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Original Article

Effects of microabrasion and bleaching on color and shear bond strength of simulated stained-remineralized caries lesions

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ABSTRACT

Purpose: This study investigated the effects of bleaching and microabrasion on the color and shear bond strength (SBS) of stained-remineralized caries-like lesions (s-RCLs).**Methods:** Human enamel specimens were demineralized, stained (coffee/tea), then remineralized (2% NaF and artificial saliva [AS]) to create s-RCLs. Specimens were randomly divided into five groups (n = 18): G1, demineralized/AS; G2, s-RCLs/AS; G3, s-RCLs/at-home bleaching (15% carbamide peroxide [CP]), 6 h/d×7/AS; G4, s-RCLs/microabrasion (6.6% hydrochloric acid, [1min/3cycles]/AS; and G5, s-RCLs/microabrasion and at-home bleaching/AS. Color was spectrophotometrically measured at baseline, after demineralization, after staining, and after treatment. After two weeks, the SBS was tested using a universal testing machine. Outcomes were analyzed using ANOVA models followed by Tukey's test ($\alpha = 0.05$).**Results:** The mean colors (ΔE) for demineralization and staining were significant ($\Delta E \leq 5.9$ and ≤ 14.4 , respectively). G3 (ΔE 23.9) and G5 (ΔE 25.2) were significantly improved compared to G4 (ΔE 12.3). The SBS in G5 had the highest significant value (25.2 MPa), followed by G4 and G3 (21.5–20.6 MPa), which were significantly higher than G2 (16.8 MPa). G2, in turn, was more significant than G1 (10.9 MPa).**Conclusion:** At-home bleaching (15% CP) for seven days eliminated and improved organic stains on RCLs. Faster results were achieved when combined with microabrasion. All surface treatments resulted in high SBS.

1 Introduction

With the improvement of dental materials and technologies, minimally invasive (MI) approaches have shifted clinical focus from surgical intervention to conservative esthetic modalities (bleaching, microabrasion, resin infiltration, etc.) (Al-Angari, 2021; Jingarwar et al., 2014). The MI approach to non-cavitated caries lesions, such as white spot lesions, includes remineralization therapy (fluoride-containing products) and behavioral modifications to halt the caries process (Pitts et al., 2017; Yu et al., 2021). Arrested caries lesions (ACLs) are highly mineralized surfaces with an unesthetic dark discoloration due to pigment incorporation during the remineralization process (coffee, tea, chromogenic bacteria, etc.) (Watts and Addy, 2001), however, they are otherwise inactive and require no surgical treatment unless they affect form, function and esthetics (Al-Angari, 2021).

The unesthetic appearance of the stained ACLs is a concern for some

patients (Ahmad, 2010). In such cases, clinicians perform surgical intervention by removing a significant amount of sound tooth structure to restore and mask the discoloration (Holmgren et al., 2014). The unnecessary removal of tooth substrate is considered aggressive. Furthermore, inadequate restorations may result in poor bonding strength, microleakage, secondary caries, and possible periodontal diseases (Blum and Özcan, 2018). Therefore, it is sensible to take conservative approaches as the first line of treatment to improve the tooth's color and allow for a good bonding seal.

Bleaching and microabrasion are MI procedures indicated to improve the color of stained teeth (Perete-de-Freitas et al., 2017). Bleaching is a simple and affordable MI method proven successful in treating stained ACLs (Al-Angari and Eisa, 2020; Al-Angari et al., 2021, 2019a; Al-Angari and Hara, 2016). It is attributed to the action of oxidative free radicals, which break the double bonds of the ACLs' chromophore molecules, alter their structures, and improve their color

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lightness, which appears more pronounced as the ACL gradually blends with its unstained surrounding enamel (Al-Angari and Eisa, 2020; Al-Angari et al., 2021, 2019a; Al-Angari and Hara, 2016). Microabrasion, on the other hand, improves the color outcome by eliminating the porous subsurface enamel layer and its entrapped stains, resulting in a more regular, lustrous surface. (Pini, 2015). However, the effects of these procedures on restorative materials' shear bonding strength (SBS) to treated stained-remineralized caries-like lesions (s-RCLs) have not been explored. The present *in-vitro* study investigated the effects of the MI approaches (bleaching and microabrasion) on s-RCLs' color and SBS. The null hypothesis tested was that MI approaches do not affect s-RCLs' color or SBS.

2. Materials and methods

2.1. Experimental design

This study created artificial enamel caries-like lesions, followed by staining and remineralization to create s-RCLs. The experimental factor was esthetic treatments at five levels: negative control, positive control, at-home bleaching, microabrasion, microabrasion and at-home bleaching. The outcomes were color change (ΔE) and SBS (MPa). Color measurements were performed using spectrophotometry, SBS was done using a universal testing machine, and Scanning Electron Microscopy (SEM) was utilized to assess the surface topography (Fig. 1).

2.2. Specimen preparation

Enamel slabs ($4 \times 4 \times 2$ mm) were chosen from the buccal and lingual surfaces of human molars after approval by the King Saud University Institutional Review Board (IRB# E-22-6639). Specimens ($n = 90$), sound and free of cracks or defects, were sectioned 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 slabs were ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, Struers Inc., Cleveland, OH, USA). Then specimens were embedded in an acrylic resin (Varidur; Buehler, Lake Bluff, IL, USA), and their surfaces were flattened and grounded using #500-, 1200-, 2400- and 4000-grit silicon carbide papers (MDFuga, Struers Inc., Cleveland, OH, USA). After which, they were polished with 1- μ m diamond suspension (DP-Suspension P, Struers Inc., Cleveland, OH, USA) and sonicated in a detergent solution (Micro-90, International Products Corporation, Burlington, NJ, USA). Then placed under running deionized water for 3 min. and stored in moist conditions at 4 °C in a refrigerator (Kenmore; Whirlpool, Benton Harbor, MI, USA).

2.3. Caries-like lesion creation

The specimens were placed in a carboxymethylcellulose demineralizing solution, as described by Lippert *et al.* (2011) (Al-Angari et al., 2019a). All specimens were demineralized for seven days in a solution containing 0.1 M lactic acid, 4.1 mM Ca (as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 8.0 mM PO_4 (as KH_2PO_4), and 1.0 % w/v carboxymethylcellulose (Sigma-Aldrich Co., St. Louis, MO, USA) with pH adjusted to 5.0, at 37 °C.

2.4. Staining, remineralization, and treatment

Following demineralization, Group 1 (negative control) received no treatment and was stored in artificial saliva (AS); 2.20 g/l gastric mucin, 1.45 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5.40 mM KH_2PO_4 , 28.4 mM NaCl, 14.9 mM KCl, pH 7.0) (Al Dehailan et al., 2016; Al-Angari et al., 2019a;). Groups 2–5 were subjected to a previously described protocol to create the s-RCLs (Al-Angari et al., 2019a, 2019b). Specimens were incubated in coffee (The Folger Coffee Company Inc., Orrville, OH, USA) and tea (Nestea, Nestle Inc., Glendale, CA, USA) solutions prepared based on the manufacturer's instructions and used immediately after preparation (Al-Angari et al., 2019b, 2019a). They were kept in a stirring staining

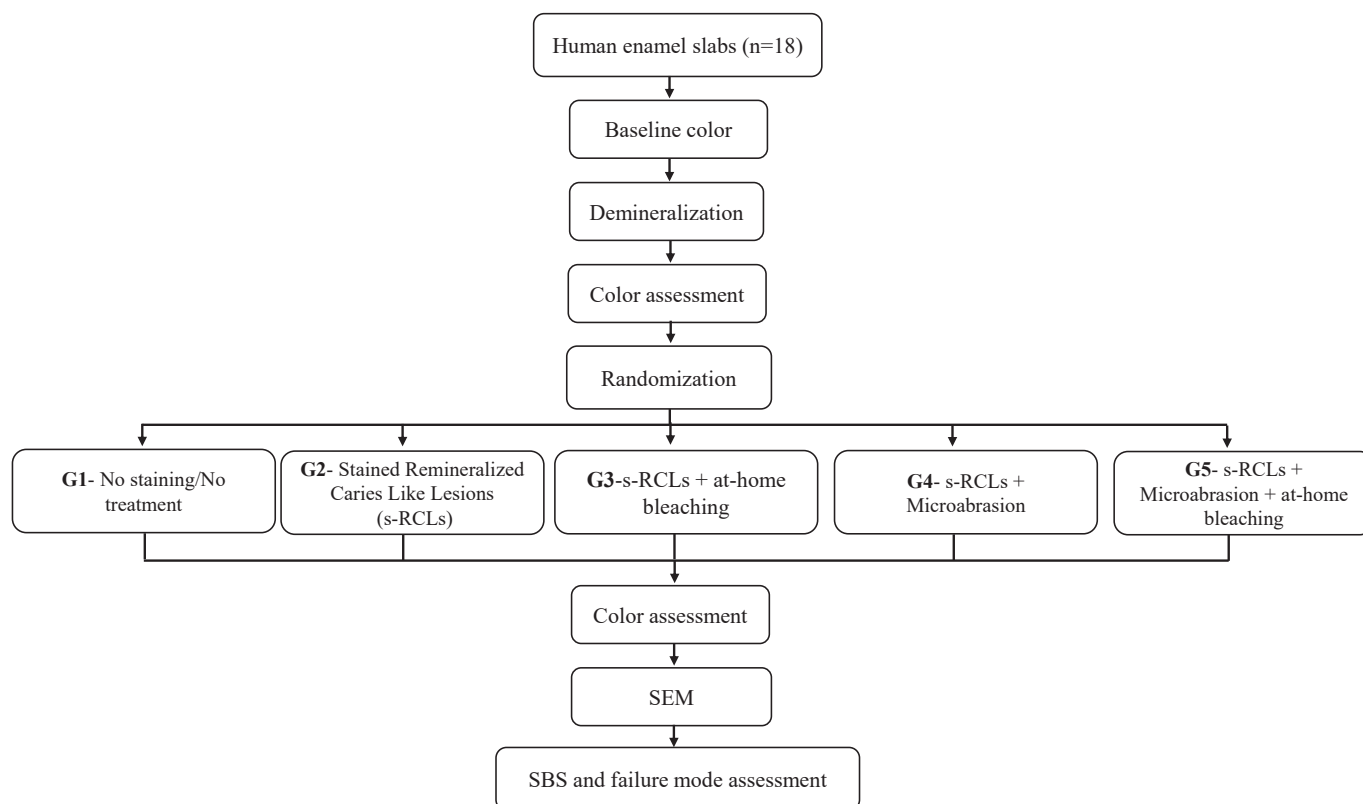


Fig. 1. Flow chart presenting the study design.

solution at ~ 37 °C for 8 h, rinsed, allowed to dry, and treated with 2 sodium fluoride (NaF) gel (Medicom, New York, NY) for 4 min, then immersed in AS overnight for five consecutive days (Al-Angari et al., 2019a). Subsequently, specimens were randomly divided into five groups based on the treatment protocol (Table 1). Group 1; (negative control), demineralized only, group 2; (positive control) s-RACL, group 3; at-home bleaching protocol, group 4; microabrasion, and group 5; microabrasion and at-home bleaching protocol. All specimens were kept in AS throughout the experiment, which was changed daily (Table 2).

2.5. Color assessment

$L^*a^*b^*$ values (Commission Internationale de l'Éclairage) were taken for each specimen at baseline, after demineralization, staining, and after the assigned treatments. One examiner performed all measurements, which were repeated three times, using a spectrophotometer (ColorEye 7000A, GretaMacBeth LLC, NY USA; light beam diameter of 0.3 mm). The color difference (ΔE) was calculated after demineralization (ΔE_{Demin} : demineralization-baseline), after staining ($\Delta E_{\text{Staining}}$: staining-demineralization), and after the assigned treatments ($\Delta E_{\text{Treatment}}$: treatment-staining):

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

2.6. Dental microabrasion test

Microabrasion (G4 and G5) was performed prior to bleaching (Sundfeld et al., 2014a, 2007) by the application of the microabrasive slurry (6.6% hydrochloric acid, Opalustre, Ultradent Products Inc, South Jordan, UT, USA), using a prophylaxis rubber (1.0 mm thick) three times per specimen at 60-second interval (Wang et al., 2020). Then, specimens were polished with a fluoridated prophylaxis paste (Vigodent Coltene SA Indústria e Comércio; Rio de Janeiro, RJ, Brasil) and a 2% neutral-pH NaF gel (Medicom Denticare, Pointe-Claire, Montreal, Quebec, Canada) was applied for 4 min based on the manufacturer's instructions (Sundfeld et al., 2014a).

2.7. Dental bleaching efficacy

Groups 3 and 5, bleaching was performed immediately after microabrasion, were bleached using 15 % CP (pH 6.5; Opalescence PF, Ultradent Products, Inc., South Jordan, UT, USA). The gel was applied on the surface (0.5–1.0 mm thick) and kept for 6 h daily in an incubator at 37 °C for seven days. After each bleaching cycle, specimens were rinsed with running distilled water for 1 min to remove the bleaching agents, blot-dried, and immersed in AS overnight for the next day's treatment.

2.8. Shear bond strength testing

Two weeks after the experiment, resin-based composite (Filtek™ Z350, Shade A2, 3M ESPE, Neuss, Germany) was incrementally built on the enamel's surface using a silicone mold (Ø: 3 mm; height 2 mm).

Table 1

Group definitions based on the treatment protocol used.

Groups	Treatment protocol
G1	Demineralized (negative control)
G2	s-RCLs (positive control)
G3	s-RCLs + At-home bleaching
G4	s-RCLs + Microabrasion
G5	s-RCLs + Microabrasion + At-home bleaching

*At-home bleaching: 15% Carbamide peroxide bleaching gel, Microabrasion: 6.6% hydrochloric acid/pumice. s-RCLs; Stained Remineralized Caries-Like Lesions.

Table 2

Color change (ΔE) means (standard deviation) after demineralization, staining, and treatment.

Groups	ΔE_{Demin} ⁺		$\Delta E_{\text{Staining}}$ ⁺		$\Delta E_{\text{Treatment}}$ ⁺	
G1	5.1 (1.6)	A,a	6.7 (1.6)	A,a	6.6 (1.4)	A,a
G2	5.5 (1.9)	A,a	14.4 (3.1)	B,b	14.8 (1.2)	B,c
G3	5.3 (1.7)	A,a	14.1 (2.5)	B,b	23.9 (9.7)	C,d
G4	5.9 (3.0)	A,a	14.2 (3.2)	B,b	12.3 (2.5)	B,b
G5	5.6 (2.2)	A,a	12.8 (4.2)	B,b	25.3 (8.7)	C,d

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

ΔE_{Demin} ⁺: demineralization-baseline, $\Delta E_{\text{Staining}}$ ⁺: staining-demineralization, and $\Delta E_{\text{Treatment}}$ ⁺: treatment-staining.

G1: Negative Control (demineralized), G2: Positive control (Stained Remineralized Caries-Like Lesions s-RCLs), G3: s-RCLs + At-home bleaching, G4: s-RCLs + Microabrasion, G5: s-RCLs + Microabrasion + At-home bleaching.

Enamel was etched for 15 s (37 % phosphoric acid, N-Etch Etching gel, Ivoclar Vivadent, Schaan, Liechtenstein), then the bonding agent (Tetric N-Bond Universal, Ivoclar Vivadent, Schaan, Liechtenstein) was light-cured for 20 s (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with a diameter of 9 mm, at a light irradiance of 1,000 mW/cm² measured by a radiometer (Bluephase meter, Ivoclar Vivadent, Schaan, Liechtenstein). After removing the mold, additional light curing was carried out for 20 s on all sides of the build-up at zero mm distance from the sample.

Specimens were stored in distilled water for 24 h at 37 °C, then mounted on the universal testing machine (Instron 5965, Instron Corporation, Norwood, MN, USA) and subjected to shear force using a knife edge chisel at a cross-speed of 0.5 mm/min until failure occurred. After debonding, the failure modes were assessed using a digital microscope at ×50 magnification (Hirox Co. Ltd., Tokyo, Japan). It was classified as follows: adhesive, failure between the resin-based composite and the enamel surface; cohesive, failure within the resin-based composite; and mixed failure that is partially adhesive and partially cohesive.

2.9. Scanning electron microscopy (SEM)

Three specimens/group were randomly selected to evaluate the surface topography of enamel after the assigned treatment protocols and before the SBS test. Specimens were irrigated with 5 mL of distilled water, sonicated in deionized water for 10 min, desiccated for 48 h, and sputter-coated for 2 min with gold/palladium; then images were taken (JEOL 6390 LV SEM, Peabody, MA, USA) at ×13, ×75, and ×200 magnification.

2.10. Statistical analysis

The color change (ΔE), within time points and among groups, was analyzed using one-way ANOVA repeated measurement, followed by Tukey's as a pairwise multiple comparison test ($\alpha = 0.05$). The SBS was analyzed using one-way ANOVA ($\alpha = 0.05$), followed by Tukey's multiple comparison test and Chi-square test for the failure mode. Statistical analysis was performed using SPSS software (IBM SPSS Statistics v.26, IBM, Armonk, NY, USA). Prior to the study, a sample size calculation of 15/group had a power of 90 % to detect a 0.47 difference between groups, assuming a 5% significance level, with an additional 15 specimens for the SEM test.

3 Results

The Shapiro-Wilk test showed that normality was satisfied ($p > 0.05$), and homogeneity of the variance test (Levene test) was satisfied ($p = 0.169$). The mean color change after demineralization was significant, indicating the successful creation of white spot lesions, with no significant difference among groups. All groups (G2-G5) were

significantly ($p < 0.001$) stained compared to the control group G1, with no significant difference among them. Regarding the treatments, G3 and G5 had the highest significant response ($p < 0.05$), as they eliminated the stains and increased their color lightness, compared to their staining status and to G4, which improved the discolorations but to a lesser extent.

The SBS in G5 had the highest significant ($p = 0.002$) bond strength compared to G4 and G3, which were not significant from each other ($p = 0.821$), yet significantly ($p < 0.001$) higher than G2, which was significant than G1 ($p < 0.001$) (Table 3). According to the Chi-square test, the failure mode assessment showed no correlation between the groups and failure level. Furthermore, the failure mode level distribution was the same across all groups ($p = 0.603$).

The SEM analysis revealed that all treatments affected the surface topography. G1 showed exposed enamel pores compared to G2, which had fewer pores due to the remineralization process. However, G3 had microporosities, pitted and rough surfaces. While G4 presented surface strokes, and G5 had pitted surfaces and visible surface strokes. Qualitatively, the specimens had less pitted and smoother surfaces in the control groups compared to the treatment groups (Fig. 2).

4. Discussion

This study created *in-vitro* s-RCLs models, which allowed for better standardization of the demineralization process (lesions depth), the stains type (organic, i.e., coffee and tea), and the remineralization method (2 %NaF and AS) to minimize variability. Furthermore, the color measurements (L^* coordinate) after demineralization were used for stratified randomization to produce systematic results and limit the influence of color differences among groups (Al-Angari, 2021). The color was objectively evaluated using the CIE $L^*a^*b^*$ system, a standard method for characterizing colors based on human perception and eliminating subjective errors (Hafez et al., 2010). Coffee and tea were chosen as the staining media due their popularity and their intense discoloration effects (Al-Angari et al., 2019a; Grosso et al., 2016). It is known that ΔE values ≥ 3.3 are considered clinically perceptible (Hafez et al., 2010). The color change after staining ($\Delta E \leq 14.4$) was greater than 3.3 units, indicating that our staining process produced distinct visual discoloration (Johnston and Kao, 1989). Furthermore, the color results were similar to the s-RCLs model replicated from previous studies (Al-Angari et al., 2019a, 2019b).

At-home bleaching was chosen as it has clinically perceptible color improvement of organically stained RCLs and is superior to in-office bleaching (Al-Angari, 2021). Bleaching s-RCLs for seven days (42 hrs) showed a remarkable clinical color improvement ($\Delta E = 23.9$) (Al-Angari and Eisa, 2020; Al-Angari et al., 2019b). The substantial increase in ΔE from staining ($\Delta E 14.4$) to bleaching ($\Delta E 23.9$) signifies a remarkable improvement in both stain removal and overall tooth whitening. Bleaching exceeded the initial staining level by a significant margin ($\Delta E = 9.5$), suggesting that bleaching penetrates deep into the dental

structure and acts on internal pigments. This response was attributed to the stain type; coffee and tea consist of organic chromophores, which have light molecular weight and are water-soluble, thus can easily be oxidized by peroxides (Al-Angari, 2021). Furthermore, the longer exposure time of the peroxides, generated more reactive oxidative free radicals, allowing deeper diffusion of the bleaching agent into the enamel (Alqahtani, 2014). This resulted in further breakdown of the chromophores' double-bond conjugations to single bonds (stain oxidation), altered the chromophores' configurations, and subsequently changed their optical properties to a lighter color (Al-Angari and Hara, 2016; Alqahtani, 2014). Moreover, hydrogen peroxide caused surface modification by etching and demineralizing the surface, changing the tooth's mineral content, increasing surface porosity, and altering the enamel's structure (Alqahtani, 2014). This crystal structure change allowed light to scatter more effectively, leading to an improved color outcome. These findings were based on a significant increase in the lightness (L^*) and a decrease in the greenness (a^*) and blueness (b^*) coordinates, which directed the ΔE change towards lighter color improvement (Ahrari et al., 2015; Al-Angari and Eisa, 2020). It is worth mentioning that all our ΔE_{ab} values exceed the established thresholds for acceptability and perceptibility (Paravina et al., 2015). Consequently, the color differences observed in our study were not only perceptible to the human eye but also fall beyond the range of acceptability.

Bleaching combined with microabrasion had the highest results ($\Delta E = 25.3$). Microabrasion minimizes the enamel's thickness, allowing the transmitted light to reveal the underlying yellowish dentin (Perete-de-Freitas et al., 2017). Therefore, combining it with bleaching produces efficient results (Bezerra-Junior et al., 2016; Sundfeld et al., 2014a, 2011). Specimens treated with microabrasion significantly improved in color ($\Delta E 12.3$), yet did not exceed the staining value ($\Delta E 14.1$). This aligns with the manufacturer's recommendation that microabrasion works on superficial stains (25–200 μm) but does not eliminate deeper ones (Castro et al., 2014; Pini, 2015; Sundfeld et al., 2014b). It also verifies that the s-RCL model we created was successful, as the stains penetrated deeper within the demineralized surface during the cyclic process and were incorporated into the lesioned structure (Al-Angari et al., 2019b, 2019a).

Despite the superior physical, mechanical, and esthetic properties of resin-based composite, its long-term success depends on the effectiveness of the SBS in restorative materials (Gurgan et al., 2009; Lai et al., 2002). This may concern clinicians, especially as regards esthetically treated (bleaching/microabrasion) s-RCL surfaces. For this study, a nanohybrid resin-based composite (shade A2) was chosen, as it is the most frequently used clinical material (Alsayed et al., 2022; Rodrigues et al., 2012). Bonding was done two weeks after treatment, as SBS was reported to decrease upon immediate bonding to bleached surfaces and to improve when delayed (average of two weeks) (Gurgan et al., 2009; Imani et al., 2020). The reduced SBS was attributed to the oxygen-free radicals on the enamel's surface, that adversely affect the diffusion of the bonding agent into the tooth and inhibit its complete polymerization (Lai et al., 2002).

All treatment groups had higher significant SBS values (20.6–25.2 Mpa) than the controls (10.9–16.8 Mpa). G5's high value is attributed to the use of two-surface treatments: microabrasion and bleaching. Microabrasion works on two levels: its erosive/chemical effect due to the use of hydrochloric acid, and its abrasive/mechanical effect caused by rubbing silica-carbide microparticles against the enamel (Sundfeld et al., 2014b). The latter initiated the exposure of the enamel's pores and facilitated the peroxide's acid penetration and subsequent chemical reaction, which resulted in morphological and structural changes. These results correspond with the SEM findings, such as increased microporosity, surface roughness, and erosion (Azrak et al., 2010; Pini et al., 2014). The two-surface treatment in G5 facilitated the resin penetration into the surface irregularities, forming more resin tags and a stronger SBS than G3 and G4. The latter two groups received one form of surface treatment and were significantly less than the controls, which received

Table 3

Shear bonding strength (MPa) means (standard deviation) after the designated treatments.

Groups	Shear bonding strength (MPa)
G1	10.9 (1.7) a
G2	16.8 (2.1) b
G3	20.6 (2.6) c
G4	21.6 (3.4) c
G5	25.2 (2.2) d

Lowercase letters indicate significant difference among treatments (column, $p < 0.05$).

G1: Negative control (demineralized), G2: Positive control (Stained Remineralized Caries-Like Lesions s-RCLs), G3: s-RCLs + At-home bleaching, G4: s-RCLs + Microabrasion, G5: s-RCLs + Microabrasion + At-home bleaching.

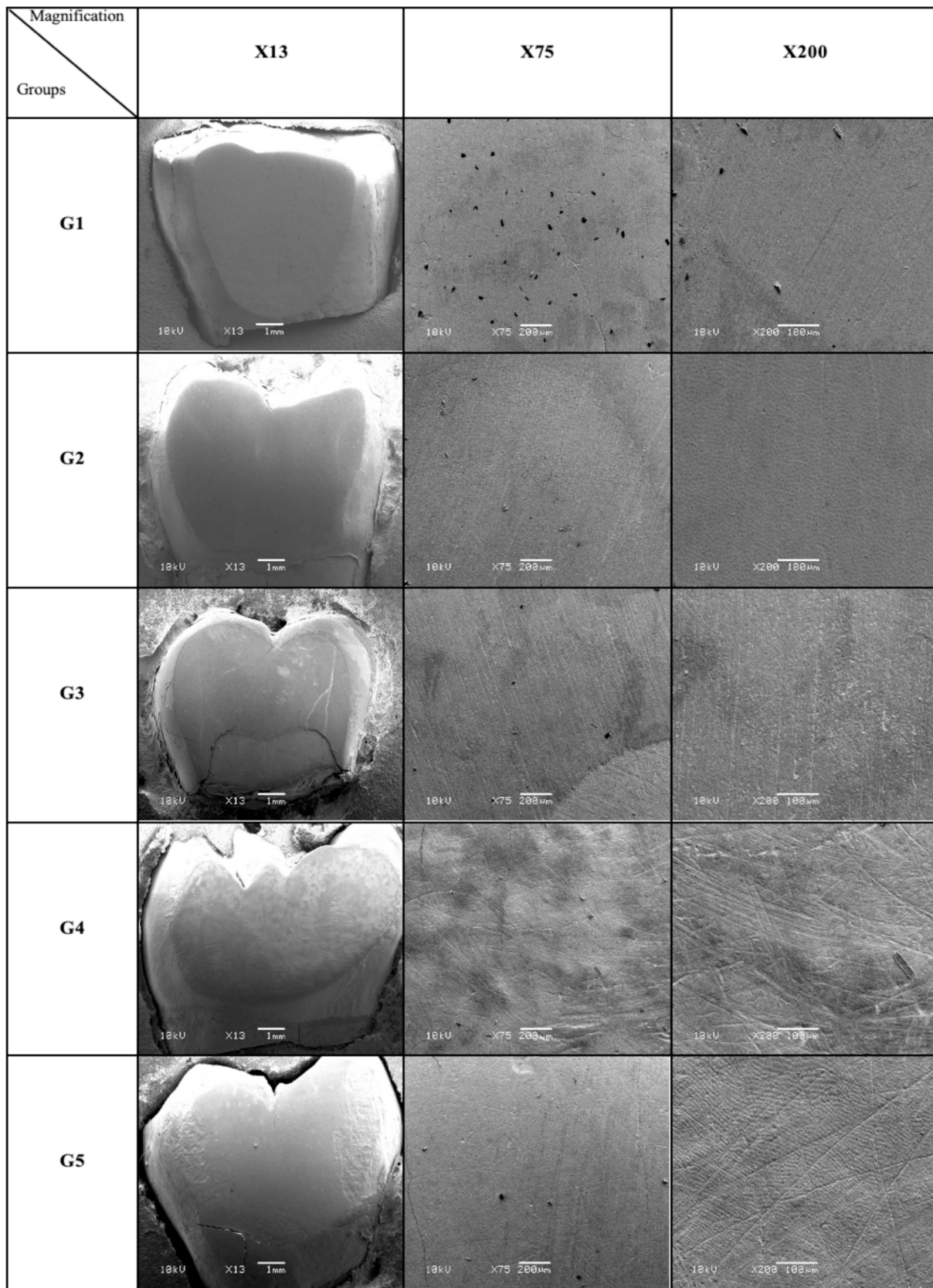


Fig. 2. Scanning electron microscopic images ($\times 13$, $\times 75$, $\times 200$) of enamel surface for all groups after designated treatment protocol. (G1) Negative control (demineralized); (G2) Positive control, Stained Remineralized Caries-Like Lesions (s-RCLs); (G3) s-RCLs + at home bleaching (G4) s-RCLs + microabrasion, (G5) s-RCLs + microabrasion and at home bleaching.

no surface treatments. Despite being more physically destructive, microabrasion was not significantly different from bleaching. As microabrasion works on a superficial level, the immediate polishing and remineralization (2 % NaF gel) following the treatment may have helped minimize the surface roughness and improved smoothness (Sundfeld et al., 2014b).

G2's higher SBS (16.8 Mpa) compared to G1(10.9 Mpa) can be explained by the combination of two remineralization sources, which supports the success of the remineralization process (5 % NaF/AS) in our model. This corresponds with previous findings that SBS is higher in remineralized groups than in demineralized ones (Rizvi et al., 2016; Uysal et al., 2011). G1's weak bond strength was attributed to the demineralized enamel's inferior structural quality, which formed inadequate resin tags that resulted in poor mechanical interlock (Uysal et al., 2011).

The use of bleaching and microabrasion to improve s-RCLs' color and SBS resulted in significant color improvement and high SBS. Thus, the null hypothesis was rejected. Although *in-vitro* models are highly standardized, with better control over variables (lesion type, depth, stain), they are unable to simulate the complex biological processes involved in s-RCL formation. Furthermore, despite their esthetic benefits, they may alter the enamel's morphological structure and make it more prone to plaque accumulation and reactivation of the caries lesion (Preston et al., 2008). Hence, implementing preventive measures based on topical fluoride application and polishing may be necessary (Preston et al., 2008). Further studies should be conducted to evaluate the effects of different adhesive systems on the SBS of s-RCLs treated with different MI techniques.

Understanding the nature of s-RCLs/ACLs (lesion depth and stain history), MI techniques, and the chosen materials is crucial to achieving optimal clinical results. Clinicians should be encouraged to use at-home bleaching as the first line of MI s-ACL treatment. Furthermore, as, in the present study, combining at-home bleaching with microabrasion showed faster color outcomes and stronger SBS results. Additionally, if restorations such as resin infiltration and orthodontic bracket attachment are required, it would create surfaces with high bonding strength.

5. Conclusion

In conclusion, treating s-RCLs using MI esthetic methods showed promising results, as at-home bleaching (15% CP) for seven days eliminated the stains and improved their color outcomes. However, microabrasion alone succeeded in partially improving the color. Furthermore, bleaching combined with microabrasion presented faster results and had the highest SBS. All tested surface treatments resulted in high SBS.

Ethics approval

All human teeth used in this study were approved by the King Saud University Institutional Review Board (IRB# E-22-6639).

Credit authorship contribution statement

Sarah S. Al-Angari: Conceptualization, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Project administration, Supervision. **Nassr S. Al-Maflehi:** Validation. **Alhanouf AlNowaiser:** Writing – original draft, Writing – review & editing, Project administration. **Jawaher AlSenaiddi:** Writing – original draft, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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