1-year In Vitro Evaluation of Tooth Discoloration Induced by 2 Calcium Silicate–based Cements

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Abstract

Introduction: The purpose of this study was to compare tooth discoloration that occurs in teeth filled with ProRoot MTA (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) or Biodentine (Septodont, Saint Maur des Fossés, France) over the course of 1 year. Methods: Twenty-eight intact premolars were resected 2 mm apical to the cementoenamel junction and the pulp tissues extripated via the cervical cut. After the preparation of occlusal access to the pulp chamber, specimens were assigned into 4 groups according to a stratified randomization sampling process: group 1, negative control (dry sterile cotton pellet); group 2, positive control (blood-moistened cotton pellet); group 3, ProRoot WMTA (DENTSPLY Tulsa Dental Specialties); and group 4, Biodentine. The experimental materials were condensed into the crowns and the access sealed with glass ionomer restorative cement. Color was assessed at baseline (before placement of the materials), immediately after material filling, after 6 weeks of storage, and after 1 year using the Commission International de I'Eclairage L*a*b* system. Change in color, \( \Delta E \), was compared among groups and over time using analysis of variance.

Results: The 4 groups showed a significant decrease in \( L^* \) values over time. Differences between Biodentine and WMTA were detected after 1 year, with the greater variation associated with WMTA (\( P = .001 \)). The 4 groups presented a significant increase in \( \Delta E \) from baseline to 1 year. All groups revealed perceptible color changes (\( \Delta E > 2.3 \)) between immediately after material filling and after 6 weeks and after 6 weeks and 1 year. After 1 year, no differences could be detected between Biodentine and WMTA. Conclusions: Delayed tooth discoloration was detected for the 2 materials at the 1-year evaluation, but it was more evident for ProRoot MTA than Biodentine. Luminance was the most affected parameter, with a higher decrease for ProRoot MTA.

Key Words

Biodentine, calcium silicate–based cements, mineral trioxide aggregate, tooth discoloration

Currently, the choice of a specific material for endodontic use should not rely solely on biological and functional aspects but also on the esthetic considerations. Thus, biomaterials used in endodontics should be chromatically stable, present optical properties similar to dental structures, and exhibit no staining effects on hard dental tissues over time (1). Mineral trioxide aggregate (MTA) has been successfully used for endodontic procedures such as apexogenesis, pulp capping procedures, and root resorption or perforation repair (2,3) because of the excellent biocompatibility and bioactivity. However, some disadvantages of MTA mainly related to the discoloration potential, presence of toxic elements in the material composition, demanding handling characteristics, long setting time, absence of a known solvent, and difficulty of removal after cure (4–6) should inspire some improvements in the material or the development of new biomaterials in this field of applications.

Biodentine (Septodont, Saint Maur des Fossés, France) is a calcium silicate–based material (GSM) that exhibits physical and chemical properties similar to those described for certain Portland cement derivatives (7). This material claims dentinlike mechanical properties and can be used as a dentin substitute on crowns and roots. Compared with MTA cements, Biodentine handles easily and has a significantly shorter setting time (8). On the biological level, it appears to be biocompatible (9) and capable of inducing dentin apposition by stimulating odontoblast activity (10). Unlike other Portland cement–based products, it is sufficiently stable so that it can be used both for pulp protection and temporary fillings (11). However, data are sparse concerning color stability and tooth pigmentation induced by Biodentine. The aim of the present in vitro study was to compare tooth discoloration that occurs in human teeth filled with ProRoot MTA (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) or Biodentine over the course of 1 year.

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Tooth Discoloration by Calcium Silicate Cements
Basic Research—Technology

Materials and Methods

Specimen Preparation

The study used 28 premolars extracted for orthodontic purposes. Only teeth clinically and radiographically free of caries, cracks, restorations, and pathologic discolorations were selected for the experimental procedures. All external surfaces of each tooth were visually inspected and meticulously cleaned with periodontal scalers and polished with pumice and water to remove organic materials, calculus, and extrinsic staining. Root resection was performed 2 mm apical to the cementoenamel junction and the pulp tissues extirpated via the cervical cut with Hedström files.

To make a stable sealed base for the tooth crown, a single-step self-etching dental adhesive (Xeno III; Dentsply DeTrey, Konstanz, Germany) was applied to the cervical surface and cured for 10 seconds. Then, each specimen was inserted in a standardized silicone elastomer matrix filled with a fluid composite resin (Sinergy D6 Flow; Coltène Whaledent, Gayahoga Falls, OH) and polymerized for 40 seconds.

Access occlusal cavities were then prepared in all specimens under copious water coolant with a cylindrical bur to expose the entire pulp chamber. The elimination of all pulp remnants was confirmed under 40× magnification with a dental microscope (Leica M300; Leica Microsystems [Schweiz] AG, Heerbrugg, Germany). The samples were stored hydrated until the experimental procedures.

Experimental Setup

The specimens were stratified by value and then randomly assigned to 4 groups (stratified random sampling) according to the material used to fill the standardized cavities. Groups 1 and 2 were the negative control (NCtrl) and positive control (PCtrl), respectively. Groups 3 (WMTA) and 4 (Biodentine) were the experimental groups, receiving ProRoot MTA and Biodentine, respectively. Further details are described in Table 1.

After preoperative color measurement of each specimen, the materials used for the experimental groups were mixed homogenously and according to the instructions provided by the manufacturers. Uniform plugs of approximately 15 mm³ of each material (roughly a 2-mm diameter and 5-mm height) were then inserted into the cavities using a carrier and posteriorly compacted into the pulp chamber. For the control groups, cotton pellets, dry or moistened with fresh blood collected by pricking the finger of a volunteer for the negative and positive controls, respectively, were placed into the cavities.

Cavities were posteriorly sealed with a restorative glass ionomer cement (Ketac Fil Plus Aplicap; 3M ESPE, Neuss, Germany) shade A1. Immediate postoperative color measurements were recorded, and specimens were stored in the dark in a 100% humidity environment at 37°C with normal atmospheric gas levels until the subsequent color measurement points.

Tooth Color Measurement

Color was recorded at 4 time points: T₀: baseline (after preparation of the cavities but before placement of the materials), T₁: immediately after placement of the filling material and provisional restoration, T₆: after 6 weeks of storage, and T₅₂: after 52 weeks (1 year) of storage.

Tooth measurements were performed by a single operator using a colorimeter (PR-650 SpectraScan Colorimeter; PHOTO RESEARCH Inc, Chatsworth, CA) in a custom-built measuring station composed of a scone with a 6-W light-emit diode light of 5500 K and a white board for greater light reflection during the measurements in a dark room as represented in Figure 1.

Color was consistently measured in a circle centered at the midbuccal point of the cervical third of the anatomic crown, occupying the mesiodistal distance of the tooth. Measurements were performed using the color space defined by the Commission International de l’Eclairage in 1931 (CIE XYZ), with X, Y, and Z coordinates and lightness value Y (cd/m²) and posteriorly converted to the CIE L*a*b* system. In the CIE L*a*b* perceptual color space, ΔL corresponds to the change in lightness or luminance (from 0 [black] to 100 [white]), Δa* corresponds to the change in the red/magenta (positive a*) to green (negative a*) channel, and Δb* is the change in the yellow (positive b*) to blue (negative b*) channel position.

The total color difference between 2 objects (ΔE) is numerically expressed by their Euclidean distance given by

\[
\Delta E = \sqrt{(\Delta L')^2 + (\Delta a')^2 + (\Delta b')^2}
\]

For each coordinate (L*, a*, b*), delta values were calculated based on the difference between baseline and each of the following evaluations (T₁, T₆, and T₅₂). Perceptible chromatic alterations for the human eye were defined at the threshold value of ΔE ≥ 2.5.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics version 20 (IBM, Armonk, NY). Related samples Friedman 2-way analysis of variance by rank was applied to evaluate ΔE (dependent variable) variation over time within each group. For each time point, nonparametric 1-way analysis of variance with Tukey post hoc tests considered the ranked values to verify differences between groups. A similar approach was applied for the analysis of variation of the chromatic coordinates L*a*b* over time. The significance level was set at α < 0.05.

Results

Table 2 represents the descriptive statistics obtained for the CIE L*a*b* coordinates after conversion from the CIE xyY space for all

| Table 1. Description of the Control and Experimental Groups and Respective Material Composition |
|---|---|---|---|
| Group | Material Filling | Manufacturer | Composition |
| 1. Negative control | Dry sterile cotton pellets | — | Fresh human blood obtained from one of the researchers |
| 2. Positive control | Blood-moistened sterile cotton pellets | — | 75% Portland cement |
| 3. WMTA | ProRoot MTA | DENTSPY Tulsa Dental Specialties | 20% bismuth oxide (Bi₂O₃) |
| 4. BD | Biodentine | Septodont, France | 5% calcium sulphate dehydrate powder: tricalcium silicate (C₃S), dicalcium silicate (C₂S), calcium carbonate and oxide, iron oxide, zirconium oxide |
| | | | Liquid: calcium chloride, hydrosoluble polymer |
groups at each period of evaluation considered (baseline, immediately postoperative, 6 weeks, and 1 year).

As a consequence of the stratified random sampling technique based on specimen value, all groups presented similar initial luminance values ($L^*$ at $T_{BL}$). The 4 groups showed a significant decrease in $L^*$ values over time. The PCtrl group revealed the highest decrease from $T_{BL}$ to $T_{S2}$ with a mean difference of $12.59 \pm 1.52$ points ($P = .02$). For the same interval, the NCtrl and the Biodentine groups presented similar reductions in luminance ($4.61 \pm 1.40$ and $4.74 \pm 0.97$ points, respectively). ProRoot WMTA (WMTA) showed an intermediate decrease in luminance with an $8.81 \pm 1.19$ points reduction. Differences between groups were detectable from 6 weeks onward ($P = .002$ and $P < .01$ at $T_6$ and $T_{S2}$, respectively); the PCtrl group was statistically different from the NCtrl and Biodentine groups in both evaluations. After 1 year of storage ($T_{S2}$), it was possible to observe significant differences between WMTA and BD, with the greater $L^*$ variation associated with WMTA ($P = .001$). Regarding variation in the magenta/green channel, even though both groups 2 and 4 showed statistically significant differences over time, only the PCtrl group revealed a clear change toward the positive portion of the spectrum. All groups also showed a significant change toward the positive portion of the yellow/blue channel.

Total color variation from baseline to each of the following evaluations is represented in Figure 2. The 4 groups presented a similar behavior over time with a statistically significant increase in $\Delta E$. Perceptible color variations were detected for the PCtrl group in all periods. All other groups only presented perceptible color changes at $T_6$ and $T_{S2}$.

One-way analysis of variance revealed statistically significant differences among groups for all periods. Pair-wise comparisons revealed that at the immediate postoperative moment, the PCtrl group presented color variation superior to that of the NCtrl and Biodentine groups, which was extended to the WMTA group at the 6-week evaluation. At the 1-year evaluation, all groups revealed significant chromatic changes. At this point, statistically significant differences could only be found between the PCtrl and the Biodentine groups.

Discussion

Dental discoloration subsequent to pulp capping or root canal filling procedures has been previously reported with the use of different materials, namely CSMs (1, 12, 13). The present study addressed tooth discoloration induced by 2 CSMs (ProRoot WMTA and Biodentine) on human premolars using specimen preparation similar to that of Kang et al (14) and Valles et al (15). However, this study differed from the cited studies because it extended to re-evaluation at 1 year and used occlusal access cavities to fill the pulp chamber as reported by Shokouhinejad et al (16) to approximate the setup to the clinical conditions.

Even though total color variation ($\Delta E$) was consistently higher for WMTA than Biodentine, the results revealed no statistically significant differences between the 2 test groups in any of the evaluation periods. Because the changes in the $a^*$ and $b^*$ patterns were similar for the 2 groups, the differences in discoloration patterns could be mainly attributed to the higher decrease in luminosity of the WMTA specimens, as reported by the $L^*$ parameter (Table 2).

In fact, WMTA showed discoloration similar to that of the PCtrl group in which the cavity was filled with blood-moistened cotton pellets, whereas Biodentine approximated the behavior of the NCtrl group. These results are consistent with those presented by Shokouhinejad et al (16), Valles et al (15), and Kohli et al (17), who found significant WMTA-induced color changes increasing over time, contrasting with the performance of Biodentine, which could be paired with that of the control group. In fact, other studies comparing bioceramic cements using bismuth oxide as a radiopacifier with others using zirconium or tantalum oxide (13, 14, 18–23) also point to the greater potential of the former to cause tooth discoloration. The radiopacifier bismuth oxide has been associated with different chemical transformations that result in darkening of the material and subsequent staining of the tooth structure.

**Figure 1.** A schematic representation of the setup for color measurement under standardized conditions. For all evaluations, specimens were illuminated with a 5500-K light-emitting diode light and placed over a white cardboard exactly at 69.1 cm from the colorimeter.

**Table 2.** Mean Values of Each of the $L^*a^*b^*$ Coordinates of Each Group for All Periods of Evaluation

<table>
<thead>
<tr>
<th>Group</th>
<th>Coordinates</th>
<th>$T_{BL}$</th>
<th>$T_{PO}$</th>
<th>$T_6$</th>
<th>$T_{S2}$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCtrl</td>
<td>$L^*$</td>
<td>90.08 ± 2.10</td>
<td>89.39 ± 2.34</td>
<td>89.42 ± 3.46</td>
<td>85.47 ± 3.24</td>
<td>.026*</td>
</tr>
<tr>
<td></td>
<td>$a^*$</td>
<td>0.58 ± 0.63</td>
<td>0.49 ± 0.64</td>
<td>0.76 ± 0.72</td>
<td>0.77 ± 1.01</td>
<td>.212</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>7.01 ± 3.69</td>
<td>7.51 ± 3.04</td>
<td>10.66 ± 3.61</td>
<td>17.26 ± 4.46</td>
<td>.011*</td>
</tr>
<tr>
<td>PCtrl</td>
<td>$L^*$</td>
<td>90.03 ± 2.02</td>
<td>88.17 ± 0.63</td>
<td>83.77 ± 1.31</td>
<td>77.43 ± 0.98</td>
<td>.011*</td>
</tr>
<tr>
<td></td>
<td>$a^*$</td>
<td>0.48 ± 0.26</td>
<td>1.36 ± 0.35</td>
<td>1.92 ± 0.50</td>
<td>2.09 ± 0.29</td>
<td>.013*</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>6.79 ± 3.16</td>
<td>4.96 ± 1.57</td>
<td>7.08 ± 1.06</td>
<td>13.74 ± 3.02</td>
<td>.013*</td>
</tr>
<tr>
<td>WMTA</td>
<td>$L^*$</td>
<td>89.28 ± 2.32</td>
<td>88.94 ± 1.65</td>
<td>86.56 ± 2.07</td>
<td>80.47 ± 2.17</td>
<td>&lt;.01*</td>
</tr>
<tr>
<td></td>
<td>$a^*$</td>
<td>0.79 ± 0.72</td>
<td>0.59 ± 0.69</td>
<td>0.95 ± 0.91</td>
<td>0.71 ± 0.87</td>
<td>.266</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>7.52 ± 2.87</td>
<td>7.34 ± 2.46</td>
<td>10.10 ± 1.98</td>
<td>16.39 ± 3.02</td>
<td>&lt;.01*</td>
</tr>
<tr>
<td>BD</td>
<td>$L^*$</td>
<td>89.89 ± 1.48</td>
<td>89.11 ± 1.74</td>
<td>89.02 ± 1.74</td>
<td>85.14 ± 2.03</td>
<td>&lt;.01*</td>
</tr>
<tr>
<td></td>
<td>$a^*$</td>
<td>0.62 ± 0.40</td>
<td>0.76 ± 0.40</td>
<td>1.01 ± 0.48</td>
<td>0.84 ± 0.79</td>
<td>.013*</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>6.21 ± 1.91</td>
<td>6.70 ± 2.24</td>
<td>9.78 ± 1.70</td>
<td>15.94 ± 2.71</td>
<td>&lt;.01*</td>
</tr>
</tbody>
</table>

BD, Biodentine; NCtrl, negative control; PCtrl, positive control; $T_6$, after 6 weeks of storage; $T_{S2}$, after 52 weeks of storage; $T_{BL}$, baseline; $T_{PO}$, immediately after placement of the filling material and provisional restoration.
Some authors claim that the discoloration of MTA-type materials may be produced by the exposure of bismuth oxide to formaldehyde released from zinc oxide–eugenol sealers (24) or from the polymerization of methacrylate-based resins (25) but not from the direct contact with methacrylate (22). In the present study, WMTA discoloration could not be attributed to the formaldehyde-induced reduction of bismuth oxide to metallic bismuth or even to polymerization under anaerobic conditions (26) because no endodontic sealers were used and the resin base of each specimen was polymerized before plug insertion in the cavity. This is in line with the findings of Ioannidis et al (27), who were able to detect coronal discoloration by the use of MTA without the exposition to methacrylate-based composite resins or polymerization lights. Accordingly, Marciano et al (22) detected evident discoloration of WMTA concentrated close to the dentin interface but not in contact with composite, which was not observed with other CSMs. The effect was attributed to the interaction between the radiopacifier bismuth oxide and the dentin matrix collagen with production of a black precipitate, similarly to the reported reaction of WMTA with sodium hypochlorite solutions (28, 29). Actually, the majority of studies using either human or bovine teeth complement the mechanical debridement of the pulp tissues with sodium hypochlorite (12–15, 17, 22, 23, 27, 30), which could be a confounder regarding the origin of the discoloration. Sodium hypochlorite penetrates the dentin matrix up to 300 μm (31) and not only damages collagen and contributes to opening of the lumen of the tubules (32) but also may endure in the tooth structure (20). As a consequence, the residual hypochlorite and the extended surface of collagen can get into contact with the CSMs and, in the presence of bismuth oxide, suffer greater discoloration. In the present study, even though no attempt was made to chemically remove pulp tissues or the smear layer over the dentin walls, WMTA-induced discoloration was evident (ΔE = 12.73 ± 2.29) but inferior to the values reported in other studies with hypochlorite irrigation and shorter follow-ups (14, 17). Furthermore, the fact that no smear layer was removed could justify the less evident discoloration of the specimens filled with blood-embedded cotton pellets and the reason for the delayed discoloration of the WMTA group (33) after the 6th week of control as opposed to the results of Marciano et al (22, 34) and Valles et al (15).

Regarding Biodentine, results of the 6-week measurements are in accordance with previous reports in the literature (17, 35), revealing generally acceptable color change. However, the majority of discoloration of Biodentine specimens was detected at the 1-year evaluation and to a greater extent than the ΔE values determined by Shokouhinejad et al (16) and Vales et al (15) after 6 months of follow-up, suggesting that discoloration of Biodentine could take place in the period between 6 months and 1 year, parallel to that of the specimens with no filling. Regrettably, the present study lacks an evaluation after 6 months of storage in the dark to confirm the inference.

**Conclusion**

Delayed tooth discoloration was detected for the 2 CSMs at the 1-year evaluation, but it was more evident for ProRoot MTA than Biodentine. Luminance was the most affected parameter, with a statistically higher decrease for ProRoot MTA than Biodentine.

**Acknowledgments**

The authors deny any conflicts of interest related to this study.

**References**