with MTA it is quite possible that this causes discoloration of MTA. No literature or research has assessed the effect of chitosan irrigating solution on the color stability of wMTA. On visual examination, it caused a slightly yellowish discoloration of wMTA [Figure 1]. The inherent color of 0.2% Chitosan irrigant used in this study was light yellowish-brown, which could be the causative factor of a similar color imparted onto the sample. The mean $\Delta E$ value of Group F was 10.86 [Table 1]. When compared with the $\Delta E$ of wMTA in all the groups individually, there is a highly statistically significant difference, $P < 0.001$ [Table 3].

Visual spectrophotometry is considered the gold standard for the evaluation of color. The CIE $L^*a^*b^*$ system is a three-dimensional, uniform color space designed to approximate the perceptual response of the human eye to all perceivable colors. The location of a color in the CIE $L^*a^*b^*$ color space is explained by using 3 chromatic parameters. The value of $L^*$ indicates lightness where as $a^*$ and $b^*$ values indicate the location on the green to red and blue to yellow gradient, respectively. The extent of color difference between 2 objects can be expressed numerically in their Euclidean distance in $\Delta E$ values. A $\Delta E$ value $>3.7$ is considered to be perceivable color change.[7] In the present study all the groups except group A (dry) presented with mean $\Delta E$ values $>3.7$.

The only limitation of this study is that the wMTA discs are immersed in irrigating solutions for a period of 24 h, wherein all surfaces of the sample are in contact with the irrigants. Clinically, the surface in contact is comparatively lesser as it depends on the amount and concentration of residual irrigant in contact. Also, longer contact durations can induce greater discoloration. The extent of discoloration in the present study would imply the maximum possible discoloration potential of wMTA.

The present study validates the results obtained in similar studies and thereby rejects the null hypothesis that no irrigating solutions affects the color stability of wMTA. The present study is the first of its kind to assess the change in color when wMTA is treated with 0.2% Chitosan. wMTA is discolored most by 2% CHX followed by 5% NaOCl, 0.2% Chitosan, and 17% aqueous EDTA.

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Conflicts of interest
There are no conflicts of interest.

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