The Effect of Various Remineralizing Agents on Post-bleaching Shade Stability: A Randomized Double-blind Clinical Study

Keywords

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Authors

Bassant Ismail * §
(BDS, MSc)

Zainab Soliman ‡
(BDS, MSc, DDSc)

Bahaaeldeen Ismail ^
(MD, MSc)

Farid El-Askary †
(BDS, MSc, DDSc)

Address for Correspondence

Bassant Ismail * §

Email: BassantIsmail@dent.asu.edu.eg

* *Ph.D. Student, Operative Dentistry Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt*

§ *Assistant Lecturer, Operative Dentistry Department, Faculty of Dentistry, Badr University in Cairo, Egypt*

‡ *Assistant Professor, Operative Dentistry Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt*

^ *Assistant Professor of Medicine, University of Kentucky Chandler, Lexington, Kentucky, USA*

† *Professor, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.*

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ABSTRACT

Objectives: This study aimed to compare the effects of four remineralizing agents on post-bleaching shade stability and to evaluate the agreement between objective and subjective shade assessments. Methodology: Forty-eight eligible patients underwent in-office bleaching with 32% hydrogen peroxide, followed by one of the following: 1. potassium nitrate/sodium fluoride (After Whitening Mousse, AWM, n=11), 2. casein phosphopeptide-amorphous calcium phosphate/fluoride (MI Paste Plus, MI, n=13), 3. nano-hydroxyapatite/fluoride (Remin Pro, RP, n=11), or 4. PolyAmidoamine Dendrimer (PAMAM, n=13). Stability was assessed using ΔE, ΔSGU, ΔL^*, Δb^*, and ΔWI_D at regular follow-ups over one year. Statistical analyses were performed using a Linear Mixed-Effects Model, Kendall's W, and Cohen's Kappa ($\alpha = 0.05$). Results: Based on linear mixed-effects model, remineralizing agents did not significantly affect ΔE, ΔSGU, ΔL^*, Δb^*, or ΔWI_D ($p>0.05$). The follow-up time had a significant effect on these color parameters ($p<0.05$). No post-bleaching shade relapse was observed in any of the experimental groups. There were slight to moderate agreements between the two evaluation methods (Kappa=0.106, W=0.504). Conclusions: None of the remineralizing agents compromised post-bleaching shade stability. Accurate post-bleaching shade stability assessment requires both objective and subjective methods. Clinical relevance: Bleaching stability was maintained with all remineralizing agents evaluated in this study.

INTRODUCTION

Teeth discoloration is a common concern among patients seeking dental treatment.[1] This has led to a growing demand for dental bleaching, which is considered one of the most conservative and safest treatment options[2]

Chemical in-office bleaching is a quick and controlled procedure using high concentrations of hydrogen peroxide; however, it is often associated with color rebound.[3] Previous studies have reported several adverse effects of bleaching, including tooth hypersensitivity, changes in enamel surface texture, increased enamel porosity, and surface roughness.[4,5] Alterations in enamel texture can make bleached teeth more susceptible to staining, highlighting the need to evaluate shade stability in controlled clinical trials.[6,7]

To mitigate the negative effects of bleaching, the use of remineralizing agents is recommended. These agents alleviate bleaching-induced hypersensitivity, help remineralize enamel defects, and reduce enamel surface roughness,[8] with maintaining a brighter tooth shade over time by reducing stain uptake.[9,10]

Fluoride is commonly used due to its effectiveness in enhancing enamel remineralization.[11] Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) binds to the enamel pellicle and acts as a mineral reservoir during demineralization.[12,13] Nanotechnology has led to the development of nano-hydroxyapatite (n-HAP), which has been integrated into various dental products. These products are used alongside bleaching to repair enamel surface porosities caused by the bleaching process.[14] Polyamidoamine (PAMAM), a biomimetic remineralizing agent, effectively remineralizes damaged enamel by mimicking the regulatory roles of enamel matrix proteins in hydroxyapatite formation.[15] It has shown promising *in vitro* results in remineralizing artificially induced initial carious lesions,[16] and has demonstrated clinical effectiveness in treating white spot lesions.[17]

Objective and subjective methods are commonly used to assess bleaching shade change and stability over time. The objective method employs a digital spectrophotometer, while the subjective method uses commercially available dental shade guides.[18,19]

The objective method extracts color parameter data to detect differences using formulas, including ΔEab, the most widely used and clinically supported equation for color difference evaluation.[20] The ΔE calculation enables quantitative analysis of color changes in dental research.[21,22] However, this method may lack clinical relevance, fail to indicate the direction of color change, and lead to misleading results. Errors may also impact the accuracy of shade measurements.[20]

Visual thresholds, such as perceptibility (PT) and acceptability (AT), help link objective data to clinical outcomes.[23] The AT defines the acceptable level of color difference, with values typically ranging from 1.7 to 5.6 for ΔE, although many studies suggest a value of 3.7.[21,24] The AT serves as a reference for evaluating bleaching efficiency and stability.[25,26] Evaluating changes in individual color parameters, such as ΔL, Δa, and Δb*, offers additional insight into the direction of color change. These parameters correspond to variations in brightness, the green-red axis, and the blue-yellow axis, respectively.[24] Reference values of 1.25 for ΔL* and Δa*, and 2.8 for Δb*, are considered the AT.[23]

In contrast, the subjective method, which measures the difference in shade guide units (ΔSGU) may offer a more accurate reflection of the clinical situation compared to ΔE measurement.[18] However, this method cannot extract color parameter values for precise color difference calculation.[27] Additionally, the subjective method can be inconsistent due to observer-related factors such as gender, clinical experience, eye fatigue, and visual deficiencies.[28,29]

Whiteness indices have been developed to provide objective and quantitative assessment of whiteness. This is particularly important for monitoring bleaching effectiveness in clinical and laboratory settings, where a white material has very high lightness or value and very low saturation.[29] A relatively new index, the Whiteness Index for Dentistry (WI_D), proposed in 2016, is based on the CIELAB color space and has been found to correlate more closely with the human visual perception of color.[30] Recently, whiteness PT of 0.7 and AT of 2.6 were suggested based on the WI_D.[30]

Several *in vitro* studies have investigated the effects of remineralizing agents on post-bleaching shade stability.[31,32] However, clinical studies with similar objectives are scarce. Most clinical trials focus on comparing bleaching techniques, formulations, or pH levels.[3,7,33,34] Additionally, two clinical studies evaluating the impact of remineralizing agents on post-bleaching shade stability or bleaching efficiency had short follow-up periods, limiting definitive conclusions.[13,35] Tawfik *et al*.,[13] assessed the effect of amorphous calcium phosphate on tooth shade over 6 months, while Moharam *et al*.,[35] evaluated various remineralizing agents on in-office bleaching efficacy over 3 weeks, without investigating their long-term effects on shade stability.

This randomized clinical study aims to assess and compare the effects of various remineralizing agents applied post-bleaching on bleaching shade stability over a 1-year follow-up period, with the primary outcome being the post-bleaching shade stability. Additionally, the agreement between the objective and subjective methods of shade analysis will be evaluated as a secondary outcome. The null hypotheses being tested are: 1. The stability of the bleaching shade over the entire follow-up period will not differ among the different remineralizing agents. 2. The results of the objective shade analysis will align with those of the subjective analysis.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee and Institutional Review Board, Faculty of Dentistry, Ain Shams University Research Ethical Committee (FDASU-REC), approval number: FDASU-RecID032004. The study was registered at ClinicalTrials.gov website, ClinicalTrials.gov Identifier: NCT06358924.

TRIAL DESIGN AND SETTING

A randomized, parallel, prospective, double-blind clinical study was conducted with a follow-up of 1 year. Neither the participants nor the outcome assessors were aware of the type of remineralizing agent allocated to each participant. The recruitment of patients and all clinical procedures were conducted at the dental clinic of the Operative Dentistry Department at the Faculty of Dentistry, Ain Shams University in Cairo, Egypt, from November 2021 to August 2023. The study design is shown in Figure 1 and follows the CONSORT (Consolidated Standards of Reporting Trials) recommendations. All patients who participated in the study were fully informed about the trial steps and duration before starting any procedures and signed an informed consent form.

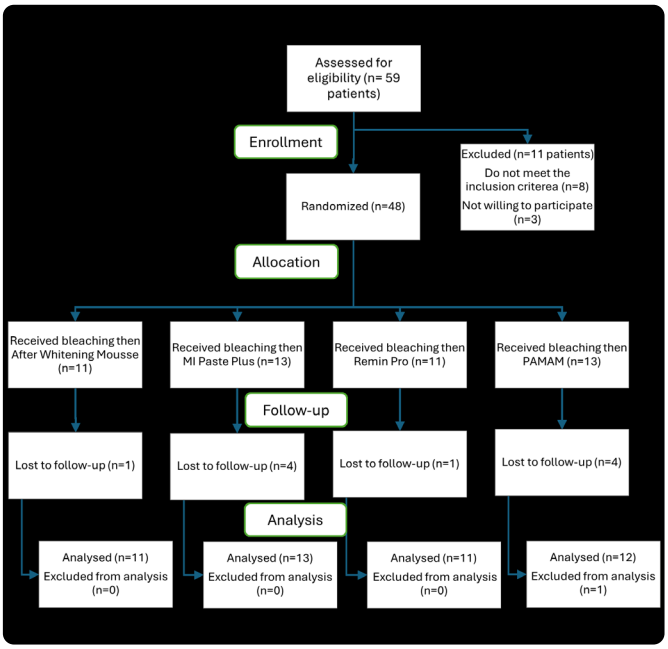

Figure 1: Flowchart of the clinical trial.

ELIGIBILITY CRITERIA

Male and female patients were selected according to the following inclusion criteria: age between 18 and 40 years old, the presence of at least 14 sound teeth in each of the upper and lower arches including the anterior teeth and the first premolars on both sides, having low caries risk, having a VITA-shade of A2 or darker, and being cooperative and medically free.[6,21] Patients were excluded from the study if they were seriously medically compromised, smokers or alcoholics, had hypersensitivity to any of the materials used, were pregnant or lactating, displayed any signs of serious oral diseases, enamel cracks, enamel hypoplasia or hypomineralization, periodontal disease, severe dental hypersensitivity, or had any previous bleaching treatments.[21]

SAMPLE SIZE CALCULATION

The sample size was calculated using PS Power and Sample for Windows, version 3.1.6 using an independent t-test based on a previous study by Nassar *et al.* in 2018,[36] with a power of 80% and a 95% confidence level ($\alpha = 0.05$). To reject the null hypothesis that the experimental and control groups' population means are equal, 40 patients were needed (n=10).

Patients who dropped out of the study before the 3-month post-bleaching follow-up time were counted, recorded, and subsequently substituted. In total, 48 patients completed the bleaching/remineralization treatment.

RANDOMIZATION AND ALLOCATION CONCEALMENT

Simple randomization was implemented using a Microsoft Excel-generated computer program, with only one unit of randomization, which was the remineralizing agent type. An independent researcher who was unaffiliated with the clinical treatment, nor shared in the evaluation procedure was responsible for the randomization process, and the allocation sequence remained confidential.

PATIENT ALLOCATION

Patients were randomly assigned into four experimental groups according to the remineralizing agent: After Whitening Mousse (AWM, Manufacturer's remineralizing agent, Control group), MI Paste Plus (MI), Remin Pro (RP), or Poly(Amidoamine) dendrimer (PAMAM). Products used in the study, batch numbers, active compositions, manufacturers, and modes of application are summarized in Table 1.

STUDY INTERVENTION

Medical and dental histories were taken, and all patients underwent full mouth scaling using a non-fluoridated polishing paste (i-FASTE Prophy Paste, Douromed, EU), and received full oral hygiene instructions.

Pre-bleaching shade was objectively recorded using the clinical spectrophotometer, VITA Easyshade V (VITA Zahnfabrik, Germany). The shades of the upper two central incisors were recorded under standardized light conditions. Each tooth was evaluated twice, and the L*, a*, and b* values were recorded and averaged. The shade was also subjectively recorded by matching the upper two central incisors to the closest VITA classical shade guide tab (VITA Zahnfabrik, Germany).[21,36]

Before the application of the in-office chemical bleaching agent, proper soft tissue isolation was achieved through the placement of a cheek retractor, and cotton rolls in the labial, lingual, and buccal vestibules. A light-cured gingival barrier (WHITEsmile gingiva protector) was applied to cover approximately 0.5–1 cm of the labial gingival tissue and about 0.2-0.3 cm of cervical enamel. After light curing, the gingival barrier was carefully inspected for any areas of leakage.

The clinical application of the bleaching gel is summarized in Table 1. The number of applications depended on the desired outcome by the patient unless sensitivity occurred, and the dental shade was assessed after each application. If teeth sensitivity persisted, the treatment was discontinued. After the last application, the gel was removed by high-volume suction and thoroughly rinsed with a water spray. The remineralizing agent was applied immediately after the bleaching procedures according to the randomization sequence. Detailed application procedures for each remineralizing agent were summarized in Table 1. If an additional bleaching session was desired, the entire bleaching and remineralization procedures were repeated after 1 week.

Patients were given post-bleaching instructions, and their normal oral hygiene routines of regular tooth brushing using a fluoridated non-whitening toothpaste twice daily were continued. Patients were scheduled for follow-ups at regular intervals of 24 hours, 3 days, 1 week, 1 month, 3 months, 6 months, 9 months, and 1 year after bleaching. The shade of the upper two central incisors was assessed objectively and subjectively by two external assessors, who had clinical experience exceeding 8 years and were not involved in the prior study procedures. Before their involvement in the evaluation, the methodology of shade assessment was discussed in detail to ensure standardized shade assessment each time.

Table 1. Products used in the study, their lot numbers, compositions, manufacturers, and application modes.

Product name (lot #)	Active composition	Manufacturer	Mode of application
Power Whitening YF (20044, 22062)	40% hydrogen peroxide (upon mixing hydrogen peroxide concentration reaches 32%).	WHITEsmile GmbH, Germany.	The auto-mixing tip was connected to the upper part of the dual-chamber syringe. The blended gel was then injected through the tip onto the teeth that needed bleaching, creating a uniform 1-2 mm thick layer on their facial surfaces. After 15-20 minutes, the gel was removed from the teeth using a surgical suction tip. This process was repeated with a fresh mixture for a total of 2 to 3 applications during each visit.
WHITEsmile gingiva protector (20040)	A mixture of acrylic resins, fillers, and initiators.	WHITEsmile GmbH, Germany.	Applied through the special tip onto the gingival tissue, then light cured for 20 seconds.
After Whitening Mousse (20056)	30 % Xylitol, 4.2 % potassium nitrate, and 1450 ppm fluoride.	WHITEsmile GmbH, Germany.	Applied after bleaching and left undisturbed for 10 minutes followed by rinsing with water.
MI Paste Plus (328619)	Casein-phosphopeptide-amorphous calcium phosphate (CPP-ACP) (10% wt./vol) with 0.2% wt./vol sodium fluoride (900ppm).	GC America Inc., USA.	Applied after bleaching and left undisturbed for 3 minutes followed by removal of the excess and further distribution of the cream by the patient's cheeks and tongue for 2 minutes. The patient was not allowed to eat or drink for 30 minutes after application.
Remin Pro (327472)	Hydroxyapatite (calcium and phosphate), ethanolic colophony, fluoride (1450 ppm NaF), and xylitol.	VOCO-GmbH, Germany.	Applied after bleaching and left undisturbed for 3 minutes followed by removal of the excess the patient was not allowed to eat or drink for 30 minutes after application.
PAMAM (MKCL9988)	Poly (Amidoamine)-succinamic acid dendrimer, 1,4-diaminobutane core, generation 4 solution. 10 wt.% in water.[37]	Sigma-Aldrich Chemie GmbH, Germany.	Applied by a micro-brush and left for 30 minutes (according to previous studies),[38,39] followed by thorough rinsing with water.

SHADE EVALUATION

An evaluation was objectively carried out through color difference (ΔE) calculations using the mean L*, a*, and b* values of the upper two central incisors for each patient obtained from the VITA EasyShade V device at each follow-up time, according to the following equation: $[\Delta E_{ab} = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ (Commission Internationale de l'Eclairage).20,21 The difference in the L* and b* color parameters (ΔL* and Δb*) was also calculated, by subtracting the pre-bleaching values from the post-bleaching ones.

Subjectively, the shade was evaluated through the difference in shade guide units (ΔSGU) using the VITA classical shade guide. The 16 tabs of the classical shade guide were arranged in descending order according to value as follows: B1, A1, B2, D2, A2, C1, C2, D3, A3, D4, B3, A3.5, B4, C3, A4, and C4, and were assigned numbers from 16 for B1 until 1 for C4.[40,41] Teeth with shades that appeared lighter than the B1 shade of the VITA Classical shade guide were given the Bl (Bleached) symbol, and the number 17 was assigned to them.

Additionally, the difference in the Whiteness Index for Dentistry (ΔWI_D), was calculated at each follow-up time according to the formula: "$\Delta WI_D = WI_{D\ (post\text{-}bleaching)} - WI_{D\ (pre\text{-}bleaching)}$", where "$WI_D = 0.511L^* - 2.324a^* - 1.100b^*$",[29,42] with 0.511 is the constant for lightness, 2.32 is the constant for hue and chroma on the green-red axis, and 1.100 is the constant for hue and chroma on the blue-yellow axis.[43]

The VITA shades recorded at each visit using the VITA Easy-Shade device, alongside those obtained by matching teeth shades to the nearest VITA Classical shade guide tab, were documented. These records were utilized to assess both the absolute agreement between the objective and subjective methods, as well as the quantification of the degree of concordance between them based on the order of the shades according to the value as described above.

STATISTICAL ANALYSIS

Shapiro-Wilk test was used to check the normality of numerical data. To evaluate inter-rater reliability between the two observers, the intraclass correlation coefficient was calculated, and statistical analysis was performed using the average of the two measures. The means and standard deviations (SD) of the parametric variables (ΔE, ΔSGU, ΔL*, Δb*, and ΔWI_D) were reported. Both the overall effects of remineralizing agents and time, as well as the differences between agents at each follow-up time and between follow-up times within each group, were evaluated using

a linear mixed-effects model. The Kruskal-Wallis test with Dunn's post-hoc tests was used to evaluate the non-parametric variables (patients' ages and number of applications), which were reported as medians and interquartile ranges (IQR). Fisher's exact test was used to assess categorical variables, such as the patient's gender, which were reported as frequencies and percentages. Kendall's W coefficient was used to quantify concordance, while Cohen's Kappa was used to estimate the absolute agreement between the objective and subjective shade evaluations. The p-value threshold for statistical significance was set at 0.05 for every test. SPSS (25th edition, IBM Corporation, New York, USA) and R Statistics software 4.3.0 were used for the statistical analysis.

RESULTS

Forty-eight patients were enrolled, with 38 completing the study. Ten patients withdrew due to inability to attend follow-ups or unwillingness to continue *(Figure 1)*. In the AWM and RP groups, one patient per group was lost after the 1-week follow-up. In the MI group, two patients were lost after the 1-week follow-up, one after the 1-month follow-up, and one after the 3-month follow-up. In the PAMAM group, one patient was lost after the 24-hour follow-up, one after the 1-week, one after the 1-month, and one after the 9-month follow-ups. Table 2 shows no significant age difference ($p > 0.05$). A significant gender difference ($p < 0.05$) was found with more females overall (77.1%). Only the AWM group had significantly more male patients compared to the other groups ($p < 0.05$). No significant difference was observed in the number of bleaching agent applications ($p > 0.05$).

Excellent reliability was revealed by the two observers' intraclass correlation coefficient (0.924).

Using the Shapiro-Wilk test, it was shown that ΔE and ΔSGU for pre-bleaching/24-hour and pre-bleaching/3-day were normally distributed across all groups ($p > 0.05$). While the pre-bleaching/3-day ΔE was 8.6±4.1, showing significant shade changes, the pre-bleaching/24-hour mean ΔE was 3.4±2.2, within the AT. Pre-bleaching/24-hour and pre-bleaching/3-day had ΔSGU values of 4.6±2.5 and 4.3±2.5, respectively. Pre-bleaching/3-day post-bleaching ΔE and ΔSGU were used as the reference values for comparing ΔE and ΔSGU at subsequent follow-up times since considerable shade change was observed.

According to the linear mixed-effects model, the four remineralizing agents did not significantly affect ΔE, ΔSGU, ΔL*, Δb*, and ΔWI_D color parameters ($p > 0.05$), however, they were significantly affected by follow-up time ($p < 0.05$).

Regarding the effect of remineralizing agents, for both ΔE and ΔSGU, Tables 3 and 4 do not demonstrate any significant differences at individual follow-up times ($p > 0.05$). Additionally, no significant differences were seen in ΔL*, Δb*, and ΔWI_D.

Table 3 shows that there were no significant differences between the AWM, MI, and PAMAM groups' post-bleaching follow-up times (3 days to 1 year) with respect to the effect of time on ΔE ($p > 0.05$). In contrast, the RP group's ΔE after 3 months was significantly lower than that at 3 days ($p < 0.05$). In comparison to the 3-day follow-up, Table 4 demonstrates that ΔSGU decreased significantly beginning at 6 months for AWM, MI, and PAMAM, and at 3 months for RP ($p < 0.05$). There was no shade relapse, as evidenced by the mean ΔE and ΔSGU values between pre- and post-bleaching shades for all groups remaining above the AT of 3.7 and one SGU.

The linear mixed-effects model revealed that follow-up time significantly reduced ΔL* from 1 month in the AWM group and 9 months in the RP group, compared to the 3-day follow-up ($p < 0.05$, Figure 2). There were no significant differences in follow-up times in the MI group ($p > 0.05$; Figure 2). The ΔL* in PAMAM significantly decreased at 6 months and 1 year compared to the 3-day follow-up time ($p < 0.05$).

Figure 2 demonstrates a significant rise in Δb* at 1 month and 3 months compared to 3 days in the AWM group ($p < 0.05$). There were no significant differences in MI and PAMAM groups across the follow-up times ($p > 0.05$). The RP group showed significantly higher Δb* in the 1 and 3 months compared to 3 months ($p < 0.05$).

Table 2. Summary of the number of patients per group and intergroup statistical comparisons of demographic data and the number of bleaching agent applications.

Parameter		Overall	Control (AWM)	MI	RP	PAMAM	P-value
Number of patients		48	11	13	11	13	
Gender [n (%)]	Male	11 (22.9%)	6 (54.5)	1 (7.7%)	1 (9.1%)	3 (23.1%)	<0.001
	Female	37 (77.1%)	5 (45.5%)	12 (92.3%)	10 (90.9%)	10 (76.9%)	
Age (median [IQR])		24.00 [21.00, 26.00]	24.00 [21.00, 24.00]	23.00 [22.00, 26.00]	24.00 [23.50, 38.50]	22.00 [21.00, 26.00]	0.391
Number of applications (median [IQR])		3 [3, 3]	3 [3, 3]	3 [3, 3]	4 [2, 5]	3 [3, 3]	0.051

p-values < 0.05 indicate significant differences

Table 3. Objective shade change (ΔE) of the study groups at different follow-up times expressed as means ± standard deviations, as well as their statistical analysis, for the evaluation of bleaching stability.

Evaluation point	Control (AWM)	MI Paste Plus	Remin Pro	PAMAM
Pre/3 days	9.9±3.4^{Aa}	7±3.4^{Aa}	9.1±3.4^{Aa}	8.7±3.4^{Aa}
Pre/1 week	9.9±3.4^{Aa}	6.4±3.4^{Aa}	8.7±3.4^{Aab}	9.1±3.4^{Aa}
Pre/1 month	7.8±3.3^{Aa}	6±3.2^{Aa}	6.8±3.3^{Aab}	7.1±3.3^{Aa}
Pre/3 months	7.7±3.3^{Aa}	5.3±3.1^{Aa}	6.7±3.3^{Ab}	7.5±3.1^{Aa}
Pre/6 months	7.8±3.3^{Aa}	5.5±3^{Aa}	7±3.3^{Aab}	7.5±3.1^{Aa}
Pre/9 months	7.8±3.3^{Aa}	6±3^{Aa}	8.3±3.3^{Aab}	7.5±3.1^{Aa}
Pre/1 year	8±3.3^{Aa}	6.1±3^{Aa}	6.8±3.3^{Aab}	7.9±3.1^{Aa}

Means with different capital superscript letters within each row, and small superscript letters within each column indicate statistically significant differences between experimental groups at p = 0.05.

Table 4. Subjective shade change (ΔSGU) of the study groups at different follow-up times expressed as means ± standard deviations, as well as their statistical analysis, for the evaluation of bleaching stability.

Evaluation point	Control (AWM)	MI Paste Plus	Remin Pro	PAMAM
Pre/3 days	3.8±2.3^{Aa}	3.2±2.3^{Aa}	5.2±3.2^{Aa}	5±2.3^{Aa}
Pre/1 week	3.7±2.3^{Aa}	2.9±2.3^{Aab}	4.6±3.2^{Aab}	4.4±2.3^{Aab}
Pre/1 month	3.4±2.2^{Aab}	2.8±2.1^{Aab}	4±2.2^{Aab}	4.2±2.2^{Aab}
Pre/3 months	3.3±2.2^{Aab}	2.3±2.1^{Aabc}	3.6±2.2^{Ab}	4.1±2.1^{Aab}
Pre/6 months	3.1±2.2^{Ab}	2.1±2^{Abc}	3.6±2.2^{Ab}	3.5±2.1^{Ab}
Pre/9 months	3±2.2^{Abc}	1.8±2^{Ac}	3.6±2.2^{Ab}	3.4±2.1^{Ab}
Pre/1 year	2.5±2.2^{Ac}	1.7±2^{Ac}	3.6±2.2^{Ab}	3.3±2^{Ab}

Means with different capital superscript letters within each row, and small superscript letters within each column indicate statistically significant differences between experimental groups at p = 0.05

Figure 2 reveals no significant differences in ΔWI_D over follow-up times in AWM, MI, and PAMAM groups ($p > 0.05$). However, the RP group had significantly reduced ΔWI_D at 1 month compared to 3 days and 9 months ($p < 0.05$). A numerical drop in mean ΔWI_D, suggesting reduced whiteness, was found starting at 1 month in the AWM, MI, and PAMAM groups, and 1 week in the RP group.

Bleaching stability was confirmed with ΔL*, Δb*, and ΔWI_D values, which remained above the AT (1.25, 2.8, and 2.6, respectively) of these parameters in all groups and follow-up times, indicating no shade relapse to pre-bleaching values.

Figure 3 presents representative clinical photographs of patients treated with AWM (*Figures 3a and 3b*), MI (*Figures 3c and 3d*), RP (*Figures 3e and 3f*), and PAMAM (*Figures 3g and 3h*), captured prior to bleaching (*Figures 3a, 3c, 3e, and 3g*) and at the 1-year post-bleaching follow-up (*Figures 3b, 3d, 3f, and 3h*). The images demonstrate that, regardless of the remineralizing agent applied, the tooth shade did not return to its original pre-bleaching shade after one year of follow-up.

Limited to moderate agreements between the objective and subjective shade assessment methods were observed with a Kappa value of 0.106 and Kendall's W of 0.504 respectively.

DISCUSSION

The remineralizing enamel treatment applied after dental bleaching can help restore the enamel's natural translucency and repair enamel defects caused by bleaching. This results in a reduction in surface roughness and maintains teeth whiteness through the decrease of stain reuptake.[9,10]

Figure 2: Bar charts showing means and standard deviations of the ΔL* (blue chart), Δb* (yellow chart), and ΔWI_D (red chart) in relation to the pre-bleaching shade. Different small letters indicate statistically significant differences at p = 0.05 within each remineralizing agent.

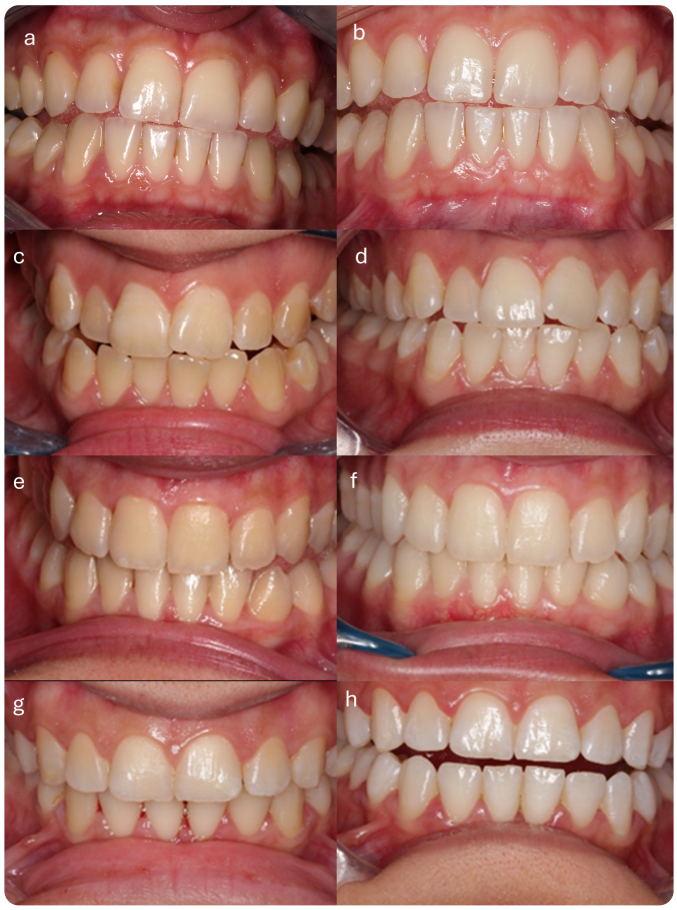

Figure 3: Representative photos of patients treated with: After Whitening Mousse (a and b), MI Paste Plus (c and d), Remin Pro (e and f), and PAMAM (g and h). Figures 3a, 3c, 3e, and 3g represent photos before bleaching, while Figure 3b, 3d, 3f, and 3h represent photos of 1-year post-bleaching follow-up.

Based on the results of the objective analysis, the 3-day post-bleaching shade was chosen as the reference shade for evaluating bleaching stability. Results of the 24-hour post-bleaching shade indicated a minimal color change, making this follow-up time not a suitable reference point. This may be due to dehydration and demineralization of the teeth, causing an increase in enamel surface porosities, which could lead to inaccurate VITA EasyShade readings due to the edge-loss effect.[44] On the other hand, the 3-day post-bleaching follow-up times indicated a significant shade change. This may be due to enamel rehydration and reduction of surface porosities through the effect of remineralizing agents and saliva, leading to more precise shade assessment.[9] This finding agreed with a previous study that reported higher ΔE values within a week from in-office bleaching.[45] The prolonged action of the perhydroxyl radical remnants in tooth structure may also explain the continued increase of ΔE.[45] Furthermore, although the ΔSGU showed a higher mean value at the 24-hour follow-up compared to the 3-day follow-up, reduced enamel translucency and increased opacity due to dehydration, along with increased surface roughness, may lead to false shade measurements. These measurements can rapidly change once normal enamel translucency is restored.[46]

The findings of this study confirmed the first null hypothesis regarding the effect of remineralizing agents on post-bleaching shade stability as no differences were observed between the four remineralizing agents, both objectively and subjectively, at any of the post-bleaching follow-up times. However, the second null hypothesis, which concerned the agreement between the objective and subjective shade evaluation methods, was rejected.

Objective analysis demonstrated that bleaching stability was maintained following the application of remineralizing agents, irrespective of the agent used. Although ΔE values decreased numerically over the follow-up period, the reduction remained within the acceptability threshold (AT), indicating no clinically significant color change.[26]

The remineralizing properties of all the agents used can explain their similar behavior regarding bleaching shade stability. The AWM, MI, and RP all contain sodium fluoride in their compositions, which could repair post-bleaching enamel defects through surface deposition of minerals and enhancement of fluorapatite formation.[5,10] In addition to sodium fluoride, MI contains CPP-ACP, which binds to enamel and provides a source of minerals needed for remineralization.[15] RP contains hydroxyapatite, which could repair enamel porosities due to its similarity to the composition of human enamel.[15] Conversely, PAMAM encourages enamel remineralization in a manner close to that of enamel proteins, acting as a scaffold and utilizing minerals found in saliva, forming highly organized HAP crystals.[16] Additionally, the bleaching agent used in this study had a slightly alkaline pH (pH=8-8.6 according to the product's safety data sheet), which is likely to cause less demineralization of enamel and possibly less susceptibility to re-staining, maintaining the bleached shade stability.[34]

The bleaching stability observed in the current study is consistent with several previous clinical studies.[7,34] Although hydrogen peroxide in-office bleaching was employed in the studies of Estay *et al.*[7] and Bersezio, *et al.*,[34] their main objective was to compare different hydrogen peroxide concentrations and bleaching gel pH levels without considering the impact of applying remineralizing agents. The results of this study were aligned with those of Tawfik *et al.*[13] They found that the use of a remineralizing agent containing amorphous calcium phosphate (ACP) in combination with bleaching resulted in better color stability up to 6 months after bleaching. However, these findings contrast with those of Alghonaimy *et al.*,[8] who reported shade relapse at 3 months following chemically activated in-office bleaching combined with a desensitizing agent containing potassium nitrate and sodium fluoride. This discrepancy may be attributed to differences in the concentration and composition of the bleaching agent, which can affect the degree of shade stability.

Although the ΔSGU results showed no relapse to the pre-bleaching shades, the numerical and statistical decrease in ΔSGU indicated a noticeable darkening of the shade. The ΔSGU values for MI at 9 months and 1 year were very close to the perceptibility threshold,[29] suggesting that bleaching stability was questionable subjectively for this agent. This highlights the limited agreement between the objective and subjective methods, indicating potential limitations in one or both methods of shade analysis. Nonetheless, none of the evaluation methods in this study showed a relapse to pre-bleaching shades up to the 1-year follow-up period.

An analysis of ΔL*, Δb*, and ΔWI_D was carried out to address one of the shortcomings of ΔE, which is the inability to identify the direction of color change.[23] Analysis of ΔL* and Δb* color parameters is especially crucial because most bleaching-related changes are caused by changes in these two parameters.[46,47] A slight reduction in lightness was observed after one week, possibly due to ongoing tooth rehydration and remineralization, which may increase enamel translucency and disclose the underlying dentin color.[48] The reversal of the bleaching process through the reduction of previously oxidized compounds, which could restore the tooth's original color, and the gradual re-staining of teeth over time from external chromogens present in the oral environment, could also be other possible explanations.[46] The negative Δb* values throughout the follow-up period indicated that the hydrogen peroxide bleaching might permanently break down the organic chromogens responsible for the increased chroma within the tooth structure.[46]

The numerical decrease in ΔWI_D may be attributed to enamel rehydration and remineralization following initial dehydration and demineralization, clinically perceived as shade darkening.[3,47] However, ΔWI_D values remained above the acceptability threshold (AT), further supporting the stability of the bleaching shade.

The limited to moderate agreement between the objective and subjective shade evaluation methods complicated the results interpretation, as the objective measurements indicated consistent bleaching stability, whereas ΔSGU values suggested reduced stability in some groups. This discrepancy, consistent with previous findings,[26,44] highlights potential limitations inherent in one or both assessment methods. Sensitivity to lighting conditions, difficulty in positioning the guide tip on convex surfaces, sensitivity to changes in tooth surface texture, and translucency can all affect the results of the objective method using the spectrophotometer.[27,49] The subjective method may be biased and fail to detect subtle color changes,[22,28] suggesting that the ΔSGU method may be inaccurate.[50] In addition, the differences between shades with the scale used in this study are not linear, so interpreting them as continuous data may not produce accurate findings.[26,40] Furthermore, it was reported that the VITA classical shade guide had coverage errors as high as 4.4 ΔE_{ab}, which is higher than the AT.[44] Despite their simplicity of use, the present shade guides do not cover the entire range of tooth colors, which makes it more difficult to match teeth shades exactly.[44,51]

One of the limitations in this study is the use of a single bleaching gel with a specific concentration and alkaline pH, which does not encompass the diversity of bleaching agents available on the market and may not fully reflect clinical conditions where multiple formulations are employed. The properties of the bleaching agent can influence shade stability. Nevertheless, the primary objective was to compare the effects of different remineralizing agents. Introducing multiple bleaching agents could compromise comparability, as their interactions with remineralizing agents may vary. Future studies should investigate how remineralizing agents interact with various bleaching formulations to better reflect clinical variability. Another limitation is the relatively short follow-up period. Although longer than in many previous studies, extending the follow-up beyond the one-year period used in this study could provide more comprehensive insights into the long-term stability of bleaching outcomes.

CONCLUSIONS

Based on the study's limitations, the following conclusions were drawn:

1. During the one-year follow-up period, no remineralizing agents compromised the post-bleaching shade stability.
2. The subjective and objective methods have limited to moderate agreement and cannot be utilized independently to measure shade stability in clinical settings.
3. Nevertheless, the use of remineralizing agents appears to be a critical post-bleaching clinical step, not only for their potential to reduce post-bleaching hypersensitivity, but also for maintaining shade stability over the follow-up period evaluated in this study.

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