Head & Face Medicine

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Effect of plaque-disclosing agents on biofilm removal: single-center randomized trial in fourth-year dental students

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Abstract

Background To assess whether plaque-disclosing agents (PDA) improve self-performed mechanical plaque removal and gingival health compared with standard instruction alone in dental students.

Design/Setting Single-center, parallel-group randomized controlled trial (1:1), Istanbul Medipol University (Oct 2023–Jan 2024).

Participants 124 fourth-year dental students (age 20–30 years) randomized to test ($n=62$) or control ($n=62$).

Interventions Both groups received standardized oral-hygiene instruction (Modified Bass; interdental cleaning guidance). The test group additionally received PDA before brushing as a visual aid; all outcomes were measured after triple rinsing.

Primary outcome (prespecified) Between-group difference in change (Δ) in Plaque Index (PI) from baseline to week 1.

Secondary outcomes ΔPI baseline→post-brushing and baseline→month 1; ΔGingival Index (GI) baseline→week 1 and baseline→month 1.

Masking Outcome assessor blinded at the time of measurement (after rinsing).

Results All 124 randomized participants completed follow-up and were analyzed. Both groups showed short-term improvement (immediate reduction in PI post-brushing; GI improvement at week 1). The primary outcome showed no significant between-group difference in ΔPI from baseline to week 1. Secondary analyses similarly found no significant between-group differences in ΔPI or ΔGI at month 1.

Conclusions In a population with high baseline oral-hygiene proficiency, adding PDA did not confer a measurable advantage over standardized instruction alone on PI or GI change. PDA may be more informative in lay populations with lower oral-health literacy.

Trial registration ClinicalTrials.gov, NCT06080672, registered on October 12, 2023.

Keywords Plaque-disclosing agents, Dental education, Plaque index, Gingival index, Oral hygiene

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Introduction

Dental plaque is a significant contributing factor to the development of white spot lesions, periodontal issues, and dental caries [1]. In particular, dental plaque biofilm plays a key role in the onset of periodontal diseases. Effectively reducing dental plaque biofilm can lower the incidence of tooth loss and prevent periodontal conditions such as gingivitis, highlighting the critical importance of patient motivation in oral health management. Patients must understand the importance of plaque removal and be adequately motivated to maintain good oral hygiene [2].

Oral biofilm, which is typically colorless [3], can be challenging to detect without assistance. Disclosing agents, available in solution, swab, and tablet forms, are used to visualize biofilm by staining it, thereby making it easier to identify and remove [4]. Since the coloration of teeth and dental plaque is often similar, plaque removal can be difficult. Plaque-disclosing agents (PDA) have been employed since the early 20th century to address this issue by rendering the plaque visible. These agents include a range of specific chemicals, such as iodine, Bismarck brown, erythrosine, gentian violet, malachite green, methylene blue, basic fuchsin, and two-tone solutions. Substantial evidence supports the use of PDA for professional plaque detection in clinical settings, as well as for enhancing patient education and motivation [5]. By making biofilm visible, these agents can improve patient oral hygiene practices and provide guidance for personal oral care [4]. The color intensity of PDA corresponds to the thickness of the plaque, making them highly effective in assessing a patient's oral hygiene status. These agents help raise awareness about the importance of biofilm removal, offer personalized instructions and motivation for better oral hygiene, facilitate self-assessment, evaluate the effectiveness of oral hygiene practices, and contribute to the success of prevention and training programs. Additionally, they support studies focused on biofilm identification. Visualization of dental plaque using disclosing solutions serves as an auxiliary method in dental prophylaxis, potentially enhancing the quality of the procedure [6].

The application of PDA is crucial in encouraging patients to maintain effective oral hygiene routines [7]. Dentists should utilize these agents to visually demonstrate the presence of bacterial plaque on and around the teeth, enabling patients to identify areas of plaque accumulation and remove it using appropriate dental tools [2].

This study aims to evaluate the impact of visual guidance provided by PDA on plaque removal efficacy, determine whether these agents positively influence oral care, and assess their effectiveness in improving overall oral health. Additionally, the study will investigate whether the application of dental PDA by dental professionals in oral health programs contributes to reducing gingival and/or plaque indices. The study hypothesizes that the use of PDA in oral health programs significantly enhances plaque removal efficacy and improves overall oral health outcomes.

Materials and methods

Ethical considerations

This study was conducted at the Department of Pedodontics, Istanbul Medipol University, between October 2023 and January 2024. Ethical approval was obtained from the Istanbul Medipol University Ethics Committee (Reference No: E-10840098-772.02-2361). All participants were fully informed about the study, and written informed consent was obtained prior to their inclusion. The study adhered to the Principles of the Declaration of Helsinki and was registered at ClinicalTrials.gov (Registration No: NCT06080672, 12/10/2023).

Inclusion and exclusion criteria

The inclusion and exclusion criteria, along with the study procedure, are presented in Fig. 1. Inclusion criteria included being systemically healthy, drug-free, and having a minimum of 20 teeth. Exclusion criteria were smoking, undergoing orthodontic treatment, experiencing pain or infection that could impede brushing, pregnancy, breastfeeding, and unwillingness to participate.

Study design

This interventional prospective study involved 124 fourth-year dentistry students, aged 20 to 30 years, from Istanbul Medipol University. Participants were selected using a convenience sampling method and were drawn from the Preventive Dentistry course. As part of the course, they practiced brushing techniques on themselves to prepare for teaching these methods to patients during their clinical internships.

Sample size, study population

Based on data from previous research [8], the plaque reduction rate through manual tooth brushing was approximately 70%. The sample size for this study was calculated to be 124, assuming that the application of the Modified Bass technique in conjunction with a plaque-disclosing agent could increase plaque removal efficacy to 90%, with a significance level (alpha) of 0.05 and a power of 80%. The final study sample included 124 students who completed all measurements.

At the outset, participants completed a questionnaire covering their demographic data and oral health behaviors, including tooth brushing frequency, use of dental floss, use of mouthwash or mouth spray, and gum bleeding.

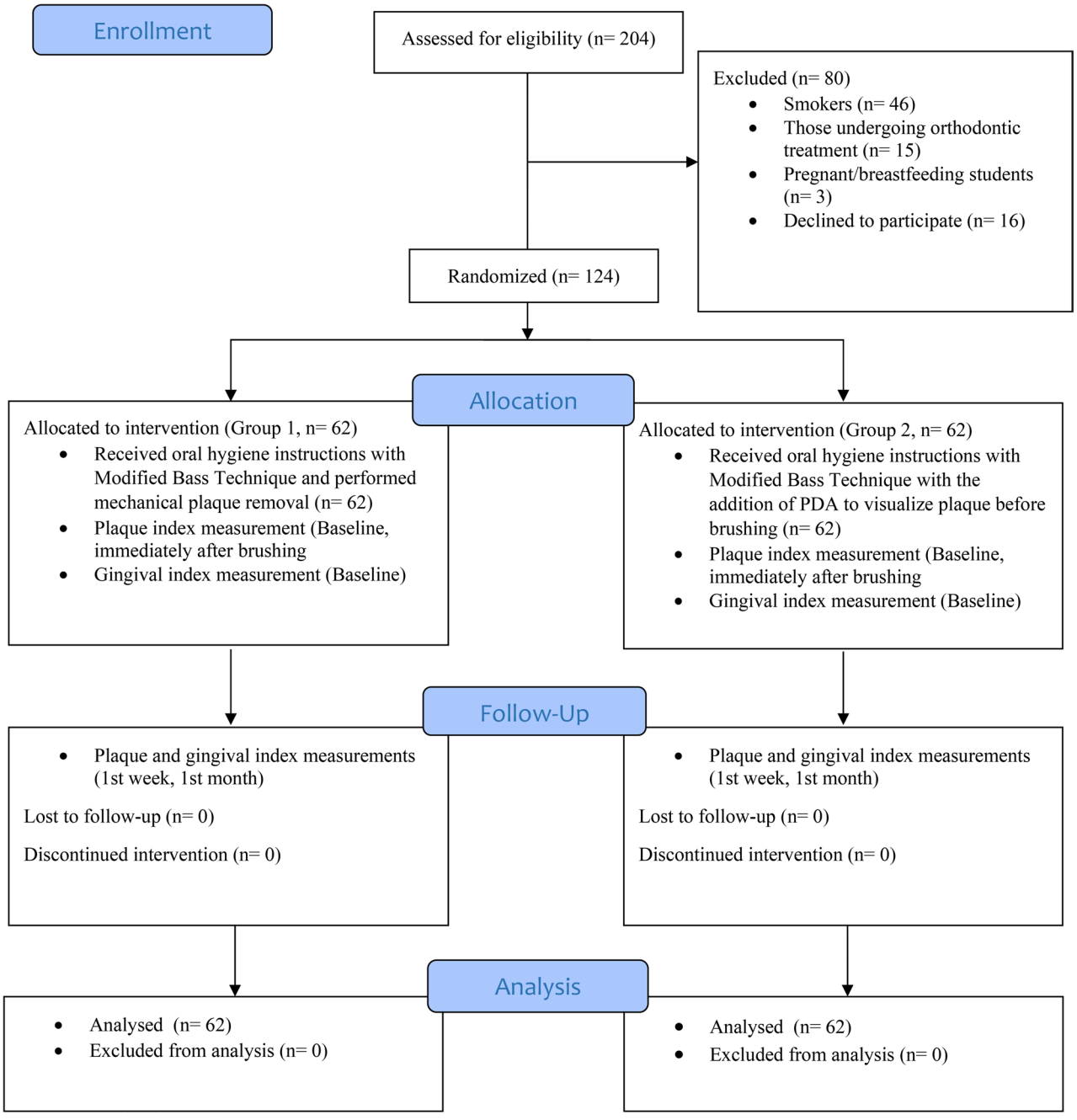

Fig. 1 Flow chart of the application procedure

Randomization and allocation concealment, measurement methods and data collection

Sequence generation

Participants were randomized in a 1:1 ratio using a computer-generated sequence created with an online randomization tool (GraphPad QuickCalcs). No stratification was used.

Allocation concealment mechanism

The sequence was transferred to sequentially numbered, opaque, sealed envelopes (SNOSE) prepared by an independent researcher (M.B.) who had no role in enrollment, intervention delivery, or outcome assessment.

Implementation

Participants were enrolled and assigned to study arms by N.Ü. and B.B.A., who opened the next envelope in numerical order after baseline procedures and

immediately before the intervention. Outcome measurements were performed by N.A. with 8 years of experience in periodontology, who was blinded at the time of assessment after the standardized triple-rinse procedure.

- *Group 1 (Control Group; n* = 62): Students received instruction on the Modified Bass Technique and the use of dental floss or interdental brushes and then brushed their teeth.
- *Group 2 (Test Group; n* = 62): In addition to the instruction provided to Group 1, students' teeth were stained with a plaque-disclosing agent, and the stained areas were shown to them in a mirror before they brushed their teeth.

Clinical examinations were conducted to record the Decayed, Missing, and Filled Teeth (DMFT) index, baseline gingival index (GI, Löe and Silness, 1963 [9]) and plaque index (PI, Silness and Löe, 1964 [10]). In the test group, a plaque-disclosing solution was applied before brushing; after brushing, participants rinsed thoroughly with water three times to remove any residual dye. GI and PI were then recorded. Because no visible dye remained and the assessor had no access to the allocation list, the outcome assessor was blinded to group allocation at the time of measurement. GI and PI measurements were taken on four surfaces using a Williams periodontal probe (mesiobuccal, midbuccal, distobuccal, midlingual) of each tooth, excluding third molars, and averaged to obtain the mean GI and PI for each participant.

The GI index (GI, Löe and Silness, 1963 [9]) and PI index (PI, Silness and Löe, 1964 [10]), which were used to determine the oral hygiene level of the individuals included in the study, were evaluated as follows:

"GI index

0. Healthy gingiva.
1. There is mild inflammation, discoloration and edema, no bleeding on probing.
2. Moderate inflammation, marked redness, edema, and bleeding on probing.
3. Severe inflammation, prominent redness and edema, ulceration, spontaneous bleeding tendency.

PI index

0. No bacterial plaque on the tooth surface.
1. There is plaque in the form of film and can be seen with the help of a probe.
2. There is moderate soft attachment visible to the naked eye.
3. There is a visible dense soft appendage filling the gingival sulcus on the tooth surface."

Participants received standardized training in the Modified Bass technique via a video and model demonstration delivered by the same instructor. They were instructed to brush twice daily for ~2 min per session and to perform interdental cleaning (dental floss or interdental brushes) once daily between visits. Both groups received identical instructions. Standard toothbrushes (Banat Deep Clean, Turkey) and toothpaste (Colgate Total 12, Colgate-Palmolive Company, Turkey) were provided to all participants.

- *Control Group*: Students brushed their teeth using the instructed technique.
- *Test Group*: Students' teeth were stained using Mira 2-tone Solution (Hager & Werken, Duisburg, Germany) to visualize plaque, which was then shown to them in a mirror. After this, they brushed their teeth using the Modified Bass Technique.

Participants were not supplied with or instructed to use plaque-disclosing agents at home. Between visits, they maintained their usual oral-hygiene routines. Both groups received identical home-care instructions: brush twice daily (~2 min per session) using the Modified Bass technique and perform interdental cleaning (dental floss or interdental brushes) once daily. No other adjunctive products were prescribed.

Following baseline measurements, PI was measured immediately after brushing, one week and one month later, whereas GI was measured one week and one month later. Since no immediate change in the gingival index was expected post-brushing, only the plaque index was re-measured.

Protocol deviation

The prespecified Green & Vermillion OHI-S was not collected due to time constraints during the course session; therefore, only GI and PI were analyzed as hygiene outcomes.

Follow-Up measurements

At one week and one month, plaque and gingival indices were re-measured using the same protocol as at baseline. In the test group, a plaque-disclosing solution was applied before brushing to visualize deposits; participants then brushed and rinsed thoroughly with water three times to remove any residual dye, and GI/PI were recorded only after rinsing. In the control group, participants brushed and rinsed in the same manner (without disclosing solution). Thus, outcome measurements in

Table 1 Demographic data and oral health behaviors among the groups

		Group 1	Group 2	
		n (%)	*n* (%)	*p*
Age $_{Mean\pm SD}$		23.35 ± 1.65	22.91 ± 1.23	[1]0.094
Gender	Male	23 (37.1%)	32 (51.6%)	[2]0.104
	Female	39 (62.9%)	30 (48.4%)	
Brushing frequency	Once a day	10 (16.1%)	12 (19.4%)	[3]0.273
	Two or more times a day	49 (79%)	50 (80.6%)	
	Infrequently	3 (4.8%)	0 (0%)	
Use of dental floss	None	15 (24.2%)	16 (25.8%)	[3]0.880
	Once a day	12 (19.4%)	15 (24.2%)	
	Two or more times a day	4 (6.5%)	3 (4.8%)	
	Infrequently	31 (50%)	28 (45.2%)	
Use of mouth-wash or mouth spray	Yes	32 (51.6%)	33 (53.2%)	[2]0.857
	No	30 (48.4%)	29 (46.8%)	
Gum bleeding	Yes	10 (16.1%)	23 (37.1%)	[4]0.015*
	No	52 (83.9%)	39 (62.9%)	

[1]Student t test, [2]Chi-square test, [3]Fisher Freeman Halton Exact Test, [4]Continuity (yates) correction, *$p<0.05$

both groups were performed at the identical post-brushing time point, ensuring equivalent measurement timing across arms.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 22. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistics (minimum, maximum, mean, standard deviation, median, frequency) were used alongside inferential statistical methods. The Kruskal-Wallis test was applied to compare non-normally distributed quantitative data between groups. The Student's t-test was used to compare normally distributed data between two groups, while the Mann-Whitney U test was used for non-normally distributed data. The Friedman test (post hoc Wilcoxon signed-rank test) was used for intra-group comparisons of non-normally distributed parameters. Qualitative data were compared using the Chi-square test, Fisher Freeman Halton Exact test, and Continuity (Yates) Correction. Statistical significance was set at $p < 0.05$.

In addition to unadjusted analyses, adjusted group comparisons were performed using univariate ANCOVA models to account for baseline imbalance in self-reported gum bleeding and potential sex differences. For each time point (baseline, week 1, month 1), a separate ANCOVA model was constructed with group as the fixed factor and sex and gum bleeding status as covariates. Adjusted means and 95% confidence intervals were obtained via the estimated marginal means (EMMEANS) procedure.

Table 2 Baseline characteristics and clinical parameters by group (mean ± SD [median])."

	Group 1	Group 2
	Mean ± SD (median)	Mean ± SD (median)
DMFT	6.02 ± 4.15 (5)	5.92 ± 4.76 (5)
GI.1	0.95 ± 0.44 (0.97)	0.82 ± 0.46 (0.86)
PI.1	0.59 ± 0.32 (0.53)	0.63 ± 0.41 (0.54)

Footnote: GI.1 = baseline; PI.1 = baseline

Because GI and PI values were not normally distributed, within-group repeated-measures analyses were conducted using the Friedman test with Bonferroni-adjusted Wilcoxon signed-rank tests.

Results

Participant flow

Of 204 students screened for eligibility, 80 were excluded (46 smokers, 15 undergoing orthodontic treatment, 3 pregnant/breastfeeding, and 16 who declined). The remaining 124 participants were randomized 1:1 to the control ($n = 62$) or test ($n = 62$) group. There were no losses to follow-up and no post-randomization exclusions; all randomized participants completed all assessments and were included in the analysis. No serious adverse events potentially related to treatment procedures were expected.

The study included 124 participants, comprising 55 male (44.4%) and 69 female (55.6%), with ages ranging from 19.5 to 30.7 years. The mean age was 23.13 ± 1.47 years. Table 1 presents demographic data and oral health behaviors, including the frequency of tooth brushing, use of dental floss, mouthwash or mouth spray, and the incidence of gum bleeding among the groups based on the questionnaire. Statistical analysis revealed no significant differences between the groups regarding mean age, gender distribution, brushing frequency, or the usage rates of dental floss, mouthwash or oral sprays ($p > 0.05$).

According to the data from the questionnaire in which students subjectively evaluated themselves in response to the question, 'Do your gums bleed?', the incidence of bleeding in Group 2 (37.1%) was significantly higher than in Group 1 (16.1%), with statistical significance ($p = 0.015$; $p < 0.05$, Table 1)

Baseline clinical parameters

Baseline clinical parameters (DMFT, GI.1, PI.1) did not differ meaningfully between groups (Table 2).

Intergroup comparison of change from baseline (primary effectiveness)

Table 3 represents intergroup comparisons of clinical indices at follow-up time points. We compared ΔGI and ΔPI between groups at key intervals:

Table 3 Intergroup comparisons of change scores and observed clinical indices at follow-up time points. Values are mean ± SD (median). 95% CI shows the between-group difference; cohen's d is the effect size; p values from Mann–Whitney U test. Within-group change was evaluated with the Friedman test

	Group 1	**Group 2**	**Between-group difference (Δ)**	**95% CI of the difference**			
	Mean ± SD (median)	**Mean ± SD (median)**		**Lower**	**Upper**	**Cohen's d**	***p*[1]**
ΔGI (Week 1 – Baseline)	-0.40 ± 0.48 (-0.46)	-0.44 ± 0.50 (-0.37)	0.04	-0.14	0.21	-	0.887
ΔPI (Week 1 – Baseline)	0.04 ± 0.38 (0.03)	-0.05 ± 0.45 (-0.02)	0.09	-0.05	0.24	-	0.281
GI.1	0.95 ± 0.44 (0.97)	0.82 ± 0.46 (0.86)	-	-0.028	0.291	0.29	0.129
GI.2	0.55 ± 0.49 (0.45)	0.38 ± 0.37 (0.27)	-	0.013	0.322	0.53	0.053
GI.3	0.58 ± 0.40 (0.59)	0.50 ± 0.40 (0.42)	-	-0.062	0.224	0.20	0.215
Partial Eta Squared	0.506	0.465					
p^2	0.001*	0.001*					
PI.1	0.59 ± 0.32 (0.53)	0.63 ± 0.41 (0.54)	-	-0.165	0.096	0.11	0.942
PI. post-brushing	0.48 ± 1.53 (0.17)	0.23 ± 0.29 (0.13)	-	-0.143	0.638	0.23	0.435
PI.2	0.63 ± 0.43 (0.58)	0.66 ± 0.73 (0.52)	-	-0.233	0.192	0.05	0.595
PI.3	0.55 ± 0.33 (0.51)	0.58 ± 0.40 (0.50)	-	-0.164	0.094	0.08	0.863
Partial Eta Squared	0.071	0.580					
p^2	0.001*	0.001*					

Footnote. Δ indicates change from baseline

Primary endpoint: ΔPI (baseline → week 1)

GI.1 = baseline; GI.2 = week 1; GI.3 = month 1

PI.1 = baseline; PI.post-brushing = immediately after brushing; PI.2 = week 1; PI.3 = month 1

[1]Mann–Whitney U (between groups); [2]Friedman (within-group change); $p<0.05$

Table 4 Adjusted comparisons of GI and PI between groups at each time point (ANCOVA adjusted for sex and baseline gum bleeding). Adjusted means were derived from EMMEANS. Different superscript letters within columns indicate significant within-group differences after bonferroni correction

	Group 1	Group 2	
	Mean ± SD (median)	Mean ± SD (median)	[1] Adjusted *p*
GI.1	0.98 ± 0.44 (0.98)[a]	0.81 ± 0.46 (0.86)[a]	0.047*
GI.2	0.57 ± 0.47 (0.46)[b]	0.36 ± 0.35 (0.27)[b]	0.009*
GI.3	0.60 ± 0.39 (0.60)[b]	0.49 ± 0.40 (0.42)[c]	0.142
[2]p	0.001*	0.001*	
PI.1	0.62 ± 0.32 (0.55)[a]	0.62 ± 0.41 (0.54)[a]	0.928
PI. post-brushing	0.32 ± 0.37 (0.17)[b]	0.23 ± 0.29 (0.13)[b]	0.176
PI.2	0.66 ± 0.43 (0.60)[a]	0.56 ± 0.36 (0.52)[a]	0.229
PI.3	0.55 ± 0.33 (0.52)[a]	0.58 ± 0.39 (0.50)[a]	0.686
[2]p	0.001*	0.001*	

Footnote. GI.1 = baseline; GI.2 = week 1; GI.3 = month 1;

PI.1 = baseline; PI.post-brushing = immediately after brushing; PI.2 = week 1; PI.3 = month 1;

$p<0.05$. Different superscript letters within columns indicate significant within-group changes (Bonferroni-adjusted Wilcoxon test)

- ΔGI (week 1 – baseline) and ΔGI (month 1 – baseline): Both groups showed improvement at week 1 with partial rebound at month 1, but between-group differences in change were not statistically significant.
- ΔPI (post-brushing – baseline), ΔPI (week 1 – baseline), ΔPI (month 1 – baseline): Both groups showed an immediate reduction post-brushing and no sustained advantage for the test group at week 1 or month 1; between-group differences in change were not statistically significant.

After adjusting for sex and baseline gum bleeding using ANCOVA, Group 1 showed significantly higher GI scores than Group 2 at baseline (adjusted $p = 0.047$) and week 1 (adjusted $p = 0.009$), whereas no significant adjusted difference was found at month 1 ($p = 0.142$). For PI, no significant adjusted between-group differences were observed at any time point (all $p > 0.05$). These findings were consistent with the unadjusted analyses (Table 4). Within-group changes across time remained significant for both groups (Friedman $p < 0.001$).

Within-group change (supporting)

Within each group, GI decreased from baseline to week 1 (both $p < 0.01$) and partially increased by month 1 while remaining close to baseline; PI dropped immediately after brushing (both $p < 0.01$) and rose thereafter toward week 1 and month 1 (Table 4). These patterns indicate short-term improvements without a differential sustained effect attributable to the plaque-disclosing agent.

Discussion

This study evaluated whether using a plaque-disclosing agent before brushing as a visual aid improves mechanical plaque removal—and thereby reduces gingival inflammation over time—compared with standard instruction alone, using between-group differences in change (ΔPI, ΔGI) as the primary evaluation.

Proper tooth brushing techniques are crucial for maintaining oral hygiene, and as such, a dedicated course on tooth brushing was included as an integral component of the Preventive Dentistry curriculum. Preventive Dentistry, a mandatory and specialized course for dental students, is introduced in the spring term of the third-year undergraduate program at Istanbul Medipol University. At this stage, students possess only basic knowledge of dentistry. Therefore, the significance of the tooth brushing course extends beyond technique. It requires students to engage in hands-on learning, compare different methods, and critically evaluate their findings. Additionally, it provides aspiring dentists with their first opportunity to develop professional advice and treatment plans for future patients. In the fourth year, as part of the Preventive Dentistry course, it is of critical importance for dentistry students to first practice the brushing techniques on themselves before instructing patients during their clinical internship. This approach is essential for achieving the educational objectives of the course.

The Gingival Index (GI) showed no significant between-group differences in change across time points (baseline, week 1, month 1). This pattern paralleled the Plaque Index (PI), which likewise showed no differential change between groups. Our findings are consistent with prior reports [4, 11] but differ from Peng et al. [12], who observed increases in GI in both arms at three months. Peng et al. attributed this rise to biofilm retention with fixed appliances and the absence of ongoing reinforcement (participants were not required to continue disclosing), concluding that a single educational session is insufficient and repeated instruction is needed.

Although the GI scores in both groups decreased significantly after one week, measurements taken one month later revealed an increase in GI scores, albeit not to the levels observed at baseline. This suggests that students' brushing motivation may decline over time as the interval between assessments increases. We believe that the periodic repetition of standardized training on the Modified Bass Technique, delivered through video presentations and model demonstrations, would be beneficial in maintaining and enhancing students' motivation over time. The repeated assessment of PI and GI scores may have acted as a motivational factor [13]. This phenomenon is referred to in the literature as the Hawthorne effect, which suggests that individuals modify their behavior when they are aware of being observed

or evaluated [14, 15]. This effect was first identified during a series of experiments conducted in the 1920s at the Hawthorne Works factory in Illinois. Researchers observed that workers exhibited increased productivity when they were aware of being observed, irrespective of any modifications to their working conditions, such as adjustments in lighting levels [16]. The fundamental premise of the Hawthorne effect is that the awareness of observation can induce behavioral changes. Individuals may modify their actions to align with perceived expectations or due to the increased attention received from researchers [17, 18]. In practical applications, the Hawthorne effect has been observed across various domains. For example, in clinical trials, patients tend to demonstrate improved adherence to treatment regimens upon realizing they are under observation [19]. Similarly, research has indicated that healthcare professionals exhibit higher compliance with hygiene protocols when they are aware of being monitored [20]. In educational environments, students have been found to achieve better academic performance when they are conscious of their performance being assessed [21]. Another example of the Hawthorne effect in the present study is observed in the gum bleeding scores, which were derived from a self-reported questionnaire and showed a significant difference among groups, whereas the GI scores showed no significant difference at baseline. Since self-assessments can be influenced by personal perception and bias, there is a possibility of misjudgment or over/underestimation of symptoms. In contrast, the gingival index was objectively measured by a single investigator with eight years of experience in periodontology, ensuring a standardized and unbiased assessment of gingival health. To mitigate the Hawthorne effect and sustain adherence, future trials should incorporate objective adherence monitoring (e.g., toothbrush timers/app logs, brief home diaries, or counts of returned disclosing tablets when applicable) and use minimal, prespecified, and identical contact in both arms—such as weekly SMS reminders or a 2–3-minute micro-refresher video—to avoid differential motivation. Reinforcement content, frequency, and delivery should be standardized in the protocol and reported transparently. Outcome assessments should remain masked and scheduled independently of reminder delivery to reduce reactivity to observation. Together, these steps can limit Hawthorne-related inflation of short-term effects while providing reliable adherence data.

In the present study, no statistically significant differences were observed between the groups in terms of PI scores across the measurement intervals. The application of conventional oral hygiene instructions and chairside motivational techniques, including the use of plaque disclosing agent, did not result in a reduction of PI scores among the groups. These findings are consistent with previous studies [4, 11], which reported no significant changes in PI scores over a three-month period among orthodontic patients utilizing conventional plaque control techniques. In contrast to our results, Marini et al. [22] demonstrated that patients who received repeated oral hygiene motivation and chairside motivational techniques, including plaque indicator solution and manual toothbrushing, exhibited significant reductions in PI scores after three months. Similarly, Peng et al. [12] observed a significant increase in PI scores in both their control group (routine oral hygiene instructions) and biofilm-disclosing group (biofilm-disclosing tablets) after three months compared to baseline. These discrepancies may be attributed to variations in patient demographics, measurement methodologies, and motivational techniques employed across the studies.

PDA were utilized in Group 2 to visualize plaque localization and assess brushing quality in the clinic. However, no significant differences in PI scores were observed between Groups 1 and 2. This outcome may be attributed to the study population, as the participants were fourth-year dental students who likely possessed sufficient knowledge of proper brushing techniques, potentially rendering additional education on brushing techniques redundant, given their previous training. Previous studies employing PDA as chairside motivational tools have yielded mixed results. For instance, Boyd [23] demonstrated that using a disclosing solution for motivational purposes was more effective in overall plaque removal than providing plaque control instructions alone. In contrast, Peng et al. [12] and Acharya et al. [11] found that the use of disclosing agents was not significantly more effective as a motivational technique for plaque control compared to control groups.

PDA, available in tablet and liquid forms, are well-established tools used to help patients visualize oral plaque and improve their self-performed hygiene and compliance, both in professional and home settings [12, 24]. They have also been demonstrated to ensure thorough cleaning of molar occlusal surfaces before fissure sealing [25] and to enhance biofilm control on dentures [26]. In the context of professional oral hygiene, it can be hypothesized that PD agents are beneficial not only for patients but also for clinicians, serving as a guide for biofilm removal by providing immediate feedback, particularly for hard-to-reach areas and for individuals at high risk for carious or periodontal disease [27]. The effectiveness of plaque-disclosing agents as teaching tools in dental education has been demonstrated in various studies [6, 28–32], emphasizing their role in enhancing learning outcomes and improving clinical practices. Hoskin et al. explored dental educators' perceptions of learning domains and emphasized that practical, hands-on experiences, such as the use of disclosing agents, are essential

for effective learning in dental education, improving student engagement and clinical performance [28]. Similarly, a systematic review by Fasoulas et al. concluded that disclosing agents, when combined with oral hygiene instructions, effectively improve self-performed plaque control, reinforcing their value as educational aids [6]. Kawanishi et al. highlighted the importance of visual aids in training dental hygienists, demonstrating that disclosing agents provide immediate visual feedback, which is critical for developing effective clinical skills [29]. Kim et al. further confirmed the effectiveness of natural disclosing agents in reinforcing proper brushing techniques and enhancing students' understanding of plaque control [30]. In addition, Buischi et al. found that integrating disclosing agents into oral health education programs for adolescents significantly improved their comprehension of plaque accumulation and its consequences, emphasizing their relevance in fostering oral hygiene awareness among dental students [31]. Provenzano's study on adolescents undergoing orthodontic treatment revealed that the visualization of plaque retention through disclosing agents significantly improved oral hygiene practices, further supporting their educational utility [32]. The literature consistently supports the effectiveness of plaque-disclosing agents as valuable teaching tools in dental education. These agents not only enhance students' understanding of plaque control but also serve as motivational aids, encouraging better oral hygiene practices. By providing immediate visual feedback, disclosing agents bridge the gap between theoretical knowledge and practical application, fostering improved clinical outcomes in educational settings. In the present study, a significant reduction in plaque levels was observed immediately post-brushing compared to the baseline measurement. However, no significant changes were noted between the first and second measurements, or between the first and third measurements in Group 2. These findings suggest that the use of plaque-disclosing agents (PDA), when combined with self-performed mechanical plaque removal and repeated oral hygiene motivation, may contribute to improved oral hygiene and increased student motivation. However, our results indicate that the application of disclosing agents alone, without consistent motivational reinforcement, may be less effective as an oral hygiene intervention.

These findings align with previous studies such as those by Provenzano [32] and Buischi et al. [31], which emphasize the role of repeated educational interventions in enhancing the effectiveness of plaque-disclosing agents. Similarly, our study supports the conclusions drawn by Kim et al. [30], who found that natural disclosing agents significantly improve plaque control when used alongside structured oral hygiene programs. However, unlike the study by Fasoulas et al. [6], which highlighted the long-term benefits of disclosing agents in preventive oral hygiene training, our findings suggest that short-term use without continued reinforcement may yield limited benefits. This discrepancy underscores the importance of ongoing education and motivational support to sustain oral hygiene improvements over time.

This study has several limitations that should be considered when interpreting the findings.

A key limitation of this study is the homogeneity of the sample, which consisted exclusively of dental students. Given their advanced oral hygiene knowledge and frequent brushing habits, this group may have reached a ceiling effect in oral hygiene performance, thereby masking any potential incremental benefits of plaque-disclosing agents. Consequently, the generalizability of these findings to broader populations is limited. While our results suggest that plaque-disclosing agents may provide limited additional benefit in populations with high baseline oral hygiene proficiency, they may still hold significant value in lay populations or individuals with lower oral health literacy.

The relatively short follow-up period of one month limits the ability to capture long-term behavioral changes and clinical outcomes. Short-term observation also increases the likelihood of transient behavioral modifications, such as the Hawthorne effect, which may overestimate the immediate impact of the intervention. A previous study [14] suggests that follow-up periods of six months or longer are more appropriate to mitigate this effect and provide a more accurate evaluation of long-term outcomes.

Future studies should include more diverse populations and be conducted in real-world clinical settings, encompassing patients with varying levels of oral health knowledge, adherence behaviors, and oral health conditions. Larger sample sizes, randomized controlled trial designs, and objective measures of plaque (e.g., digital plaque quantification) would provide stronger evidence. Furthermore, exploring psychological determinants such as motivation, self-efficacy, and perceived susceptibility to oral disease could deepen understanding of how plaque-disclosing agents influence oral hygiene behavior.

Nevertheless, a residual risk of detection bias cannot be completely ruled out if minimal staining persisted or if participants inadvertently revealed their assignment.

Additionally, a protocol deviation occurred because OHI-S, prespecified in the registry, was not collected due to time constraints; this is disclosed here and will be updated in the registry.

Conclusion

This study evaluated the impact of PDA combined with oral hygiene instructions on the efficacy of mechanical plaque removal and gingival health in dental students. Although both groups showed improvements in PI for

post-brushing and GI for first follow-up, no statistically significant differences were found between the test group (using PDA) and the control group. As a result, the study hypothesis—that PDA significantly enhance plaque removal efficacy and improve oral health outcomes—was rejected. These findings suggest that dental students, due to their prior knowledge and training in proper brushing techniques, may not experience significant additional benefits from PDA alone.

These findings should be interpreted with caution, as the study population consisted of dental students with advanced oral hygiene knowledge. Future research should focus on lay populations with varying oral health literacy to better evaluate the broader applicability of plaque-disclosing agents.

While PDA have been shown to be effective in various clinical settings, their role in improving oral hygiene outcomes may be less pronounced when used without complementary motivational techniques. This study underscores the importance of continued motivation and reinforcement in maintaining oral hygiene, particularly as the observed GI and PI scores increased over time, indicating potential declines in brushing motivation.

Abbreviations

PDA	Plaque disclosing agents
DMFT	Decayed, Missing, and Filled Teeth
GI	Gingival index
PI	Plaque index

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
BBA conceptualized the manuscript. BBA, NU, NA carried out methodology; MB carried out data analysis, drafted and edited the manuscript. All authors subsequently revised the drafts. All authors reviewed and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

Declarations

Ethics approval and consent to participate
Ethics committee approval was received for this study from the Ethics Committee of Istanbul Medipol University (REF: E-10840098-772.02-2361). The research was carried out in compliance with the policy set out in the Declaration of Helsinki, and informed consent inwritten form was obtained from the participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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