Tooth Discoloration Induced by Different Calcium Silicate–Based Cements: A Two-Year Spectrophotometric and Photographic Evaluation *in Vitro*

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Objective: *Calcium silicate-based cements (CSCs) may lead to coronal staining in young permanent teeth over the time. The purpose of this study was to evaluate and compare the long-term tooth discoloration induced by different CSCs.* ***Study Design:*** *Ninety freshly-extracted human molars were assigned randomly into 6 groups (n=15/group) according to the CSC used as a pulpotomy material: ProRoot MTA, MTA Angelus, NeoMTA, EndoSequence Putty, Biodentine and Negative control (No cement). The color was assessed at baseline, and thereafter at 3, 6, 12 and 24 months by using both a spectrophotometer and digital images taken with and without a cross-polarizing filter. The time-dependent changes in color (ΔE) were compared within and among groups using Analysis of Variance.* ***Results:*** *Angelus MTA and ProRooT MTA showed severe coronal discoloration (p>0.05) starting at 3 months. ΔE values of NeoMTA, EndoSequence Bioceramic Putty and Biodentine were below the perceptibility threshold, with Biodentine showing greater ΔE values than NeoMTA and EndoSequence Putty in the absence of statistical significance (p>0.05).* ***Conclusions:*** *Discoloration elicited by CSCs may develop soon after placement, and continue to increase for up to two years. Angelus MTA and ProRooT MTA cannot be recommended for vital pulp therapies in the esthetic zone of young individuals.*

Keywords: *Endodontics, Mineral Trioxide Aggregate, Tricalcium Silicate, Pulpotomy, Tooth Discoloration*

INTRODUCTION

Originally introduced as a root repair material, mineral trioxide aggregate (MTA) has received widespread acceptance for use in a variety of endodontic procedures in young permanent teeth including pulpotomies and direct pulp capping, apical closure, and more recently, regenerative endodontic procedures. [1-3] MTA has demonstrated superior sealing properties, good biocompatibility and biomechanical properties, but tooth discoloration associated with its use in the esthetic zone continues to be major concern.[4,5] Over the years, some calcium silicate-based cements have been introduced as non-staining biomaterials that do not incorporate bismuth oxide, a radiopacifier that has been held responsible for MTA-related discoloration.[6-9] Biodentine (Septodont, Saint-Maur-des-Fossés, France), Neo MTA (NuSmile Ltd., Houston,TX) and EndoSequence Bioceramic Root Repair Material (Brasseler, Savannah,GA) are such non-staining materials, and possess biological properties similar to those of MTA.[10–12] These products have been shown to prevent discoloration for up to few months *in vitro*,[13-15] but the long-term stability of their non-staining properties have remained unknown. Only one study[4] has compared the discoloration of White MTA and Biodentine and has demonstrated delayed tooth discoloration for up to 12 months, suggesting that coronal staining induced by calcium silicate-based cements might increase even after 1 one year.

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Spectrophotometry and digital image analysis have been commonly used to measure tooth discoloration induced by endodontic biomaterials.[16] Spectrophotometers are considered as gold standard in the field of color science as well as in dentistry,[17] and use the Commission International de I'éclair age's (CIE's) L*, a*, b* system for measurements. Likewise, digital cameras can provide images for color measurements using CIE L*a*b units at much lower costs in pediatric practice, but their reliability has not been compared with dental spectrophotometers.

The aim of the present study was to evaluate and compare the long-term coronal tooth discoloration induced by original white MTA formulations and non-staining calcium silicate cements when used in a pulpotomy procedure. The tested null hypothesis was that, none of the tested cements induced clinically perceptible crown discoloration over 2 years as measured by spectrophotometry and computer analysis of digital images.

MATERIALS AND METHOD

Freshly-extracted human third molars were collected and stored in 1% chloramine-T solution and were evaluated under a dental operating microscope (Carl Zeiss OPMI ProErgo;Carl Zeiss, Oberkochen,Germany) at 20X to exclude those with restorations, caries, cracks, and/or enamel defects. The study protocol involving the experimental use of extracted human teeth was approved by the Local Ethics Committee. The teeth were cleansed with an ultrasonic scaler and polished with pumice and water to remove soft tissue remnants, calculus, and extrinsic stains.

Following endodontic access with water-cooled high-speed diamond burs, the coronal pulp was removed, and the teeth were randomly assigned to 6 groups (n=15/group) according to the cement used: group 1: ProRoot MTA (Dentsply Tulsa Dental,Tulsa, OK); group 2: MTA Angelus (Angelus, Londrina, Brasil); group 3: NeoMTA; 4: EndoSequence bioceramic putty; group 5: Biodentine and group 6: Intact teeth (negative control). All materials were prepared according to the manufacturers' recommendations and placed as a 3mm-thick pulpotomy base on the pulp chamber floor. Then, a thin layer of glass ionomer (ChemFil Rock; Dentsply/Caulk, Milford, DE) was placed over each material to prevent washout of the cements during subsequent adhesive procedures. The remaining access cavity was restored with acid-etch universal composite (Filtek Ultimate, 3MEspe, St.Paul, MN) whose shade matched with the coronal tooth structure as confirmed with a spectrophotometer (VITA EasyShade; VITA Zahnfabrik, BadSackingen, Germany). Each sample was stored separately in phosphate buffered saline solution at 37°C in 100% humidity throughout the study.

Spectrophotometry and Digital Image Analysis

The coronal color shade was recorded before endodontic access (baseline), immediately after material placement and after 3, 6, 12 and 24 months by using a spectrophotometer (Spectroshade MHT S.P.A., Verona, Italy). The measurements were performed under constant laboratory illumination by positioning the spectrophotometer at the occlusal, middle, and cervical thirds of the crown. The measurements were carried out by a single operator and repeated three times for each sample and the mean values were calculated.

The same tooth specimens were also tested for accuracy of color analysis using a digital camera. A digital photograph was taken from the buccal aspect of molars using a Canon EOS 650D (Canon Inc., Japan) camera equipped with a 100mm macro lens and 14MR-EX ring flash (Both Canon). The images were obtained at the same time intervals and laboratory conditions with Spectroshade measurements with and without the use of a cross polarization filter to reduce glare. The images were then transferred to Adobe Photoshop CS6 (Adobe, San José,CA) software, and CIE lüL*a*b* system was used for color assessment at the occlusal, middle and cervical one thirds of molar crowns. The data were transformed into 3 parametric values of the CIE L*a*b color system. Accordingly, the amount of "L" ranges from 0 (black) to 100 (white) and indicates the rate of lightness. "a" and "b" represent the color change in green-red and blue-yellow axes, respectively. The discoloration was calculated using following formulation: [18]

$$\Delta E = \{(L_2-L_1)^2 + (a_2-a_1)^2 + (b_2.b_1)^2\}^{1/2}$$

Statistical Analysis

The time-dependent change in color, (ΔE), was compared within and among the test groups using univariate analysis of variance. Bonferroni correction was used as post-hoc analysis. All statistical analysis was performed with SPSS software (version 16.0, SPSS Inc.,Chicago, IL). The level of statistical significance was set at $p<0.05$.

RESULTS

The results of mean (SD) total color differences (ΔE) of the test groups are shown in Table 1. All test materials showed a significant increase in ΔE at 3 months ($p<0.05$). Both ProRoot MTA and MTA Angelus exceeded the perceptibility threshold (ΔE ≥3.3), displaying the most severe coronal discoloration in comparison with remaining test materials and negative control ($p<0.05$). MTA Angelus showed greater ΔE values than ProRoot MTA in the absence of statistical significance ($p>0.05$). Both ProRoot MTA and MTA Angelus showed a significant increase in ΔE in the first three months ($p<0.05$), followed by a stable increase for two years ($p>0.05$). There were no significant differences between the coronal discolorations of NeoMTA, Endosequence bioceramic putty, Biodentine and Negative control groups at any examination period ($p>0.05$) and none of those four groups exceeded the perceptibility threshold, despite a statistically-insignificant increase in ΔE over the time. Representative photographs of time-dependent color changes from each group are presented in Figure 1.

The spectrophotometric measurements indicated significantly lower ΔE values than those obtained with computer analysis of digital images ($P<0.05$). However, there was no significant difference between both techniques in terms of L*a*b* measurements ($P>0.05$). Finally, no significant differences were found between the computer analysis of digital images obtained with and without a cross-polarizing filter ($P>0.05$).

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Figure 1. Time-dependent changes in tooth color as viewed on digital photographs. B=Baseline.

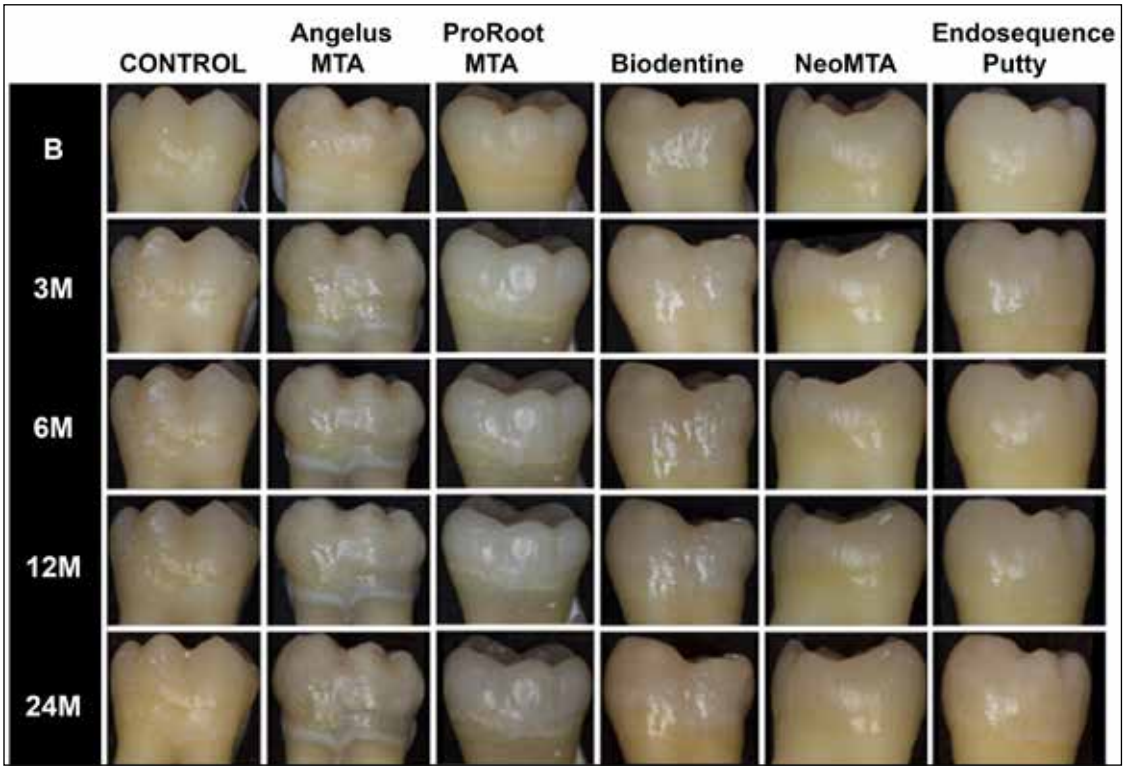

Table 1. Mean ΔE and standard deviation (SD) values of experimental groups at 3, 6, 12 and 24 months. (S) Spectrophotometer, (DC-) Digital Camera without a cross- polarizing filter, (DC+) Digital Camera with a cross- polarizing filter.

		Baseline	3m	6m	12m	24m
Negative control	S	1,34 (0,24)	1,35 (0,46)	1,58 (0,32)	1,61 (0,47)	1,68 (0,53)
	DC-	5,54 (0,91)	6,51 (0,94)	7,16 (1,06)	7,17 (0,96)	7,26 (0,88)
	DC+	6 (1,11)	6,05 (1,15)	7,28 (1,26)	8,23 (0,80)	8,35 (0,89)
ProRoot MTA	S	1,86 (0,24)	3,37 (0,32)	3,97 (0,47)	4,13 (0,45)	4,50 (0,52)
	DC-	3,70 (0,93)	5,85 (0,91)	5,93 (1,06)	6,14 (0,96)	6,20 (0,89)
	DC+	4,37(1,11)	6,99 (1,15)	7,25 (1,25)	7,70 (0,80)	8,05 (0,90)
Biodentine	S	1,80 (0,24)	1,83 (0,46)	1,89 (0,53)	2,04 (0,47)	2,28 (0,32)
	DC-	5,45 (0,93)	5,66 (0,91)	7,07 (1,06)	7,39 (0,96)	7,40 (0,88)
	DC+	6,35 (1,11)	6,58 (1,15)	6,93 (1,26)	7,08(0,80)	7,23 (0,89)
MTA Angelus	S	3,01 (0,24)	5,39 (0,32)	5,98 (0,46)	6,81 (0,53)	6,94 (0,47)
	DC-	6,69 (0,93)	9,38 (0,91)	9,44 (0,96)	9,50 (0,88)	11,19 (1,06)
	DC+	4,25 (1,11)	9,58 (0,80)	10,16 (0,89)	10,42 (1,15)	11,31 (1,26)
EndoSequence Bioceramic Putty	S	1,18 (0,24)	1,51 (0,32)	1,61 (0,46)	1,76 (0,53)	1,79 (0,47)
	DC-	5,90 (0,93)	6,26 (0,91)	6,47 (1,06)	6,50 (0,96)	6,64 (0,88)
	DC+	5,67 (1,11)	6,73 (1,15)	6,90 (1,26)	7,10 (0,80)	7,32 (0,89)
Neo MTA	S	1,62 (0,23)	1,76 (0,32)	1,78 (0,46)	1,92 (0,53)	2,04 (0,47)
	DC-	4,44 (0,93)	4,80 (0,91)	5,10 (1,06)	6 (0,96)	6,17 (0,88)
	DC+	6,06 (1,11)	6,44 (1,15)	7,15(1,56)	7,48 (0,80)	7,70 (0,89)

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DISCUSSION

Over the two-year observation period, the negative control group (sound tooth) showed a slight increase in coronal discoloration that was below the perceptibility threshold.[19] This finding can be explained by gradual necrosis of the pulp, which is known to cause discoloration in both permanent and primary dentitions,[20, 21] without necessarily requiring the diffusion of blood derivatives into dentinal tubules.[21] Such necrosis-associated discoloration was not regarded as a confounder to the origin of discoloration in the experimental groups, because the coronal pulp was removed in a vital state soon after extraction. Because the experimental pulpotomy procedures took place while the teeth were disconnected from active blood supply, the possible contribution of active bleeding during coronal pulp removal[22] and hemostatis[4] steps to cause coronal discoloration were also discarded. Although the aim of this study was to assess the color changes in a pulpotomy scenario, an attempt to simulate hemostasis with the frequently used sodium hypochlorite was not made, because bismuth oxide is known to exhibit discoloration upon contact with residual sodium hypochlorite.[4,13] Finally, the teeth received resin composite fillings as final restorations, because they do not interfere with the process of discoloration caused by calcium silicate–based cements.[14]

In the present study, the null hypothesis was rejected for ProRoot MTA and Angelus MTA, since both materials showed severe, clinically perceptible coronal discoloration over two years. Previous studies on the discoloration potential of calcium silicate-based cements are limited to shorter observation periods, and the only longer in vitro study of 12 months[4] has indicated that ProRoot MTA and Biodentine caused 'delayed' discoloration, which justifies the need for studies with longer observation periods, utilizing a larger spectrum of calcium silicate-based products. The authors of the one-year study[4] were not able to were determine the approximate time of the 'delayed discoloration', because their observation periods were set to baseline, 6 weeks and 1 year, and significant discolorations were only recorded for both materials at 1-year measurements. Because the present study utilized two additional measurement periods at 3- and 6-months using similar methodology, it was possible to observe that the discoloration induced by ProRoot MTA and Biodentine, as well as all other tested cements herein were not 'delayed'. In fact, they did occur as early as three months (Table 1), followed by a smaller and insignificant increase throughout the 2-year experimental period. Here, it is important to emphasize that irrespective of the discoloration being above or below the perceptibility threshold, all test groups showed significantly greater ΔE values at 3 months, which corroborates and adds to the findings of Forghani *et al* ,[23] who reported progressive tooth discoloration during the first 3 months. According to Partovi *et al* ,[24] the relative decrease in the level of tooth discoloration after 3 to 6 months could be regarded as the time for the stains to spread through the dentinal tubules. Collectively, these findings indicate that cervical tooth discoloration is a dynamic process in both conventional and non-staining MTA formulations and calcium silicate-based cements, which starts as early as a few months after placement and continues in a decreasing trend over the time.

In pediatric endodontic procedures, it is important to record and document initial and progressive coronal discolorations induced by trauma and biomaterials. Although spectrophotometers are the most reliable instruments for clinical use,[25] they can be quite costly for a pediatric practice. Therefore, this study compared spectrophotometry with computer analysis of digital images, a more cost-effective solution due to the availability of digital cameras in many practices and the reported effectiveness of digital pictures for color measurements in dentistry.[26,27] Here, digital images were obtained with and without a cross-polarization filter for comparisons, because spectrophotometers already have built-in cross-polarization filters to reduce glare that might interfere with readings. Although there is no defined threshold value for digital camera, our results indicate that a basic digital camera without a cross-polarization filter could perform as effectively as a dental spectrophotometer to determine the discoloration of the teeth adjunct with software, which are available in all operating systems as freeware or open-source tools. These findings are in line with previous studies,[27-29] demonstrating the reliability of digital cameras in the assessment of tooth discoloration.

In the present study, NeoMTA, Endosequence bioceramic putty and Biodentine showed visually undetectable color changes that were only measurable by spectrophotometry and digital image analysis. Such negligible discolorations can be attributed to the incorporation of the non-staining radiopacifiers tantalum oxide and zirconium oxide into the latter three formulations. Biodentine showed greater ΔE values than NeoMTA and EndoSequence Putty, respectively at all observation periods in the absence of statistical significance.

CONCLUSIONS

Within the experimental limitations of an *in vitro* study, the following conclusions were drawn:

1. Discoloration elicited by calcium silicate-based biomaterials may become visually perceptible as early as 3 months after placement, and may continue to increase for up to two years.
2. The tested calcium-silicate based products exhibited different staining properties, with ProProot MTA and MTA Angelus revealing severe discoloration that could compromise esthetics in young individuals. NeoMTA, EndoSequence bioceramic putty, Biodentine did not exhibit clinically perceptible color changes, and thus might be considered for use in the esthetic zone.
3. The use of digital photographs can be considered a practical and cost-effective solution to document coronal discolorations in pediatric endodontic procedures.

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