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ORIGINAL RESEARCH

Effects of silver diamine fluoride combined with potassium iodide and diode laser irradiation on antibacterial activity and microleakage: an *in vitro* study

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Abstract

Background: Silver diamine fluoride (SDF) is widely used in minimally invasive caries management; however, limited evidence exists regarding the combined use of potassium iodide (KI) and diode laser irradiation, particularly concerning their effects on antibacterial efficacy and microleakage of glass hybrid restorations. **Methods**: This *in vitro* study included 144 sound permanent molars. For antibacterial evaluation, a dual-species biofilm of *Streptococcus mutans* and *Lacticaseibacillus casei* was established on 72 dentin disks (2 mm thick), randomly assigned to six groups (n = 12): SDF + KI, SDF only, SDF + KI + diode laser, SDF + diode laser, diode laser only, and control. Antibacterial activity was assessed using confocal laser scanning microscopy and scanning electron microscopy. For microleakage evaluation, 72 standardized Class V cavities were prepared and assigned to the same six experimental groups using identical treatment protocols. All cavities were restored with a glass-hybrid restorative material and subjected to thermocycling. Dye penetration was evaluated using basic fuchsin under stereomicroscopy. Antibacterial outcomes were analyzed using generalized estimating equations with pairwise comparisons, while microleakage outcomes were analyzed using the Kruskal-Wallis test. **Results**: A statistically significant difference in antibacterial activity was observed between the control group and all experimental groups ($p < 0.05$). The SDF + KI + diode laser group demonstrated significantly greater antibacterial activity than the SDF group and diode laser only group ($p < 0.05$). No statistically significant differences were detected among the groups with respect to microleakage; however, KI-containing groups showed higher median microleakage values, whereas the lowest median value was observed in the control group ($p > 0.05$). **Conclusions**: Within the limitations of this *in vitro* study, the combination of SDF, KI, and diode laser irradiation demonstrated the most pronounced antibacterial effect among the tested protocols, without significantly affecting microleakage performance of glass-hybrid restorations under the present experimental conditions.

Keywords

Silver diamine fluoride; Diode laser; Potassium iodide; Microleakage; Glass hybrid; Antibacterial efficacy

1. Introduction

Conventional caries management is often challenging in young or special needs children due to limited cooperation, behavioural issues, and dental anxiety [1]. Invasive procedures such as drilling may further exacerbate this anxiety, leading to treatment avoidance and progression of dental diseases [2]. Therefore, minimally invasive approaches, such as silver diamine fluoride (SDF) and laser-assisted therapies, have gained increasing popularity over the past decade, as they reduce the risk of dental trauma and improve treatment acceptance in children [3].

SDF is favored for its ease of application, affordability, and the fact that it does not require mechanical removal of decay [4]. The most notable drawback of SDF is the discoloration it causes on enamel and dentin, primarily caused by residual silver ions [4–7]. Potassium iodide (KI) application following SDF has shown promising results in reducing black staining [8]. This effect is attributed to the deposition of a water-soluble, yellowish compound (silver iodide) through the reaction of KI with residual silver ions, thereby minimizing visible discoloration in clinical practice [5].

In addition to chemical strategies such as KI application, laser-based approaches have also been investigated as adjunctive methods in SDF therapy. Laser irradiation following SDF or topical fluoride application may facilitate fluoride ion uptake by dentin, resulting in the formation of fluoridated hydroxyapatite [9, 10]. Diode laser irradiation has been reported to enhance fluoride uptake and improve enamel resistance to acid demineralization through physicochemical modifications of the dental surface [11]. Additionally, their antimicrobial action results from laser energy absorption at the bacterial site or direct absorption by microorganisms, leading to bacterial damage [12].

The combined use of SDF with various laser systems has been reported in previous studies [10, 13–15]; however, evidence regarding its application with diode lasers remains limited [10], and the evaluated outcomes have varied.

Most previous studies specifically investigating SDF in combination with diode laser irradiation have mainly focused on microhardness, remineralization potential, and fluoride uptake. In contrast, the simultaneous evaluation of antibacterial efficacy and restorative sealing performance has been largely overlooked. Furthermore, data regarding the combined use of SDF, KI, and diode laser irradiation remain scarce. The potential influence of KI on the antimicrobial efficacy of SDF and on restorative sealing is still controversial. Moreover, adjunctive diode laser irradiation, intended to enhance antibacterial activity, may also influence restorative integrity.

Accordingly, this study investigated the combined effects of SDF, KI, and diode laser application on antibacterial efficacy in dentinal specimens and on microleakage in Class V cavities restored with a glass hybrid restorative material. The null hypothesis was that the combined use of SDF, KI, and diode laser irradiation would not significantly affect antibacterial efficacy or microleakage.

2. Materials and methods

2.1 Study design and sample size

This *in vitro* study was conducted between April 2025 and November 2025 at the Department of Pediatric Dentistry, Marmara University. An a priori power analysis was conducted using G*Power (3.1.9.7, Heinrich Heine University Düsseldorf, Düsseldorf, NRW, Germany) based on the data reported by Al-Hamdan *et al.* [16]. The analysis used a mean difference of 6.370 and a standard deviation of 3.490. Assuming a two-tailed test, an alpha level of 0.05, and a power of 0.95, it was determined that a minimum of 8 samples per group would be sufficient. To increase statistical reliability and compensate for potential specimen loss, 12 specimens were included in each group.

2.2 Sample preparation

Ethical approval was obtained from the Ethics Committee for Non-Drug and Non-Device Clinical Research at the Faculty of Medicine, Marmara University (Approval No: 09.2024.662), and written informed consent was obtained from all saliva donors and from all patients whose extracted teeth were used in the study. A total of 144 sound, non-carious human permanent molars extracted for orthodontic or periodontal reasons were collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Marmara University. Teeth presenting cracks, restorations, caries, or other visible structural defects were excluded. Soft tissue remnants and periodontal debris were removed with a periodontal scaler, and polished with a pumice-free prophylaxis brush. The specimens were stored in 0.1% thymol solution until the experimental procedures. The maximum time between extraction and experimentation was three months.

For the antibacterial test, 72 teeth were removed from 0.1% thymol solution three days prior to the experiment and stored in deionized water to minimize possible residual effect of thymol. During this period, specimen preparation procedures were performed. The teeth were embedded in acrylic resin using a silicone mold (Akrodent Acrylic, Koca Kimya ve Dental, Turkey). Each tooth was sectioned horizontally with a precision cutting machine (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) using a diamond saw blade (15 hc, no: 11-4244, Isomet, Buehler, USA) to obtain 2-mm-thick dentin disks. Dentin disks were stored in distilled water until testing.

2.3 Collection of saliva samples

Saliva specimens were obtained in under resting conditions from 14 healthy volunteers (9 females, 5 males) between 18–35 years of age who had no systemic diseases, were non-smokers, and had not used any medication within the previous three months. Saliva collection was performed between 10:00 and 15:00 at least 1.5 hours after any oral activity such as eating, drinking, or tooth brushing. The saliva samples were centrifuged at 15,000g for 15 minutes at 4 °C, and the supernatant was sterilized using a 0.22 μm filter. After processing, saliva specimens were kept at −20 °C before further use.

2.4 Preparation of bacterial suspensions

Following 24-hour incubation of *Streptococcus mutans* (*S. mutans*) American Type Culture Collection (ATCC) 25175 and *Lacticaseibacillus casei* (formerly Lactobacillus casei) ATCC 4646, 10 mL of each culture grown in Brain Hearth Infusion (BHI) broth supplemented with 5% sucrose was standardized to a turbidity equivalent to 0.5 McFarland standard ($\approx 10^8$ CFU/mL) and subsequently diluted 1:100. The final bacterial concentration was adjusted to 10^6 CFU/mL. A bacterial suspension containing *S. mutans* and *L. casei* was prepared in a 3:2 ratio. Bacterial quantities were confirmed by quantitative plating of 10-fold dilutions onto agar plates.

2.5 Biofilm formation

For each group, sterile 24-well culture plates were used. One dentin specimen was placed in each well. To allow salivary pellicle formation, 1000 μL of prepared saliva was added onto each specimen, and the plates were incubated at 37 °C on a shaker for 1 hour. Then, the saliva was aspirated and the specimens were rinsed with phosphate-buffered saline. The specimens were returned to the wells, and 1.6 mL of BHI broth supplemented with sucrose and 200 μL of the bacterial suspension were added. The plates were incubated under anaerobic conditions at 37 °C on a shaker for 24 hours to allow biofilm formation.

2.6 Application of treatment protocols on biofilm-covered dentin surfaces

To remove non-adherent bacteria, the 72 dentin disks with established biofilms were subjected to three consecutive rinses with sterile saline (5 mL each). The specimens were then randomly allocated into six groups using a computer-generated sequence (Microsoft Excel) (n = 12). Randomization was performed by an independent researcher not involved in outcome assessment.

- Group 1 (SDF + KI): Following the SDI manufacturer's instructions, one drop of SDF (38% silver diamine fluoride, Riva Star Bottle 1 and 2, 1240751, SDI, Bayswater, Victoria, Australia) and two drops of KI were dispensed into a dappen dish. SDF was applied to each specimen in a circular motion for 1 minute, followed immediately by KI application for 20 seconds. The specimens were then rinsed with sterile saline for 10 seconds.
- Group 2 (SDF): SDF (Riva Star Bottle 1, SDI, Victoria, Australia) was applied for 1 minute. The specimens were then rinsed with sterile saline for 10 seconds.
- Group 3 (SDF + KI + Laser): SDF and KI (Riva Star Bottle 1 and 2, SDI, Victoria, Australia) were applied following the same protocol described for Group 1. Subsequently, laser irradiation was performed using a 980 nm diode laser (Wiser Laser Evolution, Doctor Smile, Vicenza, Italy) with a 400 μm therapy tip (spot area $\approx$ 0.00126 cm^2) at 1 W output power in continuous contact mode (tip-to-surface distance: 0 mm) for 60 seconds, in accordance with the parameters used by Cirdei *et al.* [17]. The procedure was performed without water cooling. The laser tip was moved uniformly across the dentin surface with horizontal and vertical scanning motions to ensure complete coverage of the treated area. The total delivered energy was 60 J. Based on the fiber tip diameter, the approximate energy density was calculated as 47,770 J/cm^2.
- Group 4 (SDF + Laser): SDF (Riva Star Bottle 1, SDI, Victoria, Australia) was applied as described in Group 2. Immediately after, laser irradiation was performed using the same parameters and technique as described for Group 3.
- Group 5 (Laser): Laser irradiation was carried out according to the protocol as described for Group 3, without any prior surface treatment.
- Group 6 (Control): No treatment was applied.

2.7 Antibacterial evaluation with confocal laser scanning microscope (CLSM)

60 specimens, 10 per group, were allocated for evaluation with CLSM (Leica TCS SPE, Leica Microsystems, Wetzlar, HE, Germany). Twenty microliters of Live/Dead Bacterial Viability Stain (1910798, Invitrogen, Thermo Fisher Scientific, Eugene, OR, USA) (prepared in dimethyl sulfoxide (DMSO) to contain 0.4 μL/mL of Compounds A and B) were applied onto the biofilm-covered dentin specimens placed in sterile Petri dishes. The samples were incubated in the dark for 15 minutes.

Images were obtained from three different randomly selected areas of each specimen at 5× magnification using excitation wavelengths of 488 nm and 532 nm. Bacterial viability and biofilm density were analysed using ImageJ software (ImageJ, National Institutes of Health, USA). Live bacteria exhibited green fluorescence, while dead bacteria exhibited red fluorescence.

2.8 Antibacterial evaluation with scanning electron microscope (SEM)

12 specimens, 2 per group were prepared for SEM analysis through fixation and dehydration procedures. For fixation, two dentin disks from each group were immersed in 4% paraformaldehyde solution within a 24-well plate. Following a 36-hour fixation period, dehydration was performed using a graded ethanol concentration (25%, 50%, 75%, 2 × 96%, and 2 × 100%), with each step lasting 15 minutes. After dehydration was completed, the samples were air-dried for 24 hours.

For SEM analysis, the specimens were sputter-coated with a coating device (SC7620, Quorum Technologies Ltd., Laughton, East Sussex, UK) with an alloy consisting of 80% gold and 20% palladium at 18 mA for 330 seconds. Argon gas was used during the coating process. The dried specimens were then examined under a SEM (Zeiss EVO MA10, Carl Zeiss Microscopy GmbH, Jena, BW, Germany). Images were obtained at 15,000× magnification for qualitative evaluation.

2.9 Microleakage experimental procedure

One day prior to the experimental procedures, 72 molars were transferred into deionized water. Each tooth was mounted in cold acrylic using a silicone mold, ensuring that the crown remained above the surface. Standardized Class V cavities (n = 72) measuring 4 × 2 × 2 mm (length × width × depth) were prepared using a fissure diamond bur in a high-speed handpiece under water cooling on the buccal surfaces of the teeth. A Williams periodontal probe was used to ensure standardization of cavity dimensions. All cavity preparations were performed by a single operator.

For the microleakage evaluation, the same treatment protocols described in the antibacterial experiment were applied to standardized Class V cavities: Group 1, SDF + KI; Group 2, SDF only; Group 3, SDF + KI + laser; Group 4, SDF + laser; Group 5, laser only; and Group 6, no treatment. Following the respective procedures, the specimens then proceeded to the restoration phase.

2.10 Restorative procedure

Equia Forte HT Fil (GC Corp., Tokyo, Japan), a glass hybrid restorative material selected for its improved mechanical properties, enhanced esthetics, broad clinical indications, and ease of application, was used in this study [18]. Capsules were activated by pressing the plunger on the back of the capsule and mixed for 10 seconds using an amalgamator. The material was placed into the cavities within 10 seconds using its dedicated applicator. Subsequently, Equia Forte Coat (GC Corp., Tokyo, Japan) was coated using the bonding brush provided in the product kit and light cured for 20 seconds (LED curing unit, Vega, Dentac, Istanbul, Turkey) in standard mode as recommended by the manufacturer. All restorations were polished using a low-speed handpiece under light pressure. This restorative procedure was standardized and performed identically for all specimens.

2.11 Microleakage evaluation

The teeth were subjected to thermal cycling between 5 °C and 55 °C for 2000 cycles. Following thermal cycling, the teeth were immersed in a 0.5% basic fuchsin dye solution for 24 hours to assess dye penetration. After dye immersion, the teeth were sectioned longitudinally in a buccolingual direction, from the coronal to the apical region, using a low-speed saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) equipped with a 15 HC diamond disk (No: 11-4244). Images of the specimens were recorded under a stereomicroscope (Leica MZ75, Wetzlar, HE, Germany) at 25× magnification with a 0.63× zoom setting. Dye penetration was assessed independently by two calibrated evaluators according to the scoring system proposed by Araujo CS *et al.* [19]. The examiner assessing the outcomes was blinded to group allocation. The microleakage scoring for dye penetration was as follows:

0: No leakage;
1: Leakage extending to half the depth of the cavity;
2: Leakage extending beyond half the depth of the cavity;
3: Leakage extending to the bottom of the cavity.

2.12 Statistical analysis

The antibacterial effects were evaluated by analyzing the ratio of viable to non-viable (dead) bacteria. The data were analyzed using the Generalized Estimating Equations (GEE) method to account for clustering at the tooth level and the potential correlation among the three measurements obtained from each dentin surface. This population-averaged approach provides robust standard error estimates for correlated data. A linear model assuming a normal distribution with an identity link function was applied. An exchangeable working correlation structure was selected, and robust (sandwich) estimators were used to obtain standard errors. Pairwise comparisons between groups were performed to assess differences in antibacterial outcomes. Group comparisons were based on estimated marginal means derived from the GEE model. Statistical analyses were conducted using appropriate statistical software, and the level of significance was set at $p < 0.05$.

The statistical microleakage evaluation was performed using the R programming language (version 4.4.1). The normality of distribution was assessed using the Kolmogorov-Smirnov test. Numerical variables with normal distribution were expressed as mean ± standard deviation, whereas those without normal distribution were presented as median (Q1–Q3). Categorical variables were expressed as frequencies (percentages). When the number of groups exceeded two, differences among groups were analyzed using the Kruskal-Wallis test for ordinal variables that did not follow a normal distribution. Inter-rater agreement was analyzed using the Weighted Kappa method. Both the Weighted Kappa analysis and its graphical representation were performed in R using the "irr", "ggplot2", "reshape2", and "vct" packages. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1 CLSM results

CLSM analysis demonstrated a statistically significant difference in antibacterial outcomes among the experimental groups based on the live-to-dead bacterial ratio (Wald $\chi^2 = 22.92$, $df = 5$, $p < 0.001$) (Table 1). Pairwise comparisons revealed that Group 6 (control) exhibited significantly higher live-to-dead bacterial ratios than all experimental groups (all $p < 0.05$), indicating the lowest antibacterial effect.

TABLE 1. Comparison of antibacterial efficacy among groups based on CLSM-derived live/dead bacterial ratios.

	Mean ± Std. Error	95% CI (Lower–Upper)
Group 1 (SDF + KI)	0.76 ± 0.09	0.57–0.94
Group 2 (SDF)	0.99 ± 0.08	0.83–1.14
Group 3 (SDF + KI + Laser)	0.59 ± 0.10	0.39–0.79
Group 4 (SDF + Laser)	0.91 ± 0.19	0.53–1.28
Group 5 (Laser)	0.87 ± 0.09	0.71–1.04
Group 6 (Control)	1.52 ± 0.19	1.14–1.89

The GEE analysis revealed a significant overall group effect (Wald $\chi^2 = 22.92$, $df = 5$, $p < 0.001$).
Group 6 demonstrated significantly higher live/dead bacterial ratios than all experimental groups ($p < 0.05$).
SDF: Silver diamine fluoride; KI: potassium iodide; Std. Error: Standard error; CI: Confidence interval.

When mean values were compared (Table 1), the groups were ranked in descending order as follows: Control > SDF only > SDF + Laser > Laser only > SDF + KI > SDF + KI + Laser. The lowest live-to-dead bacterial ratio, reflecting the highest antibacterial activity, was observed in the Group 3 (SDF + KI + Laser).

Pairwise comparisons (Table 2) showed that the combined SDF + KI + Laser (Group 3) demonstrated significantly greater antibacterial activity than SDF only (Group 2) and laser only (Group 5). Additionally, all treatment groups showed significantly lower bacterial viability compared with the control group.

CLSM analysis was performed to evaluate the distribution of live and dead bacteria within the biofilms of all experimental groups (Fig. 1). Visual examination of the CLSM images revealed differences in the distribution and density of live and dead bacteria among the groups. The groups treated with SDF in combination with laser irradiation (with or without KI) exhibited increased red fluorescence and reduced green fluorescence compared with the other groups, suggesting enhanced antibacterial activity. In contrast, the control group demonstrated higher levels of green fluorescence, indicating greater bacterial viability. The SDF, SDF + KI and laser only groups showed a mixed distribution of live and dead bacteria.

TABLE 2. Pairwise comparisons of antibacterial outcomes between groups based on GEE analysis.

	Group	Mean Difference ± Std. Error	p	95% CI (Lower–Upper)
Group 1 (SDF + KI)				
	2	−0.23 ± 0.12	0.060	−0.47–0.01
	3	0.17 ± 0.14	0.224	−0.10–0.44
	4	−0.15 ± 0.21	0.484	−0.56–0.27
	5	−0.12 ± 0.13	0.362	−0.37–0.13
	6	−0.76 ± 0.21	<0.001*	−1.18–−0.34
Group 2 (SDF)				
	3	0.40 ± 0.13	0.002*	0.15–0.65
	4	0.08 ± 0.21	0.685	−0.32–0.49
	5	0.11 ± 0.12	0.325	−0.11–0.34
	6	−0.53 ± 0.21	0.011*	−0.93–−0.12
Group 3 (SDF + KI + Laser)				
	4	−0.33 ± 0.21	0.141	−0.74–0.10
	5	−0.28 ± 0.13	0.032*	−0.54–−0.02
	6	−0.93 ± 0.22	<0.001*	−1.35–−0.50
Group 4 (SDF + Laser)				
	5	0.03 ± 0.21	0.879	−0.38–0.44
	6	−0.61 ± 0.27	0.023*	−1.14–0.08
Group 5 (Laser)	6	−0.64 ± 0.21	0.002*	−1.05–−0.23

*$p < 0.05$.
*Additional GEE parameter estimates confirmed lower live/dead bacteria ratios in all treatment groups compared with the control group, with the largest reduction observed in Group 3 (**Supplementary Table 1**).*
SDF: Silver diamine fluoride; KI: potassium iodide; Std. Error: Standard error; CI: Confidence interval.

3.2 SEM results

SEM images were obtained at 15,000× magnification and evaluated for all groups (Fig. 2a–f).

• Groups 1 (SDF + KI), 2 (SDF only), and 3 (SDF + KI + Laser) showed dentin surfaces predominantly covered with granular precipitates, with no evidence of a continuous biofilm layer or clearly identifiable bacterial morphology (Fig. 2a–c).

• Group 4 (SDF + Laser) exhibited an irregular surface with scattered deposits and limited bacterial presence, with some cells showing altered morphology (Fig. 2d).

• Group 5 (Laser only) demonstrated bacterial cells on the dentin surface, including recognizable coccoid and rod-shaped forms, although the biofilm was not uniformly organized (Fig. 2e).

• By contrast, the Group 6 (Control) demonstrated dense and well-organized biofilm formation, with abundant coccoid and rod-shaped bacteria embedded in an extracellular matrix and preserved cellular morphology (Fig. 2f).

3.3 Microleakage results

A total of 72 specimens were evaluated for microleakage across six groups (n = 12 per group) using a predefined ordinal scoring system. Representative dye penetration patterns for each score category are presented in Fig. 3.

All specimens were independently assessed by two calibrated evaluators. Inter-rater agreement was excellent, with a weighted kappa value of 0.94 ($p < 0.001$). For Rater 1, no statistically significant differences were observed among the groups ($p = 0.589$). Similarly, the analysis for Rater 2 revealed comparable results, with no statistically significant differences detected among the groups ($p = 0.852$).

As shown in Table 3, median microleakage scores were comparable between the two raters across all groups. Group 3 (SDF + KI + Laser) demonstrated the highest median values for both evaluators, whereas the lowest median scores were observed in the Group 6 (control). However, these differences were not statistical significance.

4. Discussion

This study evaluated the antibacterial efficacy and microleakage behaviour of SDF, KI, and diode laser activation when used individually and in combination. While the effects of SDF and KI have been widely investigated, limited data are available regarding the influence of KI on microleakage and the interaction between SDF and diode laser activation. Therefore, the findings of the present study provide additional insight into the combined effects of these approaches on antibacterial performance and marginal integrity.

SDF has received increasing attention as a non-invasive caries-arresting agent in contemporary dentistry. Despite its

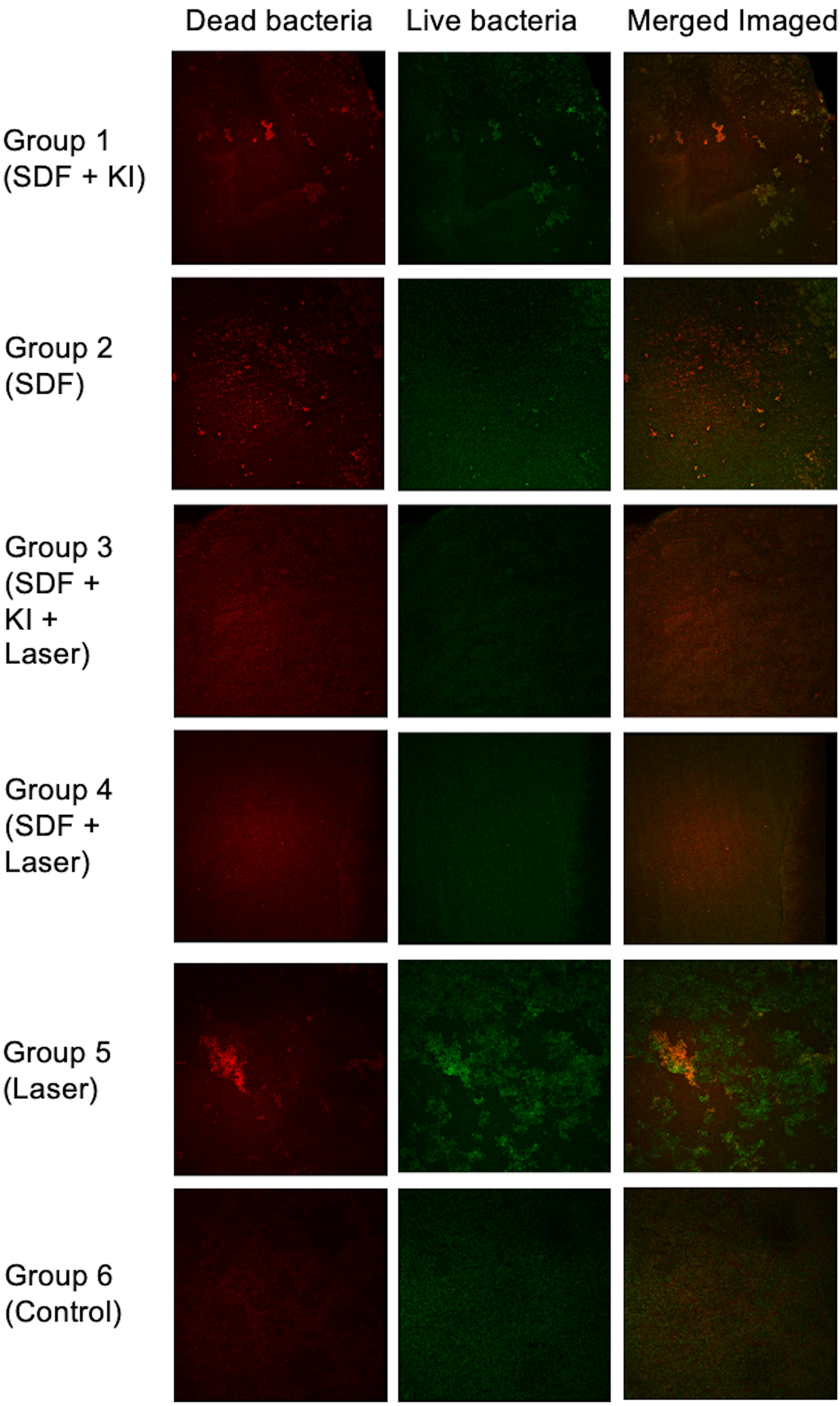

FIGURE 1. Representative confocal laser scanning microscopy (CLSM) images of biofilms from each experimental group showing live bacteria/dead bacteria/merged images. Within each panel, tiles represent Dead (red), Live (green), and Merged (red + green) views (lower-right tile left blank per export). Dead cells = red; live cells = green. Scale bar: 100 μm. SDF: Silver diamine fluoride; KI: potassium iodide.

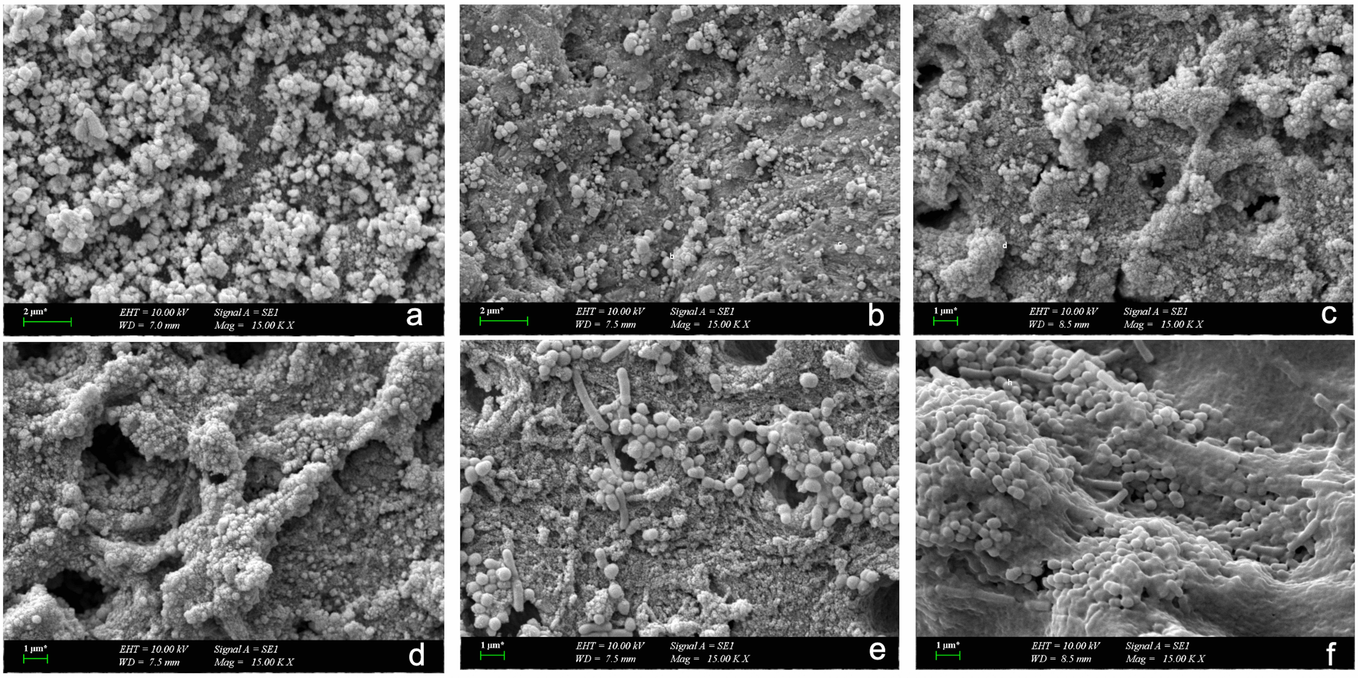

FIGURE 2. Representative SEM images of dentin surfaces obtained at 15,000× magnification. (a) Group 1(SDF + KI); (b) Group 2 (SDF only); (c) Group 3(SDF + KI + Laser); (d) Group 4 (SDF + Laser); (e) Group 5 (Laser only); (f) Group 6 (Dentin surface with biofilm). EHT: Electron high tension; WD: Working distance; SEI: Secondary electron image.

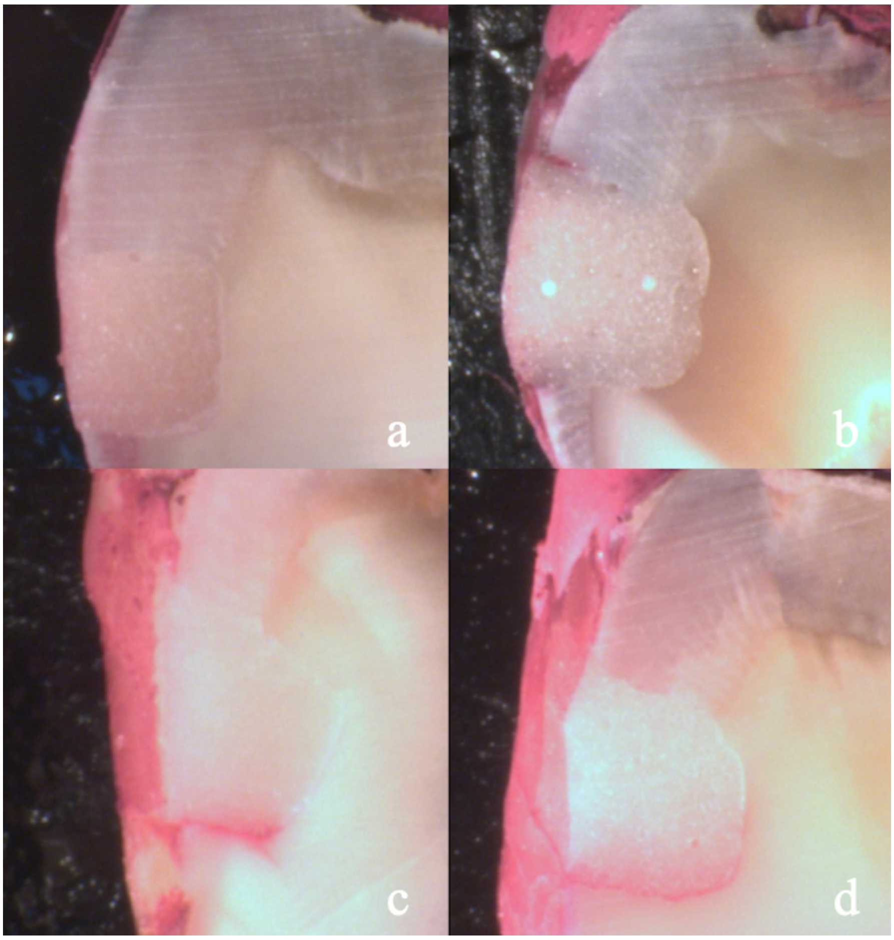

FIGURE 3. Representative images of dye penetration scores in Class V restorations. (a) score 0: No leakage; (b) score 1: Leakage extending to half the depth of the cavity; (c) score 2: Leakage extending beyond half the depth of the cavity; (d) score 3: Leakage extending to the bottom of the cavity.

TABLE 3. Median (Q1–Q3) microleakage scores assessed by two independent raters across the study groups.

Group	Rater 1	Rater 2
Group 1 (SDF + KI)	2 (1–2.75)	1.5 (0.25–2.75)
Group 2 (SDF)	0.5 (0–3)	1 (0–3)
Group 3 (SDF + KI + Laser)	2 (1–3)	2 (0.25–3)
Group 4 (SDF + Laser)	1 (0–3)	1 (0–3)
Group 5 (Laser)	1 (0.25–3)	1 (1–3)
Group 6 (Control)	0.5 (0–2)	0.5 (0–2.75)

Kruskal-Wallis test revealed no statistically significant differences among groups ($p > 0.05$). Inter-rater agreement between the two evaluators was excellent, with a weighted kappa value of 0.94 ($p < 0.001$).
Since the overall intergroup comparison for microleakage was not statistically significant, no post hoc pairwise comparisons were performed.
SDF: Silver diamine fluoride; KI: potassium iodide.

proven antibacterial and remineralizing effects, SDF causes permanent discoloration, particularly in caries-affected dentin [20]. This staining may negatively influence parental acceptance and clinicians' willingness to recommend SDF as a treatment option [20, 21].

KI is the most commonly used agent to reduce SDF-induced staining. Previous studies have demonstrated that the addition of KI does not adversely affect the remineralizing or mechanical properties of dental hard tissues. Cömert *et al.* [22] showed that KI decreased SDF-related discoloration without impairing remineralization, while Gadallah *et al.* [23] observed no significant difference in surface hardness between SDF alone and SDF + KI applications on primary enamel. In addition, Karaduran *et al.* [24] reported that KI did not alter the antibacterial efficacy of SDF, suggesting that KI can be incorporated into SDF protocols without compromising its primary biological effects.

Consistent with previous reports, the present study confirmed that SDF-containing protocols exhibited significantly higher antibacterial activity compared with the control group. Mei *et al.* [25] demonstrated a marked antibacterial effect of SDF against *S. mutans* and *Lactobacillus acidophilus* biofilms formed on dentin surfaces, and Vinson *et al.* [26] reported that both SDF alone and SDF/KI significantly reduced *S. mutans* counts compared with a control solution (sterile distilled water). Notably, Vinson *et al.* [26] also observed a higher antibacterial potential for SDF/KI compared with SDF alone, which aligns with the trends observed in the present study.

The highest antibacterial efficacy in this study was observed in Group 3 (SDF + KI + diode laser). This finding may reflect a synergistic interaction of chemical and physical antibacterial mechanisms. While SDF exerts antimicrobial activity primarily through silver ion-mediated bacterial inhibition, diode laser irradiation contributes through photothermal effects and biofilm disruption. The combination of these modalities appears to enhance antibacterial efficacy beyond that achieved by either approach alone. In addition, although KI is primarily used to control discoloration, its incorporation may have contributed to the antibacterial response observed in the combined protocol, particularly when used in conjunction with diode laser activation.

In this investigation, a 980 nm diode laser was selected due to its practicality, portability, cost-effectiveness, and clinical applicability. This wavelength has been commonly utilized for caries management and has been reported to be non-invasive, painless, and safe [27–29]. Diode lasers, in particular, produce controlled increases in dentin temperature that promote mineral deposition while preserving collagen integrity [30]. Although diode lasers are generally less effective in increasing microhardness compared with carbon dioxide (CO_2) or Neodymium-doped Yttrium Aluminium Garnet (Nd:YAG) lasers, their ability to enhance fluoride accumulation and retention has been well documented [31, 32]. Previous studies have demonstrated that diode laser utilization with fluoridated agents enhances calcium and phosphorus content without inducing enamel cracks or structural damage [17, 27, 28].

The laser parameters applied in the present study were selected in accordance with previously reported protocols [17]. In that study, the use of a 980 nm diode laser demonstrated favorable remineralization outcomes, supporting the clinical relevance of the selected wavelength. The selected parameters (1 W, 60 s, continuous, contact-mode) were considered appropriate for providing controlled energy delivery while minimizing excessive thermal effects. Furthermore, the 400 μm therapy tip was preferred to allow precise application and uniform scanning across the dentin surface. Guarato *et al.* [33] reported that 980 nm diode lasers offer greater clinical versatility compared with Erbium, Chromium-doped Yttrium Scandium Gallium Garnet (Er,Cr:YSGG) lasers. They also observed that although the order of fluoride and laser application did not significantly affect outcomes, fluoride-first protocols yielded more favorable results. In the present study, a 980 nm diode laser was likewise used, and SDF was applied prior to laser irradiation in accordance with this approach.

Recent studies have further supported the adjunctive use of diode lasers following SDF application. Laser-assisted SDF protocols have been reported to increase dentin microhardness, enhance fluoride uptake, and promote remineralization compared with SDF alone [34–36]. These effects may be attributed to the controlled thermal action of diode lasers, which may improve mineral deposition and fluoride retention within dental hard tissues. Such findings further support the potential beneficial interaction between SDF and diode laser irradiation.

With respect to microleakage, Class V cavities were selected due to their high configuration factor and clinical relevance [37]. The dye penetration method was used, as it remains a commonly accepted and practical technique for evaluating microleakage [38].

Recent evidence has also emphasized the importance of preserving marginal integrity in glass ionomer-based systems. Al Qassar *et al.* [39] reported reduced microleakage and improved bond integrity in conventional and resin-modified glass ionomer cements, supporting the relevance of interfacial adaptation in restorative sealing performance. Similarly, Khan *et al.* [40] demonstrated lower microleakage values in glass hybrid restorative systems compared with conventional and resin-modified glass ionomer cements. Collectively, these findings indicate that glass hybrid restorative materials have shown promising sealing performance in various *in vitro* settings, which supports the use of this material in the present study.

The present findings demonstrated no statistically significant differences in microleakage among the experimental groups. This observation is consistent with previous studies reporting that SDF application does not significantly compromise marginal sealing in glass ionomer-based restorations [5, 41]. Similarly, Powell *et al.* [42] reported that SDF application did not increase microleakage in high-viscosity glass ionomer restorations.

Although no statistically significant differences were detected, higher median microleakage values were observed in the SDF + KI and SDF + KI + laser groups. This trend may be attributed to the formation of additional reaction products or precipitate layers following KI application, including the possible formation of silver iodide precipitates after reaction with silver ions from SDF. Such reaction products may have influenced surface characteristics and affected the interaction between the restorative material and tooth structure, which may help explain the findings observed in the KI-containing groups [35]. Meraji *et al.* [43] similarly reported increased microleakage when SDF and KI were combined in Class V composite restorations. The addition of diode laser activation may further enhance surface reactions, potentially influencing the bonding substrate, although this effect did not reach statistical significance. However, surface alterations associated with KI application do not necessarily indicate compromised interfacial performance. Mohammed *et al.* [44] reported that the application of KI following SDF treatment positively influenced the bond durability of resin composites bonded to demineralized dentin, suggesting that KI-related surface interactions may not be detrimental to bonding outcomes. Conversely, the relatively lower median values observed in the control group may be related to the absence of any surface pretreatment, thereby preserving the original dentin surface characteristics and allowing more favorable adaptation of the restorative material. This finding may support the view that dentin surface modifications caused by pretreatment protocols can influence microleakage. In the present study, no cavity conditioner was applied prior to placement of the restorative material. Cavity conditioners are commonly used before glass hybrid or glass ionomer restorations to reduce the smear layer and enhance adhesion to tooth structure [45]. However, conditioner use was omitted because the experimental protocol already involved multiple surface pretreatment steps, and its use is not mandatory for the selected restorative material. Nevertheless, the use of a cavity conditioner might have influenced the microleakage outcomes by improving marginal adaptation.

Although the investigated protocol involves multiple pretreatment steps, procedures such as SDF application and diode laser irradiation are relatively quick, non-invasive, and clinically practical. In the present study, the combined protocols did not adversely affect microleakage outcomes and may offer additional antibacterial benefits, which may support their consideration in selected pediatric cases where caries control is prioritized.

Several limitations of this study should be considered. As this was an *in vitro* study, the findings do not fully reflect the complex biological conditions of the oral environment, including saliva, temperature fluctuations, masticatory forces, and host-related factors. Although a dual-species biofilm model was used, it does not fully reproduce the complexity of natural multispecies oral biofilms, including interspecies competition, extracellular matrix dynamics, and ecological shifts over time. In addition, saliva samples used for biofilm formation were obtained from adults, and the microbial profile may differ from that of pediatric populations. CLSM-based observations should be interpreted cautiously, as fluorescence intensity may depend on biofilm structure and image acquisition conditions. Donor age information was not available, which should be considered, as age-related variations in enamel and dentin properties may influence outcomes. While a standardized experimental design was adopted for the microleakage assessment, future studies using carious tooth substrates may provide a closer approximation to clinical conditions. Despite these limitations, the findings may provide insight into optimizing minimally invasive caries management strategies in clinical settings. Further clinical investigations would also be beneficial to support the applicability of these results.

Overall, the findings of the present study suggest that while SDF-based protocols do not significantly compromise marginal integrity, the combined use of SDF, KI, and diode laser activation may enhance antibacterial efficacy. These results suggest that such minimally invasive combinations have the potential to provide effective caries control without adversely affecting the sealing performance of glass hybrid restorations.

5. Conclusions

Within the limitations of this *in vitro* study, all treatment protocols exhibited antibacterial activity compared with the control group, with the SDF + KI + laser combination showing the highest antibacterial efficacy among the experimental groups. With regard to microleakage in glass hybrid restorations, no statistically significant differences were observed among the treatment groups, suggesting that the evaluated antibacterial protocols did not adversely affect the sealing ability of the restorations. Overall, the findings suggest that the combined use of SDF, KI, and diode laser activation may offer antibacterial benefits without compromising microleakage performance under the present *in vitro* conditions.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

DDA, NT and MAY—designed the research study. DDA, NT and DLC—performed the research. EGÖ—performed the formal analysis. DDA and MYÜ—analyzed the data. DDA—wrote the manuscript. MAY—supervised the study and contributed to review and editing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee for Non-Drug and Non-Device Clinical Research at the Faculty of Medicine, Marmara University (Approval No: 09.2024.662). Written informed consent was obtained from all saliva donors for sample collection and all patients for the use of their extracted teeth for research purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jocpd.com/files/article/2095406614431318016/attachment/Supplementary%20material.docx.

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