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ORIGINAL ARTICLE

Plaque disclosing agent as a guide in biofilm removal in patients with fixed orthodontic appliance: a randomized clinical trial

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Abstract

Effective professional plaque removal is of major importance in the prevention of white spot lesions and gingivitis in patients with fixed orthodontic appliances. However, visual identification of plaque can be difficult, especially around brackets, ligatures and wires. The purpose of the present randomized clinical trial was to evaluate the effectiveness of a plaque disclosing agent (PDA) as a visual guide for biofilm removal. Thirty-two systematically and periodontally healthy adults with fixed orthodontic appliances and Plaque Index (PI) > = 25% were enrolled from October 2020 to May 2022, the subjects were equally randomized into test and control group. Primary outcome was the change in the difference in percentage of residual plaque area (RPA) between the two study groups. In the test group, a PDA was applied before professional oral hygiene, whilst the control group received a hygiene session without disclosing. The PDA was then re-applied at the end of the treatment in both groups, and the RPA was assessed via Image-J software analysis of standardized frontal photos and compared between groups. The average RPA in the test group was 3.9% (CI 95% 2.6%; 5.1%), which resulted significantly lower than in the control group, where it reached 12.0% (CI 95% 8.0%–16.0%) (p-value < 0.001). The percentage of area with residual plaque was modelled using a beta-regression model. The use of plaque disclosing agents as guidance for professional oral hygiene treatment leads to improved plaque removal in patients with fixed orthodontic appliances. NCT05428189, 2022-06-08, retrospectively registered.

Keywords Orthodontics · Dental biofilm · Plaque disclosing · Oral hygiene

Introduction

Identification of plaque deposits on the tooth surface during hygiene procedures is a complex process for patients and clinicians due to the chromatic similarity between dental enamel and the biofilm. A plaque disclosing agent is a dye that contains chromophoric molecules capable of binding to bacterial products and interacting with visible light [1], therefore, aiding plaque visualization. Plaque disclosing agents are available in various forms such as tablets, solutions or gels. After application the agent is rinsed off leaving only biofilm-covered areas stained. The application of plaque disclosing agents proved helpful within professional oral hygiene protocols as a visual guide for clinicians and a tool for effective patient education and motivation [2–4].

Patients with fixed orthodontic appliances pose extra difficulties to oral hygiene, as it has been highlighted that brackets, bands, and ligatures can limit the physiological self-cleansing mechanisms carried out by the tongue, cheeks and saliva, thus allowing the growth of more bacterial biofilm [5–7]. Several scientific studies have indeed found a higher prevalence of acidogenic bacteria in orthodontic patients, in particular of Streptococcus *mutans* and *Lactobacilli spp,* which increases the risk of gingival inflammation and White Spot Lesions (WSL) or tooth decay [8–13]. Therefore, to prevent the onset of caries and gum disease, it is essential to include orthodontic patients in tailored professional oral hygiene programs, and plaque disclosing could

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play a useful role in effective biofilm removal [4, 5, 14]. Considering the operator's inherent difficulty in completely removing biofilm deposits from and around the complex fixed orthodontic components, some scientific studies have proposed alternatives to traditional prophylaxis with rubber cups and abrasive paste such as employing air-polishing devices. The evidence shows that air-polishing might be more effective and faster than use of pumice on rubber cups [15–17].

Based on the findings reported by Ramaglia et al. [15], air-polishing systems demonstrate superior efficacy in the removal of supragingival plaque including in patients undergoing fixed orthodontic treatment. For this reason the team selected air polishing as the professional plaque-removal method in the present study and investigated whether the use of a colorimetric guide (disclosing agent) prior to therapy could enhance the accuracy of biofilm removal. This approach not only facilitates patient motivation and oral hygiene instruction but is also hypothesized to reduce the amount of residual plaque remaining on tooth surfaces after treatment. For that reason the method was selected and the investigative team attempted to improve the results using the disclosing agent.

However, despite the documented benefits of both plaque disclosing agents and air-polishing devices, no study to date has systematically evaluated their combined use in improving biofilm removal in orthodontic patients. The plaque disclosing agent is an essential aid for patient education and motivation in home oral hygiene, and numerous studies have confirmed its effectiveness in orthodontic patients; however, to date no study has evaluated its use as a guide for the professional removal of biofilm [4].

The present study aimed to quantitatively assess the completeness of biofilm removal by evaluating the residual plaque area (RPA) after professional hygiene performed with an air-polishing device in patients with fixed orthodontic appliances. Specifically, the study investigated whether the use of a plaque disclosing agent as a visual guide influences the amount of residual plaque. The primary outcome was RPA that has been measured through ImageJ software analysis following professional hygiene procedures performed better with a plaque disclosing agent. The null hypothesis was that the use of a plaque disclosing agent could improve the professional removal of supragingival biofilm in patients undergoing fixed orthodontic treatment.

Materials and methods

Study design and population

The present study is a single-blind, controlled, randomized, monocentric clinical trial conducted with two parallel groups. It was conducted in accordance with the Declaration of Helsinki and reported following CONSORT guidelines (Consolidated Standards of Reporting Trials) and approved by the Ethics Committee of Spedali Civili di Brescia (protocol number 3925) and registered on ClinicalTrials.gov (NCT05428189).

Study patients were selected from the population attending the Dental School "Clinica Odontoiatrica Lidia Verza," University of Brescia, Department of Radiological and Public Health Sciences, within the ASST Spedali Civili di Brescia, Department of Dentistry (Brescia, Italy) from October 2020 to May 2022. Verbal and written informed consent were obtained from all participants.

The inclusion criteria were:

- Systemically healthy;
- Age between 18 and 75 years;
- Fixed orthodontic appliances on the upper and lower arches in the anterior sextants;
- Presence of all teeth in the anterior sextants;
- Plaque index above 25%;
- Smoking less than 10 cigarettes per day.

The exclusion criteria were:

- Chronic obstructive pulmonary disease (COPD) or asthma;
- Patients with periodontal disease;
- Upper and/or lower fixed retainers;
- Presence of prosthetic elements in the anterior sextants;
- Tumors and/or oral cavity diseases;
- Recent chemotherapy or radiation therapy;
- Pregnant or breastfeeding;
- Allergy to erythritol, chlorhexidine and adverse reactions to lactose or its derivatives.

Interventions

Initial screening and assessment were performed by same blind examiner (ES), including recording of pocket probing depths (PPD), plaque index (PI), and bleeding on probing (BOP) using a millimetre-marked periodontal probe (PCP-UNC 15).

If the patients were eligible for the study according to the inclusion criteria, they were informed about the protocol and potential risks and a consent form and privacy form were provided and signed.

Thirty-two patients were then divided according to a randomization list and enclosed numbered envelopes. All treatments were performed by the same experienced operator (MM).

Patients assigned to the test group underwent the following treatment: a lips and cheeks retractor was applied

(OptraGate®, Ivoclar Vivadent), followed by a plaque disclosing agent (Biofilm Discloser®, EMS, Nyon, Switzerland), then rinsed with water (Fig. 1).

Removal of supra-gingival biofilm was performed using an air-polishing device (Airflow® Prophylaxis Master EMS, Nyon, Switzerland) with erythritol (14 µm) powder (PLUS powder® EMS, Nyon, Switzerland) and the same settings: 5 mm distance from the tooth, 30°/60° angulation and 5 bar pressure.

Subsequently, calculus deposits were removed using a piezoceramic handpiece (Piezon®, Airflow® Prophylaxis Master EMS, Nyon, Switzerland) with Perio Slim tip (PS® EMS, Nyon, Switzerland) (Fig. 2).

The treatment ended when all the PDA has been removed. No fixed or standardized instrumentation time was imposed, as the effectiveness of the procedure relied on cleaning each site until complete removal of visible PDA.

Finally, the plaque disclosing agent was re-applied and frontal photographic images were taken (Fig. 3).

Patients assigned to the control group underwent the same protocol without the initial application of the disclosing agent. As for the test group, the treatment ended when the clinician judged that all visible plaque/calculus had been removed, with no standardized treatment duration and no recording of procedure time, since timing cannot be fixed without compromising clinical efficacy.

Primary outcome was the change in the difference in percentage of RPA between the two study groups.

The final plaque disclosing application in both groups aimed at highlighting the post-treatment RPA.

An extraoral camera was used for the photographs (Nikon D90 with AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED) with standard position and focus (the subjects open their mouth slightly until all the dental crowns are visible, the photo includes from upper canine to upper canine and the photo is taken parallel to the chewing surface), standard camera settings (focus distance 40 cm from the subject, f/36, 1/160 s) and flash settings (Metz Mecablitz 15 MS-1 Digital Flash Anular, 1/8 power). The images were then analysed using ImageJ software analysis by ES.

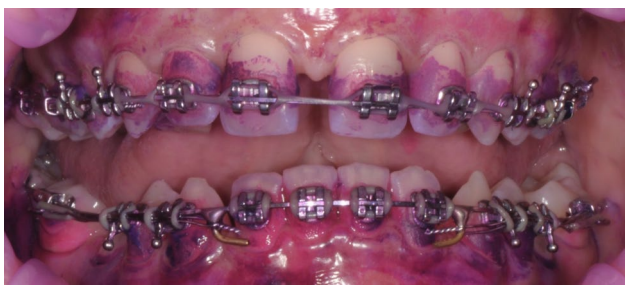

Fig. 1 Test Group: Pre-treatment photograph of the anterior sextants, taken after application of the plaque disclosing agent

Fig. 2 Test Group: Post-treatment photographs of the anterior sextants

A second method for the assessment of residual plaque was considered to evaluate the reliability of and consistency with the selected digital method. The Orthodontic Plaque Index (OPI) [18] was recorded by two calibrated operators and reported by sextants, as described by the authors.

The complete study protocol is illustrated in Fig. 4.

Software image analysis

Photographic analysis was performed by the blinded examiner (ES) to the patients group allocation using the software Image-J, which allows calculation of the RPA as a percentage of the total area of the tooth. After manually selecting the area of interest (vestibular sextants II and V) with particular attention to interproximal spaces and the precise path delimited by the gingival margin (Fig. 5), the space occupied by the orthodontic appliance (brackets, orthodontic wire, and ligatures) was delimited and excluded from the analysis process (Fig. 6). The color threshold range between 175 and 200 was selected because it corresponds to the chromatic variation of the plaque-disclosing agent used (Mira-2 Ton), which produces staining ranging from pink to deep violet. After application of the disclosing agent, plaque covered areas consistently exhibited color values within this range and were clearly distinguishable from the surrounding tooth surface. This marked chromatic contrast allowed for

Fig. 3 Test Group: Post-treatment photograph of the anterior sextants, taken after re-application of the plaque disclosing agent

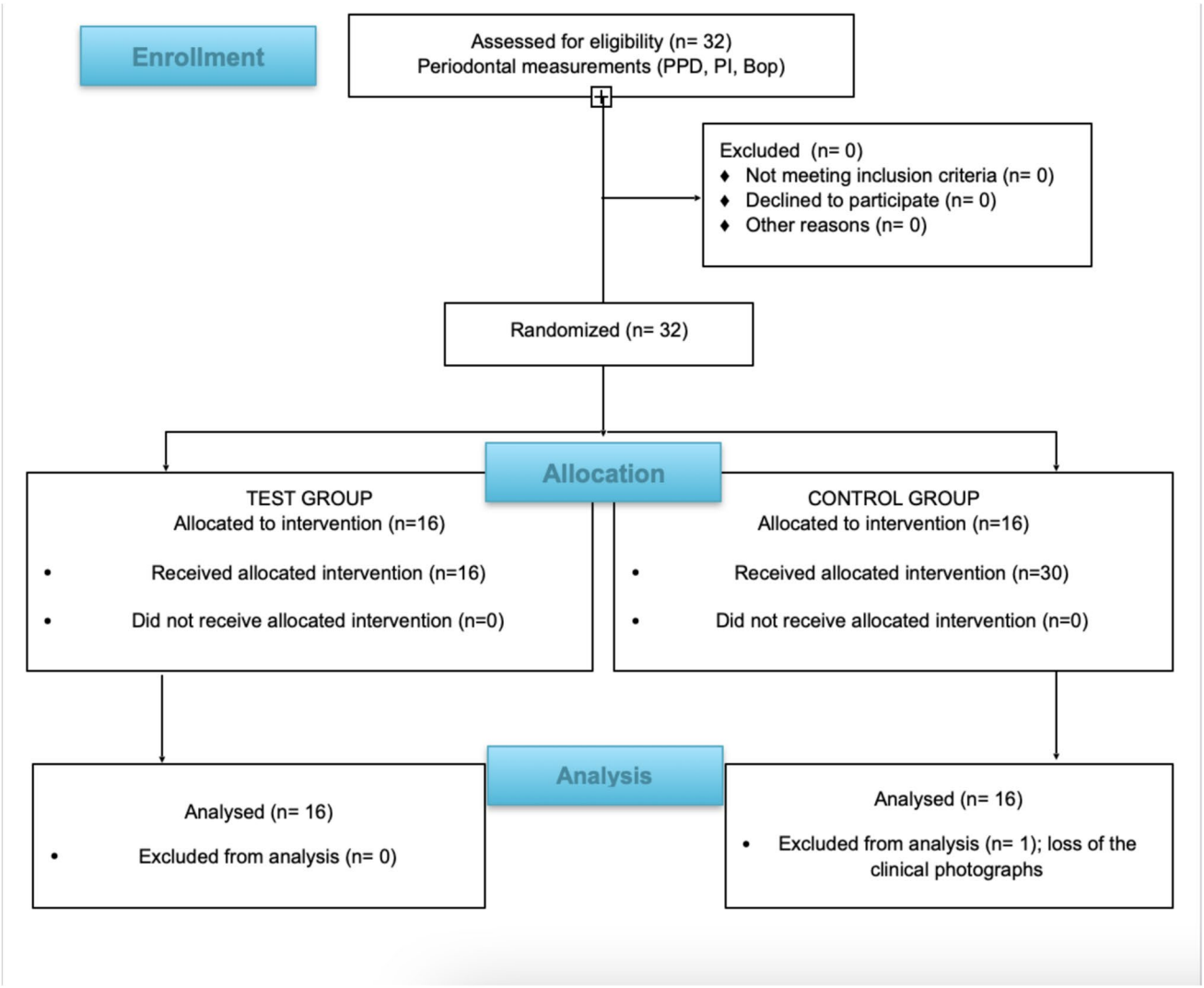

Fig. 4 Study CONSORT flow diagram (*PPD* Pocket Probing Depth, *PI* Plaque Index, *BoP* Bleeding on Probing)

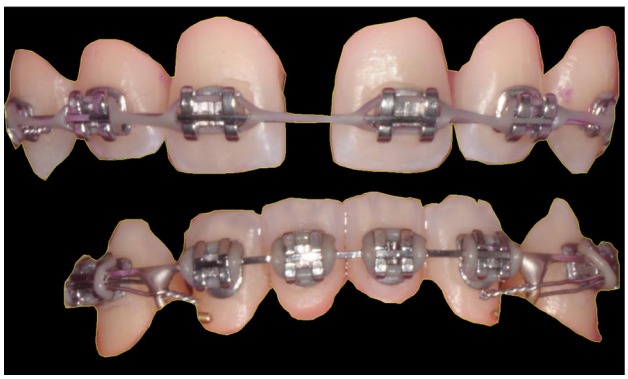

Fig. 5 Software Image analysis. The clinical image was uploaded on a drawing tablet and the clinical crowns were selected

Fig. 6 Software Image analysis. The areas corresponding to the orthodontic appliance were cropped out

selective and reproducible identification of the areas of interest using a predefined threshold. With regard to methodological reliability, all operators follow a standardized protocol for region of interest (ROI) selection, based exclusively on the color difference between the tooth surface and the plaque-stained areas. The clear visual distinction between unstained dental surfaces and pigmented regions minimized subjectivity in manual ROI selection and contributed to consistency among examiners [2].

Using the Adjust-Color Threshold command, the chromatic range corresponding to the plaque disclosing colour was selected (value range 175–220), allowing for the identification of the RPA (Fig. 7) [2].

Calibration

Clinical calibration: the examiner (ES) underwent a calibration session measuring PPD in one quadrant with at least 6 teeth in 10 patients. Measurements were repeated after one hour and variability was assessed. To ensure reliability of measurements, the single rater was calibrated by repeated assessments (two assessments) on ten patients, both of Area (two different sectors) and PPD, with repeatability evaluated using the Intraclass Correlation Coefficient (ICC). The achieve ICC was significantly higher than 95% (ICC = 1, IC 95% 0.999; 1).

Measurement calibration: the examiner underwent a calibration session measuring OPI and RPA in ten pictures. Measurements were repeated on some pictures the day after, and variability was assessed.

Sample size determination and randomization

The sample size was computed assuming a two-independent group comparison based on a t-test allowing for different variances (Welch's test). We assumed 5% and 10% residual plaque (% of plaque is over total teeth inspected area), respectively, and a 60% coefficient of variation for both groups. Considering an 80% power and a 5% significance level, we computed a total sample size of N = 32 (16 for each group). To allow for potential deviations from normality assumption for percentages, we also computed sample size using a Wilcoxon-Mann–Whitney simulation based on 2000 Monte Carlo samples from the null distributions (with parameters as specified above), achieving a consistent (software: PASS 15) sample size. Patients were randomized using a computer-generated randomization list. The random allocation sequence was generated using uninformative labels (A – test and B—control) and a random block size algorithm [19].

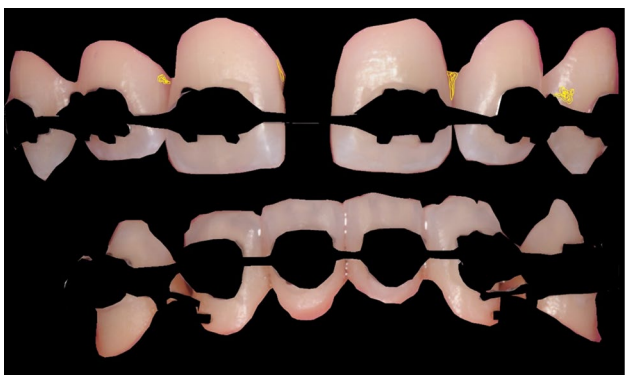

Fig. 7 Software Image analysis. Using the Adjust-Color Threshold command, the chromatic range corresponding to the plaque disclosing colour was selected (value range 175–220), allowing for the identification of the RPA

Statistical analysis

The study's primary endpoint was the change in the percentage of RPA between the two study groups.

All data analyses were carried out according to a pre-established analysis plan by a biostatistician blinded to group allocation. The percentage of area with residual plaque was modelled using a beta-regression model, which allows modelling a response variable constrained in a (0,1) range as a proportion [20]. OPI was modelled as an ordinal variable using a cumulative logit model (CLM) [18]. Estimates are reported as class mean values.

Results were reported as estimates and corresponding 95% confidence intervals. All model fits were checked for assumptions using graphical procedures. All the analyses were performed using R (version 4.5.1), assuming a 5% level of significance.

Figure 8 represents a boxplot with points comparing distribution among treatments, as well as a Posterior Predictive distribution plot (Fig. 9), showing how the model mimics the real data distribution (supplementary).

Results

A total of 32 patients were selected, with 16 subjects assigned to randomization test group and 16 to control group, with comparable demographic characteristics and baseline plaque index (Tab. 1). One patient from the control group was excluded from the study due to technical complications which caused the loss of clinical photographs.

After treatment, in test group the RPA was 3.9% (CI 95% 2.6%; 5.1%), whereas in control group it was 12.0% (CI 95% 8.0%; 16.0%), showing a statistically significant difference (control—test) of 8.1% (CI 95% 4.4%; 11.9%) in favour of test group (p-value < 0.001) (Table 2).

After treatment, in test group the OPI was 2.9 (CI 95% 2.5; 3.2), whereas in control group it was 3.6 (CI 95% 3.2;

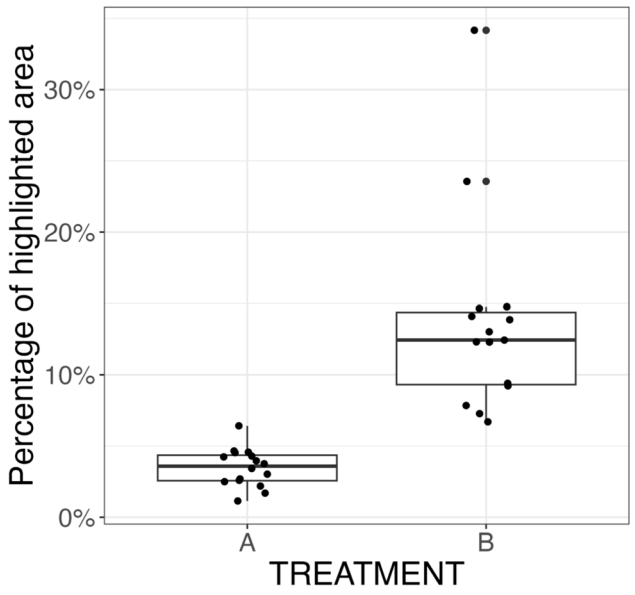

Fig. 8 Boxplot with points comparing distribution among treatments

4.0), showing a statistically significant difference (control—test) of 0.7 (CI 95% 0.3; 1.2) in favor of test group (p-value < 0.001) (Table 3).

Discussion

This study was designed to evaluate the effectiveness of a plaque disclosing agent as a guide for the operator during professional oral hygiene sessions in patients with fixed orthodontic appliances. The results show a significant reduction in post-treatment plaque in both groups, with a significantly lower (RPA) in the disclosed group compared to the control group, demonstrating the usefulness of the plaque disclosing agent in achieving better biofilm removal. The improved biofilm removal allowed by the disclosing agent was shown by the same authors in a previous study on patients without orthodontic appliances [2].

The literature describes several methods for plaque detection, as well as various indices commonly used in orthodontics [18, 21, 22]. In this study, we selected a method similar to that proposed by Azevedo CL et al. [23], as it is considered more objective than visual indices such as OPI [18]. Nevertheless, the OPI was also recorded to enable comparison and to allow a more detailed assessment of plaque accumulation specifically around the brackets, where demineralization, white spot lesions, and caries are more likely to develop.

Interestingly, the RPA obtained in the present study in the disclosed group has reached similar values to the previous study [4.0% (CI 2.8–5.2) and 4.8 (CI 3.3–6.8)], demonstrating that even in the presence of additional plaque retentive factors such as a fixed orthodontic appliance, the guidance of the PDA can lead to satisfactory biofilm removal. The same

Fig. 9 Posterior Predictive distribution plot

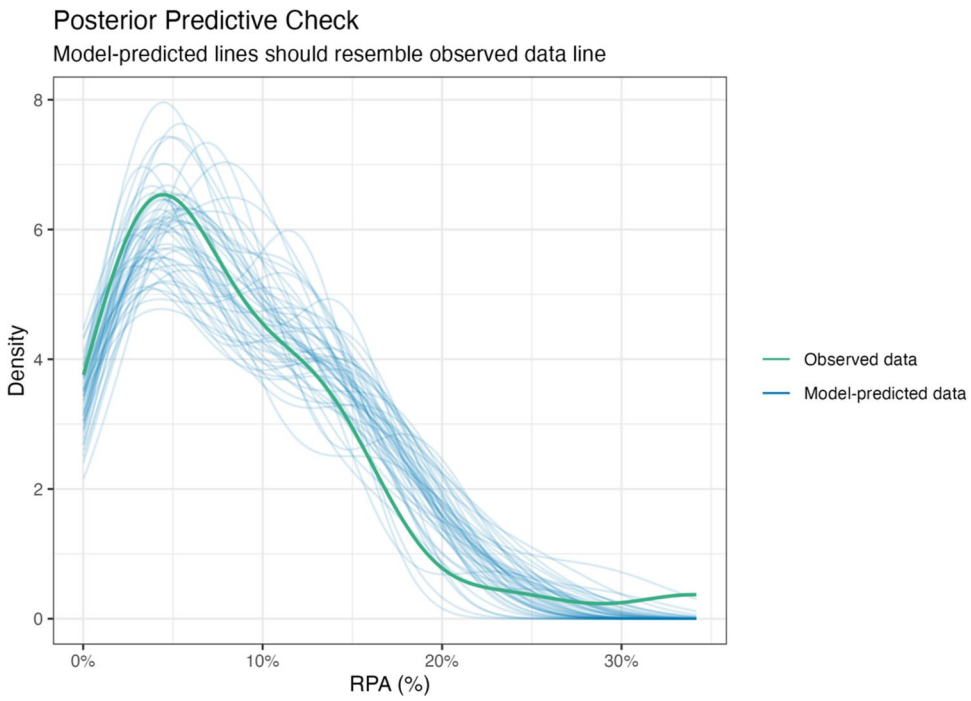

Table 1 Demographic characteristics of the selected population: age, sex, baseline plaque index (PI) at baseline and smoke status

		Overall (N = 32)	Test (N = 16)	Control (N = 16)	p- value[1]
Age Mean (SD)		22.6 (4.7)	22.6 (4.7)	20.7 (2.1)	0.14
Sex N. (%)	Female	19 (61%)	8 (50%)	11 (73%)	0.2
	Male	12 (39%)	8 (50%)	4 (27%)	
PI Mean (SD)		0.8 (0.3)	0.8 (0.3)	0.7 (0.3)	0.8
Smoking N. (%)	No	26 (84%)	11 (69%)	15 (100%)	0.043
	Yes	5 (16%)	5 (31%)	0 (0%)	

PI Plaque Index, *SD* Standard Deviation, *N* Number, % Percentage

[1]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Table 2 RPA estimate (%) in test and group control

Group	RPA % (CI)	*p*-value[1]
Test	3.9 (2.6; 5.1)	
Control	12.0 (8.0; 16.0)	
Control—Test	8.1 (4.4; 11.9)	**< 0.001**
Smoke (Yes vs No)	−1.7 (−6.2; 2.9)	0.51

RPA Residual Plaque Area, *CI* Confidence Interval, % Percentage

[1]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Bold value indicates statistical significant result

Table 3 OPI estimate in test and group control

Group	OPI (CI)	*p*-value[1]
Control	3.6 (3.2; 4.0)	
Test	2.9 (2.5; 3.2)	
Control—Test	0.7 (0.3; 1.2)	**< 0.001**
Smoke (Yes vs No)	−0.31 (−0.88; 0.255)	0.28

CI Confidence Interval,

[1]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Bold value indicates statistical significant result

cannot be said about the control groups: it can be observed that not applying the PDA in orthodontic patients resulted in a higher level of residual plaque with an RPA of 13.3% (CI 11.1–15.6) compared to 9.0% (CI 6–13.1) in patients without fixed orthodontic appliances.

It is interesting to note that, even when using the OPI index, the difference between the two groups favored the test group, with a statistically significant difference ($p < 0.001$), with values of 3.8 in the control group and 3.0 in the test group, respectively.

Further analysis would be necessary to determine if this difference is clinically significant.

There are no other publications where the disclosing agent has been used before the treatment as a guide of professional mechanical plaque removal compere to the same method of treatment without using the disclosing agent. So we cannot compare data. But if we look at the results of Ramaglia in the group 1 where they used APP (Air Powder Polishing) without chlorhexidine mouthwash the results were superior then RC (Rubber Cups) but non significantly we were able to make this difference significant using the APP with the disclosing agent as a guide comparing the same method without a disclosing agent.

In terms of clinical significance, there is no definitive answer as to whether we can determine a cut-off value for sufficient biofilm removal and acceptable RPA due to individual variation in the microbiome and immune response, where the critical mass of biofilm can vary over time depending on the patient's systemic conditions and local predisposing factor, leading to the initiation, progression, or quiescence of the disease [24, 25]. In fact, the new periodontal classification defines health on intact periodontium based on probing attachment loss, pocket depths, bone loss and bleeding on probing, with no set cut-off for plaque index [25]. However, some studies on surgical periodontal intervention state that patients should ideally not have a Full Mouth Plaque Score (FMPS) exceeding 15% to increase the chances of post-surgical success [26]; therefore, we could consider a Full Mouth RPA (FMRPA) of 15% as the acceptable value. In light of this speculation, the residual RPA in patients of the control group where the PDA was not applied at the beginning of the therapy, despite the poorer outcome, is still within this cut-off.

Patients undergoing fixed orthodontic treatment are at increased risk of developing enamel demineralization, particularly white spot lesions (WSLs) and early caries, due to the presence of brackets, bands, and auxiliaries that create plaque-retentive niches. These appliances hinder natural cleansing mechanisms and make mechanical plaque removal more challenging, resulting in greater biofilm accumulation around bracket margins. Several studies have demonstrated that WSLs may develop rapidly—sometimes within the first month of treatment—when plaque control is inadequate, highlighting the clinical relevance of early and continuous intervention [27, 28].[1,2]

The pathogenic potential of persistent biofilm is primarily related to the metabolic activity of cariogenic microorganisms such as Streptococcus mutans, which lower the local pH and initiate subsurface enamel demineralization. Without effective mechanical disruption, these ecological changes promote lesion progression and increase the likelihood of developing irreversible defects [28, 29].

In this context, periodic professional oral hygiene procedures are an essential adjunct to patient-performed oral hygiene. Evidence shows that professional prophylaxis helps maintain lower plaque levels and reduces the incidence of WSLs in orthodontic populations [30]. Among the available techniques, air-polishing systems have emerged as an effective and minimally invasive method for removing supragingival biofilm around brackets. According to Ramaglia et al., air polishing demonstrates superior plaque-removal efficacy compared with conventional rubber-cup polishing in orthodontic patients, while also reducing chairside time [15]. This supports the integration of air-polishing into routine maintenance visits to enhance biofilm control in areas that are otherwise difficult for patients to manage.

Collectively, the literature reinforces that meticulous biofilm management, combining patient education with effective and minimally abrasive professional techniques, is fundamental in reducing the risk of WSLs and caries during orthodontic treatment.

It is essential to acknowledge one limitation of the present study: only two anterior sextants were considered in the image analysis due to the impossibility of standardizing posterior photographs; therefore, the results presented might not reflect the real full-mouth RPA. This limitation might explain why a similar study [3] reported a much higher residual plaque reaching well above 15%: the authors chose a clinical analysis of the residual plaque via Marginal Mean Plaque Scores (MMPS) by calibrated examiners rather than image software analysis and considered all the sextants. Posterior regions often present challenges in terms of accessibility during home oral hygiene practices and evaluating the effectiveness of the same therapy in these areas would provide valuable insights.

To our knowledge, there are currently no published studies reporting RPA values post-cleaning using the same methodology as in the present work. However, comparable post-cleaning plaque-reduction outcomes have been documented using established visual plaque indices—particularly the Quigley–Hein Index (QHI), modified by Turesky et al. —which remains one of the most widely adopted indices for quantifying supragingival plaque following professional cleaning or oral-hygiene interventions [31, 32]. The QHI scores dental surfaces from 0 to 5 based on the extent of disclosed plaque, providing a standardized and reproducible measure. Studies employing air-polishing systems, such as the clinical investigation by Ramaglia et al. [15], have demonstrated significant plaque reductions using this index, supporting the expected direction and magnitude of plaque removal outcomes following cleaning procedures—even though these studies do not report RPA specifically.

The positive results obtained in both groups in the present study might also be related to the prophylaxis technique applied, including using a low-abrasiveness air-polishing device.

A study by Ramaglia et al. [15] showed how applying air-polishing in orthodontic patients led to better and faster plaque and staining removal when compared to traditional rubber cups and pumice. The same was described by Burgess et al. [33]. In the past, air-polishing devices were merely used for stain removal and, as the name indicates, polishing. Current technology allows their use as a primary means for biofilm removal. Therefore, Divnic-Resnik et al. [34], in a study on applying such technology in periodontal patients, suggested the term "air-flow debridement" could be more suitable. A recent publication by Liu C.C. et al. concluded that air-powder water-jet technology is both effective and comfortable for use in nonsurgical periodontal and peri-implant therapy as well as in prophylactic treatment. [35]

In conclusion, it has been observed that the use of a plaque disclosing agent prior to prophylaxis therapy can offer significant benefits in biofilm removal in orthodontic patients.

The primary objective of the present study was to validate the use of a plaque disclosing agent as a practical and effective adjunctive tool to enhance mechanical plaque removal, rather than to directly assess clinical inflammatory outcomes.

Our findings demonstrate that color-guided plaque visualization significantly improves biofilm removal compared with unguided, freehand cleaning. Importantly, this effect was observed regardless of the baseline plaque levels and independently of the presence of fixed orthodontic appliances. These results suggest that the benefit of the disclosing agent is not limited to patients with high initial plaque accumulation or to specific clinical conditions, but represents a broadly applicable tool capable of enhancing oral hygiene performance across different patient profiles.

This study should therefore be interpreted as a proof-of-principle investigation aimed at validating the technical effectiveness of plaque disclosure as a guidance system for improving biofilm removal. While improved plaque removal is a prerequisite for better periodontal health, the present study was not designed to evaluate clinical inflammatory parameters as primary endpoints.

The impact of plaque disclosure on clinical outcomes such as marginal bleeding, gingival inflammation, or other periodontal indicators should be investigated in future prospective studies specifically designed with these parameters as primary outcomes and with adequate follow-up periods.

Overall, the use of a plaque disclosing agent appears to be a simple yet highly effective adjunct that significantly enhances biofilm removal compared with unguided cleaning, supporting its potential role as both a clinical and educational tool in preventive oral care.

For the future, it could be useful to analyze the FMRPA with more modern technologies that allow the standardization of the data and increase the sample on four arms basing the sample size on the type of ligatures. It would be useful consider a multicenter study to further expand the sample, include more types of patients like those in treatment with aligners and follow up the subjects for more time.

To our knowledge, this is one of the first studies to measure RPA quantitatively in orthodontic patients using image analysis software, thereby filling a methodological and clinical gap in current research. The principal limitation of this study lies in the impossibility of blinding the operator, which could lead to non-impartial performance between the study groups.

The clinical relevance of the findings emerges primarily in the message to be shared with the orthodontic community—both specialists and patients—highlighting the necessity of an effective, comfortable, and guided method of biofilm removal to prevent complications such as caries and gingival disease. Periodic recall programs should be planned in conjunction with the orthodontic treatment plan to ensure the patient's oral health. Investing in rapid and efficient technologies and protocols constitutes a necessary and ethical responsibility for the clinician.

Another limitation of the present study is that treatment time was not recorded. Although the primary aim was to evaluate the effectiveness of plaque removal rather than procedural efficiency, information on chairside time would have provided additional insight into the potential clinical advantages or disadvantages of using a disclosing agent as a guide. In particular, it remains unclear whether the improved RPA in the disclosed group is associated with a substantial increase in treatment duration or whether the guidance provided by the plaque disclosing agent may actually streamline the procedure by allowing more focused instrumentation. Future studies should therefore include standardized time measurements to clarify the impact of plaque disclosure on treatment efficiency.

A further consideration would be to compare multiple operators with different levels of clinical experience and assess potential differences when using or not using a plaque disclosing agent, while also accounting for plaque-retentive factors in order to perform multilevel analyses.

Furthemore future trials should be specifically designed and powered to investigate the effect of ligature type, under study designs capable of isolating its impact on biofilm accumulation and RPA.

Future multicentre studies with larger and more heterogeneous samples including posterior sextants and comparing different types of plaque-disclosing agents are needed to confirm and extend our findings.

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Data availability Data is available upon reasonable request to the authors.

Declarations

Conflict of interest Dr. Mensi reports Personal fees from EMS, personal fees from KULZER, personal fees from SUNSTAR outside the submitted work. Dr. Garzetti has nothing to disclose. Dr. Scotti has nothing to disclose. Dr. Marchetti has nothing to disclose. Dr. Venturi has nothing to disclose. Dr Di Monda has nothing to disclose. Dr. Sordillo reports personal fees from EMS, during the conduct of the study outside the submitted work. Dr. Calza has nothing to disclose.

Ethical approval The present study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Spedali Civili di Brescia (protocol number 3925).

Informed consent Informed consent was obtained from all subjects involved in the study.

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