Natural and synthetic photosensitizers activated by photodynamic therapy on enamel reconditioning rebonded to metallic brackets: an *in vitro* study

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Abstract. – OBJECTIVE: To evaluate the bond efficacy and failure rates of rebonded metallic brackets after enamel reconditioning with chemical 37% phosphoric acid (PA) and natural and synthetic photosensitizers activated by PDT.

MATERIALS AND METHODS: A total of 50 non-cavitated, and cautiously extracted human premolars were congregated after sample size calculation. The enamel exterior was etched, washed, dried for adhesive application, and cured. Metallic brackets were then oriented and adapted to enamel surface using composite. Later, brackets were debonded from the surface via a Weingart plier. Enamel was finished for ensuing surface reconditioning. Ultimately, specimens were randomly distributed into five groups (n=10). Enamel surface before rebonding was reconditioned with curcumin photosensitizer (CP), riboflavin photosensitizer (RP), rose bengal photosensitizer (RBP), methylene blue photosensitizer (MBP), and 37% PA (control) respectively. After following reconditioning protocol, brackets were rebonded to the enamel exterior employing a composite adhesive system. Then, specimens were subjected to the universal testing machine for analyzing shear bond strength (SBS), and bond failures were predicted using an ARI index. One-way ANOVA and Tukey multiple comparison tests were used for statistical analysis at a variance value of $p < 0.05$.

RESULTS: Enamel reconditioned with 37% PA demonstrated the highest SBS for bracket rebonding, and the lowest SBS was presented by CP actuated by PDT. Enamel reconditioned with RP and RBP corroborated the analogous SBS outcome to 37% PA. Likewise, enamel surface treatment with MBP revealed a statistically significant result to CP for metallic bracket rebonding. The most prevalent failure scores anticipated among groups were 0 and 1 indicating an adhesive failure with the exemption of group 5 (control) that encountered more score 2 cohesive failure on debonding metallic brackets from enamel exterior.

CONCLUSIONS: Rose bengal and riboflavin photosensitizers activated by photodynamic therapy with low ARI scores have the potential to be used as viable enamel reconditioning alternatives to 37% phosphoric acid for rebonding metallic brackets.

Key Words:
Enamel-reconditioning, 37% phosphoric acid, Curcumin, Methylene blue, Rose Bengal, Riboflavin, PDT, Metallic bracket rebonding, Shear bond strength.

Introduction

Malalignment of teeth is deliberated as a ubiquitous aberrant of dentofacial growth that exhibits a detrimental effect on a patient's masticatory function, psychosomatic health, and quality of life[1]. Consequently, to redress this concern, the inclination is acknowledged toward the pursuit of orthodontic treatment with fixed appliances that are bonded specifically to the enamel exterior to optimize facial aesthetics[2,3]. Noticeably, bond failure is an inevitable factor that can jeopardize clinical efficiency and treatment duration in fixed orthodontics owing to substandard clinical expertise, inadvertent bracket debonding or positioning, and deficient adhesive use; therefore, may demand bracket rebonding for precise tooth movement[4,5]. Nevertheless, in an anticipation of procuring effective rebonding; the metallic bracket's high binding capacity (SBS) to the tooth surface is a prerequisite to resisting elevated occlusal and shear

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stresses, and should be detached easily after treatment; thereby securing enamel anatomy with no adhesive remnants[6,7].

Rationally, for ensuring optimal rebonding strength of metallic brackets to the enamel surface, the adhesive remnants should be eradicated prudently by using diverse enamel reconditioning strategies manifesting nominal destructive effects to the enamel framework[5,8,9]. An accustomed enamel-reconditioning method as designated by Buonocore[10] 37% phosphoric acid (PA)' may enticingly augment the adhesive capacity of metallic brackets to the enamel exterior by surface demineralization to several microns irrevocably, thus generating microporosities and massive hydrophilicity that elicits adhesive infiltration progressing micromechanical adhesion[10,11]. This modality has been extensively deemed as a decisive paradigm in all aspects of dentistry[10].

Reliably, further encroachments in the realm of orthodontics may adjudicate a utilization of an innovative 'Photodynamic therapy (PDT)' as an alternative approach for enamel reconditioning that employs various natural and synthetic photosensitizers (PS) that when actuated at anticipated wavelengths of light, emits reactive oxygen species (ROS) on reaction with molecular oxygen, in turn, prompting microbial cell death by undergoing oxidation phenomenon[12,13]. Among varied PS, curcumin (CP) and riboflavin (RP) are natural PS, portraying effectual surface augmentation properties[14]. CP 'a polyphenolic derivative of turmeric' may encompass antimicrobial, anti-inflammatory, and antineoplastic properties by absorbing blue light at a wavelength range of 400 to 450 nm[14,15]. Moreover, it acquires an expedient implementation in dentistry by the possession of modifying the tooth's mechanical properties and collagen-substrate bond fortification due to its anti-oxidant effect, anionicity, and hydrophobicity[16,17]. Although, it displays a few precincts such as discoloration and protracted photo-irradiation time[18]. Likewise, RP 'a water-soluble vitamin' has been asserted as a dentin advancement PS that holds the capacity to reinstate the bond strength of tooth tissues by the process of crosslinking and ROS emanation, in turn revitalizing collagen and resin-infiltrated layer formation, therefore validating laudable antibacterial and bond reinforcement propensity[13,19,20].

Besides natural PS, synthetic PS in particular methylene blue (MBP) and rose bengal (RBP) may also divulge eminent enamel reconditioning properties. MBP 'a hydrophilic PS' may affirm the amended effects of bond strength escalation due to its anionicity and oxidative stress[21]. Correspondingly, RBP 'a halogenated iodine fluorescein derivative' has embraced a sustainable and compelling bond intensification affinity of tooth structure with potent antimicrobial efficacy at a concentration of 5-10 μg/ml[22].

Nevertheless, undeniable confirmation on the implementation of 37% PA, different natural and synthetic PS as enamel reconditioners with their implications on the rebound capacity of the metallic bracket to enamel surface may remain uncharted and unprecedented. Meaningfully, repetitive bracket debonding inclines to upsurge the risk of enamel fracture and patient dissatisfaction, hence significant bond strength is enforced for the cessation of unsolicited bracket bond failure[23]. Therefore, as per relevance, it has been hypothesized that the enamel surface when reconditioned with 37% PA will exhibit better SBS for metallic bracket rebonding in contrast to other examined reconditioning agents (RBP, MBP, CP, and RP). Therefore, the current *in vitro* study predicted to evaluate the bond efficacy (SBS) and failure rates of rebonded metallic brackets after enamel reconditioning with chemical 37% PA and diverse natural and synthetic photosensitizers (RBP, MBP, CP, and RP) activated by PDT.

Materials and Methods

The current *in vitro* study conformed to the checklist for reporting *in vitro* studies guidelines (CRIS) and was permitted by an ethical committee of King Khalid University Kingdom of Saudi Arabia (KSA).

Calculation of Sample Size

The sampling procedure for this *in vitro* evaluation designated that 10 stainless steel brackets would be adequate per group to determine a disparity of 2 MPa among diverse groups with 90% statistical power and a 5% level of significance, as assessed by descriptive statistical analysis software 'Minitab Version 17'. (Minitab Inc., State College, PA, USA).

Sample Preparation

Over ninety days, a total of 50 non-cavitated and cautiously extracted human premolars were congregated for the orthodontic *in vitro* scrutinization of the bracket rebonding process. Teeth were collected from the orthodontic department of King Khalid University. Evaluation of samples was performed using a stereomicroscope (NBHG, Zheijiang, China) at a 20x magnification for intact enamel tissue on the buccal wall, enamel splintering, hypoplasia, abrasive wear, and rehabilitations. Successively, specimens were polished for 15 seconds after diligent surface cleansing employing a non-fluoridated pumice slurry and rubber cup using a slow-speed handpiece (dental hand Piece, Mascot, Anhui, China). The samples were later submerged in 0.1% thymol solution (Thymol, Shanghai, China) approximately for a week and then preserved in distilled water at 37°C for further trialing.

Enamel Surface Preparation and Bracket Bonding Process

Ensuring specimen preparation, samples were decontaminated and polished again for 10 seconds with pumice (Americos Industries, Inc., Ahmedabad, India) and prophylactic rubber cups (Chromadent Dental Equipments, Mumbai, India) at speed of 2,500 rpm. For exploration, each premolar's radicular segment was fixed vertically to a slab of auto-polymerizing acrylic resin (Cosmos Plastics and Chemicals, Mumbai, India).

Sequentially, in compliance with the manufacturer's recommendations, execution of the bracket's adhesion technique was performed, commencing by determining the size of the bracket orientation area *via* a Vernier Calliper (OEM Digital caliper, Shanghai, China). As per guidelines, etching of discernible enamel exterior was performed for 30 seconds with 37% PA-gel (Prime dental products, Thane, India) then scrupulously rinsed with a copious volume of water for 30 seconds and later air-dried revealing a chalky-white etched exterior. Uninterruptedly, the application of the bonding agent was done on the surface with a bonding brush then air-dried, and cured for 10 seconds by an LED light (Curing Light, Leomed, China) at 420 to 480 nm wavelength range. Later, Transbond XT (3M Unitek, Monrovia, CA, USA) composite was utilized for bonding stainless steel orthodontic bracket to the adhesive-cured enamel surface and the bracket was hard-pressed against the tooth surface before cement curing. Ultimately, the composite material was photo-cured from proximal walls and occlusal surface for 20 seconds with an LED curing light (Curing Light, Leomed, China) placed at an angulated position of 45°.

Bracket Debonding Technique

The bracket debonding process was executed by an application of subtle forcing motion with an orthodontic Weingart plier (Rs Medico, Zhejiang, China) that held the bracket's mesial and distal wings together for detachment[24]. Following detachment, any perceptible composite remnants were cleared by employing a 12-fluted finishing carbide bur (Essen Engineers, Mumbai, India), operating at optimum speed, clearing the enamel surface to be spotless and restoration-free for performing surface reconditioning for subsequent rebonding[25]. To elude any technical discrepancies, carbide burs were replaced after preparing every five teeth.

Experimental Groups for Enamel Surface Reconditioning

A sum of 50 premolars (*n* = 10) was allocated arbitrarily to five groups, each comprising 10 samples that were subjected to different enamel surface reconditioning techniques.

Group 1: The debonded buccal enamel exterior of the specimens was treated with 40 µM CP (Sigma, Burlington, MA, USA) solution for 120 sec and activated for 60 seconds by an LED radiation (Curing Light, Leomed, China) with a power density of 1,000±100 mW/ cm^2 at a distance of 1 mm from the surface[17].

Group 2: 100 µM RP (Sigma, Burlington, MA, USA) was smeared to the sample's enamel surface for about 120sec and photo-irradiated for 60 seconds by an LED (Curing Light, Leomed, China) with a power density of 1000±100 MW/cm^2, and the peak wavelength of 460 nm at a distance of 1 mm from the specimen exterior[13,26].

Group 3: Specimens were exposed to 5 µM RBP (Brisben Chemicals, Mumbai, India) for 120 sec and photo-irradiated for 60 seconds with red-light emitting diode at a wavelength of 480 nm, power density of 526 mW/cm^2 and power output of 200 mW[13,27]

Group 4: The enamel surface of the samples was subjected to a 2% aqueous solution of 100 mg/L MBP (Brisben Chemicals, Mumbai, India) for 5 minutes and activated by a diode laser at a wavelength of 810 nm for 60 seconds of photo-irradiation time to activate oxygen species for its action[28].

Group 5: Samples were exposed to 37% PA (Prime dental products, Thane, India) for 15 seconds ensuing surface etching complemented by surface rinsing for 15 seconds to acquire enamel reconditioned surface.

Bracket Rebonding Technique

Succeeding sequential reconditioning protocol, the specimen's enamel surface was decontaminated with distilled water (Astra chemicals, Chennai, India) and air-dried then constrained proportion of Transbond XT (3M Unitek, Monrovia, CA, USA) adhesive system was tinted with an applicator brush to the reconditioned substratum and was cured for 10 seconds after careful removal of superfluous adhesive. The brackets were aligned on the specified enamel portion with composite (Transbond XT, 3M Unitek, Monrovia, CA, USA) and adapted for final orientation, and finally cured for 20 seconds from distinct angles after excess composite removal present around the bracket boundary. All specimens were preserved in distilled water for about 24 hours at 37°C and thermocycled for 500 cycles at 5-55°C with 30 seconds dwell time then evaluated for SBS analysis.

Shear Bond Strength Analysis (SBS)

A force at the occluso-gingival direction was exerted on the enamel-bracket interface until bond failure employing a universal testing machine (UTM) (ZME, Guangdong, China) at a crosshead speed of 0.5 mm/min to assess SBS. The requisite force for bracket displacement was determined by calculating SBS in Megapascals (MPa) and designated in Newton (N). SBS was calculated using the following equation:

$$\text{SBS (MPa)} = \frac{\text{Force (N)}}{\text{Bracket-base area (mm}^2\text{)}}$$

$$\left(1\ MPa = \frac{1\ N}{mm^2}\right)$$

Bond Failure Analysis (Adhesive Remnant Index)

After bracket debonding under UTM, the brackets were inspected under 10x magnification using a stereomicroscope (NBHG, Zheijiang, China) for the concentration of residual resin bonded to the substratum. Particularly, the adhesive remnant index[29] was used for the description and categorization of the magnitude of residual resin left on the enamel surfaces, following the location of the bond failure. The ARI scores range from 0 to 3 and are demonstrated as follows:

Score 0: Absence of any adhesive remnants on the tooth's enamel surface.

Score 1: Less than half of the residual adhesive persisted on the enamel bonding place.

Score 2: More than half of the adhesive remnants present on the enamel adherent site.

Score 3: The enamel adherent region was wholly masked with an adhesive.

Statistical Analysis

Statistical Package for Social Sciences Windows, version 10.0 (SPSS Inc., Chicago, IL, USA) was used to calibrate descriptive statistics (mean and standard deviation) of SBS of each reconditioning group. Bond strength comparisons were performed among different groups utilizing ANOVA and Tukey multiple comparison tests at a statistically significant level of $p<0.05$.

Results

SBS Analysis

The means and Standard deviation (SD) of SBS (MPa) of orthodontic brackets rebonded to the enamel surface on treatment with varied reconditioning groups using ANOVA and Tukey multiple comparison tests are displayed in Table I. Interpreting the decree of the study, the highest bond strength (SBS) was demonstrated by group 5 (control) i.e. 37% PA application on enamel surface for reconditioning and bracket

Table I. Shear bond strength of orthodontic brackets after rebonded to the enamel surface.

Different tested groups		Mean ± SD (MPa)	*p*-value
Group 1	CP	10.21 ± 0.47*	< 0.05
Group 2	RP	$14.17 \pm 0.05^{\#}$	
Group 3	RBP	$14.91 \pm 0.60^{\#}$	
Group 4	MBP	11.24 ± 0.39*	
Group 5	37% PA Control	$15.55 \pm 0.11^{\#}$	

Curcumin Photosensitizer (CP); Riboflavin photosensitizer (RP); Rose bengal Photosensitizer (RBP); Methylene blue photosensitizer (MBP); Phosphoric acid (PA). ∞Different superscript characters denote statistically significant difference. !Showing significant differences among study groups (ANOVA). (Tukey multiple comparison tests).

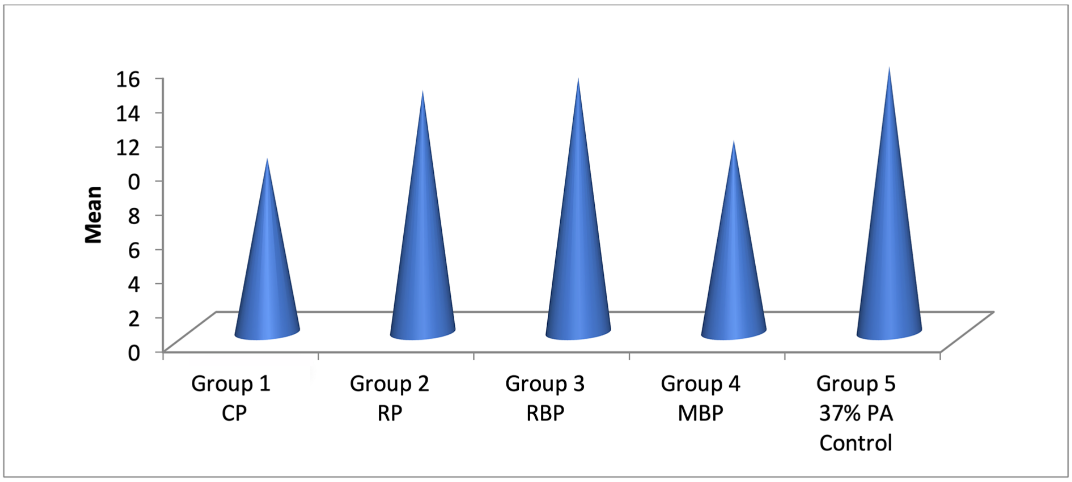

Figure 1. Shear bond strength of orthodontic brackets after rebounded to the enamel surface after different surface treatment regimes. Curcumin Photosensitizer (CP); Riboflavin photosensitizer (RP); Rose Bengal Photosensitizer (RBP); Methylene blue photosensitizer (MBP); Phosphoric acid (PA).

rebonding (15.55 ± 0.11 MPa). Whereas, the lowest SBS was presented by group 1: enamel reconditioning with CP actuated by PDT (10.21 ± 0.47 MPa) respectively.

However, when enamel reconditioned by RP in group 2 (14.17 ± 0.05 MPa) and group 3 RBP (14.91 ± 0.60 MPa) validated the comparable SBS outcome to group 5 (control) reconditioned with 37% PA ($p<0.05$). Likewise, enamel surface reconditioning with MBP (11.24 ± 0.39 MPa) was comparable to group 1 surface reconditioned with CP (10.21± 0.47 MPa) ($p>0.05$) (Figure 1).

Failure Rate Analysis

Bond failure percentages amongst trial groups utilizing adhesive remnant index (ARI) for the rebonded metallic bracket are exhibited in Table II, Figure 2. Inferring the finding of the study as per the ARI index, the most prevalent failure scores anticipated among groups were recorded as 0 (absence of adhesive remnant on the enamel exterior) and 1 (persistence of less than half of the residual adhesive on the enamel adherent site) indicating an adhesive failure with the exemption of group 5 (control) that encountered more score 2 failure (more than half of the adhesive remnant was present on the enamel bonding region) on debonding metallic brackets, denoting as a cohesive failure.

Discussion

The contemporary *in vitro* exploration was designed to assess the SBS and bond failure rates of rebonded metallic brackets after enamel surface-reconditioning with varied natural and synthetic PS activated by PDT and etching with 37% PA. Systematically, the current data was assembled on the conjecture that enamel surface when reconditioned with 37% PA will authenticate better SBS for metallic bracket rebonding in contrast to other examined reconditioning agents. Distinctly, as per validation

Table II. Percentages of failures among trial groups using adhesive remnant index (ARI).

Experimental groups		0	1	2	3	N
Group 1	CP	35%	30%	25%	10%	10
Group 2	RP	45%	35%	15%	5%	10
Group 3	RBP	30%	60%	5%	5%	10
Group 4	MBP	55%	20%	25%	-	10
Group 5	37% PA control	5%	30%	45%	20%	10

Curcumin Photosensitizer (CP); Riboflavin photosensitizer (RP); Rose bengal Photosensitizer (RBP); Methylene blue photosensitizer (MBP); Phosphoric acid (PA).

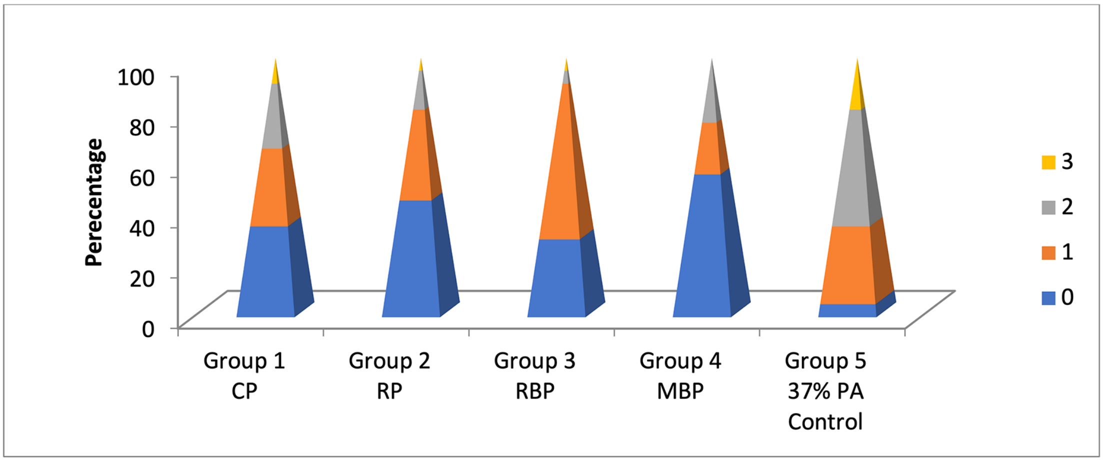

Figure 2. Percentages of failures among experimental groups using (ARI). Curcumin Photosensitizer (CP); Riboflavin photosensitizer (RP); Rose bengal Photosensitizer (RBP); Methylene blue photosensitizer (MBP); Phosphoric acid (PA).

from our *in vitro* study's finding, the hypothesis was incredibly accredited as 37% PA (control) offered the highest SBS of rebonded metallic brackets to reconditioned enamel in contrast to other groups. Congruently, RBP and RP activated by PDT corroborated the equated SBS result to 37% PA.

Bracket bonding failure serves as a consistent insuperable impediment that depicts inclination towards lengthy treatment duration, subnormal treatment strategy, enamel surface variability, moisture contamination, patient behavioral patterns, masticatory, and shear stresses[5,6,30]. However, incapacitating the aforesaid debonding factors is a foremost task to demonstrate effective bond efficacy along with further attribution towards potent enamel reconditioning agents, bracket-base design, and doable adhesive usage[31-34]. In our study, to analyze the durable and stress-disseminating 'SBS' approach of rebonded brackets, a universal testing machine was employed that emulates oral ambiance[35]. Clinically, as experimented by Reynolds et al[3] brackets should exhibit SBS of 6-8 MPa for optimal orthodontic treatment, fatigue, and debonding resistance.

The requirement for enamel surface reconditioning before the rebonding metallic bracket is to attain transformed enamel surface topography proficient in retaining the cement as enamel gradually loses its properties due to the reaction with various salivary ions or components and lodgement of foreign particles or adhesive cement in enamel pores[5,7]. Nevertheless, adhesion to tooth enamel is an imperative research direction in orthodontics, ensuring the proper attachment of the bracket to the enamel while displaying stress resistance, required for the execution of orthodontic movements at any treatment phase; thus, reliant on the use of a sustainable resin-based adhesive system with minimal bond inaccuracies and esthetical reliability[36-38].

According to the assertion of the study, etching the enamel exterior with 37% PA displayed the highest adhesive bond capacity for rebonding metallic brackets. This micro etching method serves as a chemical enamel surface modifier that dissolves smear layer and enamel prisms forming heterogeneous microporosities providing surface roughness, and wettability by enhancing surface energy, non-reactive mineral reconfiguration, and decalcification up to 10µm, consequently on adhesive application reinforces the reconditioned enamel for bracket placement by forming resin-invigorated layer ensuing micromechanical retention[39-41]. Studies conducted by Arakawa et al[42] and Asmussen[43] inferred similar results to our outcome and exhibited that through acid-etching only inter-prismatic enamel shows dissolution effect, therefore should be performed watchfully and proficiently.

Likewise, RBP and RP activated by PDT validated the comparable consequences of SBS to the micro acid-etching technique. The conceivable reason behind this could be due to their anti-oxidant and crosslinking properties that amend the enamel exterior by preventing collagen degradation and promoting surface roughness forming a honeycomb-structured porous layer for adhesive infusion, therefore authenticating esteemed antibacterial and mechanical bond reinforcing disposition for rebonding metallic bracket[13,26,44,45]. This is compatible with the study performed by Mirhashemi et al[18] and Alqerban[13]. Nevertheless, in fixed orthodontics, PDT has been portrayed for its enhanced antimicrobial, anti-inflammatory, and potent enamel reconditioning implications but should be scrutinized for further insinuation[18,26].

Reconditioning of enamel exterior with CP and MBP unveiled the lowest SBS for stainless steel bracket rebonding. This is due to the provenance of the oxidation process of PDT that emits free radicals in turn impeding the composite's polymerization, hence lowering bond integrity[46]. Practically, CP's hydrophobic nature may also account for bond failure as it displays incompetency to enhance the hydrophilicity of the enamel framework[17]. Moreover, recent work by Al Deeb et al[47] declared that PDT with MBP authenticated the least SBS due to the inhibition of formation of honeycombed porous structure, displaying significantly less permeable and less irregular enamel exterior. Baeshen[48] clinched affiliated fallouts as well. However, PDT's oxidative and CP's hydrophobic effects on rebonded brackets may demand further consideration for an absolute judgment.

Explicating the failure analysis among probationary groups utilizing the adhesive remnant index (ARI), adhesive failure prevailed the most, scored as 0 and 1, deliberating outright and judicious enamel reconditioning with natural and synthetic PS activated by PDT, in turn, screening practicable extermination of composite from the enamel exterior deterring enamel wear and chipping. Therefore, this anticipates prevention of enamel impairment *via* instrument overuse and may need less finishing, restricting chair side time. However, these judgments corroborate with recent work by Mirhashemi et al[49] and Baeshen[48]. Furthermore, 37% PA showed a higher rate of ARI score 2 which signifies inordinate composite persisted over the enamel surface that may extend treatment due to the need for unrestrained finishing leading to enamel framework deterioration. Conversely, the ARI score acts as a synergistic of diverse parameters comprising bracket-base configuration, enamel reconditioning effect, adhesive nature, capacity and location of force applied, plier torque, cross-head speed for debonding, and bond competency at the adhesive-bracket-enamel interface as directed by Z Cai et al[50] investigation[32,50,51].

Limitations

Undeniably, within the limitations of the present *in vitro* exploration, PDT has become mainstream in dental practice, principally for periodontal and mucosal diseases, expediting tooth movement and for enamel surface reconditioning to rebond metallic brackets but should be probed further *via* testing *in vivo*. However, 37% of PA and PDT utilizing natural and synthetic PS necessitate auxiliary penetration by executing scanning electron microscopy (SEM) and Atomic force microscopy (AFM) as bracket bond failure may differ clinically from *in vitro* results due to the influence of multifaceted oral forces, pH deviation and bracket deterioration (corrosion and cyclical fatigue) at interface[52,53]. Ultrastructural enamel changes and debonded surfaces employing various PS concentrations and exposure time should also be assessed *via* dispersive spectroscopy.

Conclusions

Rose Bengal and riboflavin photosensitizers activated by photodynamic therapy with low ARI scores have the potential to be used as viable enamel reconditioning alternatives to 37% phosphoric acid for rebonding metallic brackets.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval

This study was permitted by the Ethical Committee of King Khalid University Kingdom of Saudi Arabia (KSA).

Authors' Contribution

Conceptualization, M.A. Kamran and S. Almoammar; methodology, A.A. Alnazeh, and S. Almoammar; software, A.A. Alnazeh and S. Almoammar; validation, S. Almoammar, A.A. Alnazeh, and A.Alshahrani.; formal analysis, M.A. Kamran; investigation, A. Alshahrani; I.Alshahrani resources, A.A. Alnazeh; data curation, A. Alshahrani; M.A. Kamran writing original draft preparation, S. Almoammar, and A.H.A. Alhaizaey; writing, review and editing, A.S.A; visualization, A.H.A. Alhaizaey; supervision, I.A., M.A. Kamran and I.A.; project administration, A.H.A.

Alhaizaey; funding acquisition, A.H.A. Alhaizaey. All authors have read and agreed to the published the version of the manuscript.

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