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ORIGINAL RESEARCH

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# Spectrophotometric evaluation of tooth discoloration caused by ProRoot® MTA, Biodentine™, and NeoPutty® in regenerative endodontics: an *in vitro* study

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## Abstract

**Background**: Regenerative endodontic procedures (REPs) offer biologically based treatments for immature teeth but may result in tooth discoloration, compromising esthetic outcomes. This study aimed to evaluate the color stability of three commonly used materials in REPs—ProRoot MTA (PMTA), Biodentine, and NeoPutty—with or without blood contamination. **Methods**: A total of 96 extracted human maxillary anterior teeth were standardized and randomly assigned into eight experimental groups (n = 12), based on the material used and presence or absence of blood contamination. Two additional control groups (saline only and blood only) were included. REP was simulated following the American Association of Endodontists protocol, including canal preparation, calcium hydroxide disinfection, and placement of the test material at the cemento-enamel junction. Color measurements ($\Delta$E) were obtained using a VITA Easy Shade® spectrophotometer at baseline and after 1, 3, 6 and 12 months. **Results**: Statistical analysis revealed significant effects of both time and material type on color change ($p < 0.05$). After 12 months, the highest discoloration was observed in the PMTA/Blood group ($\Delta E = 7.42 \pm 0.56$), while the lowest discoloration occurred in the Biodentine/Saline ($\Delta E = 2.18 \pm 0.43$) and NeoPutty/Saline ($\Delta E = 2.26 \pm 0.51$) groups. Blood contamination significantly increased discoloration across all materials, with PMTA demonstrating the least color stability. **Conclusions**: Blood contamination adversely affects the color stability of materials used in regenerative endodontic procedures. Biodentine and NeoPutty exhibited superior esthetic outcomes compared to PMTA and may be more suitable for use in anterior teeth where esthetics is a priority.

## Keywords

Regenerative endodontic procedures; Color stability; Discoloration; Spectrophotometric analysis; NeoPutty

## 1. Introduction

Regenerative endodontic procedures (REPs) represent a groundbreaking advancement in endodontics, marking a paradigm shift from traditional approaches. REPs are most commonly performed in children and adolescents with immature permanent teeth. In such cases, especially in anterior teeth, esthetics are a critical concern due to the young patients' high cosmetic expectations and long-term outcomes [1]. These procedures aim to preserve tooth vitality and function by stimulating tissue regeneration [1, 2]. To achieve this, the selection of suitable biomaterials plays a critical role in creating a biologically favorable environment for tissue ingrowth and healing. Among these materials, calcium silicate-based cements (CSCs) have gained prominence due to their bioactivity, biocompatibility, and ability to support regenerative outcomes [2, 3]. Mineral trioxide aggregate (MTA), developed in the early 1990s, is a CSC widely used in endodontics due to its biocompatibility, sealing ability, antibacterial properties, and capacity to set in moist environments [3]. Initially MTA was introduced as gray MTA (GMTA), and later, a white version (WMTA) was introduced in 2002 to address aesthetic concerns by reducing magnesium, iron, and aluminum content, and its commercial form was marketed as tooth coloured ProRoot® MTA (Dentsply, Ballaigues, Switzerland). However, it has drawbacks including extended setting time, handling difficulties, and most notably, tooth discoloration [4–6].

To reduce discoloration, newer calcium silicate based-materials have been introduced with alternative radiopacifiers such as zirconium oxide, tantalum oxide, or calcium tungstate [7–9]. However, they can also affect other properties of the


  

material including the setting time, solubility, or biological properties of the material [3, 10, 11].

Several studies investigated the effects of different radiopacifiers on tooth discoloration. Kohli *et al*. [5] reported that materials containing bismuth oxide caused greater color change compared to those with zirconium oxide. Shokouhinejad *et al*. [10] also found that bismuth-free cements showed better esthetic outcomes in regenerative procedures. These findings suggest that the choice of radiopacifier is critical when aesthetics are important.

Biodentine™ (Septodont, Saint-Maur-des-Fossés, France) is a bioactive substance composed of calcium silicate, intended to serve as a substitute for dentin. Its advanced hydration properties improve mechanical performance, accelerate setting time, and enhance resistance to discoloration [12]. The material is available as a powder and liquid, with the liquid contained in an ampoule and the powder packaged in a capsule for mechanical mixing. The inclusion of zirconium oxide reduces the discoloration potential [13, 14]. Compared to PMTA, Biodentine has been reported to exhibit greater resistance to compressive and flexural forces, lower solubility, enhanced structural integrity, and superior capping performance [15]. However, its powder-liquid formulation presents certain limitations, including the need for manual liquid addition and the excessive material volume per capsule, which may not be ideal for single-tooth applications. In addition to these drawbacks, its radiopacity is not optimal compared to WMTA, potentially affecting its radiographic visibility and clinical assessment [12, 16].

To overcome the challenge of variability in material uniformity observed in traditional CSCs that require water mixing, premixed CSCs have been introduced [17, 18]. One development in this category is NeoPutty™ (Nusmile Inc., Houston, TX, USA), a newly introduced premixed bioactive paste comprising nano-sized inorganic tricalcium/dicalcium silicate particulate suspended in an organic medium [19]. This ready-to-use formulation is designed to set upon exposure to moisture from the oral environment [20]. NeoPutty incorporates tantalum oxide as the radiopaque component and is designed with an anhydrous water-compatible carrier to enhance its manipulation and application characteristics [21, 22].

The absence of metals such as iron and the use of zirconium or tantalum oxide as radiopacifiers in place of bismuth oxide are responsible for the enhanced color stability seen in new-generation CSCs [23]. Nonetheless, when these components are used in clinical applications such as vital pulp therapy or REPs, they inevitably interact with blood, increasing the potential for tooth discoloration [24, 25]. Up to date, the information available for the discoloration potential of newly introduced materials such as NeoPutty, on different conditions is scarce.

This study aimed to compare the color stability of three calcium silicate-based materials—NeoPutty, Biodentine, and PMTA—when used in REPs, with and without blood contamination. Color changes were evaluated over a 12-month period using spectrophotometric analysis.

## 2. Materials and methods

The present study was conducted using an *in vitro* experimental design.

### 2.1 Sample size calculation

Ethics committee approval was given to conduct this study by the Health Sciences University.

Hamidiye Scientific Resarch Ethics Committee (Approval No: 7/37). The sample size was determined using G*Power (Version 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, NRW, Germany) and calculated as 10 samples per group to ensure sufficient statistical power ($\alpha$ = 0.05, power = 0.80, effect size = 1.374) for detecting a significant effect, in accordance with the methodology described by Chen *et al*. [25]. However, the sample size was increased to 96 samples, with 12 samples per group, considering potential sample losses. The study design and color measurement protocol were developed with reference to the methodology reported by Chen *et al*. [25].

A total of 96 freshly extracted, intact human permanent maxillary anterior teeth were used in this *in vitro* study. The teeth were collected from adult individuals (ages 18–35) undergoing extractions for orthodontic or periodontal reasons. It was confirmed by a dental operating microscope (Leica Microsystems, Wetzlar, HE, Germany) that all teeth had no caries, cracks, fractures, and that there were no restorations on the teeth.

### 2.2 Inclusion and exclusion criteria

The study included permanent maxillary anterior teeth that were free of carious cavities and restorations. Teeth exhibiting any level of discoloration (intrinsic or extrinsic), structural defects such as cracks or fractures, or any other anomalies that undermine their integrity were excluded.

All specimens were kept in distilled water at a temperature of 4 °C until they were included in the study. An ultrasonic scaler (Cavitron, Dentsply, York, PA, USA) was used to remove soft tissue remains on the study samples as well as calculus, and surface stains.

To establish color uniformity among the samples at baseline, the Whiteness/Yellowness Index was measured (refer to Eqn. 1). The mean difference across all groups was determined to be ±0.2, ensuring minimal variation. Following this assessment, the samples were categorized and evenly distributed into groups based on their initial color similarity (n = 12 per group).

$$WI_D = \left[(L^* - 100)^2 (a^*)^2 + (b^*)^2\right]^{1/2} \quad (1)$$

### 2.3 Sample preparation

To achieve a standardized length of 10 ± 1 mm from the buccal cementoenamel junction, the root end was resected using a high-speed fissure bur. A high speed diamond round bur was used for access cavity preparation. In the middle third of the crown, a digital caliper was used to adjust the enamel-dentin thickness at the labial wall to 2 ± 0.3 mm.

Root canal preparation was performed by first establishing patency using a #10 stainless steel hand K-file (Dentsply Sirona, Tulsa, OK, USA), ensuring apical patency and facilitating subsequent instrumentation. This was followed by initial shaping with sequentially larger K-files to progressively enlarge the canal. The preparation was then completed using a #60 stainless steel hand K-file. Then, the canals were instrumented with Peeso reamers (Dentsply Sirona) from size 1 up to size 6 (1.7 mm).

REPs were performed based on the guidelines of the American Association of Endodontists (revised 2021) [26]. The root canals were then subjected passive ultrasonic irrigation with 5 mL of 1.5% sodium hypochlorite (050325055, Microvem, Istanbul, Turkey). The last irrigation was done with 3 mL 17% Ethylenediaminetetraacetic acid (EDTA) solution (Saver, Prime Dental Pvt Ltd, 070225078, Maharashtra, India), and then 5 mL of distilled water was used. After that, sterile paper cones were used to dry the canals. Following the sealing of the root ends with resin composite material (Grandio, Voco, Germany), the adequacy of the seal was verified through visual inspection under magnification (M320 dental microscope, Leica Microsystems, Wetzlar, HE, Germany) range of 8× to ensure the absence of any extrusion of the irrigation solution from the apical part.

To simulate the disinfection phase of REPs, calcium hydroxide paste (18010014, Pulpdent Corporation, Watertown, MA, USA) was placed into the root canals using a lentulo spiral. The canals were then sealed with a cotton pellet and temporary restorative material (Cavit™; 3M ESPE). All samples were stored in an incubator at 37 °C and 100% humidity for 7 days. This duration was selected based on standard REP protocols and was applied uniformly across all specimens to avoid bias in dentin permeability and discoloration outcomes. After incubation, the calcium hydroxide was removed by irrigating with 15 mL of 17% EDTA followed by saline. The canals were then dried with sterile paper points prior to placement of the test materials.

All REPs were performed under magnification using a dental operating microscope (M320 dental microscope, Leica Microsystems, Wetzlar, HE, Germany) at a magnification range of 8× to 12× to ensure precision and procedural accuracy. In order to prevent irrigating solutions from contacting the external root surface and to avoid potential undesirable chemical interactions with the materials used in this study, all procedures were performed under rubber dam isolation.

## 2.4 Experimental procedure

An online randomization tool (www.randomizer.org/) was used for the distribution of the study samples among different groups. Blood was taken from one of the authors by a trained professional and stored in sterile tubes (Vacuette®; Greiner Bio-One, Kremsmünster, Austria) containing $K_3$EDTA anticoagulant to prevent coagulation.

A sterile absorbent sponge (cut to 3 mm thickness) was placed below the cemento-enamel junction and saturated with either blood or saline using an insulin syringe to avoid contamination in the access cavity. Each group was then separated into three subgroups based on the cement type utilized: PMTA group, Biodentine group, NeoPutty group.

After that, the following six experimental subgroups were allocated, with 12 samples per group: PMTA/Blood group, Biodentine/Blood group, NeoPutty/Blood group; PMTA/Saline group, Biodentine/Saline group, NeoPutty/Saline group.

Additionally, twenty-four teeth served as controls, equally divided into two groups: twelve samples with a blood clot placed up to the cemento-enamel junction served as the positive control, and twelve samples with saline only served as the negative control.

The test cements were mixed in accordance with the manufacturers' instructions prior to their placement in the root canals. Following material placement, the cements were applied up to the cementoenamel junction (CEJ) with a standardized thickness of 3 mm. Any excess material within and around the access cavity was carefully removed using a microbrush and a cotton pellet to ensure a clean coronal surface. The access cavities were temporarily sealed with a restorative material (Cavit™; 3M ESPE, St. Paul, MN, USA). The thickness and location of the test materials were confirmed radiographically using periapical imaging. Specimens were then incubated at 37 °C in 100% humidity for 72 hours. During incubation, each specimen was stored individually in a sealed container.

A conventional glass ionomer cement liner (GC Fuji I, GC Corporation, Tokyo, Japan) was placed prior to adhesive application. A 2-step self-etch adhesive system (Clearfil SE Bond®, Kuraray, Tokyo, Japan) was applied using a selective enamel etching technique. Enamel margins were etched with 35% phosphoric acid gel (Scotchbond Etchant Gel, REE90, 3M ESPE, St. Paul, MN, USA) for 15 seconds and rinsed thoroughly with water for 10 seconds. Both enamel and dentin were gently air-dried for 5 seconds to maintain proper moisture control. Primer and bonding agent were then applied as per the manufacturer's instructions, and light curing was performed for 20 seconds using an LED curing unit (Valo® Cordless LED curing light, Ultradent Products Inc., South Jordan, UT, USA). Definitive restoration of the access cavities was performed using an A1-shaded nanohybrid resin composite (Grandio, Voco, Cuxhaven, Germany), applied in ≤2 mm-thick increments, with each layer light cured for 20 seconds. The shade of the resin composite was matched to the coronal tooth structure measured with a spectrophotometer (Vita Easy Shade® digital spectrophotometer, VITA Zahnfabrik, Bad Säckingen, BW, Germany). After restoration, the specimens were stored under ambient laboratory conditions (approximately 23 °C, 50% relative humidity) for the 12-month observation period. Throughout the storage period, all specimens were kept in light-proof containers.

All experimental procedures, including sample preparation, placement of test materials, and restoration steps, were performed by specialist dentists (SÖ and ŞTK) with a minimum of 5 years of clinical and research experience.

## 2.5 Spectrophotometric analysis

Color measurements were conducted performed on 96 teeth under standardized lighting conditions using a VITA Easy Shade® spectrophotometer (VITA Zahnfabrik, Bad Säckin-

gen, Germany). All color measurements were conducted by an experienced investigator (ED) with at least 5 years of research experience in dental materials research.

To standardize the measurement process, an 8-mm custom-made silicone jig was fabricated for each sample. This jig covered the entire buccal wall and one-third of the incisal edge, ensuring repeated and consistent spectrophotometric readings. Before each measurement session, the spectrophotometer was calibrated according to the instructions provided by the manufacturer.

Color assessments were conducted at the following time points: Baseline before access preparation, 24 hours after material placement and thereafter 1, 3, 6, 12 months post-procedure. The Commission Internationale de l'Éclairage (CIE) Lab color scale was used for color analysis.

The following formula was used to record color parameter changes for each group (refer to Eqn. 2).

$$\Delta E = \left([Li - L0^*]^2 + [ai - a0^*]^2 + [bi - b0^*]^2\right)^{1/2} \quad (2)$$

Brightness changes on a scale that ranges from black (0) to white (100) are represented by ΔL. Color changes from red (−80) to green (+80) are represented by Δa. Color changes from blue (−80) to yellow (+80) are represented by Δb. Discoloration was deemed clinically significant when the ΔE value was ≥3.7 [27].

All teeth was photographed using a digital camera (Canon EOS R7; Canon Inc., Tokyo, Japan) equipped with a flash. This process facilitated the comparison of these images. Visual evaluation of photographs is intended to be used as an additional, non-statistical element for illustrative purposes only. All ΔE values were systematically recorded and documented in an Excel spreadsheet, ensuring a structured and reliable data collection process.

### 2.6 Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics for Windows (Version 29.0, IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was used to assess how materials and conditions affect color stability. The significance level was established at $p < 0.05$ for both intragroup and intergroup comparisons using Tukey's *post hoc* test.

## 3. Results

The Whiteness/Yellowness Index measurements showed no significant differences among the groups ($p > 0.05$), indicating initial color uniformity across all samples. Table 1 displays the ΔE values for all groups throughout each time interval.

### 3.1 ΔE for saline groups

Fig. 1 shows the mean ΔE values for the saline groups at the four different time intervals. Statistical analysis showed that both the type of material used and the observation time had a significant effect on color change (ΔE) ($p < 0.05$). However, there was no significant interaction between material type and time, which means that the color change over time was similar for all materials ($p > 0.05$).

Among the groups, PMTA/Saline group showed the greatest time-dependent changes, consistently exhibiting higher ΔE values at all time intervals in comparison to other groups. No significant differences were detected among the NeoPutty/Saline, Biodentine/Saline, and Control/Saline groups, indicating comparable color stability ($p > 0.05$) (Table 2). When analyzing the time-dependent changes, a significant difference was observed between the values at the 12-month follow-up and those at earlier time points ($p < 0.05$). However, no significant differences were found between the 1, 3 and 6-month time points ($p > 0.05$) (Table 2). Overall, ΔE values increased significantly over time with the most substantial change occurring at the 12-month interval (Fig. 1).

### 3.2 ΔE for blood groups

Fig. 2 shows the average ΔE values for the blood groups at the four different time intervals, providing a comparative analysis of color changes over time in the presence of blood contamination. Groups, time and their interaction are all statistically significant ($p < 0.05$). Across all time periods, the results showed that Control/Blood had significantly higher ΔE values than the other groups (Table 2). PMTA/Blood caused the least color stability, whereas NeoPutty/Blood and Biodentine/Blood demonstrated the most color stability and performed similarly (Fig. 2). There is a significant difference in ΔE values between the 1-month and 12-month intervals (Table 2).

### 3.3 Effect of blood on color change by different materials

Blood contamination significantly increased ΔE* values across all materials, after adjusting for both incubation time and material type ($p < 0.05$). A significant correlation was found between time and ΔE* values ($p < 0.05$), indicating progressive discoloration over time, irrespective of material type and blood presence (Table 2).

In both blood-contaminated and non-contaminated conditions, the Biodentine and NeoPutty groups exhibited significantly more color stability in comparison to the PMTA group ($p < 0.05$) (Table 2).

At the 12-month assessment, among all experimental groups, the greatest color change was observed in blood-contaminated PMTA, whereas the least discoloration occurred in the Biodentine and NeoPutty groups (Fig. 3).

## 4. Discussion

Tooth discoloration following REPs remains a key esthetic concern, especially when treating anterior teeth in young patients. As REPs gain popularity for preserving the vitality of teeth, clinicians must navigate the balance between functional success and aesthetic outcomes [12, 24]. Tooth discolouration following REPs has been recorded in clinical trials and *in vitro* studies [7, 8, 28]. The primary objective of the present study was to assess the color stability of NeoPutty, a premixed CSC, following REPs in anterior teeth. The results demonstrated

**TABLE 1. Compositions of all the materials.**

| Product | Manufacturer and lot number | Composition | Setting mechanism |
|---|---|---|---|
| ProRoot® MTA | DENTSPLY, Tulsa, OK, USA<br>Powder: 12002493<br>Liquid: 120407 | Bismuth oxide, tricalcium silicate, dicalcium silicate, calcium dialuminate, and calcium sulfate dehydrated | Mixed powder/liquid ratio: 1/3 |
| NeoPutty® | Nusmile Inc., Houston, TX, USA<br>2022080402 | Tantalite, Tricalcium silicate Dicalcium silicate, Calcium aluminate, Grossite, Tricalcium aluminate, Calcium sulfate, Di- and tricalcium silicate/calcium aluminate/tantalum oxide/tricalcium aluminate/calcium sulfate/proprietary organic liquid and stabilizers | Pre-mixed material and ready to apply single paste chemically cured by hydration tricalcium silicate<br>Premixed putty syringe |
| Biodentine™ | Septodont, Saint-Maurdes-Fosses Cedex, France<br>B27049 | Powder: tricalcium silicate, dicalcium silicate, calcium carbonate, oxide filler, iron oxide shade, and zirconium oxide<br>Liquid: water, calcium chloride, and hydrosoluble polymer | Chemical cured by hydration tri-calcium silicate<br>Powder capsule + vial of special liquid<br>Mixing premeasured unit dose capsules in a high-speed amalgamator for 30 s |

*ProRoot® MTA: ProRoot® Mineral Trioxide Aggregate.*

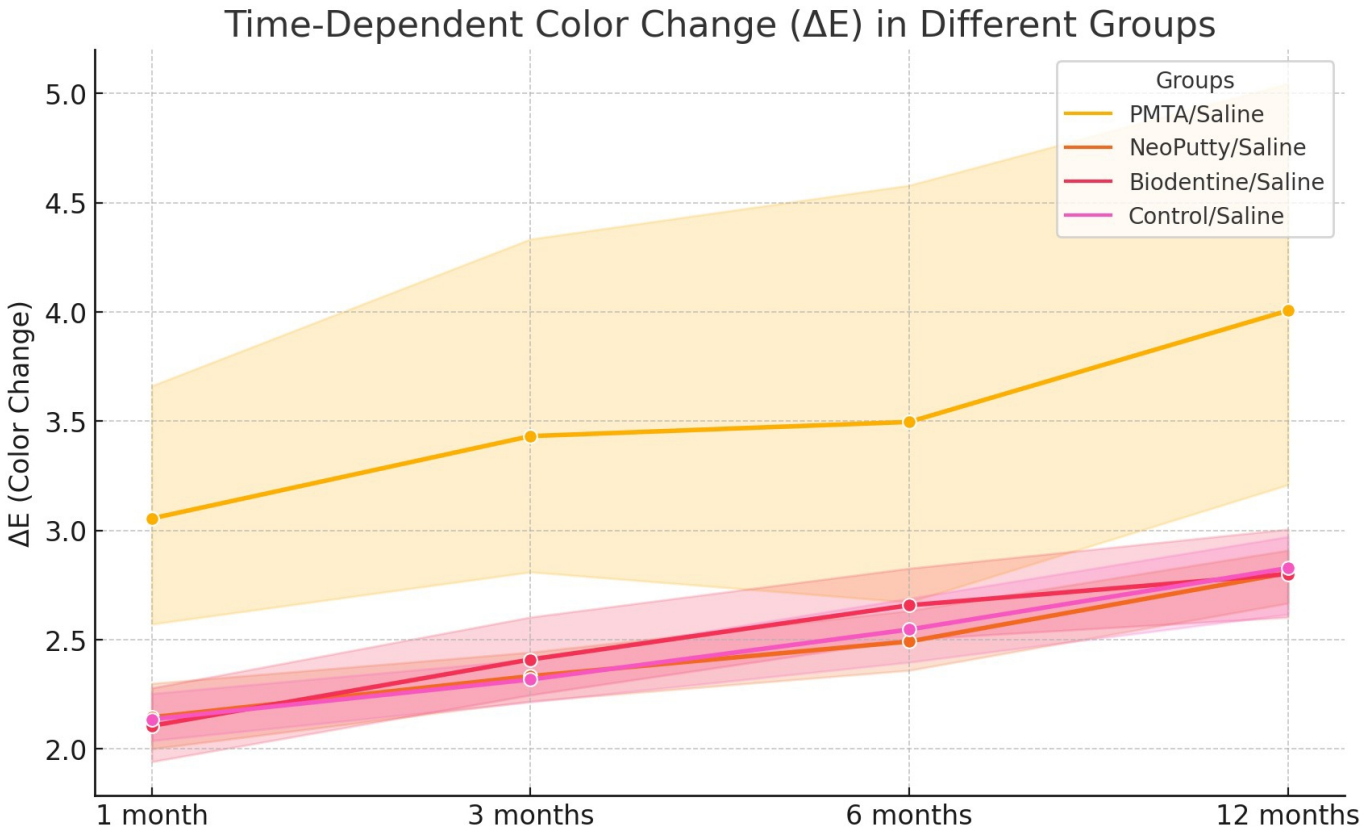


**FIGURE 1. Changes in ΔE over time by group saline.** PMTA: ProRoot MTA.

**TABLE 2. Mean (±SD) ΔE values for saline and blood groups over time.**

| | PMTA | | NeoPutty | | Biodentine | | Control | |
|---|---|---|---|---|---|---|---|---|
| | Saline | Blood | Saline | Blood | Saline | Blood | Saline | Blood |
| 1 M | 3.05 ± 1.08 | 4.25 ± 2.11 | 2.14 ± 0.28 | 2.43 ± 0.29 | 2.10 ± 0.30 | 2.50 ± 0.37 | 2.13 ± 0.14 | 9.45 ± 2.41** |
| 3 M | 3.43 ± 1.49 | 5.23 ± 3.40 | 2.33 ± 0.22 | 2.73 ± 0.33 | 2.41 ± 0.32 | 2.71 ± 0.41 | 2.32 ± 0.13 | 11.09 ± 2.13** |
| 6 M | 3.50 ± 1.69 | 6.17 ± 4.23 | 2.49 ± 0.27 | 3.01 ± 0.33 | 2.66 ± 0.31 | 2.86 ± 0.40 | 2.55 ± 0.19 | 15.59 ± 3.61** |
| 12 M | 4.01 ± 1.79* | 7.05 ± 5.08*,** | 2.80 ± 0.24 | 3.19 ± 0.34 | 2.80 ± 0.36 | 3.06 ± 0.35 | 2.83 ± 0.24 | 18.08 ± 3.89** |
| | *$p$ = 0.048 | | | | | | | |
| | *$p$ = 0.036; **$p$ = 0.022 | | **$p$ = 0.598 | | **$p$ = 0.804 | | **$p < 0.001$ | |

**Statistically significant difference in 12-month values ($p < 0.05$). **Statistically significant difference between Blood and Saline groups and time ($p < 0.05$). PMTA: ProRoot MTA; M: Month; SD: Standard Deviation.*

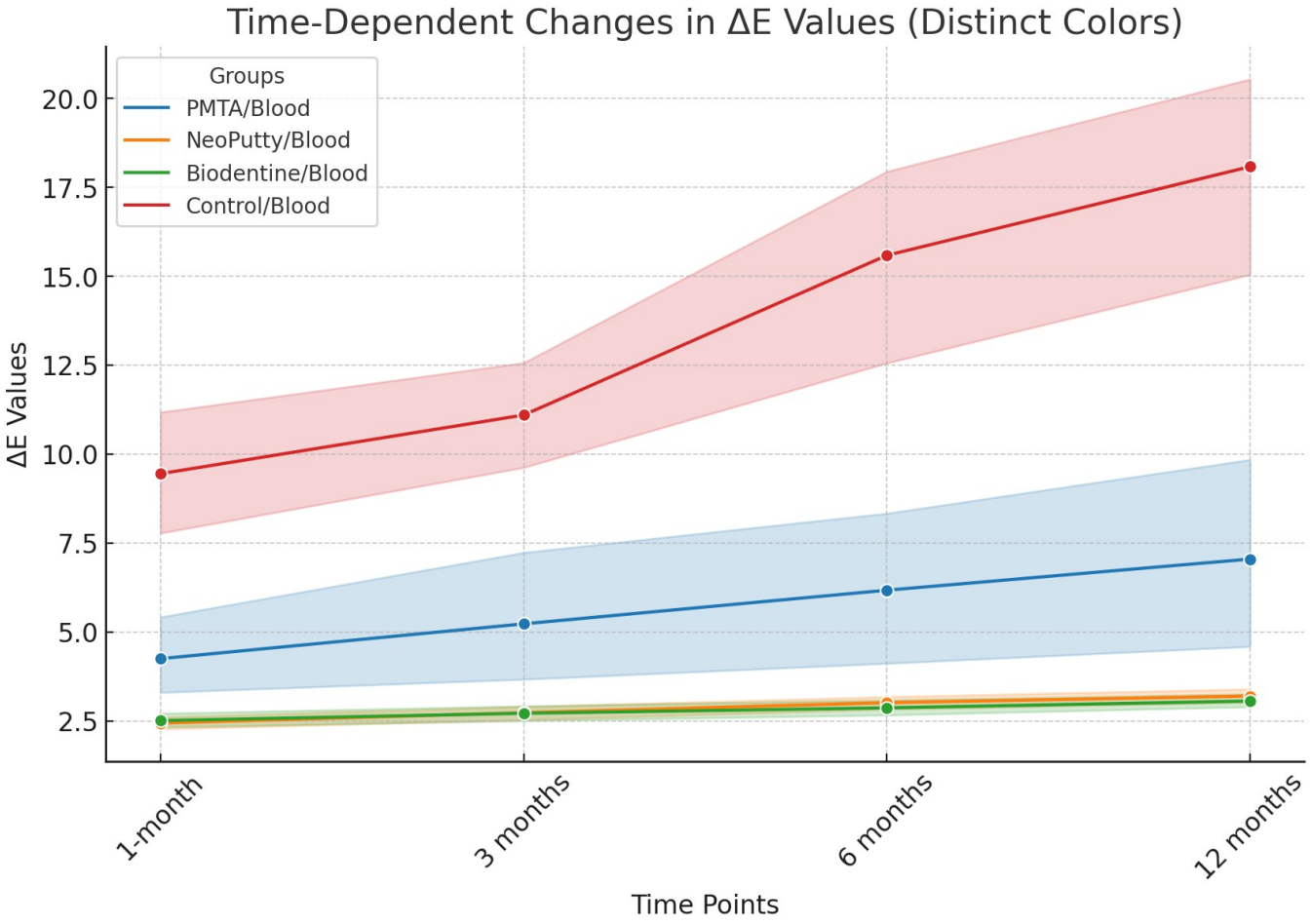


**FIGURE 2. Changes in ΔE over time by group blood.** PMTA: ProRoot MTA.

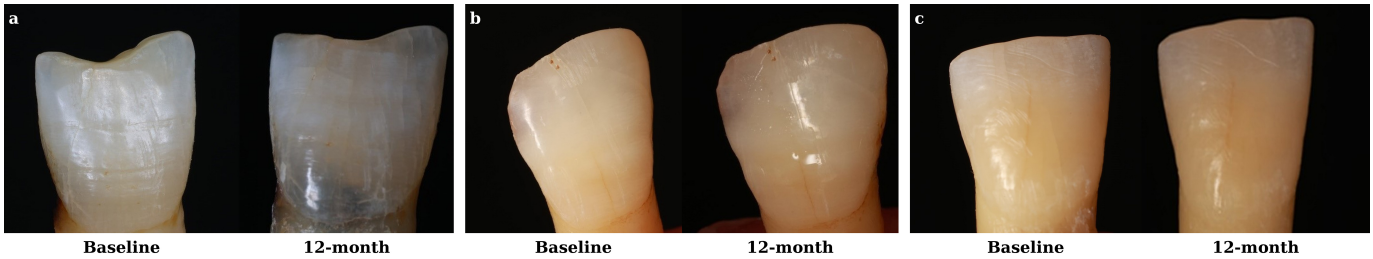


**FIGURE 3. Representative photographs taken at baseline and after 12 months, illustrating the discoloration patterns associated with each material: (a) PMTA, (b) Biodentin, and (c) NeoPutty.**

notable differences in color stability across the three materials evaluated, resulting in the rejection of the null hypothesis.

Research on the color stability of CSCs typically focuses on either intrinsic material discoloration or its interaction with tooth structure. However, the influence of these materials on tooth color changes in blood contamination has not been thoroughly investigated. NeoPutty is a premixed bioactive repair material based on tricalcium silicate (NuSmile, Houston, TX, USA) which, according to the manufacturer, has been specifically designed to minimize discoloration.

The findings of this study indicate that PMTA exhibited significantly lower color stability compared to other tested CSCs, even in the absence of blood contamination. Previous studies have confirmed the discoloration associated with PMTA [14, 16, 17]. Bismuth oxide utilized in PMTA as an opacifier has demonstrated significant chromogenic changes upon irradiation with light in an oxygen-free environment. A condition established within the tooth crown upon the placement and sealing of the material [14].

Literature shows that the color changing potential of new CSCs that do not contain bismuth oxide is significantly lower compared to other bismuth-containing CSCs. Al-Hiyasat *et al*. [17] and Chen *et al*. [25], who investigated Biodentine discoloration in the absence of blood following REPs, did not find discoloration exceeding the detectability threshold at all time periods, consistent to the present study. This finding is consistent with other studies [5, 14, 23].

Blood plays an important role in the discoloration potential of materials to the coronal dentin. Although the exact mechanism underlying blood-induced discoloration remains incompletely understood, it is primarily attributed to the hemolysis of erythrocytes, hematin molecules deposited in the dentinal tubules and their subsequent penetration into the dentin structure [25]. Additionally, discoloration intensity is most pronounced in the dentin closest to the pulp chamber, with a gradual decrease towards the outer dentinal layers [12, 25].

Previous REPs studies showed that blood contamination increases the discoloration potential of MTA and CSCs [14, 25]. Shokouhinejad *et al*. [10] and Chen *et al*. [25] found the PMTA/Blood group resulted in more significant discoloration compared to Biodentine/Blood group. The possible mechanism may be due to the slow hydration process of PMTA, allowing erythrocytes to penetrate the tooth structure. Conversely, blood may lead to cavities and pores that entrap blood components, resulting in discoloration of the material [6, 16]. A slow setting time may lead to prolonged porosity of the material, which can increase blood absorption and consequently result in an increased discoloration [29]. Similarly, other studies have shown that Biodentine demonstrates higher color stability than MTA in the presence of blood [8, 14]. However, at different time intervals, Biodentine has been reported to undergo a color change perceptible to the human eye in the presence of blood [14].

One study compared the discoloration potential of premixed CSC (TotalFill), PMTA and Biodentine, and found that Biodentine/Saline showed the most color stability among the groups, while Biodentine/blood demonstrated more color stability than TotalFill/Blood [17]. On the contrary, Carvalho *et al*. [18] demonstrated that coronal discoloration was increased over time in all experimental groups including PMTA, Biodentine, TotalFill and this was consistent among groups. The present study demonstrated that NeoPutty provides similar color stability to Biodentine over time.

Both NeoPutty and Biodentine possess compositional features that may contribute to their superior color stability compared to conventional MTA. NeoPutty includes a proprietary organic matrix that acts as an anhydrous carrier, replacing water in the initial hydration process. This may influence the setting kinetics and reduce early-stage porosity, potentially limiting fluid or blood absorption, which is often linked to discoloration [22, 25]. While the exact mechanism remains unclear, this carrier system may help improve esthetic outcomes. On the other hand, Biodentine contains approximately 5–15% zirconium oxide by weight, which not only ensures sufficient radiopacity but also provides chemical stability with reduced chromogenic potential. Unlike bismuth oxide, zirconium oxide is less likely to undergo color-altering interactions in the presence of light or moisture [29]. These compositional differences may explain why both materials exhibited significantly less discoloration than PMTA in the present study.

Variations in translucency within the residual tooth structure may conceal or amplify the discoloration effects of different materials. A previous study showed that teeth with buccal wall thicknesses of 2.8–4.5 mm and 1–2.7 mm differed statistically significantly in color change [2]. The labial wall thickness was consistently maintained at $2 \pm 0.3$ mm to guarantee uniformity and consistency in the current investigation.

Although the present *ex vivo* study does not fully replicate clinical conditions such as mechanical forces, thermal fluctuations, saliva, and oral hygiene practices, it utilized an effective model for assessing tooth discoloration. The color change observed in this study, similar to previous research, was quantified using a spectrophotometer, which may not directly correlate with clinically significant discoloration. Therefore, applying these results to clinical practice requires further research to compare spectrophotometer measurements with visually perceptible color changes that are clinically meaningful. Another limitation of this study is that blood used for contamination was obtained from a single donor. While this approach ensured standardization, it does not account for inter-individual variability in blood composition, which could potentially influence discoloration outcomes. Future studies should aim to examine other physical and biological properties of the tested materials, and validate the color change findings under clinical conditions by incorporating long-term follow-up data. The use of thermocycling to simulate intraoral temperature fluctuations and mechanical stresses may better reflect the aging behavior of the materials. In addition, including patient-based esthetic evaluations alongside spectrophotometric analysis could offer a more comprehensive assessment of discoloration that aligns with clinical perception and patient satisfaction.

From a clinical standpoint, the findings of this study underscore the importance of material selection in regenerative endodontic procedures performed in the anterior esthetic zone. Discoloration of anterior teeth can have significant psychological and cosmetic implications for patients, particularly among adolescents and young adults who are common candidates for

REPs. The superior color stability observed with NeoPutty and Biodentine suggests that these materials may be more suitable than PMTA when aesthetics are a primary concern. Their reduced discoloration potential, even in the presence of blood contamination, supports their use in anterior teeth to maintain long-term esthetic outcomes following regenerative treatment.

## 5. Conclusions

Tooth discoloration remains an important concern in regenerative endodontic procedures, particularly in the esthetic zone. Within the limitations of this *in vitro* study, NeoPutty and Biodentine demonstrated superior color stability compared to PMTA, both in the presence and absence of blood contamination. These findings suggest that NeoPutty and Biodentine may be more suitable alternatives to PMTA when esthetic outcomes are a priority. Future clinical studies are warranted to confirm these results under *in vivo* conditions.

### AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding or first author on reasonable request.

### AUTHOR CONTRIBUTIONS

SÖ and HA—designed the research study. SÖ, ED and ŞTK—performed the research. HMAA—provided help and advice on methodology and interpretation of the findings. SÖ and ED—analyzed the data. SÖ and ŞTK—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study received approval from the Health Sciences University Hamidiye Scientific Resarch Ethics Committee (Approval Number: 7/37). The need for informed consent was waived by the Health Sciences University Hamidiye Scientific Resarch Ethics Committee.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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