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Color stability and solubility of Biodentine and NeoPutty in contact with different irrigation solutions

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Conceptualization: Usta SN, Keskin C; Data curation: Usta SN; Formal analysis Usta SN; Investigation: Usta SN; Methodology: Usta SN, Keskin C; Software: Usta SN; Supervision: Keskin C; Validation: Usta SN, Keskin C; Visualization: Usta SN, Keskin C; Writing -

ABSTRACT

Objectives: This study aimed to evaluate the color stability and solubility of Biodentine and NeoPutty in contact with different irrigation solutions.
Materials and Methods: Biodentine and NeoPutty were set in cylindrical molds with 7 mm diameter and 1.5 mm high and immersed in distilled water, 17% ethylenediaminetetraacetic acid (EDTA), 2% chlorhexidine (CHX), 9% 1-hydroxyethylidene 1,1-diphosphonate (HEDP), and 5% sodium hypochlorite (NaOCl) solutions for 24 hours. The color change was measured with a spectrophotometer. The solubility values were calculated as the mass loss was expressed as a percentage of the original mass using an analytical balance with 10^{-4} g accuracy. Data were analyzed with Kruskal-Wallis followed by Mann-Whitney *U* tests, and 2-way analysis of variance test followed by Bonferroni corrections for pairwise comparisons for solubility and color stability with a 5% significance threshold, respectively.
Results: Biodentine exhibited higher color changes compared to the NeoPutty contact with all solutions except distilled water ($p < 0.05$). Both hydraulic cements (HCs) showed higher discoloration values immersion in CHX followed by NaOCl. No statistically significant difference was found between Biodentine and NeoPutty regardless of irrigation solution in terms of solubility ($p > 0.05$). Solubility values were lower in the distilled water group compared to EDTA and CHX ($p < 0.05$).
Conclusions: Tested HCs showed solubility and color changes at various rates. NeoPutty could be an appropriate material in aesthetic areas. The usage of HEDP as an irrigant solution can be considered suitable for various endodontic treatments due to its relatively lower solubility and discoloration values.

Keywords: Chlorhexidine; Endodontics; Sodium hypochlorite; Solubility; Tooth discoloration

INTRODUCTION

The success of endodontic treatments relies on preventing and/or treating apical periodontitis while preserving the functional and aesthetic aspects of the tooth. Since the introduction of mineral trioxide aggregate (MTA), tooth discoloration following its use has become a crucial issue that adversely affects patients' satisfaction when used in the aesthetic region [1]. Currently, it is evident that irrigation solutions, intracanal medicaments, sealers,

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or hydraulic cement (HC) induce discoloration to varying degrees. Accurately discerning the material properties and interactions will enhance the success of the treatment and align with the patient's expectations [2].

HCs are materials containing tricalcium silicate, dicalcium silicate, and tricalcium aluminate phases and are set by reaction with water [3]. MTA, comprised of Portland cement, was the pioneering hydrophilic cement introduced into the dental market [4]. Although MTA has superior properties such as biocompatibility, bioactivity, and sealing ability, it can cause tooth discoloration due to bismuth oxide used for radiopacity. Therefore, several HCs have been introduced to overcome this limitation, along with their compelling handling and long-setting time characteristics [5,6]. Biodentine (Septodont, Saint-Maur-des-Fossés, France), one of those materials, contains tricalcium silicate instead of the Portland cement in MTA, calcium carbonate, a water-soluble polymer, calcium chloride, and zirconium oxide [4,7]. It has been reported that Biodentine can be used successfully in all endodontic procedures in place of MTA and causes minimal tooth discoloration [3,8]. Other HC, NeoPutty (NuSmile, Houston, TX, USA) presents as a premixed format and is composed of tantalum oxide, tricalcium silicate, calcium aluminate, dicalcium silicate, tricalcium aluminate, and calcium sulfate [9]. Comparable success to MTA and adequate cytocompatibility of this material have brought forward its utility, especially in vital pulp treatments and perforation repairs [9-11]. Additionally, since NeoPutty contains non-staining radiopaque agent tantalum oxide, It does not cause tooth discoloration [12].

HCs contact with irrigation solutions during various endodontic procedures, such as perforation repair, vital pulp, or regenerative treatments. Sodium hypochlorite (NaOCl) is a commonly used irrigation solution in endodontic treatments due to its antibacterial efficiency and organic tissue dissolution capacity [13]. It has been shown that NaOCl leads to discoloration in contact with HCs [14]. Moreover, chlorhexidine gluconate (CHX) is a cationic bisbiguanide that is mostly utilized as a final irrigation solution due to its lower antibacterial activity compared to NaOCl, and its absence of tissue dissolving capability [15]. Previous studies in the literature demonstrated the discoloration of the HCs when they are exposed to CHX [14,16]. Ethylenediaminetetraacetic acid (EDTA) is a chelating agent that reacts with the calcium ions in dentine and forms soluble calcium chelates. Despite evidence indicating that EDTA induces color alterations in HCs, research on this topic remains relatively scarce [14]. Another solution, 1-hydroxyethylidene 1,1-diphosphonate (HEDP), also known as etidronic acid or etidronate, has been proposed as a weak chelator [17]. Although Zollinger *et al.* [18] indicated that HEDP did not have any direct impact on blood color or NaOCl-derived bleaching, there is still a paucity of data concerning its potential for discoloration in HCs.

Solubility is a critical characteristic of HCs, especially in perforation repairs, owing to their superior long-term sealing capability [19]. The material's low solubility is linked to its enhanced stability upon encountering fluids within the root canal system or periapical tissues, reducing fluid leakage [20]. HCs could get in touch with different types of liquids during endodontic treatment procedures, and therefore, it is essential to determine the solubility of those materials.

Since several HCs have been introduced to markets with different physical and chemical properties, the interactions between those materials and irrigation solutions need to be investigated. In this sense, regarding NeoPutty as a recently produced HC, color stability and solubility have not been evaluated. There is also limited information on the effect of

HEDP and EDTA, which is recommended for use within the scope of continuous chelation, on the discoloration of HCs [21]. Thus, this study aimed to investigate the color stability and solubility of Biodentine and NeoPutty after contact with different irrigation solutions. The null hypothesis is that there would be no significant difference regarding the color stability and solubility values of these 2 HCs.

MATERIALS AND METHODS

The sample size was determined based on a similar study published previously at the significance level of 0.05, effect size of 0.54536, and power of 0.90 using G* Power v3.1 (Heinrich Heine, Universität Düsseldorf, Germany), and consequently, 8 samples were assigned to each group [22]. Eighty samples of Biodentine and NeoPutty were prepared according to the manufacturers' recommendations and transferred into cylindrical molds with 7 mm diameter and 1.5 mm height. Then, the samples were stored in an incubator at 37°C and 100% humidity for 24 hours during the setting. After setting, prepared molds were weighed 3 times using an analytical balance with an accuracy of 10^{-4} g (Mettler Toledo AT201, Mettler-Toledo GmbH, Greifensee, Switzerland). The mean values of the baseline readings were recorded. Afterwards, molds were immersed in the below-mentioned solutions for 24 hours as 8 samples for each ($n = 8$):

1. Distilled water
2. 17% EDTA (Saver, Prime Dental Products, Maharashtra, India)
3. 2% CHX (Microvem, Istanbul, Turkey)
4. 9% HEDP (Dual Rinse HEDP, Medcem, Weinfelden, Switzerland)
5. 5% NaOCl (Microvem, Istanbul, Turkey)

Spectrophotometric analysis

The color stability of the HCs was evaluated using a spectrophotometer (VITA Easyshade Compact, VITA Zahnfabrik, Bad Säckingen, Germany) under the same conditions. In order to standardize the environment, measurements were performed in a particular cabinet that blocks the exterior light under simulated natural light illumination (D65). The spectrophotometer was calibrated before each measurement. The same blind operator in the groups performed the color assessment 3 times for each sample. The color differences of the samples before and after immersion in endodontic solutions were calculated by The Commission Internationale de l'Eclairage (CIE) system according to the following formula:

$$\Delta E = \{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\}^{1/2}$$

The value of L* is the lightness from black to white, and the values of a* and b* are the red-green axis and the yellow-blue axis of the chromaticity parameter, respectively [23]. ΔE* describes the color difference between the measurements. If ΔE* exceeds 3.3 units, which means the discoloration could be detectable clinically [24]. Images of the samples were also taken before and after contact with the solutions using a digital camera (EOS 250D, Canon, Tokyo, Japan).

Assessment of solubility

All experimental set-ups were carried out following ISO 6876/2012, including preparation of apparatus and materials and calculation solubility. Following the immersion of the molds into irrigation solutions in the glass plates, they were kept in cabinets at 37°C ± 1°C. Then,

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samples were removed from plates, washed with 1 mL of distilled water to remove excess moisture, and put into desiccators containing thoroughly dry anhydrous calcium sulfate for 24 hours. Prepared molds were re-weighed 3 times using an analytical balance. The mass loss was expressed as a percentage of the original mass.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences software (SPSS 26, SPSS, Chicago, IL, USA). The Shapiro-Wilk test showed a non-normal and normal distribution for solubility and color change values, respectively. Color changes were analyzed using a 2-way analysis of variance test and Bonferroni corrections for pairwise comparisons. The Kruskal-Wallis and Mann-Whitney *U* tests were used to compare the solubility of HCs. The level of significance was set at $p < 0.05$.

RESULTS

The contact with irrigation solutions influenced the color stability of Biodentine and NeoPutty at variable rates. Digital images of the HCs before and after immersion in different irrigation solutions were demonstrated in **Figure 1**. Biodentine exhibited significantly higher color changes compared to the NeoPutty contact with all solutions apart from distilled water. For both HCs, there was a statistically significant difference between solutions for causing color change ($p < 0.05$). The distilled water and CHX caused significantly lower and higher ΔE values compared to

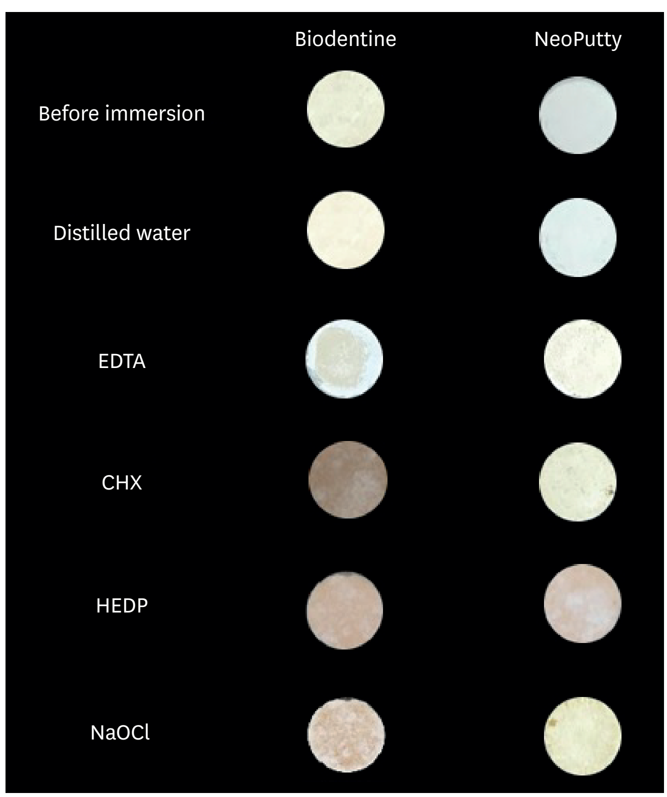

Figure 1. Images of hydraulic cements before and after immersion in different solutions.
EDTA, ethylenediaminetetraacetic acid; CHX, chlorhexidine; HEDP, 1-hydroxyethylidene 1,1-diphosphonate; NaOCl, sodium hypochlorite.

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Table 1. The median ΔE values of the hydraulic cements immersion in different irrigation solutions

Variables	Biodentine				NeoPutty			
	ΔE	*L*	*a*	*b*	ΔE	*L*	*a*	*b*
Distilled water	$1.32^{A,a}$	1.1	0.2	0.7	$1.06^{A,a}$	0.9	0.2	0.5
EDTA	$3.54^{A,b}$	2.62	0.6	2.3	$2.24^{B,b}$	1.8	0.4	1.2
CHX	$9.64^{A,c}$	8.83	2.3	3.1	$3.68^{B,c}$	2.91	0.5	2.2
HEDP	$6.86^{A,d}$	5.58	1.7	3.6	$3.11^{B,d}$	2.94	0.4	0.9
NaOCl	$6.95^{A,de}$	5.49	2.1	3.7	$3.02^{B,de}$	2.69	0.4	1.3

EDTA, ethylenediaminetetraacetic acid; CHX, chlorhexidine; HEDP, 1-hydroxyethylidene 1,1-diphosphonate; NaOCl, sodium hypochlorite.
Different superscript uppercase letters in the same row indicate a statistically significant difference ($p < 0.05$).
Different superscript lowercase letters in the same column indicate a statistically significant difference ($p < 0.05$).

Table 2. The mean percentage changes and standard deviations of solubility values along with median values of the weight loss (g) of the hydraulic cements immersion in different irrigation solutions

Variables	Biodentine (Median)	NeoPutty (Median)
Distilled water	$0.87 \pm 0.03^{A,a}$ (0.0012)	$0.14 \pm 0.01^{A,a}$ (0.0012)
EDTA	$3.54 \pm 0.11^{A,b}$ (0.0032)	$2.58 \pm 0.14^{A,b}$ (0.0029)
CHX	$3.27 \pm 0.08^{A,b}$ (0.0028)	$2.31 \pm 0.07^{A,b}$ (0.0027)
HEDP	$2.46 \pm 0.04^{A,c}$ (0.0017)	$1.62 \pm 0.02^{A,c}$ (0.0015)
NaOCl	$2.51 \pm 0.03^{A,c}$ (0.0018)	$1.73 \pm 0.05^{A,c}$ (0.0016)

EDTA, ethylenediaminetetraacetic acid; CHX, chlorhexidine; HEDP, 1-hydroxyethylidene 1,1-diphosphonate; NaOCl, sodium hypochlorite.
The same superscript uppercase letters in the row indicate no statistically significant difference ($p > 0.05$).
Different superscript lowercase letters in the same column indicate a statistically significant difference ($p < 0.05$).

the other solutions, respectively ($p < 0.05$). In addition, Biodentine and NeoPutty showed higher ΔE values immersion in NaOCl compared to EDTA. Moreover, while HEDP caused discoloration at similar rates to NaOCl ($p > 0.05$), values were lower and higher compared to CHX, and EDTA, respectively ($p < 0.05$). **Table 1** demonstrates the pairwise comparisons.

Table 2 shows the solubility results of HCs immersed in the different irrigation solutions. Regardless of the irrigation solutions, solubility was similar between Biodentine and NeoPutty ($p > 0.05$). Moreover, among the solutions, distilled water was associated with significantly lower solubility values compared to the other solutions ($p < 0.05$). Solubility values in EDTA and CHX groups were significantly higher compared to NaOCl and HEDP groups ($p < 0.05$). However, no statistically significant differences were observed between EDTA and CHX ($p > 0.05$) and NaOCl and HEDP ($p > 0.05$) groups in pairwise comparisons.

DISCUSSION

Maintaining the functions of HCs by preserving their physicochemical structure is an important point that increases the success of endodontic procedures. Especially, contact with irrigation solutions may alter the material properties of HCs and cause aesthetic or biologically based problems that affect the outcomes of treatments [25]. In this sense, reported tooth discoloration following the usage of HCs in several endodontic procedures at various rates is one of the challenges regarding these materials [4,26]. Moreover, the solubility of HCs when immersed in different solutions could affect the quality of the treatment [19,27]. Based on the above-mentioned information, this study aimed to demonstrate the color stability and solubility of NeoPutty which is a relatively new HC compared to Biodentine in contact with different irrigation solutions. Since the results significantly differed between the 2 materials, the null hypothesis has been rejected.

Due to the assessment of the color stability of NeoPutty and Biodentine, materials were immersed in distilled water, EDTA, CHX, and NaOCl, which are the most commonly used irrigation solutions in endodontic treatments along with HEDP for 24 hours. However, longer exposure periods have also been utilized in the literature [28,29]. Since HCs immediately interact with solutions following their placement in the related area, long immersion times may not accurately mimic clinical conditions [19]. Afterwards, color changes were measured using visual spectrophotometry, which is still accepted as the gold standard method in dentistry for measurements of color based on the CIE system developed for the specification of color signals [30]. Spectrophotometric analysis with the Vita Easyshade Compact was performed due to its sensitivity to small changes in color, repeatability, and objectivity [25].

The results in terms of the discoloration effect of Biodentine were very divergent. Although Biodentine contains zirconium oxide as an opacifier rather than bismuth oxide that associated with discoloration due to the dark color crystals of metallic bismuth and oxygen when exposed to visible and ultraviolet light, Biodentine exhibited higher discoloration values based on its contact solution as shown in this study [31]. Higher values compared to NeoPutty can be explained by the potential effect of different types of metal components, especially iron, in the structure of Biodentine on color change since the oxidation of the iron content remaining in the set material is considered a possible mechanism for discoloration [32]. In furtherance, the superior handling and color stability of NeoPutty could lead to its use in various endodontic treatments. Especially, during vital pulp treatments and regenerative endodontic procedures for anterior teeth, choosing the right HCs is paramount due to their potential discoloration capacity. Optimal selection could ensure the success of the treatment.

Immersion of Biodentine and NeoPutty in CHX caused significantly higher discoloration similar to other studies in the literature [14,25]. The extrinsic discoloration efficiency of CHX on silicate filling materials and dental tissues at various concentrations by influencing dental pellicles or plaque could cause this result [25]. Furthermore, increased pH and calcium ion release as a result of the contact between CHX and HCs could lead the higher discoloration values. Additionally, significantly higher discoloration values in Biodentine contact with irrigation solutions can be explained by the higher biomineralization ability of the material [19]. In addition, NaOCl was the other solution that led to visible color change on used HCs. A possible explanation for this result could be higher calcium ion release and dense calcium hydroxide and calcium phosphate crystal formation on the surface of materials after contact with NaOCl similar to the CHX [19]. In line with other studies in the literature, EDTA showed lower discoloration values than CHX in this study [1,14]. Especially in regenerative endodontic treatments, EDTA is used for removing the intracanal medicaments and therefore, it contacts with HC barrier materials. Lesser color change values in the EDTA group may promote using Biodentine and NeoPutty safely as barrier materials in regenerative procedures. Furthermore, since there is no study regarding the color stabilities of HCs when in contact with HEDP, interpreting the results is challenging. However, mixing etidronic acid with NaOCl during the preparation of HEDP may explain the similar values observed between these 2 solutions regarding color changes.

The solubility of HCs is another topic that should be investigated since it is directly related to the sealing ability of materials and consequently, the treatment outcomes. Both Biodentine and NeoPutty showed higher solubility in EDTA and CHX compared to the other solutions, while values were significantly lower in distilled water. Although water is recommended

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as an immersion solution since it is easy to standardize, it does not accurately mimic the environment due to the absence of ions and proteins [33]. Different solubility values can be also explained by the decomposition of particle-binding hydration phases by acid corrosion in acidic environments [34,35]. Used HCs may alter their structures due to increased porosity and accumulated crystalline structure in relatively low pH values [19,34]. On the other hand, NaOCl and HEDP similarly affected the solubility of HCs. This may lead to utilizing HEDP as an irrigation solution safely in various endodontic treatments along with its other superior properties. Moreover, according to the manufacturers, the solubility of NeoPutty is lower than 3% and our results support this information. However, since it was the first study that evaluated the solubility of NeoPutty, the obtained result cannot be compared directly.

Despite the use of ISO 6876 for solubility assessment provides standardization, quality assurance, and validity, test conditions always do not simulate the actual environment for HCs as retrograde filling material [33]. In this sense, micro-computed tomography might be utilized for measuring standardized specimens with a single surface exposed to solutions and could be considered more reproducible [33]. Moreover, the inability to establish the extent of the relationship between the color changes of Biodentine and NeoPutty and their effects on teeth may be noted limitation of this study. In this context, it should be taken into consideration that many factors such as tooth type and dentin thickness may affect the degree of discoloration in the teeth. Although NeoPutty exhibited lower color change and solubility values, further well-designed studies are needed to approve these results since it was the first study that evaluated the above-mentioned parameters.

CONCLUSIONS

The findings revealed that both materials exhibited variable rates of discoloration and solubility when exposed to common endodontic irrigation solutions. The superior color stability of NeoPutty in contact with irrigation solutions compared to Biodentine could enhance its clinical appeal and patient satisfaction in aesthetic areas. These findings also the use of HEDP which demonstrated similar color stability and solubility values to NaOCl, along with the NeoPutty, may significantly improve endodontic outcomes, warranting further investigation and potential widespread adoption in dental practice.

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