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Dental bleaching efficacy and impact on demineralization susceptibility of simulated stained-remineralized caries lesions

Sarah S. Al-Angari[a,b], Frank Lippert[c], Jeffrey A. Platt[b], George J. Eckert[d], Carlos González-Cabezas[e], Yiming Li[f], Anderson T. Hara[c,*]

[a] Department of Restorative Dentistry, King Saud University, Riyadh, Saudi Arabia
[b] Department of Biomedical and Applied Sciences, Indiana University School of Dentistry, Indianapolis, IN, USA
[c] Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, Indianapolis, IN, USA
[d] Department of Biostatistics, Biostatistician Supervisor, Indiana University School of Medicine, Indianapolis, IN, USA
[e] Department of Cariology, Restorative Sciences and Endodontics, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
[f] Center for Dental Research, Loma Linda University School of Dentistry, Loma Linda, CA, USA

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ABSTRACT

Objectives: To evaluate the efficacy of different bleaching systems on artificially created stained-remineralized caries lesions; and to assess the susceptibility of the bleached lesions to further demineralization.
Methods: Human enamel specimens were sectioned, polished, demineralized, and randomly divided into six groups (n = 21) to create stained-remineralized lesions, either non-metallic (non-Met: G1, G2 and G3) or metallic (Met: G4, G5 and G6). G1 and G4 received no bleaching treatment, while G2 and G5 were treated with 15% carbamide peroxide (at-home bleaching protocol; 4 h/d × 7), and G3 and G6 with 40% hydrogen peroxide (in-office bleaching protocol; 20min × 3). Susceptibility to further demineralization was tested after bleaching treatment. Lesion mineral loss and depth were measured by transversal microradiography, and color change by spectrophotometry. Outcomes were analyzed using ANOVA models followed by Fisher's PLSD tests ($\alpha = 0.05$).
Results: Metallic-stained lesions were significantly darker (all $p < 0.001$) and more resistant to bleaching ($p < 0.005$) than non-Met ones. For both stain types, the at-home bleaching protocol was more effective than the in-office ($p < 0.005$); however, it also increased the lesion susceptibility to demineralization ($p < 0.05$) [$\Delta\Delta Z$ mean ± SD ranging from 205 ± 73 to 313 ± 188 (at home) vs. 132 ± 45 to 206 ± 98 (in office); $p < 0.05$]. After bleaching, non-Met lesions were significantly more susceptible to demineralization ($p < 0.05$), with the $\Delta\Delta Z$ ranging from 206 ± 98 to 313 ± 188 compared to Met lesions ranging from 132 ± 45 to 205 ± 73.
Conclusions: At-home bleaching protocol presented greater bleaching efficacy compared to in-office bleaching protocol. After bleaching, metallic-stained lesions were more resistant to subsequent demineralization compared to non-metallic stained lesions.
Clinical significance: Bleaching stained-arrested caries lesions may improve aesthetics but also increase susceptibility to demineralization, depending on the type of stain involved and bleaching system used.

1. Introduction

Aesthetic dentistry is considered an essential part of the restorative dental practice [1], yet options regarding aesthetic treatment of stained arrested caries lesions are few and mostly confined to surgical intervention [2]. The major drawback of invasive approaches is the potential contribution to the repeat restoration cycle [3]. Therefore, the development of minimally invasive treatment options would benefit the aesthetic treatment of stained arrested caries lesions.

Dental bleaching is considered a non-invasive, effective, safe, predictable and inexpensive procedure that may be indicated for the aesthetic treatment of these discolorations [4]. Despite the benefits of color improvement, bleaching has been reported to negatively affect the physical properties of the tooth structure, by decreasing surface

* Corresponding author at: Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, 415 Lansing Street, Indianapolis, IN 46202-2876, USA.
E-mail addresses: Sangari@ksu.edu.sa (S.S. Al-Angari), flippert@iu.edu (F. Lippert), jplatt2@iu.edu (J.A. Platt), geckert@iu.edu (G.J. Eckert), carlosgc@umich.edu (C. González-Cabezas), yli@llu.edu (Y. Li), ahara@iu.edu (A.T. Hara).

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hardness and increasing surface porosity [5,6]. These changes have been shown to potentially increase the susceptibility of the bleached enamel substrate to further demineralization [7].

We have previously reported dental bleaching to successfully improve the aesthetics of naturally stained arrested caries lesions [8]. We further explored this concept by developing a stained-remineralized-caries-like lesion (s-RCL) model and characterizing some of the enamel physical properties [9]. In this study, we evaluated the efficacy of different bleaching systems on artificially created metallic and non-metallic s-RCLs, as well as their post-bleaching susceptibility to demineralization.

2. Materials and methods

2.1. Experimental design

This study investigated three experimental factors: stain type at two levels (non-metallic and metallic), bleaching treatment at three levels (control, at-home and in-office), and the susceptibility of bleached s-RCL to demineralization at two levels (yes and no). The experimental units were human enamel slabs embedded in acrylic blocks and polished (n = 21 per treatment group). The study outcomes were color change (ΔE) measured at three time points (after demineralization, staining and bleaching); mineral content (ΔZ) and lesion depth (L) measured after staining; and mineral content change (ΔΔZ) and lesion depth change (ΔL) measured after a second demineralization. Color measurements were performed by optical spectrophotometry, while mineral loss and lesion depth were measured by transverse microradiography (TMR).

2.2. Specimen preparation

Enamel slabs (4 × 4 × 2 mm) were sectioned from the buccal and lingual area of human molars using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). After collection and during the preparation process, the teeth were stored in 0.1% thymol solution. The bottom and top sides of the slabs were sequentially ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, Struers Inc., Cleveland, OH, USA). One hundred twenty six specimens were embedded in an acrylic resin (Varidur; High Performance Mounting Kit; Buehler, Lake Bluff, IL, USA). The specimen surface was flattened and sequentially ground using #500-, 1200-, 2400- and 4000-grit silicon carbide papers (MDFuga, Struers Inc., Cleveland, OH, USA), polished with 1-μm diamond suspension (DP-Suspension P, Struers Inc., Cleveland, OH, USA) and sonicated in a detergent solution (Micro-90, International Products Corporation, Burlington, NJ, USA). Following the polishing procedure, the specimens were placed under running deionized water for 3 min. Specimens were stored in moist conditions at 4 °C in a refrigerator (Kenmore; Whirlpool, Benton Harbor, MI, USA).

2.3. Caries-like lesion creation

Enamel caries-like lesions were initiated in a carboxymethylcellulose demineralizing solution as described by Lippert et al. [10]. Briefly, all specimens were demineralized for seven days in a solution containing 0.1 M lactic acid, 4.1 mM Ca (as CaCl2. 2H2O), 8.0 mM PO4 (as KH2PO4) and 1.0% w/v carboxymethylcellulose (Sigma-Aldrich Co., St. Louis, MO, USA) with pH adjusted to 5.0 using KOH at 37 °C.

2.4. Staining, remineralization and cycling

After demineralization, specimens were subdivided into six groups based on the staining, treatment and bleaching protocol (Table 1). Groups 1, 2 and 3 (non-metallic stain) were incubated in a combined coffee (Instant Folger Crystals, Classic roast, The Folger Coffee Company Inc., Orrville, OH, USA) and tea (Nestea, Nestle Inc., Glendale, CA, USA) solution prepared based on the manufacturer's instructions and used immediately after preparation. Specimens were kept in a stirring staining solution at ˜37 °C for 8 h, rinsed, allowed to air dry and treated with 2% sodium fluoride gel (NaF; Sultan Healthcare Inc., York, PA, USA) for 4 min, before being immersed in artificial saliva overnight. Groups 4, 5 and 6 (metallic stain) were stained based on the protocol described by Stookey et al. [11]. Specimens were placed on a rotating rod (˜37 °C incubator), which alternately exposed them to air and to a solution (800 ml / cycle, total of 4 l) consisting of trypticase soy broth, *Micrococcus luteus* BA13 (American Type Culture Collection, Manassas, VA, USA), coffee, tea, gastric porcine mucin (American Laboratories, Omaha, NE, USA), and ferric chloride (Fisher Scientific, Fair Lawn, NJ, USA). After each cycle (8 h/day for five days) specimens were rinsed, allowed to air dry, treated with 38% silver diamine fluoride (SDF; Advantage Arrest, Elevate Oral Care LLC, West Palm Beach, FL, USA) for 2 min [12] and immersed in artificial saliva overnight.

Artificial saliva (2.20 g/l gastric mucin, 1.45 mM CaCl2.2H2O, 5.40 mM KH2PO4, 28.4 mM NaCl, 14.9 mM KCl, pH 7.0) [10] was used as the remineralizing medium.

Table 1
Group definitions based on the staining/remineralization and bleaching protocol.

Groups	Type of staining	Remineralization protocol	Bleaching protocol	2nd Demineralization	n
G1	Non-Metallic	2% NaF	Control	Yes	11
				No	10
G2	Non-Metallic	2% NaF	At-home	Yes	11
				No	10
G3	Non-Metallic	2% NaF	In-Office	Yes	11
				No	10
G4	Metallic	38% SDF	Control	Yes	11
				No	10
G5	Metallic	38% SDF	At-home	Yes	11
				No	10
G6	Metallic	38% SDF	In-Office	Yes	11
				No	10

2% NaF: Sodium fluoride gel, 38% SDF: Silver diamine fluoride solution, At-home: 15% Carbamide peroxide bleaching gel, In-Office: 40% Hydrogen peroxide bleaching gel.

2.5. Color assessment

L*a*b* values (Commision Internationale de l'Eclairage) were acquired for each specimen at baseline, and immediately after demineralization, cycling and bleaching. All measurements were performed by one examiner using a spectrophotometer (Minolta Chromameter CR-241, Minolta Camera Co., Osaka, Japan; light beam diameter of 0.3 mm), and repeated three times.

The color difference (ΔE) was calculated using the following equation, representing color changes after demineralization (ΔEDemin: demineralization-baseline), cycling (ΔECycling: staining-demineralization) and bleaching (ΔEBleaching: bleaching-staining):

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

where ΔE is the color difference and ΔL*, Δa* and Δb* represent changes in lightness, red- green coordinate, and yellow-blue coordinate, respectively.

2.6. Lesion mineral content and lesion depth

Specimens were mounted on plastic rods and sectioned with a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe, Scientific Fabrications Laboratories, Lafayette, CO, USA).

One section, 100 µm +/- 20 µm, was obtained from each specimen. The sections were kept moist to prevent dehydration, and were mounted with an aluminum stepwedge on high- resolution glass plates Type I A (Microchrome Technology Inc., San Jose, CA, USA) then X-rayed at 20 kV and 30 mA at a distance of 42 cm for 65 min.

The exposed plate was developed (in a Kodak d-19 developer) for 3 min, placed in a stop bath (Kodak 146-4247) for 45 s, and then fixed (Kodak 146-4106) for 3 min. All plates were then rinsed in deionized water for 15 min and air-dried. Microradiographs were examined under a microscope (Zeiss EOM, Hitachi Denshi Ltd., Thornwood, NY, USA) in conjunction with the TMR software v.3.0.0.11 (Inspektor Research Systems BV, Amsterdam, The Netherlands). A region of interest (approx. 400 × 400 µm) in the central area of the lesion area and not containing any cracks, debris, or other artifacts was selected for analysis. Sound enamel was defined as 87% mineral volume [13], in order to determine overall mineral loss (ΔZ) and mean lesion depth (L).

2.7. Dental bleaching efficacy

Groups 1 and 4 (control) were kept moist in an incubator at 37 °C for 8 h, then stored moist at 4 °C. Groups 2 and 5 were treated with at-home bleaching protocol, 15% carbamide peroxide (CP) home bleaching agent (pH 6.5; Opalescence PF, Ultradent Products, Inc., South Jordan, UT, USA). The bleaching gel was applied onto the top enamel surface of each specimen (0.5–1.0 mm thick) and kept for 4 h each day [1] in an incubator at 37 °C for a total treatment of 7 days. After bleaching, specimens were rinsed with running distilled water for 1 min to remove the bleaching agents, blot dried and stored in a moist environment at 4 °C.

Groups 3 and 6 were bleached using in-office bleaching protocol, using 40% hydrogen peroxide (pH 6.0–8.5; Opalescence Boost, Ultradent Products, South Jordan, UT, USA). A 0.5–1.0 mm thick layer of the bleaching gel was applied to the specimen surface. After 20 min, the bleaching gel was wiped using a cotton pellet. This bleaching treatment was repeated in the same session two additional times (total of 60 min of treatment time, as recommended by manufacturer's instructions). After the third bleaching application each specimen was rinsed with running deionized water for 1 min, blot-dried and stored moist at 4 °C, until color measurements.

2.8. Post-bleaching demineralization

Each group (n = 21) was further divided into two subgroups, either submitted to a second demineralization (n = 11) or not (n = 10).

The sides of each sample (demineralized group) were covered by nail polish, and further demineralized utilizing the same demineralization protocol used previously for a total of seven days. TMR assessed the final mineral content (ΔZ) and depth (L) of the lesions, which were used to calculate the mineral loss change (ΔΔZ) and lesion depth change (ΔL), according to the following equations: $\Delta\Delta Z = \Delta Z_{baseline} - \Delta Z_{post}$; $\Delta L = L_{post} - L_{baseline}$.

2.9. Statistical analysis

The color changes (after demineralization, after staining, and after bleaching), mineral loss and lesion depth changes (after staining and after second demineralization) were analyzed using four-way ANOVA, with factors for stain type, bleach treatment, susceptibility to second demineralization, and time points, as well as all two-way, three-way and four-way interactions among the factors. The same specimens measured at different times were allowed to have different variances.

The mineral loss change (ΔΔZ) and lesion depth change (ΔL) were analyzed using three-way ANOVA with factors for stain type, bleach treatment and susceptibility to second demineralization, as well as two-way and three-way interactions among the factors. All pair-wise comparisons from ANOVA analyses were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5%.

Table 2
Color change (ΔE) means (standard-deviation) after demineralization, staining, and bleaching.

Groups		ΔE_{Demin} [+]		$\Delta E_{Staining}$ [+]		$\Delta E_{Bleaching}$ [+]	
Non-Metallic	G1 (Control)	4.0 (2.0)	A/ab	18.5 (3.1)	B/a	5.6 (4.0)	A/a*
	G2 (At-home)	3.0 (1.5)	A/a	16.7 (3.8)	B/a	20.6 (3.9)	C/b
	G3 (In-office)	5.0 (2.3)	A/b*	17.3 (3.6)	B/a	17.1 (3.5)	B/c
Metallic	G4 (Control)	6.4 (3.8)	A/a	58.3 (4.8)	B/a	8.3 (4.2)	A/a*
	G5 (At-home)	5.0 (3.0)	A/a	58.2 (3.2)	B/a	29.0 (4.2)	C/b
	G6 (In-office)	6.2 (2.9)	A/a*	59.1 (3.9)	B/a	23.3 (6.6)	C/c

Uppercase letters indicate significant difference within treatment (row, $p < 0.05$); while lower case between treatments (column, $p < 0.05$).

* Indicate no significant difference between stain type (Non-metallic vs. Metallic).

[+] ΔEDemin: demineralization-baseline, ΔEStaining: staining-demineralization and ΔEBleaching: bleaching-staining.

3. Results

3.1. Color

Means of color change (ΔE) were significantly different within stain types, bleaching treatments and time points ($p < 0.0001$), but were not significant within susceptibility to 2nd demineralization.

Metallic stains (G4, G5 and G6) presented significantly ($p \leq 0.001$) darker stains after staining/remineralization cycling compared to non-metallic stains, however they were significantly more difficult to bleach ($p \leq 0.005$). The at-home bleaching protocol in both non-metallic and metallic groups (G2 and G5) was more ($p < 0.005$) effective (indicating lighter color) than the in-office bleaching protocol. The mean values of ΔE and comparisons within and among treatments for stain types, bleaching treatments and time points are in Table 2.

3.2. Mineral loss and lesion depth

The mineral loss (ΔZ) after the staining/remineralization/cycling and mineral loss change (ΔΔZ) after the 2nd demineralization were significantly different regarding stain types, bleaching treatments, susceptibility to 2nd demineralization and time points ($p \leq 0.05$). The lesion depth (L) and lesion depth change (ΔL) after cycling and 2nd demineralization were significantly different regarding stain types, bleaching treatments, susceptibility to 2nd demineralization and time points ($p \leq 0.001$).

After the 2nd demineralization, the non-demineralized bleached subgroups: G2, G3, G5 and G6 (non-metallic/metallic) had significantly ($p \leq 0.05$) more mineral loss change (ΔΔZ) compared to G1 and G4 (control). After cycling, ΔZ and L were significantly ($p \leq 0.05$) lower in metallic stains compared to non-metallic stains for all groups, which indicate a better remineralization of SDF compared to NaF.

Within each treatment subgroup (G1-G6), demineralized subgroups had significant increases in ΔΔZ ($p < 0.001$) and ΔL ($p \leq 0.05$) compared to non-demineralized subgroups (more susceptible to demineralization) except for ΔΔZ in G6 and ΔL in G5. Regarding subgroups submitted to 2nd demineralization, non-metallic stains (G1, G2 and G3) had significant increases in ΔΔZ compared to metallic stains (G4, G5 and G6) in regards to in-office and at-home bleaching groups ($p < 0.05$). Within bleaching treatments submitted to the 2nd

Table 3
Lesion mineral content (ΔZ) means (standard-deviation) after remineralization/staining protocol, and mineral content change (ΔΔZ) after second demineralization.

ΔZ (vol%min × μm)			ΔΔZ (vol%min × μm)[+]			
Groups		After cycling	Non-demin		Demin	
Non-Metallic	G1 (Control)	525 (87)[§]	−11 (12)	A/a[*]	121 (72)	B/a[*]
	G2 (At-home)	564 (212)[§]	96 (91)	A/b[*]	313 (188)	B/b
	G3 (In-office)	557 (75)[§]	75 (43)	A/b[*]	206 (98)	B/c
Metallic	G4 (Control)	460 (47)[§]	−8 (42)	A/a[*]	126 (43)	B/a[*]
	G5 (At-home)	474 (49)[§]	93 (33)	A/b[*]	205 (73)	B/b
	G6 (In-office)	487 (62)[§]	70 (34)	A/b[*]	132 (45)	A/a

Uppercase letters indicate significant difference within treatment (row, $p < 0.05$); while lower case among treatments (column, $p < 0.05$).
[+] ΔΔZ = ΔZ TMR bleaching/2nd demin – ΔZ TMR cycling.
[*] Indicate no significant difference between stain type (Non-metallic vs. Metallic).
[§] Indicate significant difference between stain type.

Table 4
Lesion depth (L) means (standard-deviation) after remineralization/staining protocol, and lesion depth change (ΔL) after second demineralization.

L Lesion depth (μm)			ΔL Lesion depth (μm)[+]			
Groups		After cycling	Non-demin		Demin	
Non-Metallic	G1 (Control)	33.8 (12.7)[§]	−1.0 (2.1)	A/a[*]	16.3 (18.9)	B/a
	G2 (At-home)	32.2 (9.2)[§]	6.0 (11.0)	A/ab[*]	39.4 (17.1)	B/b
	G3 (In-office)	30.0 (6.4)[§]	8.3 (7.6)	A/b[*]	19.3 (10.1)	B/a[*]
Metallic	G4 (Control)	23.9 (5.4)[§]	−2.9 (4.4)	A/a[*]	5.8 (4.5)	B/a
	G5 (At-home)	25.3 (4.2)[§]	5.7 (6.0)	A/a[*]	9.1 (4.3)	A/a
	G6 (In-office)	24.7 (6.9)[§]	5.2 (5.3)	A/a[*]	18.0 (10.6)	B/b[*]

Uppercase letters indicate significant difference within treatment (row, $p < 0.05$); while lower case among treatments (column, $p < 0.05$).
[*] Indicate no significant difference between stain type (Non-metallic vs. Metallic).
[§] Indicate significant difference between stain type.
[+] ΔL = (L TMR bleaching/2nd demin – L TMR cycling).

demineralization, at-home bleaching protocol (metallic/non-metallic groups) demonstrated significant increase in ΔΔZ ($p < 0.001$) compared to in-office ΔΔZ ($p = 0.05$). The numerical values and comparisons within and among treatments for stain types, bleaching treatments and time points are in Tables 3 and 4 respectively.

4. Discussion

This study tested the efficacy of two different bleaching systems using a previously developed remineralized-stained caries like-lesions in-vitro model. This allowed for better standardization of the lesions, reducing variability and resulting in a well-controlled test of color change, mineral loss and lesion depth.

Color change (ΔE) was used to verify the creation of stained lesions and bleaching efficacy of the tested systems/techniques. Johnston and Kao (1989) speculated that ΔE values exceeding 3.3 units are clinically perceptible [14]. In this regard, the ΔE range in this study after staining (non metallic: 16.7–18.5; metallic: 58.2–59.1) suggests remarkable clinically visible changes. Both the Non-Met and Met groups became significantly darker, as indicated by the decrease in L* values (Supplementary data, Table) compared to the color measured before the staining/remineralizing cycles. These results indicated successful incorporation of the stains during remineralization of the created lesions.

Metallic stained lesions (grey/black) were significantly darker than non-metallic ones (orange/brown), which can be explained by their different chemical composition (metallic versus organic stains) [8]. They were also significantly more difficult to bleach, possibly due to the mechanism of action of peroxides, as they tend to oxidize organic chromogens present in non-metallic stains. Conversely, metallic compounds cannot be easily degraded by oxidation [8,15]. Furthermore, it might be explained by the reduced porosity due to SDF treatment and incorporation of silver and fluoride, resulting in a comparatively less soluble surface protective layer, which in turn caused a lower response to the bleaching agent and minimized demineralization within the lesions [16].

The bleaching products tested contained 15% CP (at-home) or 40% HP (in-office). The at-home bleaching protocol caused significant color improvement in both metallic and non-metallic s-RCLs compared to in-office, despite the difference in concentration of hydrogen peroxide [15% CP (~5% HP) versus 40% HP)]. However, it should be kept in mind that the at-home bleaching protocol had longer time exposure (4 versus 1 h) and application duration (7 versus 1 day), which resulted in a total of 28 h exposure compared to only 1 h for the in-office treatment. This result corroborates previous studies suggesting that longer enamel bleaching time caused better color improvement regardless of the concentration of bleaching agents [17–22]. In this regard, in-office bleaching systems with longer application time (more than one clinical session) might be more appropriate and should be considered in future tests.

Existing literature reports mixed findings considering the side effects of bleaching on the increase of enamel susceptibility to demineralization [5,7,15,23]. The contrasting results may be due to differences in study variables, including substrate type, the bleaching gel treatment protocol (concentration, treatment time) and the different evaluation techniques. In our study, we observed that the mineral content change (ΔΔZ) was significantly higher in bleached groups compared to control in both non-metallic and metallic groups. As the bleaching agent contacts the tooth structure it develops focal areas of very shallow demineralization [24]. This indicated that bleaching promoted mineral loss from enamel structure, which is in agreement with previous studies [6,25,26]. Some studies have shown that the increase in enamel susceptibility to demineralization after bleaching can be reduced when specimens were stored in saliva (human or artificial), simulating what would happen in the mouth [27–29]. In our study, saliva was not used during bleaching, as we focused on the effect of different bleaching systems solely by eliminating other variables.

After the 2nddemineralization, non-metallic stained lesions showed significantly higher mineral loss change (ΔΔZ) when bleached by the at-home protocol (G2) followed by in-office (G3) and then the control group, which supports the findings after bleaching. The at-home bleaching protocol had longer surface time exposure, which resulted in deeper penetration of the gel into the tooth structure and more stain oxidation. This process resulted in better color improvement, but also undesirably higher mineral loss change compared to in-office bleaching [30] as metallic groups also showed significantly more demineralization (mineral loss and lesion depth) when treated with the at-home bleaching protocol.

This study focused on selective extrinsic stains, represented in coffee and tea (non-metallic stains) along with silver and iron (metallic stains). Other types of extrinsic stains (tobacco, chlorhexidine, etc.) should be further investigated to derive more generalizable conclusions. Furthermore, despite the numerous advantages of the model used

in our study, it cannot fully reproduce the complex and diverse intra-oral biological conditions involved in the generation of stained and remineralized caries lesions in vivo. Consequently, general clinical relevance extrapolated from these in vitro models should be done with caution.

In an attempt to combine efficacy and safety, dental practitioners should carefully consider selecting appropriate cases of s-RCLs for treatment with bleaching agents. Metallic stains treated with in-office bleaching protocol might need further bleaching treatment (more than one visit) or at-home bleaching for longer time periods. Furthermore, fluoride or other remineralizing agents should be considered with such treatments to minimize the possibility of demineralization.

5. Conclusion

The results of this laboratory study suggest that at-home bleaching (15% CP) leads to greater color improvement of remineralized-stained lesions than in-office bleaching (40% HP). However, results also suggest that bleaching remineralized-stained lesions increases their susceptibility to subsequent demineralization, especially in non-met stained lesions (coffee and tea stains).

Declaration of Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jdent.2018.12.008.

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