Comparative in Vitro Assessment of Tooth Color Change under the Influence of NFC and MTA

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KEY WORDS
Tooth discoloration; MTA; Root canal therapy;

ABSTRACT
Statement of the Problem: Tooth color changes followed by treatment with bioceramic materials is always a matter of concern. In this article, NFC as a new ceramic base material assessed for its ability to change the color of teeth.

Purpose: The aim of the present in vitro study was to compare tooth discoloration that occurs in human teeth filled with ProRoot WMTA (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) or NFC over the course of 3 months.

Materials and Method: Thirty human intact premolars obtained and the root of all teeth was removed by horizontally cutting them about 2 mm below the cementoenamel junction, the pulp tissue was removed afterwards using a barbed broach (Mani, Tokyo, Japan). The teeth were randomly divided to 3 groups (n= 10 teeth per group): control (no material), ProRoot WMTA and NFC. The experimental materials were condensed into the crowns and the tooth end sealed with light-cure glass ionomer cement (GC Corporation, Tokyo, Japan). Color was assessed at TBL: baseline (after preparation of the cavities but before placement of the materials), TPO: immediately after placement of the filling material and provisional restoration, T4: after 4 weeks of storage, and T12: after 12 weeks (3 months) of storage.

Result: Discoloration was evident in all teeth, immediately (TPO) after applying MTA and NFC. The highest ΔE was noted in WMTA at 3 months, followed by NFC, but there was no significant difference between the discolorations induced by these two materials.

Conclusion: There was a similar level of clinically observable tooth discoloration detected using either WMTA or NFC.

Introduction
Mineral trioxide aggregate (MTA) is a biocompatible material with good sealing and biological properties [1]. As a root canal treatment material, it has achieved FDA approval since late 20th century [2]. Due to its exceptional sealing ability and biocompatible behavior, it has been widely implemented in various applications such as apical root-end fillings, perforation repair, direct pulp capping, apexification and pulp revascularization procedures [3-8]. It is known as the ‘gold standard’ material, despite disadvantages of its use: difficult to handle, expensive, difficult to remove, and discolors over time [9]. Of major importance esthetically, the discoloration that occurs produces results that are not satisfactory, which is particularly problematic when direct pulp capping or pulpotomy are required in the anterior teeth [1, 10]. The first developed MTA was gray, which has the potential to cause tooth discoloration. White MTA (WMTA) was developed in order to overcome this disadvantage. The major difference between WMTA and gray MTA is that WMTA contains fewer metal oxides such as Al2O3, MgO, and FeO, which were assumed to be the main
causes of discoloration, but even WMTA has been shown to cause unfavorable tooth discoloration [11-12].

Recently, new calcium silicate base cement named Nano Fast Cement (NFC) was introduced by Shiraz University researchers which demonstrated favorable properties. NFC with short setting time and high strength was obtained by modification of MTA material.

Grinding is an important operation for producing small and ultra-fine particles from solids in powder technology and pharmaceutical industries. In a milling operation, the breakage of initial particles occurs, to reduce particle size. The MTA properties vary in different milling time, because the ingredients of MTA composite have specific mechanical properties and breakage behavior.

Milling is a top-down particle-forming process, where large drug crystals and excipient are reduced to smaller particles and stabilized in crystalline or semi-disordered crystals with the excipient [13].

Wet Stirred Media Milling (WSMM) was carried out in time intervals of 5, 10, 15 and 20 h. Although decreasing MTA particle size reduces setting time significantly, it shows negative impact on both compressive and flexural strength [13].

In order to overcome this issue, the MTA was reinforced with multi-walled carbon nanotubes and significantly improved mechanical strength without affecting setting time. The multi-walled carbon nanotubes have a promising application in MTA material. According to the x-ray diffraction analysis the MTA was composed primarily of tricalcium silicate, dicalcium silicate and zirconium dioxide. The results of Particle size analysis and Scanning electron microscopy images indicate the continued influence of milling on the particle size of MTA. In comparison to MTA the size of particles were decreased and setting time and handling features were improved [13]. The tooth discoloration potential of this material however, has not yet been investigated.

Therefore, the aim of the present in vitro study was to compare tooth discoloration that occurs in human teeth filled with ProRootWMTA (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) or NFC over the course of 3 months. Our null hypothesis was that there are no differences in this regard between the two materials.

Material and Method
Preparation of Extracted Teeth for the Discoloration Experiment
The samples were 30 human premolars extracted for orthodontic purposes. Teeth with clinical and radiographic signs of caries, cracks, restorations, and pathologic discolorations were excluded from the study. The root of all teeth was removed by horizontally cutting them about 2 mm below the cement enamel junction, the pulp tissue was removed afterwards using a barbed broach (Mani, Tokyo, Japan), and any organic materials on the surface of the teeth were either physically removed by curettage or by soaking the teeth for 10 minutes in 2.5% sodium hypochlorite solution for persistent organic material. The teeth were then washed several times with normal saline and stored at 4°C until required for the experiment.

The teeth were randomly divided to 3 groups (n = 10 teeth per group) named according to the material that would be used to fill their pulp chamber: control (no material), ProRootWMTA and NFC. The materials were prepared as instructed by the manufacturer and then used to retrofit the teeth to the level of the cement enamel junction in the pulp chamber. After the early setting phase, light-cure glass ionomer cement was used to seal the lower area (GC Corporation, Tokyo, Japan). In the control group, teeth were only sealed with light-cure glass ionomer cement (GC Corporation, Tokyo, Japan). Immediate postoperative color measurements were recorded, and specimens were stored in a dark environment with 100% humidity at 37°C with normal atmospheric gas levels until the subsequent color measurement points.

Molds were fabricated for each tooth using rubber impression material (Aquasil Soft Putty, Dentsply) (Figure 1). Holes with diameters of about 6 mm were created in the molds using a biopsy punch (KAI medical, Tokyo, Japan) so that the extent of discoloration could be measured at the same site. The hole was located so that it revealed an area between the midbuccal third and cervical third to facilitate observation of any MTA-induced internal discoloration.

Measurement of Tooth Color
VITA Easyshade Compact device (Vita Zahnfabrik, Bad Sackingen, Germany) was used to measure tooth color at the following time points: $T_0$: baseline (after preparation of the cavities but before placement of the
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Figure 1: The putty mold used in this study for holding the test teeth to allow consistent measurement of $L^*$, $a^*$ and $b^*$ values.

materials). $T_{PO}$: immediately after placement of the filling material and provisional restoration, $T_4$: after 4 weeks of storage, and $T_{12}$: after 12 weeks (3 months) of storage.

The color measurement was made in triplicate for all experimental groups using the $L^*a^*b^*$ value, where $L^*$ is the light intensity, $a^*$ is the red-green parameter, and $b^*$ is the yellow-blue parameter. Using these $L^*a^*b^*$ values, the differences between the color measured at the initial time point (baseline) and those measured at the various time points ($\Delta E$) were calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Statistical Analysis

All statistical analyses for differences in color changes among the products between the initial time point and the 3 other time points after the initiation of light treatment were conducted using SPSS Statistics (versions 21.0; SPSS IBM, Armonk, NY). Nonparametric one-way analysis of variance ($p < .05$) was performed, and the Dunn’s test ($p < .05$) was used as a post hoc test.

Results

Discoloration was evident in all teeth, immediately ($T_{PO}$) after applying MTA and NFC.

MTA and NFC induced significantly more severe discoloration compared to the control group ($P<0.05$).

For these groups, the mean $\Delta E$ values increased until the third month during the period of investigation.

The highest $\Delta E$ was noted in WMTA at 3 months, followed by NFC, but there was no significant difference between the discolorations induced by these two materials. Total color change induced by MTA and NFC was higher than the perceptibly threshold (3.3) at all time periods (Table 1, Figure 2).

Table 1: Mean (SD) $\Delta E$ values of groups 1–3 in all experimental periods

<table>
<thead>
<tr>
<th>Group</th>
<th>$\Delta E_1$</th>
<th>$\Delta E_2$</th>
<th>$\Delta E_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA</td>
<td>4.7640(2.32706)</td>
<td>7.4420(2.51670)</td>
<td>9.7280(2.80679)</td>
</tr>
<tr>
<td>NFC</td>
<td>4.7000(2.02409)</td>
<td>6.4230(1.41866)</td>
<td>9.0590(1.70196)</td>
</tr>
<tr>
<td>Control</td>
<td>1.1080(.55445)</td>
<td>2.0660(.92199)</td>
<td>3.2740(.63535)</td>
</tr>
</tbody>
</table>

Discussion

Patients are commonly concerned by the unaesthetic appearance of teeth caused by certain dental materials after restorative procedures [1]. MTA is commonly used endodontic cement, which has been shown to cause tooth discoloration [2, 14].

Color measurement studies implement several devices such as colorimeter [15] and spectrophotometer [2, 10, 12, 16-19]. High resolution digital photographs and color analysis by Photoshop software can also be used for this purpose [18, 20]. In 2007, Dozic et al. [21] concluded that Easy Shade spectrophotometer and digital camera are the most reliable tools for color assessment. In the current study, a spectrophotometer was used. Spectrophotometer and spectroradiometer measure the light reflection index in the visible spectrum. As the most accurate and commonly used system for color measurement, the CIE $L^*a^*b^*$ system was used in the current study. This system quantifies the change in color.
parameters. Color change is clinically perceptible in values > 3.3 [18].

Our study investigated tooth discolorative behavior of a newly introduced root filling material, NFC and the results indicated that this material as well as WMTA, causes significant color changes, immediately after placement with consistency of discoloration in the second and third time periods. Although the highest ΔE was noted in WMTA, there was no significant difference between the discoloration induced by WMTA and NFC.

Similar to our study, most studies such as Ioannidis et al. [1], Ramos et al. [9] and Kang et al. [2] showed that discoloration increases with time after using MTA.

The discoloration observed immediately after exposure to MTA may be due to change in dental transparency, it is expected that it will resolve with removal of the material.

In contrast to the results of our study, most studies indicated that significant color changes occur, after more than four weeks. For instance, Ramos et al. [9], in 2016 detected clinically perceptible crown discoloration by WMTA in the 6th week of their experiment. Although premolars were used in this study as ours, colorimeter was implemented to detect color change which is in contrast to our study; this may have resulted in difference in the results of the two studies.

In addition, Ioannidis et al. [1], in 2013 demonstrated that GMTA and WMTA induced clinically perceptible crown color change from the first and third months of application, respectively. This difference between our studies may be due to the fact that Ioannidis evaluated color change in 3rd mandibular molars while we tested premolars and the difference in structural characteristics of these teeth may have resulted in different discoloration behaviors. In their study, GMTA resulted in greater discoloration than WMTA at all experimental time periods.

Our results however, are consistent with those presented by Esmaeili et al. in 2016 [22] and Meetu et al. in 2015 [23], who found that significant color changes occur only one week after placement of WMTA.

The same formulation of MTA resulted in variable amounts of color change in different studies. The time duration between exposure of teeth to MTA and detection of discoloration also differed between teeth. This inconsistency between studies could be the result of different thicknesses of the remaining tooth structure, variety in colorimetric methods and methods of material application [24-25].

Since NFC contains nano-sized particles, it may be expected that it would penetrate deeper in dentinal tubules and cause more severe discolorations than WMTA; in this study however, our null hypothesis was proved and no difference was observed between discolorations of the two experimental materials.

NFC has recently been introduced in literature and has not been investigated with regard to its effect on tooth color; therefore, the result of our study could not be compared with those of similar studies.

In contrast to some researchers who used composite resin to seal the root end [26-27]; glass ionomer cement was used in the current study, so that it could better seal dentinal tubules. Due to the bond of carboxylic ions to calcium ions of hydroxylapatite and chemical bond to tooth structure, this cement was also implemented in similar studies [28].

Our study included an adequate sample size. In addition, in order to more accurately compare the discoloration potential of teeth we used extracted sound human teeth similar to many previous studies [2, 15, 20].

Despite these efforts to simulate the clinical conditions, the in vitro design is subject to imperfections. Hence, generalization of the results to the clinical setting must be done with caution Future clinical studies are required to better elucidate the discoloration potential of WMTA and NFC in the oral environment [14].

Conclusion
Clinically perceptible crown discoloration was detected immediately after applying WMTA and NFC. There was a similar level of clinically observable tooth discoloration detected using either WMTA or NFC and crown discoloration demonstrated an increasing pattern with time. Application of both biomaterials should be considered with high level of caution, especially in the esthetic zones.

Conflicts of Interest
Authors have no conflicts of interest to declare.
References


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