IEJ
Iranian Endodontic Journal

A Novel Niosomal Doxycycline/Triamcinolone Medicament Matches Calcium Hydroxide for Low Tooth Staining: A 4-Week *in Vitro* Study

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Article Type: **Original Article**
Received: 29 Sep 2025
Accepted: 24 Oct 2025
Published: 04 Nov 2025
Doi: *10.22037/iej.v20i1.46288*

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Abstract

Introduction: Tooth discoloration remains a significant concern in endodontic treatment, particularly with the use of intracanal medicaments. This study investigated the discoloration potential of niosomal doxycycline and triamcinolone, a novel nanotechnology-based drug delivery system, in comparison to conventional medications. **Materials and Methods:** Fifty extracted single-rooted human teeth were assigned to five groups: (1) calcium hydroxide, (2) calcium hydroxide+triamcinolone, (3) niosomal doxycycline+triamcinolone, (4) doxycycline+triamcinolone, and control groups (positive: blood; negative: saline). All samples were prepared using standardized endodontic procedures, filled with medicaments, and incubated for one month. Discoloration values (ΔE) were measured at baseline, 1 week, and 1 month using the CIE Lab system and a spectrophotometer. A ΔE>3.3 was considered clinically unacceptable. **Results:** All groups exhibited discoloration values within clinically acceptable limits (ΔE<3.3). At one month, significant differences were observed among groups (ANOVA, $P<0.05$). The niosomal doxycycline+triamcinolone group showed the lowest ΔE values, similar to the calcium hydroxide group, while the doxycycline+triamcinolone and calcium hydroxide+triamcinolone groups exhibited higher discoloration values. **Conclusion:** Emphasize that at 4 weeks, all medicaments induced clinically acceptable ΔE, with niosomal performing similarly to calcium hydroxide alone and significantly better than the conventional doxycycline+triamcinolone combination.

Keywords: Discoloration; Nanoparticle Drug Delivery System; Root Canal Medicament

Introduction

The primary goal of root canal therapy is to eliminate microorganisms from the root canal system to prevent reinfection and promote healing. However, numerous studies have demonstrated that conventional cleaning and preparation techniques rarely achieve the complete removal of all tissues and microorganisms within the complex root canal anatomy [1]. Consequently, considerable research has focused on enhancing the antibacterial efficacy and reducing the cytotoxicity of endodontic materials to improve clinical outcomes [2-7]. In addition to these biological properties, aesthetic considerations, such as the potential for tooth discoloration, play a significant role in material selection for endodontic treatment, as patient satisfaction increasingly includes cosmetic outcomes [8, 9].

Tooth discoloration following root canal therapy is a well-recognized complication that can adversely affect patient acceptance of treatment [10, 11]. Discoloration can result from various factors, broadly categorized based on the location of pigment deposition: extrinsic discoloration affects the tooth surface, while intrinsic discoloration involves pigment incorporation within the dentin or enamel structure [12, 13]. Intracanal medicaments have been identified as significant contributors to intrinsic discoloration in endodontically treated teeth [14, 15].

Advancements in nanotechnology have enabled the development of intracanal medicaments with enhanced properties,

such as increased surface area to volume ratios and improved solubility, resulting in superior antibacterial activity and potentially reduced cytotoxicity [16, 17]. Examples include nanoparticles of silver, calcium hydroxide, and antibiotic pastes combined with anti-inflammatory agents, which have demonstrated promising results in endodontic applications [18-24].

Among these, niosomal formulations of doxycycline combined with triamcinolone represent a novel nano-based drug delivery system designed to optimize the sustained release and bioavailability of these agents. Beyond their well-established antibacterial effects, triamcinolone adds an anti-inflammatory benefit that may enhance periapical healing and reduce post-treatment pain, while the niosomal delivery system potentially decreases toxicity and enhances tissue compatibility [25]. However, both doxycycline and triamcinolone have been implicated in tooth discoloration, raising concerns about their clinical use in cosmetically sensitive cases [26, 27].

To date, no studies have specifically evaluated the discoloration potential of niosomal doxycycline and triamcinolone formulations compared to conventional intracanal medicaments. Therefore, this study aims to assess and compare the tooth discoloration effects of niosomal doxycycline and triamcinolone with calcium hydroxide, calcium hydroxide combined with triamcinolone, and the doxycycline and triamcinolone combination in extracted human teeth.

Materials and Methods

In this *in vitro* study, fifty extracted human maxillary and mandibular single-rooted incisor teeth were tested. The sample size of the study was calculated with G* Power software (Heinrich Heine University, Düsseldorf, Germany) with type one and type two errors at 5% and 20% and an effect size of 0.6 for the ANOVA test with four groups. The final sample size was estimated to be at least 9 samples in each group. Additionally, five teeth were prepared for positive and negative controls.

The teeth were initially clinically and radiographically inspected for caries, cracks, previous restorations, and calcifications. The remaining tissue and debris were mechanically removed with a curette. The teeth were then immersed in normal saline (Golrang, Tehran, Iran). The access cavity was made with a 0.8 mm diamond fissure bur (TizKavan, Tehran, Iran). Gates #2 and #3 were used to enlarge the canal's corona. A size 10 K-file was put into the canal, and its length was shortened by 1 mm when the file tip was visible at the apex. The working length was then measured. The root canals were cleaned with hand K-files and the Race rotary system, following the manufacturer's recommendations and the crown-down procedure. The apical end was enlarged to size 35, with a 4% taper. To eliminate any remaining pulp tissue, irrigation was performed using 5.25% sodium hypochlorite and normal saline. The canals were then washed with regular saline. After drying, the canals were irrigated for one minute with 17% EDTA to remove the smear layer. They were then irrigated with 5.25% sodium hypochlorite for 1 min before being rinsed with normal saline and dried using paper cones.

The samples were then randomly and blindly divided into the following groups:

- *Negative control group (n=5)*: Saline was placed in the dental samples of this group.
- *Positive control group (n=5)*: Blood was placed in the dental samples of this group.
- D1: Calcium hydroxide group (n=10): The dental samples in this group received calcium hydroxide (Merck, Darmstadt, Germany) mixed with distilled water.
- D2: Calcium hydroxide and triamcinolone combination (n=10): The samples of this group received equal amounts of calcium hydroxide and triamcinolone mixed with distilled water.
- D3: Doxycycline niosomal form and triamcinolone combination (n=10): The samples of this group received equal amounts of doxycycline niosomal form and triamcinolone mixed with distilled water.
- D4: Doxycycline and triamcinolone combination (n=10): The samples of this group received equal amounts of doxycycline and triamcinolone mixed with distilled water.

After cleaning the pulp chamber with a micro-cotton ball, a micro-cotton ball was inserted inside, and the access cavity was sealed with self-cure glass ionomer. The apical end was also sealed using self-curing glass ionomer. All samples were cultured for four weeks at 37 °C in an incubator with 100% humidity. In this study, all samples were kept under controlled conditions at a constant temperature (25 degrees Celsius). Additionally, human teeth at different age stages were used to evaluate aging effects. This helped minimize the influence of confounding factors such as aging and storage conditions.

Color measurements were taken immediately after sample preparation, one week, and four weeks following drug placement. Previous studies have selected one month as an initial time frame for evaluating tooth discoloration because early changes predominantly occur within this period[28]. To guarantee constant color measurement, a circular strip with a diameter of 6 mm was attached to the buccal surface 2 mm above the CEJ. The color of each sample was assessed using the CIE Lab system with the Easyshade Advanced spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Germany) on the buccal surface of the crown and in the circular strip area. The instrument was calibrated before spectrophotometric measurement, according to the manufacturer's recommendations. Three measurements were

obtained at each time point, and the average of the three was taken into account. To ensure measurement accuracy, calibration protocols were performed according to ISO 7491 standards. Additionally, all observations were made by two different observers using a specified spectrophotometer device.

The spectrophotometer is calibrated over a white background, according to the manufacturer's recommendations. The formula for calculating discoloration value (ΔE) was as follows [29]:

$$\Delta E=\sqrt{(\Delta L)^2+(\Delta A)^2+(\Delta B)^2},$$

where *L* represents lightness/darkness, *A* denotes measurement on the red-green axis, and *B* indicates measurement on the yellow-blue axis.

The acceptable range for ΔE is between 1 and 3.3. Values of 3.3 or higher are clinically unsatisfactory to the human eye. Therefore, the maximum allowed limit for color difference is ΔE=3.3 [30].

After determining the ΔE, ANOVA was used to compare the groups, followed by Tukey's HSD test. Assumptions of variance homogeneity and normality of residuals were checked by Q-Q plot and variance homogeneity test, and for more certainty, non-parametric tests were also used, and no discrepancy was observed in the results. All the data were analyzed by SPSS 21.0 (IBM SPSS, Chicago, USA).

Results

All discoloration values of medications were within the acceptable range except for the positive control group (Table 1). The test results revealed a significant difference in the discoloration value between the groups after one week (P<0.001). The positive and negative control groups showed a substantial difference from the other groups. The Post Hoc test findings also revealed that after one week, there was a significant difference only between groups D1 and D4 (P=0.002), with no significant difference between the remaining groups.

The findings of the ANOVA test similarly revealed a significant difference in the results between the groups after one month (P<0.001). The Post Hoc analysis revealed a significant difference between groups D1 and D4 (P<0.001), D3 and D4 (P=0.008), D1 and D2 (P<0.001), D2 and D3 (P=0.044), and D2 and D4 (P=0.004) after one month, but no significant difference between the other groups. The positive and negative control groups showed a substantial difference from the other groups.

Discussion

The purpose of this study was to compare the discoloration effects of niosomal doxycycline and triamcinolone, calcium hydroxide, calcium hydroxide and triamcinolone combination, and doxycycline and triamcinolone in extracted teeth. This study revealed that the mean ΔE for all medicines was within clinically acceptable ranges, and no substance induced an undesirable color change. Doxycycline niosomal form and triamcinolone combination, and calcium hydroxide had a lower discoloration value in terms of ΔE.

In the present study, doxycycline niosomal form and triamcinolone combination had a lower discoloration value and were in an acceptable range. Studies have shown that Ledermix, which is similar to the composition used in this research, leads to discoloration [31, 32]. The results of this study could be the effect of the niosomal form. In addition, our results revealed that the calcium hydroxide and triamcinolone combination and the doxycycline and triamcinolone combination had the most discoloration value after one month. This result was replicated in previous studies [31-33].

Thomson AD *et al.* [34] in a study investigating the staining effects of various sealers and intracanal medicaments concluded that Ledermix caused the most discoloration among intracanal medicaments over 12 months. Afkhami *et al.* [35] in a study on tooth discoloration of intracanal medicaments concluded that the triple antibiotic paste caused the most discoloration, and calcium hydroxide did not cause significant discoloration. Afkhami *et al.* [36] in a study of crown color change analysis using a spectrophotometer, after using silver nanoparticles in combination with calcium hydroxide, concluded that neither calcium hydroxide nor silver nanoparticles in combination with calcium hydroxide

Table 1. Discoloration Values (ΔE) at One Week and One Month across Experimental Groups and Controls [Mean (95% CI)]

Group	One week discoloration value (ΔE)	One-month discoloration value (ΔE)
D1 (*n*=10)	1.27 (0,3.59)[a]	1.92 (1.04,2.8)[a]
D2 (*n*=10)	1.43 (0.16,3.59)[a]	2.32 (1.81,2.83)[b]
D3 (*n*=10)	1.13 (0,3.53)[a]	1.82 (0.29,3.35)[ca]
D4 (*n*=10)	1.61 (0.22,4.18)[b]	2.79 (1.44,4.14)[d]
***P*-value**	<0.001	<0.001
Negative control (*n*=5)	0.41 (0,0.97)	0.56 (0.29,0.83)
Positive control (*n*=5)	4.04 (1.41,7.87)	5.24 (3.04,7.44)

D1: Calcium hydroxide group (n=10), D2: Calcium hydroxide and triamcinolone combination (n=10), D3: Doxycycline niosomal form and triamcinolone combination (n=10, D4: Doxycycline and triamcinolone combination (n=10). Groups with different letters have significantly different mean discoloration scores at the 5% level

did not cause a color change in the crown of the tooth. In the present study, the results also showed that drugs D1 and D3 with a calcium hydroxide base caused the least amount of color change, which is consistent with the results of Afkhami *et al.'s* studies.

One of the possible reasons for the reduced discoloration observed in the group treated with doxycycline and neosomal triamcinolone is the unique structure and function of this nanocarrier drug delivery system.[37]. Neosomes encapsulate the active drug within vesicles with a lipid bilayer membrane, thereby reducing its direct contact with dentinal tubules and limiting its interaction with the organic and inorganic components of dentin. This encapsulation also protects the drug from oxidation and photodegradation factors that are especially known as causes of discoloration in tetracycline derivatives like doxycycline. Additionally, the controlled and gradual release characteristic of the neosomal form prevents sudden saturation of the canal space with high drug concentrations, thereby limiting its potential for deposition and staining. Overall, the neosomal drug delivery system demonstrates a significant advantage in endodontic treatments regarding aesthetic outcomes by reducing drug-induced discoloration.

The negative control group (NEG) showed a gradual color change over time, staying within acceptable limits. This aligns with past research [26, 34, 38, 39]. The positive control group (POS) displayed the most immediate and significant discoloration, increasing further over one month, as expected [26, 34, 38, 39]. These results are expected from blood components entering the tooth, and prior research supports this explanation [40].

In this study, one of the primary limitations lies in the use of extracted human teeth for *in vitro* experiments. These samples may differ physiologically and anatomically from vital teeth, and as such, the results may not be fully generalizable to clinical settings. The biological environment of a living tooth, including vascularization and immune responses, cannot be completely replicated *in vitro* conditions, which might influence the behavior of materials and the degree of discoloration observed. Another notable limitation is the relatively short follow-up period of one month for assessing discoloration. While this duration may be sufficient to observe initial changes, it may not adequately reflect the long-term discoloration effects observed in real clinical scenarios. Future studies are recommended to incorporate extended follow-up periods using clinically relevant models to provide a more accurate and comprehensive evaluation of the materials' performance over time. In addition, the sample size used in this study was relatively limited. A small sample size may reduce the statistical power of the study and limit the generalizability of the findings. To address this, future research should consider employing larger sample sizes and a greater number of experimental groups. This would enhance the robustness of the data and allow for a more reliable interpretation of the results.

Conclusion

This i*n vitro* study demonstrated that all tested intracanal medicaments, including niosomal doxycycline-triamcinolone, calcium hydroxide, and their combinations, induced discoloration within clinically acceptable limits ($\Delta E<3.3$). Among the groups, niosomal doxycycline-triamcinolone and calcium hydroxide exhibited the least discoloration, while the combination of calcium hydroxide-triamcinolone and doxycycline-triamcinolone caused higher levels of color change. The findings suggest that niosomal formulations may mitigate the discoloration potential of intracanal medicaments. However, these results are limited by the *in vitro* design, and further clinical studies with long-term follow-ups are needed to confirm the outcomes in real-world settings.

Acknowledgements
None.

Conflict of interest
None.

Funding support
This research was partially funded by Kerman University of Medical Sciences, Kerman, Iran.

Authors' contributions
Conceptualization: RF/AS; Methodology: RF/AS/HM; Formal Analysis and Investigation: AS/HM; Writing-Original Draft Preparation: AS/HM/AP; Writing-Review and Editing: HM/HK/AHN; Supervision: RF/AS. All authors read and approved the final manuscript.

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Please cite this paper as: Fereidooni R, Shahravan A, Manochehrifar H, Pardakhty A, Kamyabi H, Nekouei AH. A Novel Niosomal Doxycycline/Triamcinolone Medicament Matches Calcium Hydroxide for Low Tooth Staining: A 4-Week *in Vitro* Study. Iran Endod J. 2025;20(1): e43. Doi: 10.22037/iej.v20i1.46288.