Reduction of dental biofilm cariogenicity in patients with fixed orthodontic appliances by a combined Horizontal-Charters-modified Bass brushing technique and dietary advice: A randomized clinical trial

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Research Article

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Abstract

Background: The orthodontic patients with inadequate oral hygiene have susceptibility to dental caries. Increased dental biofilm accumulation has been found in orthodontic patients. However, assessment of dental biofilm cariogenicity in these patients has not been reported. This open-label, two-arm parallel trial evaluated the effects of toothbrushing and dietary advice on dental biofilm maturity and dietary habit in patients with stainless steel (SSL) and elastomeric (EL) ligatures.

Methods: At T0, diet habits, socio-demographic, medical problems, orthodontic treatment, and dental health behavior data were collected from 70 patients. The patients were randomized (1:1 ratio) to the SSL or EL group by simple randomization at T1. Dental biofilm cariogenicity was evaluated using a three-color-disclosing dye. The patients in both groups were instructed to brush their teeth using a combined Horizontal-Charters-modified Bass technique and dietary advice. The data were reassessed at the 4-week follow-up (T2).

Results: At T1, new dental biofilm (median 50%; Q1, 32.66%; Q3, 71.13%) was highest followed by mature dental biofilm (median 26%; Q1, 20.42%; Q3, 47.93%), and cariogenic dental biofilm (median 8%; Q1, 4%; Q3, 22.92%) ($p < 0.05$), in SSL group. In the EL group, cariogenic dental biofilm (median 54.16%; Q1, 44.44%; Q3, 69.56%) was highly observed, while the median for mature and new dental biofilm was 25.0% (Q1, 18.18%; Q3, 36.18%) and 13.63% (Q1, 7.55%; Q3, 26.96%), respectively ($p < 0.05$). At T2, cariogenic dental biofilm in EL group demonstrated significantly greater reduction (47.04%) compared with the SSL group (8%) ($p < 0.05$). Cariogenic food consumption was low in two groups. However, dietary habits did not change in either group at T2.

Conclusions: Combined toothbrushing effectively reduced cariogenic dental biofilm in orthodontic patients.

Trial registration Thai clinical trials registry (TCTR20220221003)

Background

Dental biofilm is a community of microorganisms embedded in a host- and bacteria-derived polymer matrix found on the tooth surface as a biofilm. It is the primary etiologic factor contributing to dental caries [1]. The recently developed Ecological Plaque Hypothesis states that the differences seen in the microbiota from sound and carious sites are due to a change in oral environmental conditions. An increased frequency of fermentable sugar consumption selects for cariogenic bacteria at the expense of health-associated bacteria that prefer an approximately neutral pH environment [2]. The growth of bacteria associated with sound tooth surfaces is reduced, which over time results in an increase in the proportions and activity of cariogenic species at specific sites and a heightened risk of caries [3].

Previous publications revealed moderate evidence that the presence of fixed appliances influences the quantity and quality of the oral microbiota. This change might be transitional depending on oral hygiene practices [4]. The stainless (SSL) and elastomeric ligation (EL) are commonly used in orthodontic treatments. There was a 12% difference in the plaque index between the SSL and EL [5]. In addition, there were increased numbers of microorganisms around EL compared with SSL, however, this was not reflected in a shift to more cariogenic species [6].

There have been other non-randomized studies comparing EL and SSL, and some of these contradict previous studies. A split-mouth study found no significant differences in microbial growth between the EL and SSL groups [7]. Studies that quantified the presence of dental biofilm using spectrophotometry and morphologic observations by scanning electron microscopy also found no significant differences between the EL and SSL groups [8, 9].

Early carious lesions in the enamel are observed clinically as white spot lesions (WSLs) [10]. WSL development is caused by prolonged dental biofilm accumulation [11]. Orthodontic treatment significantly increases the risk of WSLs in patients with inadequate oral hygiene [12]. However, Eltayeb, MK *et al.* [13] reported a nonsignificant relationship between WSLs and oral hygiene measures. Another study reported that a higher frequency of daily tooth brushing was accompanied by a lower prevalence of WSLs [14]. Therefore, it is possible that oral hygiene and tooth brushing are not the only factors that affect WSL development.

Although numerous studies have evaluated the effect of fixed orthodontic appliances on dental biofilm accumulation, no study has reported their effects on the ecological changes in plaque. Previous reports demonstrated a significant increase in cariogenic bacteria, including *Streptococcus mutans* and Lactobacilli, in subjects treated with fixed appliances [15]. However, *S. mutans* is no longer considered a necessary dominant pathogen in dental caries [16]. The Ecological Plaque Hypothesis posits that the low pH environment generated from carbohydrate metabolism is a major factor responsible for the shifts observed in dental plaque bacteria in patients eating a high carbohydrate diet [1, 3]. Therefore, dental biofilm assessment should be based on acid production by fermentation using a sucrose challenge.

A three-color disclosing dye (GC TriPlaque ID Gel ™, GC Corporation, Tokyo, Japan) has been developed to determine the cariogenicity of dental biofilm. Pink indicates newly formed dental biofilm less than 1-day-old and older plaque is stained blue/purple because the blue dye is trapped in the three-dimensional substructure of the dental biofilm. Light blue staining reveals areas of cariogenic dental biofilm due to the fermentation that occurs because of the sucrose present in the gel [17]. A study demonstrated that the areas with light blue staining have high levels of *S. mutans*, which represent dysbiosis in the dental caries and greater caries risk [18].

The concept of an ecological catastrophe, first proposed by Marsh in 1995, forms the basis of the modern understanding of the disease process [19]. Contributing factors to dental caries that affect the cariogenicity of the dental biofilm include the frequency of performing oral hygiene, the types of oral hygiene products used, eating patterns (in terms of fermentable carbohydrates and acid challenges), and the level of fluoride exposure [20]. An excessive frequency of sugar and soft drink consumption is a major caries risk factor [21]. Many methods, including dietary history, 24-h recall, dietary record, and food frequency questionnaires, are recommended to assess dietary habits. However, a 3–7 d diet chart might provide a more complete picture of the types of food/drink a person is consuming on a regular basis [22].

Specific Objectives Or Hypotheses

The objective of this study was to evaluate the effects of a combined Horizontal-Charters-modified Bass brushing technique and diet counseling on dental biofilm cariogenicity and diet habits in patients treated with fixed orthodontic appliances (SSL or EL).

Methods

Trial design and any changes after trial commencement

This study was a single-center, open-label, two-arm parallel-group, randomized clinical trial with an allocation rate of 1:1 conducted in Thailand. The experimental procedures were in accordance with the applicable ethical standards on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013 [23]. The Institutional Review Board at Walailak University approved the study on 13 August 2020 (protocol number: WUEC 20-227-01/2) prior to selecting the patients with fixed orthodontic appliances. Written informed consents were received from all patients. No changes to the methodology occurred after trial commencement. The trial is registered at the Thai clinical trials registry (TCTR20220221003). The presentation of this report is according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting trials [24]. All methods were performed in accordance with the approved guidelines and regulations.

Participants and eligibility criteria

This study was performed at a private orthodontic clinic in Samutprakarn province, Thailand. Patients were screened for eligibility at their regular scheduled orthodontic appointments. The eligibility criteria for the patients comprised: (1) physically healthy with no medical problems, (2) > 12 years old at the commencement of treatment, (3) treatment with the same type of conventional preadjusted brackets (Roth type, Tomy International, Tokyo, Japan), (4) ability to maintain adequate oral hygiene, and (5) availability to follow-up during the 3-month period of the study. The exclusion criteria were (1) hyposalivation and (2) antibiotic therapy within 3 months prior to the trial.

Interventions

Patients were screened for eligibility at their regular scheduled orthodontic appointment. Eligible patients were informed about the study by an orthodontist. The duration of this study was approximately 5–6 months for each patient. In the beginning of the study (T0), the eligible participants were asked to complete self-administered questionnaires relating to their socio-demographic, medical problems, orthodontic treatment, and dental health behavior. The patients underwent WSL and dietary habit evaluations before tying the SSL or EL. Each patient was randomly divided into two groups at T1. In group 1 (SSL group), the patients were ligated with stainless steel ligatures (0.01 inch stainless steel wire ligature ties, Highland Metals™, IL, USA). For group 2 (EL group), the patients were ligated with elastomeric rings (Dyna stick, elastomeric ties, DynaFlex company, St. Louis, MO, USA).

Dental biofilm cariogenicity was measured in both experimental groups at T1. Oral hygiene instructions and dietary counseling were given to the SSL and EL group patients at this visit. The patients received a session of professional mechanical plaque removal guided by the application of a plaque disclosing agent. They were instructed to use a combined Horizontal-Charters-modified Bass technique to clean their teeth with manual toothbrushes (124 GUM orthodontic toothbrush, Sunstar Corporation, IL, USA) [25]. The areas around the brackets and archwires on the facial surface were cleaned using the Horizontal technique. Next, the Charters technique was used to clean the undercut below and above the wing of the brackets, occlusal, and palatal surfaces, followed by a modified Bass technique to clean the intrasulcular areas. The patients were also instructed to clean all interdental areas using dental floss and an interdental brush. After receiving the oral hygiene instructions, the patients brushed their teeth based on the protocols. To monitor the patients' tooth brushing performance, dental biofilm staining was performed using GC Tri Plaque ID Gel™. Satisfactory tooth brushing was defined as when all stained biofilms were removed.

At T1, the patients in both experimental groups received dietary advice focusing on the role of diet in dental caries development, especially the effect of sugar and acid drinks on increasing their caries risk. The diet counseling aimed to change the patients' dietary behaviors so that they chose diets with low or noncariogenic snacks, and limited sweet foods to mealtimes only. The patients were also instructed to abstain from sugar 1 h before bedtime due to the reduced overnight salivary flow rate and buffering capacity [26–28]. They were informed of the types of acidic food and drinks (soft drinks, sport drinks, energy drinks, and fruit drinks) to avoid. The patients were also advised to restrict having acidic drinks to mealtimes, if possible, and avoid them close to bedtime and to finish the beverage at one time instead of sipping the beverage over a long period. If possible, the acidic drinks should also be low in sugar to minimize dental caries. Rinsing with water after drinking these products was also recommended. The patients were requested to drink only milk or water [27, 29, 30]. The researcher used the information derived from the diet analysis to guide the conversation with the patients about their dietary habits and caries risk status.

During the follow-up period, the patients were directed to regularly brush their teeth using the assigned tooth brushing technique with fluoride toothpaste for at least 2 min after every meal. A Proxabrush (Sunstar Americas, Inc., Schaumburg, IL, USA) was used to clean the small spaces under the wires and around the bands and brackets. The patients were instructed to floss with the help of a floss threader after every meal.

The patients' compliance with oral hygiene measures was monitored via a social media (Line) application. Both experimental groups provided their self-monitoring data over the entire intervention period. Smart phone videos of the patients brushing their teeth were self-recorded at their homes and sent to an investigator weekly. The patients also used the chat room to share information and pictures regarding their oral hygiene measures. The chat room was moderated by an investigator. The investigator gave feedback concerning their oral hygiene measures after visually evaluating the patients' video and level of participation in the chat room.

To assess the participants' adherence to the dietary recommendations, the investigator called the participants once a week during the follow-up period. Semi-structured interviews were performed between the patients and the investigator. The interviews focused on how well the patients followed the diet recommendations for caries prevention. Dietary advice must be realistic and always based on the patient's current dietary behavior.

The changes in dental biofilm maturity and diet behavior in each group after the inventions were assessed after ~ 4 weeks (T2). Furthermore, to assess tooth brushing performance, the subjects in both groups were directed to brush their teeth for an additional 2 min after whole mouth biofilm staining at the T2 visit. Individual focus care parameters were designated where the examiner could monitor the patient's tooth brushing performance. The Focus Care Areas comprised the following parameters: (1) tooth contact time, (2) tooth contact on the facial, occlusal, palatal, and bracket surfaces, and (3) the sequence of brushing movement as either horizontal, Charters, modified Bass techniques, and no brushing movement at all. The focus care parameters were observed in the sequence defined above, starting with the tooth contact time and ending with the brushing movements. WSL detection, dental biofilm staining, oral hygiene instructions and diet counseling were performed in an orthodontic clinic by a single calibrated operator (T.S.).

Outcomes (primary and secondary)

The primary study outcome was dental biofilm cariogenicity and the secondary outcome was sugary and acidic food consumption between meals. These outcomes were collected through clinical examination (dental biofilm staining) and a questionnaire (dietary habits). Each participant was examined before the interventions (T1). The same evaluation process was used after 4 weeks (T2).

Sample size calculation

The minimum number of participants required was determined using the G*Power version 3.1.9.4 package. The sample size was estimated based on a medium effect size (Cohen d = 0.5) expectation for the changes in the percentage plaque maturing staining (% PMS) between T1 and T2 in each group [31]. We used the Wilcoxon signed-rank test for dependent 2 groups with a power of .80 and an error $\alpha = 0.05$, which determined that a minimum of 28 participants in each group was needed. Considering a dropout rate of 10%, the final sample size required in each group was at least 31 participants.

Interim analyzed and stopping guidelines

Not applicable

Randomization (random number generation, allocation concealment, implementation)

Randomization to the two intervention groups was performed by another staff from the orthodontic clinic. The patients were randomly selected from the eligible patients with a 1:1 allocation rate. The eligible patients were divided into two groups [Group 1 (SSL) and Group 2 (EL)]. Simple randomization was performed using the chit box method to ensure an equal number in each group [32]. We prepared 35 chits by writing No.1 (for Group1) on 35 chits and No. 2 (for Group 2) on 35 chits. After folding the chits and putting them in a box and mixing well, the first patient drew a chit, and noted the number written on it. The chit was not placed back in the box. The next patient drew the second chit first, noted it, discarded it, and the randomization proceeded similarly until the last chit was drawn.

Blinding

Blinding of the clinical investigators and participants to the intervention in each group was not possible in this trial.

Examiner training

An experienced investigator (S.K.) trained an examiner (T.S.) in the dental plaque staining and WSL examination procedures. The examiner was trained for 1 month before the start of the study. The examiner made an audiovisual presentation of 8–10 cases. Plaque staining and WSL evaluation was discussed in detail, step by step, along with a clinical photograph of each variable that can affect the evaluation. This was followed by explanation about how to calculate the WSL grades. The examiner and the investigator compared each other’s grades and any discrepancies were resolved by discussion. During the practice sessions, the experienced investigator regularly reviewed the examination data and provided instructive feedback to the examiner explained where the examiner deviated from the grading guidelines.

Measured parameters

Questionnaire and diet analysis

Self-reported questionnaires were used to obtain the patient’s socio-demographic data, medical history, dental history, and oral care practices. The socio-demographic variables were age, sex, and level of education. The medical history comprised systemic diseases and medications. The questionnaire contained dental history questions regarding dry mouth symptoms, i.e., dry feeling in the mouth, difficulty swallowing and/or eating, and feeling thirsty. Oral care practices were assessed through questions on tooth-brushing frequency, when they brushed each day and method, tooth-brushing duration, fluoride exposure, and other cleaning materials used.

It is suggested that dietary assessments should feature several additional approaches, including conducting 24-hour recall interviews and asking patients to complete three-, five- or seven-day diet diaries, especially if the dentist cannot identify dietary etiologic factors of caries development during the interview process [21, 22]. In the present study, dietary analysis was performed as described by Ngo et al. [28]. The patients were required to keep a record of their food and beverage consumption for 5 d (three weekdays and over a weekend). Dietary analysis comprises the most subjective information, because it involves patients' attitude, motivation, cooperation, and honesty. Therefore, in this study, the patients were educated and motivated to enter everything that he/she consumed from morning until bedtime in the chart. They were also requested to include any medications, chewing gum, and cough lozenges. The chart was analyzed at the T2 visit by the dentist-patient duo for the frequency of acid foods including frank or occult sugar intake between meals. The average number of exposures to sugar and acid between meals over the 5 d was calculated for each patient.

WSL detection

Clinical examination for the presence of WSLs was performed on the labial surfaces of individual teeth. The examination and scoring were performed as previously described by Gorelick et al. [33]. The lesions were scored as follows:

Score 0 = no white spot formation

Score 1 = slight white spot formation

Score 2 = severe white spot formation

Score 3 = white spot formation with cavitation

Based on the WSL score on the tooth surfaces, the percentage of WSLs in each score was obtained using the formula:

% WSL of each score = (number of teeth with each WSL score /total number of teeth examined) x100.

Assessment of dental biofilm cariogenicity

Dental biofilm cariogenicity was assessed by staining all tooth surfaces, except the occlusal surfaces, with GC Tri Plaque ID Gel™. After placing a lip and cheek retractor (OptraGate, Ivoclar, Vivadent), the gel was applied with a microbrush on all tooth surfaces and left undisturbed for 2 min. The tooth surfaces were then gently rinsed with water for 30 sec, and the changes in dental biofilm color were observed (Fig. 1). The original dark blue color changed to light blue when the plaque pH was less than 5. Immature and mature dental biofilm turned pink and purple, respectively [17]. Each color-stained dental biofilm type was scored separately. Based on the color changes on the tooth surfaces, % PMS was obtained using the formula:

% PMS = (number of teeth with each colored plaque/total number of teeth examined)

X 100 as previously described [34].

Statistical analysis (primary and secondary outcomes)

Data analysis was performed using the Statistical Package of Social Sciences version 25 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Due to our small sample size, all parameters were tested for a normal distribution using the Shapiro Wilk normality test. The skewness and kurtosis of the data were also calculated. The Shapiro Wilk test ($p < 0.05$) and the skewness and kurtosis results indicated that our data were not normally distributed. Therefore, non-parametric tests were used in this study. Descriptive statistics are presented as median, range, 25th percentile (Q1), 75th percentile (Q3), and percentage. The Wilcoxon signed-rank test was used for comparisons between two dependent groups. For more than two independent groups, the Kruskal Wallis with Dunn-Bonferroni post-hoc test were performed. Statistical significance was defined as $p \leq 0.05$.

Results

The Shapiro Wilk test results indicated that our data were not normally distributed. Therefore, non-parametric tests were used in this study.

Participant Flow

Participants were included from October 25, 2020 until April 7, 2021. Seventy patients (13–53 years old, median 28 years old) with fixed orthodontic appliances were randomized in a 1:1 allocation in this study. No patients dropped out due to adverse effects of the intervention. The patients were randomly divided into two groups: the SSL (median age 28 years old, range,13–43 years old) and EL groups (median age 28 years old, range, 15–53 years old) (Fig. 2).

Baseline data

Comparable intervention arms are important at baseline and should be presented when studies are reported. However, it is advised to look for meaningful baseline differences rather than significant differences because the trial was not powered for this purpose [35].

Complete data was obtained from 70 patients, of whom 57 were female (81.42%). The median (range) duration of the fixed orthodontic therapy in the selected patients was 3.65 (0.3–7.0) years (Table 1). The median (range) age in the SSL group was 28 (13–43) years old and 28 (15–53) years old in the EL group. There were 30 (80.71%) females in the SSL group and 27 (71.14%) females in the EL group. More than 50% of our patients (68.57%, SSL group and 74.29%, EL group) had more than a high school education (Table 1).

Table 1
Distribution of socio-demographic, orthodontic treatment and white spot lesion data based on ligature type at baseline

Variable	SSL	EL
Socio-demography		
Age (years) Median Min-max	28 13–43	28 15–53
Sex Male, n (%) Female, n (%)	5 (14.29) 30 (85.71)	8 (22.86) 27 (71.14)
Educational level, n (%) < Diploma ≥ Diploma	11 (31.43) 24 (68.57)	9 (25.71) 26 (74.29)
Duration of fixed orthodontic therapy (year) Median Min-max	4 0.5-5.0	3 0.3-7.0
Duration of ligation before the interventions (T0-T1, days) Median Min-max	30 25–44	30 21–62
Follow-up duration (T1-T2, days) Median Min-max	32 21–111	30 21–180
White spot lesion (%) No [Median (min-max)] Slight [Median (min-max)] Severe [Median (min-max)]	100 (83.33–100) 0 (0-16.16) 0 (0-4.16)	96.42 (76.16–100) 3.58 (0-16.16) 0 (0-4.16)

The median (range) duration of orthodontic treatment before participating in this study was 4 (0.5–5.0) years and 3 (0.3–7.0) years in the SSL and EL groups, respectively. The duration (median and range) of ligature placement (T0–T1) before the interventions was 30 (25–44) d) in the SSL and 30 (21–62) d in the EL. The median (range) follow-up duration (T1–T2) was 32 (21–111) d and 30 (21–180) days in the SSL and EL groups, respectively.

No WSL score 3 (cavitation) was observed in our patients. The median and range of WSL scores 0, 1, and 2 in the SSL group were 100% (83.33–100), 0% (0–16.66), and 0 (0–4.16), respectively. The detection rate was 96.42% (79.16–100), 3.58% (0–16.66), and 0 (0–4.16), respectively, in the EL group.

There were no systemic diseases or dry mouth-associated symptoms in our patients (Table 2). None of the patients in the SSL and EL groups reported experiencing dry mouth. The questionnaire data regarding oral behaviours revealed that almost all of our patients (94.28%) performed tooth brushing at least twice a day. Tooth brushing was performed most frequently in the morning, after lunch, before bedtime, and other times (> 90%). Specific tooth brushing methods, i.e., Horizontal, vertical, and circular scrubbing were found in both groups (11.43%, SSL group and 20%, EL group), whereas the combined brushing method was the most common in both groups (88.57%, SSL group and 80%, EL group). Furthermore, 22.86% of the SSL group and 14.29% of the EL group spent less than 2 min brushing their teeth. In addition to brushing, 74.29% of the SSL group used dental floss compared with 82.86% in the EL group. We found that 71.43% of patients with SSLs used interdental brushes, while this rate was 82.86% for the patients with ELs. Our results indicated that 57.14% of the SSL group and 74.29% of the EL group used mouthwash. Furthermore, 8.58% of the SSL group used other oral care products compared with 20% for the EL group. The frequency of professional fluoride application was 11.43% and 22.86% in the SSL and EL groups, respectively.

Table 2
Distribution of the medical history and dental health behaviors of patients based on ligature type at baseline

Variable	SSL	EL
Systemic disease, n (%)	0 (0)	0 (0)
Yes	35 (100)	35 (100)
No		
Medication-induced hyposalivation, n (%)	0 (0)	0 (0)
Yes	35 (100)	35 (100)
No		
Dry mouth, n (%)	0 (0)	0 (0)
Yes	35 (100)	35 (100)
No		
Difficulty swallowing, n (%)	0 (0)	0 (0)
Yes	35 (100)	35 (100)
No		
Feeling thirsty, n (%)	0 (0)	0 (0)
Yes	35 (100)	35 (100)
No		
Tooth brushing frequency, n (%)	2 (5.72)	2 (5.72)
< 2 times a day	33 (94.28)	33 (94.28)
≥ 2 times a day		
Occasion of tooth brushing, n (%)	34 (97.14)	32 (91.43)
In the morning	34 (97.14)	32 (91.43)
After lunch	34 (97.14)	32 (91.43)
Before bedtime	32 (91.43)	31 (88.57)
Other times		
Tooth brushing technique, n (%)	4 (11.43)	7 (20)
Horizontal, vertical or circular	31 (88.57)	28 (80)
Combined		

Variable	SSL	EL
Tooth brushing time, n (%)	8 (22.86)	5 (14.29)
< 2 minutes	27 (77.14)	30 (85.71
≥ 2 minutes		
Use of dental floss, n (%)	26 (74.29)	29 (82.86)
Yes	9 (25.71)	6 (17.14)
No		
Use of interdental brush, n (%)	25 (71.43)	33 (94.29)
Yes	10 (28.57)	2 (5.71)
No		
Use of other oral health-care products, n (%)	3 (8.58)	7 (20)
Yes	32 (91.42	28 (80)
No		
Use of mouthwash, n (%)	20 (57.14)	26 (74.29)
Yes	15 (42.86)	9 (25.71)
No		
Professional fluoride application, n (%)	4 (11.43)	8 (22.86)
Yes	31 (88.57)	27 (77.14)
No		

Taken together, our findings demonstrated that there were no meaningful differences in baseline characteristics that can confound the results of this trial which supported the dental trials guidelines [35].

Dental biofilm cariogenicity (primary outcome)

In the SSL group at T1, the percentage of new dental biofilm (median 50%; range 8–100%; Q1, 32.66%; Q3, 71.13%) was the highest compared with mature dental biofilm (median 26%; range 0–84%; Q1, 20.42%; Q3, 47.93%) and cariogenic dental biofilm (median 8%; range 0–54.54%; Q1, 4%; Q3, 22.92%) ($p < 0.05$, Kruskal Wallis with Dunn-Bonferroni post-hoc test) (Fig. 3). After tooth brushing with the recommended technique and dietary advice (T2), the percentage of new dental biofilm significantly increased (median 79.16%; range 16.66–100%; Q1, 62.96%; Q3, 96.15%) ($p < 0.05$, Wilcoxon signed-rank test). In contrast, the percentage of mature dental biofilm (median 20%; range 0–70.83%; Q1, 1.85%; Q3, 28.42%) and cariogenic dental biofilm (median 0%; range 0–40%; Q1, 0%; Q3, 8.01%) significantly decreased ($p < 0.05$, Wilcoxon signed-rank test). The Kruskal Wallis with Dunn-Bonferroni post-hoc test indicated a significant difference in all pair-wise comparisons of dental biofilm staining at T2 in the EEL group ($p < 0.05$).

The EL group demonstrated the highest percentage of cariogenic dental biofilm (median 54.16%; range 14.81–100%; Q1, 44.44%, Q3, 69.56%) at T1, while the median percentage was 25.0% (range 0–70.83%; Q1, 18.18%; Q3, 36.18%) and 13.63% (range 0–60%; Q1, 7.55%; Q3, 26.96%) for mature and new dental biofilm, respectively ($p < 0.05$, Kruskal Wallis with Dunn-Bonferroni post-hoc test) (Fig. 4). A decrease in cariogenic dental biofilm (median 7.14%; range, 0–50%; Q1, 0; Q3, 15.78%) was observed at T2, whereas an increase in immature dental biofilm (median 64%; range 16.7–100%; Q1, 40.40%; Q3, 82.60%) was detected (all $p < 0.05$, Wilcoxon signed-rank test). However, there was no significant difference in mature dental biofilm (median 29.62%; range 0–70.83%; Q1, 13.04%; Q3, 46.21%) after the interventions ($p > 0.05$, Wilcoxon signed-rank test). The Kruskal Wallis with Dunn-Bonferroni post-hoc test indicated a significant difference in all pair-wise comparisons of dental biofilm staining at T2 in the EL group ($p < 0.05$).

Diet analysis

A low frequency of sugary and acidic food consumption between meals was observed in the patients with SSL and EL (Table 3). In the SSL group, the median (range) score of sugar exposure between meals was 0.2 (0–2.8) times/day at T1 and 0 (0–2.4) times/day after dietary advice (T2). The median (range) score of acidic food consumption between meals was 0 (0–2.0) times/day and 0 (0–1.2) times/day at T1 and T2, respectively. However, the difference in these dietary habits was not significant ($p > 0.05$, Wilcoxon signed rank test). Similarly, a low frequency of sugary and acidic food consumption was found in the EL group. The patients were exposed to sugar (median, 0 times/day; range, 0–1.4 times/day) and (median, 0 times/day; range, 0–1.6 time/day) at baseline and follow-up, respectively, which were not significantly different ($p > 0.05$, Wilcoxon signed rank test). The median and range of acid exposure between meals at T1 and T2 was 0 (0–1.6) times/day and 0 (0–0.6) times/day at T1 and T2, respectively, in the EL group, which were similar ($p > 0.05$, Wilcoxon signed rank test).

Table 3
Median and range of the frequency of sugary and food acid intake between meals at baseline (T0) and after interventions (T2) in the SSL and EL groups

Food	T0 (times/day)	T2 (times/day)	*p* - value
Sugar			
SSL	0.2 (0–2.8)	0 (0–2.4)	0.2[a]
EL	0.2 (0 -1.4)	0 (0–2.4)	0.06[a]
Acid			
SSL	0 (0–2.0)	0 (0 -1.2)	0.06[a]
EL	0 (0 -1.6)	0 (0–0.6)	0.43[a]

[a]Wilcoxon signed rank test.

Benefits and harms

The participants in this trial might benefit from the intervention by achieving better oral health. Before the interventions and all post-treatment visits, a comprehensive assessment of the teeth and oral soft tissues was conducted via visual examination of the oral cavity. Care as usual was provided following the standard orthodontic protocols. No adverse events were noted or reported during the study. No serious harm was observed.

Discussion

This parallel-design randomized clinical trial directly evaluated the effects of the assigned tooth brushing and dietary advice on reducing cariogenic dental biofilm and cariogenic food consumption. This study design can make causal inferences, provide evidence of an intervention’s efficacy, and minimize bias. The strengths of our study include its relatively large sample size (> 30 patients), high retention rate, and good intra-examiner calibration. We adopted several strategies for reducing possible bias, including randomization, allocation concealment, and intention-to treat analysis, which are recommended in the CONSORT guidelines for reporting on randomized clinical trials [24, 36]. Furthermore, the CONSORT statement recommends a table demonstrating the baseline characteristics of the patients in each group, without testing for significant differences and *p* values [37, 38]. Therefore, significant differences in the baseline data between the SSL and El groups were not presented in this study. However, most baseline data had a non-normal distribution. Therefore, we could not use an absolute standardized difference to indicate an imbalance in randomization [39]. The quality of randomization and the imbalance of this study were assessed as described by Chen *et al.* [40]. First, we described the participant characteristics in detail. The baseline Tables 1 and 2 allow readers, especially clinicians, to judge how relevant the results of this trial might be to a particular patient. Next, to establish compliance with the study protocols: the inconsistencies between our trial protocols and the final report have been documented. For example, the CONSORT flow diagram of the participants provides the participant eligibility, trial sample size, and missing data. Furthermore, we provided the baseline information necessary for replicating this trial and for comparing it with other similar randomized control trials. Lastly, the baseline demographic and clinical characteristics of the trial are useful for evaluating this trial's generalizability. Our eligibility criteria were not very rigorous and we recruited participants from patients currently undergoing fixed orthodontic therapy.

The baseline characteristics observed in this study were similar to those reported in previous studies. Generally, the oral hygiene practices of the orthodontic patients in our study were good. This finding was expected because patients are repeatedly advised to maintain good oral hygiene while receiving any orthodontic treatment. Our results suggest that regular visits to the orthodontist is a key factor in improving oral hygiene and gingival health [41]. Many studies reported that a high percentage (> 90%) of orthodontic patients brushed their teeth at least twice a day [42, 43]. Active orthodontic patients had a higher likelihood of brushing their teeth for 2 min or more compared with the control group [44]. Horizontal, vertical, or circular scrubbing techniques were performed by the patients in the current study. The combination method was the most common brushing technique in the SSL and EL groups and our findings are in line with those of a previous report [45]. However, our brushing technique findings did not concur with those of Atassi and Awartani [46], who revealed that the horizontal method was the most commonly used brushing method.

During orthodontic treatment, ideal oral hygiene may not be achieved solely by tooth brushing. Interdental tools and daily rinsing with fluoridated mouthwash can significantly improve dental health. The use of dental floss, interdental brushes, and mouthwashes was commonly observed in our patients, which agrees with previous studies [43, 47]. The possible explanation for our findings might be that the use of mouth rinse and interdental tools was reinforced in our patients. However, some studies reported that inappropriate oral hygiene was observed in orthodontic patients. Most of their orthodontic patients did not use dental floss or interdental brushes [45, 46]. These disparate findings could be attributed to several reasons, such as differences in demographic factors or oral hygiene advice protocols implemented in different countries.

Of the 70 patients who underwent fixed orthodontic treatment for 0.3–7.0 years, 0–3.58% of the examined teeth had WSLs. The reported proportion of patients with WSLs after fixed orthodontic treatment varies widely from 2–96%. This variation can be attributed to differences in the standardization of clinical examinations and the use of various detection tools with different sensitivities and specificities in diagnosing demineralization [48]. Conversely, our results were in line with those of Gorelick et al. who found that 3.6% of teeth had WSLs after placing fixed appliances for ~ 1 year. Similar to our findings, no excessive white spots or cavitation were found in their patients. According to the WSL results in the SSL and EL groups, it was assumed that the two groups had a similar caries risk status [13, 49].

In this study, we investigated the influence of SSL and EL on dental biofilm cariogenicity. To assess caries activity based on the ecological plaque hypothesis, we used the 3-tone disclosing gel to determine plaque maturity. The ecological plaque hypothesis focuses on acidogenic bacterial species and the factors that result in the environmental change of dental biofilm. Based on the determinants-confounders' model in dental caries, socio-demographic characteristics, dental health behaviors, diet habits, and caries risk status are confounders of dental biofilm and caries formation [50]. Therefore, these confounding factors should be controlled in a randomized controlled trial. Notably, in this study, no meaningful differences in sociodemographic characteristics, dental health, behaviors, caries risk status (WSL prevalence), frequency of sugary and acidic food exposure, or duration of orthodontic treatment were observed between the SSL and EL groups at T1. These results indicated that in the present study, the above-mentioned confounders were well controlled between the two study groups.

Fixed orthodontic therapy typically causes retentive site-associated dental plaque accumulation [51]. Elastomeric rings and ligature wires are the two most commonly used techniques for tying archwires. Forsberg et al. [6] discovered higher levels of acid-producing bacteria, particularly *S. mutans* and Lactobacilli, on EL compared with SSL in 12 orthodontic patients. In contrast, Tukkahraman et al. [7] found no significant difference in the number of *S. mutans* and Lactobacilli from teeth ligated with either SSL or EL. Our findings are in line with Forsberg et al. [6] because we also found a higher percentage of cariogenic dental biofilm in the EL group compared with the SSL group. This difference might be due to an increase in the retentive area of elastomeric rings compared with stainless steel ligatures [51]. Our results, however, differ from the results of Rodrigues et al. [52] who reported no difference in the dental biofilm formation between the surfaces tied with either SSL or EL when using a plaque staining method that evaluated dental biofilm accumulation, but not its cariogenicity.

In addition to the difference in retentive areas for dental biofilm accumulation, elastomeric rings are an organic material that might be more favorable for bacterial colonization compared with stainless steel, which is an inorganic material with an inert material surface [53, 54]. Furthermore, fixed orthodontic appliances can induce oral ecologic changes, such as a low pH environment, which may lead to increased dental biofilm cariogenicity. It was found that early in orthodontic treatment, EL significantly reduced salivary pH compared with SSL, lowering it below the critical pH level [55]. Our findings confirm those of previous studies that ligature type affects dental biofilm accumulation. The results of the present study suggest that ligature type has a significant effect on the ecological environment of the dental plaque deposited on orthodontic appliances. The increased retentive areas and organic nature of elastomeric rings play important roles in increasing dental biofilm cariogenicity.

Due to the increase in dental biofilm accumulation and its ecological changes, excellent oral hygiene practice during fixed orthodontic treatment must be performed. Preventive programs must be emphasized to all orthodontic patients [56]. Mechanical plaque control is a meaningful challenge for orthodontic patients, because they face multiple difficulties, including which brushing technique to use, how to access posterior areas of the dentition, the correct frequency and duration of tooth brushing, and the best method of achieving plaque control in the interdental regions [44, 45, 57]. Fixed appliances, including brackets and archwires, impede access to the tooth surface and increase the complexity of the mechanical cleaning that the patient must perform [58].

Currently, there is a lack of sufficient scientific evidence to support any recommendations on tooth brushing techniques for patients receiving orthodontic treatment with fixed appliances [25]. A few studies compared tooth brushing techniques for orthodontic patients. For patients with fixed appliances, the Bass technique was found to be more effective than the Horizontal technique, followed by the Modified Stillman technique in improving gingival health. However, for removing dental biofilm, the Horizontal technique was found to be the most effective, while the Bass technique was the least effective. This might be because the Bass technique targets biofilm on the gingival margin and in the sulcus, but is unable to clean the areas around fixed appliances [59]. In contrast, Hussein et al. [60] reported that the roll technique had a superior cleansing effect compared with the Horizontal method. The authors suggested that although orthodontic patients practiced the Horizontal brushing technique, which is the most used technique, patients practiced it with some technical errors, especially with sulcular brushing or gingival cleansing. Moreover, patient discomfort from Horizontal scrubbing was alleviated when brushing with the roll technique, resulting in them preferring the roll technique rather than the Horizontal technique. However, a study on the effect of tooth brushing on *S. mutans* and *Candida albicans* counts suggests that the modified Bass technique is an important tool in maintaining good oral hygiene [61]. Based on the results of the present study, we recommend the Horizontal technique combined with the Charter's and modified Bass techniques to ensure the complete removal of dental biofilm around the bracket and archwire areas, the undercut below and above the wing of brackets, occlusal and palatal surfaces, and intrasulcular areas. The intended effect of the combined brushing protocol was observed; the cariogenic dental biofilm in the SSL and El groups was significantly reduced at the follow-up appointment. However, the combined tooth brushing reduced the percentage of mature dental biofilm in the SSL, but not the EL group. This discrepancy in these results might be due to the differences in new retentive areas and physical properties of the two ligatures [51, 53, 54].

A specific 3-tone plaque disclosing dye can help orthodontists quickly identify where patients are struggling with mechanical cleaning. Being able to see the metabolic activity of the dental biofilm at individual sites empowers orthodontists and motivates orthodontic patients, because both can discuss the issue with real-time assessment of bacterial acid production. Furthermore, orthodontists can demonstrate appropriate dental biofilm removal methods. In addition, applying 3-tone plaque staining is useful for monitoring diet compliance of the patients. A suitable recall period can be set to allow timely monitoring of the patients [17, 62].

This study also explored whether receiving dietary counseling from an orthodontist could improve the patient's dietary habits for preventing caries. A high frequency of sugar (> 1 time/day) and acidic food (> 2 times/day) between meals is considered a high caries risk behavior [28]. Most of our patients were exposed to sugary and acidic food between meals at a frequency of less than 1 time/day and thus were not caries risk subjects. Furthermore, there was no decrease in the frequency of sugar and acid intake after dietary counseling in the SSL and EL groups. This implies that although tooth brushing and dietary counseling were provided to our patients, dietary advice did not have a meaningful impact on oral hygiene maintenance and improvement [63, 64]. However, it is possible that the baseline exposure level of cariogenic foods in this study was too low to allow for detecting the effect of diet advice.

This study has some limitations. First, blinding of the investigator and participants was not feasible. Second, short-term follow-up was not conducted at the same period for all patients because the follow-up appointment depended on the patient's availability. The average follow-up duration was 4 weeks and our findings might be affected by habits established during their orthodontic treatment before enrolling in the study. Third, our patients were not a homogenous group with a wide age range (13–53 years old). This may affect the tooth brushing ability of the subjects. However, several adolescents participated in the study. Two patients (13 and 18 years old) and two patients (15 and 18 years old) were enrolled in the SSL and EL groups, respectively. The patients were instructed to use the tooth brushing methods that were previously recommended to fixed orthodontic appliance patients [25]. Furthermore, the tooth brushing performance between the adolescent and adult subjects in our study was similar. Finally, dental biofilm maturity was not measured at T0. This evaluation should be performed in future studies to determine the effect of ligature type on dental biofilm maturity.

Conclusions

The main objective of this study was to evaluate the effects of the assigned tooth brushing method on dental biofilm maturity after ligature tying with SSL or EL, not the effect of ligature type on dental biofilm maturity *per se*. Dental biofilm cariogenicity was measured before (T1) and after the intervention (T2). In this study, the amount of time for the ligature-associated dental biofilm accumulation before the interventions was approximately the same for both groups. The potential confounders were controlled in this study therefore, the tooth brushing intervention played an important role in reducing acidogenic dental biofilm at T2.

Abbreviations

EL
Elastomeric ligature
PMS
Percentage plaque maturing staining
SSL
Stainless steel ligature
WSLs
White spot lesions

Declarations

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Author contributions

TS contributed to conception and design of the study, acquisition of data, analysis of data. SK contributed to conception and design of the study, analysis and interpretation of data, drafting and revised the manuscript. KU contributed to conception and design of the study, analysis and interpretation of data, drafting and revised the manuscript. All authors have approved the final manuscript.

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Availability of data and materials

The datasets supporting this article are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

The study was approved by Walailak University IRB (WUEC 20-227-02). Informed consent was obtained from all individual participants or a parent of participants under 16 years old before beginning of the study. All methods were performed under the relevant guidelines and regulations according to Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors do not have a commercial interest in the 3-tone disclosing gel used in this study. The authors declare that they have no commercial or associated interest that represents a conflict of interest in connection with the manuscript.

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References

1. Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006;6(Suppl 1):14.
2. Marsh PD, Head DA, Devine DA. Dental plaque as a biofilm and a microbial community—Implications for treatment. J Oral Biosci. 2015;57(4):185–91.
3. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, et al. Dental caries. Nat Rev Dis Primers. 2017;3:17030.
4. Freitas AO, Marquezan M, Nojima Mda C, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: a systematic review. Dent Press J Orthod. 2014;19(2):46–55.
5. Skilbeck MG, Mei L, Mohammed H, Cannon RD, Farella M. The effect of ligation methods on biofilm formation in patients undergoing multi-bracketed fixed orthodontic therapy - a systematic review. Orthod Craniofac Res. 2021.
6. Forsberg CM, Brattstrom V, Malmberg E, Nord CE. Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of Streptococcus mutans and lactobacilli. Eur J Orthod. 1991;13(5):416–20.
7. Turkkahraman H, Sayin MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. Angle Orthod. 2005;75(2):231–6.
8. Condò R, Casaglia A, Armellin E, Condò SG, Cerroni CL. Traditional elastic ligatures versus slide ligation system. a morphological evaluation. Oral Implantol (Rome). 2013;6(1):15–24.
9. Sukontapatipark W, El- Agroudi MA, Selliseth NJ, Thunold K. KA. S. Bacterial colonization associated with fixed orthodontic appliances. a scanning electron microscopy study. Eur J Orthod. 2001;23(5):475–84.
10. Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: part 4. detection and diagnosis of initial caries lesions. Br Dent J. 2012;213(11):551–7.
11. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. Angle Orthod. 2011;81(2):206–10.
12. Øgaard B. White spot lesions during orthodontic treatment: mechanisms and fluoride preventive sspects. Semin Orthod. 2008;14(3):183–93.
13. Eltayeb MK, Ibrahim YE, El Karim IA, Sanhouri NM. Distribution of white spot lesions among orthodontic patients attending teaching institutes in Khartoum. BMC Oral Health. 2017;17(1):88.
14. Toti Ç, Meto A, Kaçani G, Droboniku E, Hysi D, Tepedino M, et al. White spots prevalence and tooth brush habits during orthodontic treatment. Healthcare. 2022;10(2):320.
15. Contaldo M, Lucchese A, Lajolo C, Rupe C, Di Stasio D, Romano A, et al. The oral microbiota changes in orthodontic patients and effects on oral health: an overview. J Clin Med. 2021, 10(4).
16. Simon-Soro A, Mira A. Solving the etiology of dental caries. Trends Microbiol. 2015;23(2):76–82.
17. Brostek AM, Walsh LJ. Minimal intervention dentistry in general practice. Oral Health Dent Manag. 2014;13(2):285–94.

18. Jayanthi M, Shilpapriya M, Reddy VN, Elangovan A, Sakthivel R, Vijayakumar P. Efficacy of three-tone disclosing agent as an adjunct in caries risk assessment. Contemp Clin Dent. 2015;6(3):358–63.
19. Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. Dent Clin North Am. 2010;54(3):441–54.
20. Walsh LJ, Brostek AM. Minimum intervention dentistry principles and objectives. Aust Dent J. 2013;58(Suppl 1):3–16.
21. Fontana M, Zero DT. Assessing patients' caries risk. J Am Dent Assoc. 2006;137(9):1231–9.
22. Curounanidy U, Sathyanara R. Dental caries: a complete change over (part II) in the diagnosis and prognosis. J Conserv Dent. 2009;12(3):87–100.
23. Shrestha B, Dunn L. The Declaration of Helsinki on medical research involving human subjects: a review of seventh revision. J Nepal Health Res Counc. 2020;17(4):548–52.
24. Schulz KF, Altman DG, Mother D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. J Pharmacol Pharmacother. 2010;1:100–7.
25. Bok H-J, Lee CH. Proper tooth-brushing technique according to patient’s age and oral status. Int J Clini Prev Dent. 2020;16(4):149–53.
26. van Loveren C. Sugar restriction for caries prevention: amount and frequency. which Is more [mportant? Caries Res. 2019;53(2):168–75.
27. Moynihan PJ. Dietary advice in dental practice. Br Dent J. 2002;193(10):563–8.
28. Ngo HC, Wolff MS, Hume WR. Dental caries: activity and risk assessments as a logical and effective path to both prevention and cure. In: Mount GJ, Hume WR, Ngo HC, Woff MS, editors. Preservation and restoration of tooth structure. 3rd edn. Chichester: John Wiley & Sons: 2016. p. 33–49.
29. Yip HHY, Wong R, Hagg U. Complication of orthodontic treatment: Are soft drinks a risk factor? World J Orthod. 2009;10(1):33–40.
30. Mishra M, Mishra S. Sudar-sweetened beverages: general and oral health hazards in children and adolescents. Int J Clin Pediatr Dent. 2011;4(2):119–23.
31. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 2013;4:863.
32. Singh G. Randomization made easy for small size controlled clinical trials. J Int Assoc Med Sci Educ. 2006;16(2):75–8.
33. Gorelick L, Geiger AM, Gwinnett J. Incidence of white spot formation after bonding and banding. Am J Orthod. 1982;81:93–8.
34. Widhianingsih D, Koontongkaew S. Enhancement of cariogenic virulence properties of dental plaque in asthmatics. J Asthma. 2021;58(8):1051–7.
35. Wadgave U, Khairnar MR, Kadu TS, Wadgave Y. Evaluation of dental trials comparing baseline differences using p values. Acta Odontol Scand. 2019;77(3):181–3.
36. Pandis N, Fleming PS, Hopewell S, Altman DG. The CONSORT Statement: Application within and adaptations for orthodontic trials. Am J Orthod Dentofacial Orthop. 2015;147(6):663–79.

37. de Boer MR, Waterlander WE, Kuijper LD, Steenhuis IH, Twisk JW. Testing for baseline differences in randomized controlled trials: an unhealthy research behavior that is hard to eradicate. Int J Behav Nutr Phys Act. 2015;12:4.
38. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c869.
39. Schobar P, Vetter TR. Correct baseline comparisons in a randomized trial. Anesth Analg. 2019;129(3):639.
40. Chen H, Lu Y, Slye N. Testing for baseline differences in clinical trials. Int J Clin Trials. 2020, 7(2).
41. Davies TM, Shaw WC, Worthigton HV, Addy M, Dummer P, Kingdon A. The efffect of orthodontic treatment on plaque and gingivitis. Am J Orthod Dentofac Orthop. 1991;99:155–62.
42. Al-harbi AA, Alkhulayfi AF, Alharbi AT, Al-harbi M, Al-harbi AS, Al-harbi NS. Knowledge of patients about association between orthodontic treatment and periodontal diseases. Int J Oral Care Res. 2018;6(2):43–6.
43. Baheti MJ, Toshniwal NG. Survey on oral hygiene protocols among orthodontic correction-seeking individuals. J Educ Ethics in Dent. 2015;5(1):8.
44. Aljohani SR, Alsaggaf DH. Adherence to dietary advice and oral hygiene practices among orthodontic patients. Patient Prefer Adher. 2020;14:1991–2000.
45. Guo J, Li L, Guan G, Bennani F, Mei L. Oral health knowledge and practice among orthodontic client in China and New Zealand. Can J Dent Hyg. 2020;54(3):124–32.
46. Atassi F, Awartani F. Oral hygienestatus among orthodontic patients. J Comtemp Dent Prac. 2010, 11(4).
47. Anuwongnukroh N, Dechkunakorn S, Kanpiputana R. Oral hygiene behavior during fixed orthodontic treatment. Dentistry. 2017, 7(10).
48. Heymann GC, Grauer D. A contemporary review of white spot lesions in orthodontics. J Esthet Restor Dent. 2013;25(2):85–95.
49. Brown MD, Campbell PM, Schneiderman ED, Buschang PH. A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions. Angle Orthod. 2016;86(2):181–6.
50. Usha C. R S. Dental caries - A complete changeover (Part I). J Conserv Dent. 2009;12(2):46–54.
51. Raju AS, Hegde NA, Reddy VP, Chandrashekar BS, Mahendra S, Harishkoushik SR. An *in vivo* study on bacterial colonization with metal, ceramic and self-ligating brackets: A scanning ekectron microscopy study. J Ind Orthod Soc. 2013;47(2):88–96.
52. Rodrigues CF, de Araujo Ramos Sales L, Vitral RWF, Fraga MR, Quintao CCA. Effects of orthodontic ligation-using elastomeric and stainless steel ligatures-on periodontal health. Dent Press J Orthod. 2011;16(1):48–56.
53. Harikrishnan P, Subha TS, Kavitha V, Gnanamani A. Microbial adhesion on orthodontic ligating materials: an *in vitro* assessment. Adv Microbiol. 2013;3(1):108–14.

54. Sawhney R, Sharma R, Sharma K. Microbial colonization on elastomeric ligatures during orthodontic therapeutics: an overview. Turk J Orthod. 2018;31(1):21–5.
55. Al-Haifi HAA, Ishaq RAA, Al-Hammadi MSA. Salivary pH changes under the effect of stainless steel versus elastomeric ligatures in fixed orthodontic patients: a single-center, randomized controlled clinical trial. BMC Oral Health. 2021;21(1):544.
56. Srivastava K, Tikku T, Khanna R, Sachan K. Risk factors and management of white spot lesions in orthodontics. J Orthod Sci. 2013;2(2):43–9.
57. Zhu L, Petersen PK, Wang H-Y, Bian J-Y, Zhang B-X. Oral health knowledge, attitudes and behaviorof adults in China. Int Denl J. 2005, 55.
58. Luudstrom F, Hamp S-E, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. Eur J Orthod. 1980;2:27–39.
59. Nassar PO, Bombardelli CG, Walker CS, Neves KV, Tonet KNR, Bombonatti R, et al. Periodontal evaluation of different toothbrushing techniques in patient with fixed orthodontic appliances. Dent Press J Orthod. 2013;18(1):76–80.
60. Hussein D, Hussein B, Abdul-Rahman M. Effect of tooth brushing technique on oral hygeine in patients with fixed orthodontic appliance. J Coll Dent. 2005;17(1):101–4.
61. Bhatia N, Ahluwalia R, Grewal S, Bisht D. Effect of modified bass brushing technique and habitual brushing on the carriage of oral microbes in patients with fixed orthodontic appliances-A comparative study. Eur J Pharm Med Res. 2019;6(3):418–27.
62. Walsh LJ, Healey DL. Prevention and caries risk management in teenage and orthodontic patients. Aust Dent J. 2019;64(Suppl 1):37–45.
63. Harris R, Gamboa A, D Y. A. A. One-to-one dietary interventions undertaken in a dental setting to change dietary behaviour. Cochrane Database Syst Rev 2012(3):Art.No:CD006540.
64. Soldani FA, Lamont T, Jones K, Young L, Walsh T, Lala R, et al. One-to-one oral hygiene advice provided in a dental setting for oral health. Cochrane Database Syst Rev. 2018;10:CD007447.

Figures

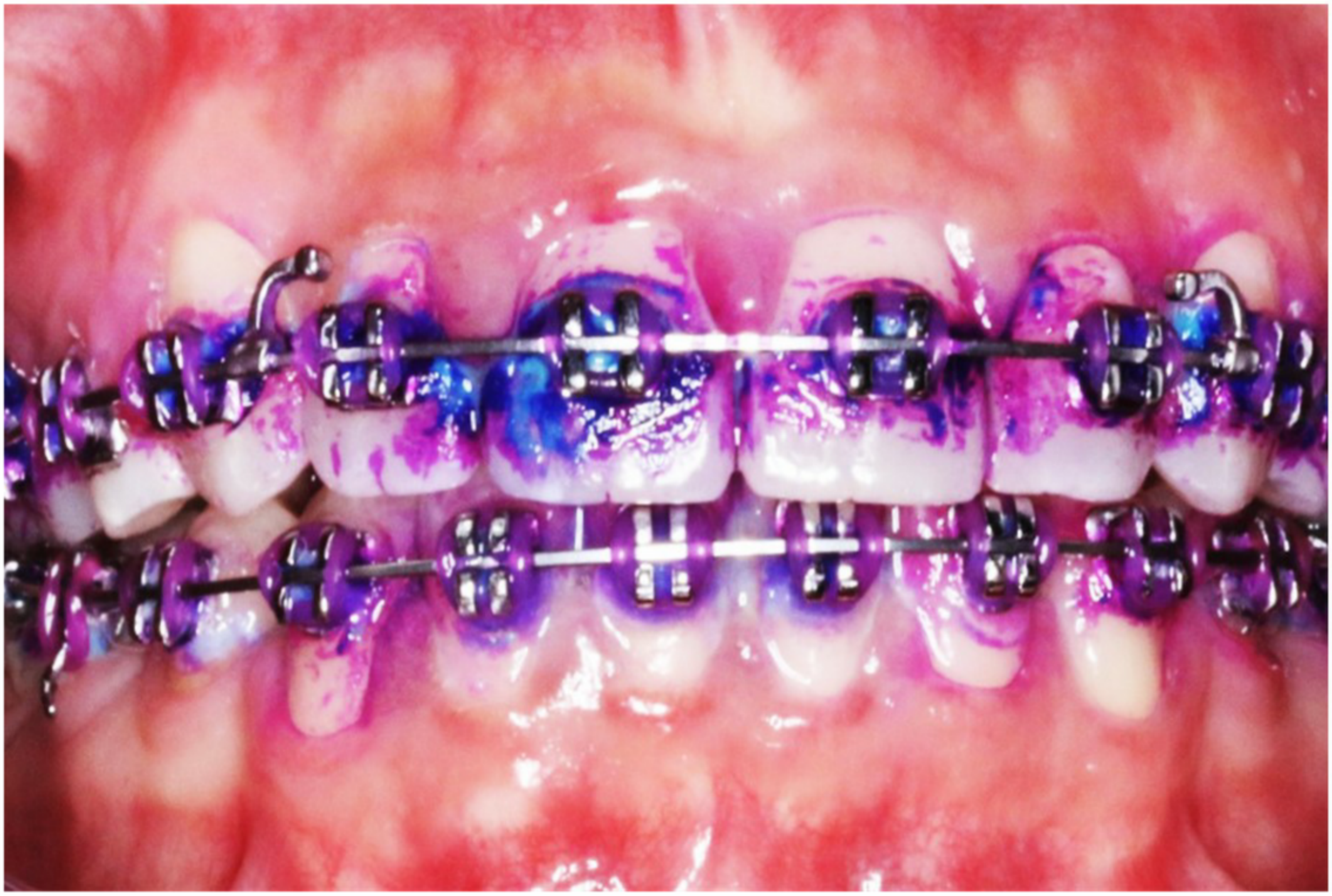

Figure 1

Common dental biofilm-related issues in an orthodontic patient. Acidogenic dental biofilm that is stained light blue is seen on 11 and 12. Mature dental biofilm is stained dark purple-blue and immature dental biofilm is stained pink

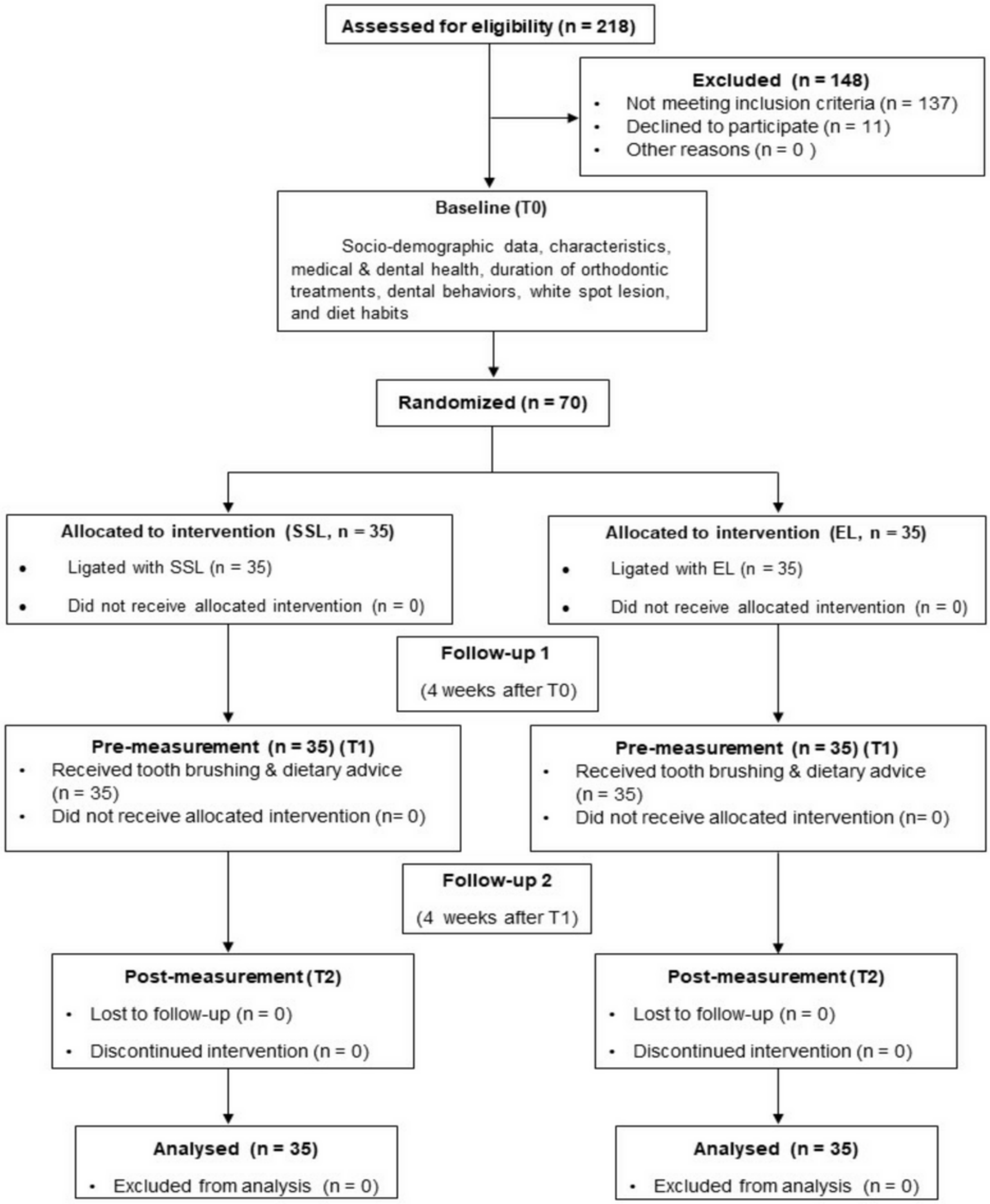

Figure 2

CONSORT flow diagram of the study participant

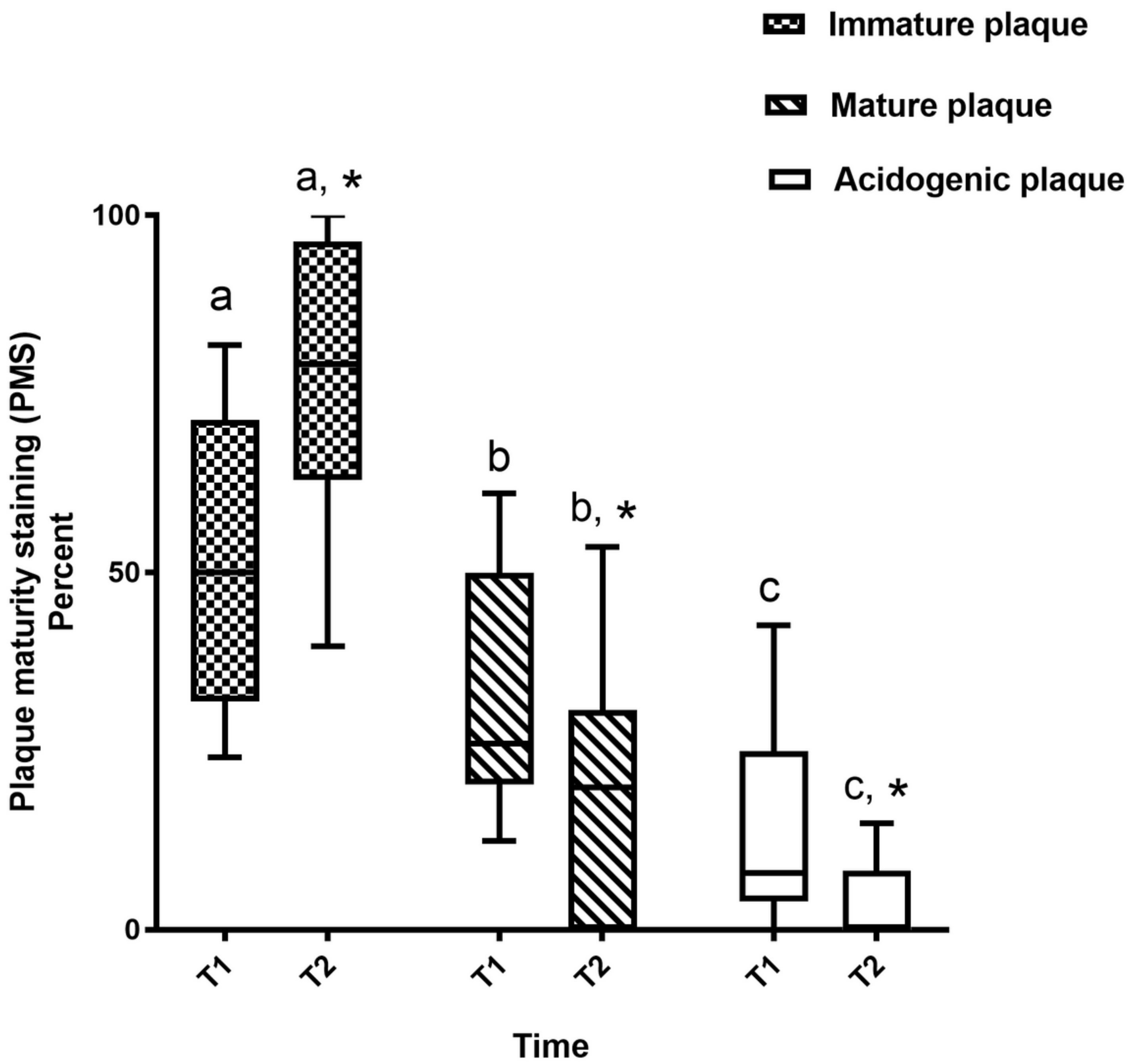

Figure 3

Frequency distribution of dental biofilm maturity in the SSL group over the course of the study. Dental biofilm staining was performed after SSL ligature tying for 30 (25-44) days (T1) and 32 (21-11) days follow-up (T2). The interventions consisted of tooth brushing with the combined Horizontal -Charters-modified Bass technique and dietary advice. The boxes represent the interquartile range and the horizontal bars within the boxes represent the median. The lower and upper ends of the vertical lines represent the 10th and 90th percentiles, respectively. The asterisks denote a significant difference in % PMS between T1 and T2 (the Wilcoxon signed-rank test, $p < 0.05$). For each time point, different superscript letters indicate significant differences (the Kruskal Wallis with Dunn-Bonferroni post-hoc test, $p < 0.05$)

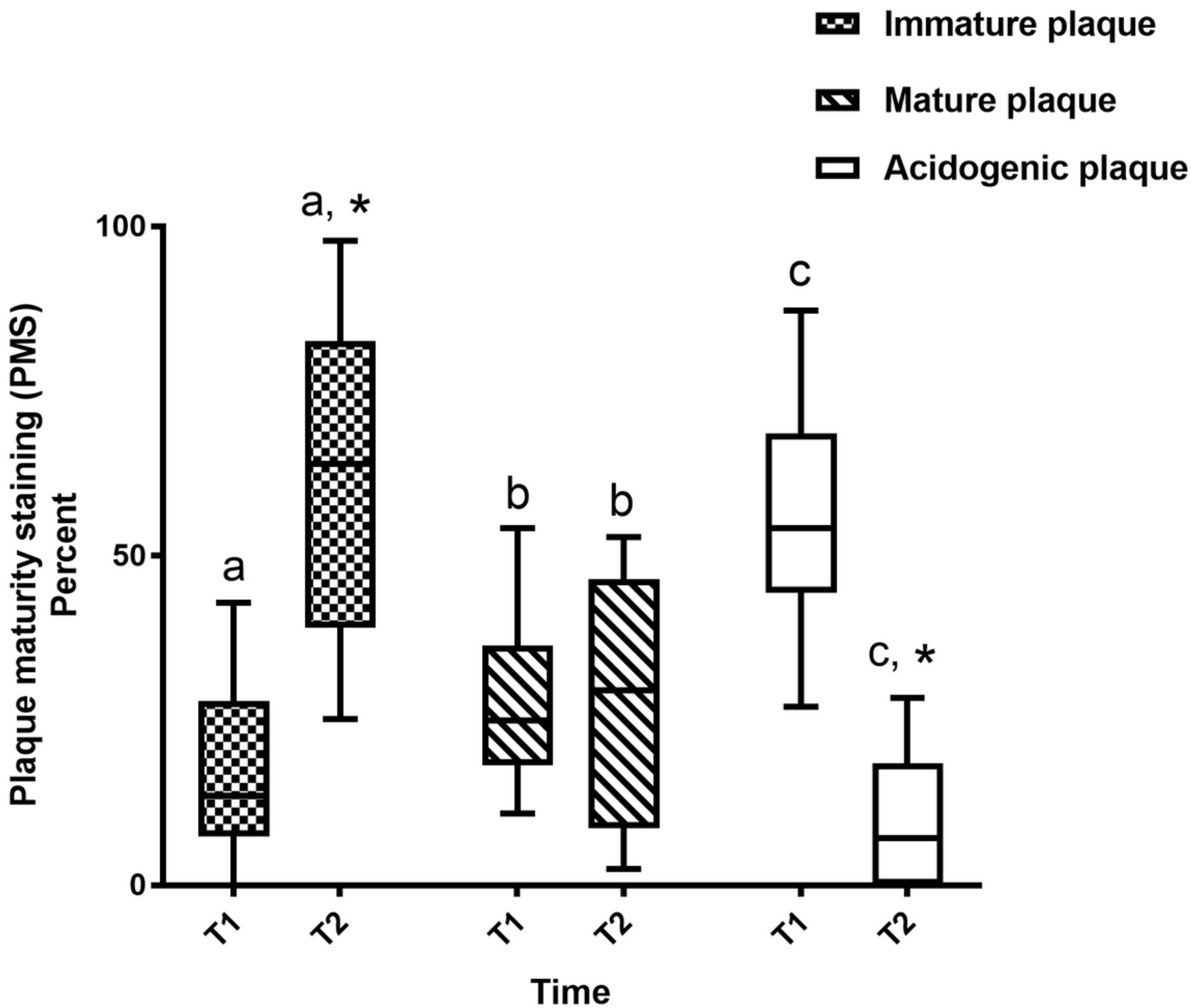

Figure 4

Frequency distribution of dental biofilm maturity in the EL group over the course of the study. Dental biofilm staining was performed after EL ligature tying for 30 (21-62) days (T1) and 30 (21-180) days follow-up (T2). The interventions consisted of tooth brushing with the combined Horizontal -Charters-modified Bass technique and dietary advice. The boxes represent the interquartile range and the horizontal bars within the boxes represent the median. The lower and upper ends of the vertical lines represent the 10^{th} and 90^{th} percentiles, respectively. The asterisks denote a significant difference in % PMS between T1 and T2 (the Wilcoxon signed-rank test, $p < 0.05$). For each time point, different superscript letters indicate significant differences (the Kruskal Wallis with Dunn-Bonferroni post-hoc test, $p < 0.05$)

Supplementary Files

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