



Original Article

Plaque Removal Efficacy of 3 Cleaning Methods for Removable Orthodontic Appliances: A Crossover Randomized Clinical Trial

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Main Points

- The microbial plaque was mostly collected in the case of using the brushing method alone.
- The use of denture cleansing tablets is recommended to enhance oral hygiene when using ROAs.
- Combination of brushing and cleansing tablets decreased the microbial biofilm.

ABSTRACT

Objective: The mechanical plaque removal methods for removable orthodontic appliances (ROAs) may damage the surface of the appliance and may not effectively eliminate the entire microbial plaque. This study aimed to compare the efficacy of brushing+denture cleansing tablets, brushing+propolis mouthwash, and brushing only, for plaque removal from the surface of each orthodontic appliance.

Methods: This crossover randomized clinical trial evaluated 32 patients aged 7-15 years with ROAs. The patients were randomly assigned to 3 groups of brushing (control), brushing + denture cleansing tablets (intervention group 1), and brushing + propolis mouthwash (intervention group 2). The plaque removal methods were switched among the groups during 3 periods, each of 1-month duration. One month after practicing a certain protocol, the plaque disclosing agent was applied on the surface of the appliance. The photographs of the appliances were analyzed by Image J software to calculate the surface area occupied by the residual microbial plaque.

Results: The ratio difference between the surface area of residual plaque to the surface area of the entire appliance was significant between the intervention group 1 and the control group ($P < .001$), while it was not significant between the intervention group 2 and the control group ($P = 0.105$). Moreover, this difference between the intervention groups 1 and 2 was statistically significant ($P < .001$).

Conclusion: The simultaneous use of toothbrush with denture cleaning tablets decreased the microbial biofilm on the surface of ROAs to a better extent, compared to the results with brushing alone. Thus, it appears that the use of denture cleaning tablets may be suitable for effective cleaning of ROAs.

Keywords: Denture cleaning tablets, microbial plaque, orthodontic appliances, propolis mouthwash

INTRODUCTION

Fixed orthodontic treatment with the use of bands and brackets decreases the efficacy of oral hygiene measures in the prevention of plaque accumulation. In contrast, removable orthodontic appliances (ROAs) allow for adequate oral hygiene and decrease the risk of dental and periodontal problems.¹ Although the use of ROAs is more limited compared with the past, they are still used for particular indications, especially in mixed dentition and in conjunction with other orthodontic treatments.²

Orthodontic appliances change the microbial ecosystem of the oral cavity by inducing bacterial growth and increasing the risk of conditions such as halitosis, periodontal disease, and caries. Moreover, being unable to clean dental plaque on the concave and hard-to-reach areas of ROAs by toothbrush can lead to the roughness of the acrylic surface and will surge plaque accumulation. In addition, using a toothbrush along with toothpaste can even result in more abrasion of the acrylic base when compared to using a toothbrush with only water or cleaning tablets.³ Therefore, some studies have considered this method obsolete and suggest chemical cleaning tablets for this purpose.⁴

To the best of our knowledge, limited published information is available regarding the efficient cleaning of resin ROAs. Thus, researchers are still seeking an ideal method for cleaning resin ROAs to minimize complications and promote the oral hygiene and satisfaction of patients.³

Dentipur tablets (Dentipur®, Helago-Pharma GmbH, Parchim, Germany) are among the materials used to improve acrylic denture hygiene. Its manufacturers claim that the advantages of these tablets, compared with toothpastes and other products, include their fast action (within 3 minutes), easy use (immersion), and not causing wear of the acrylic surfaces. Chemical cleaning tablets reduce the adhesion of microbial plaque to the surface of resin ROAs by releasing reactive oxygen species, and eliminate the microbial plaque from the surface of the appliance.³

Propolis is a natural substance derived from a plant resin that is collected by honeybees. The ethanolic extract of propolis is probably more effective than its aqueous extract to control oral biofilm and prevent the progression of dental caries.⁵ Propolis is highly effective against Gram-positive bacteria, especially *Staphylococcus aureus*, and Gram-negative bacteria such as *Salmonella*.⁶ Therefore, we decided to use propolis mouthwash to assess its effectiveness in removal of plaque accumulated on ROAs.

This study aimed to assess and compare the efficacy of 3 methods—brushing, brushing + denture cleaning tablets, and brushing + propolis mouthwash—for cleaning of ROAs, to find an efficient method for optimal plaque removal.

METHODS

The present study was conducted from May 2019 to January 2020. This crossover randomized clinical trial was approved by the University ethics committee (REC.1397.513) and registered in the Registry of Clinical Trials (CT20190106042253N2).

The patients were randomly selected among 7-15-year-olds presenting to the Dental Clinic of Hamadan University of Medical Sciences School of Dentistry, who were under orthodontic treatment with a removable appliance for a minimum of 1 and a maximum of 3 months. According to the prior studies^{7,8} in the field of microbial culture of dental plaque in ROAs, and comparing the measured biofilm level after using different cleaning methods, and using Giradueau et al.'s study⁹ with $\sigma^2_x = 0.02$, $\mu^{(1)} - \mu^{(2)} = 0.03$,

$1 - \rho = 0.09$, $Z_{\alpha/2} = 0.84$, and $Z_\beta = 1.96$, the appropriate sample size for this study was considered as 32. However, assuming an attrition rate of 20%, it was estimated that 37 patients were required to achieve 80% power to detect a difference between treatment methods, with an α level of .05.

The patients were randomized into 3 groups using balanced block randomization, and were assigned to each of these groups for the first month: brushing alone (control group), brushing + denture cleansing tablets (Dentipur®, Helago-Pharma GmbH, Parchim, Germany) (intervention group 1), or brushing + propolis mouthwash (intervention group 2). For the next 2 months, the allocation of interventions was switched for the patients in the 3 groups such that all patients received all 3 interventions by the end of 3 months.

Prior to the commencement of the study, the removable appliances were used by patients for a minimum of 1 month. During this period, the patients were requested to clean the appliance with a toothbrush and toothpaste every night.

After briefing the patients and their parents about the study and obtaining their written informed consent, an experienced clinician assessed the oral hygiene of patients by measuring their plaque index. The inclusion criteria were (I) requiring resin maxillary orthodontic appliances, and (II) plaque index < 30%. Immunocompromised patients and patients with systemic diseases, or those with improper use of the appliance, poor oral hygiene, and inappropriate cleaning of the appliance, were excluded.

The patients were told that they must use their maxillary appliance for a minimum of 10 hours during a 24-hour period and must adhere to the hygienic measures as instructed. The patients received instructions regarding oral hygiene and cleaning of their orthodontic appliance, both verbally and written in the form of a brochure.

The patients in all of the groups were requested to brush their teeth and their appliances with a medium toothbrush of any commercial brand using the Bass technique, 2 or 3 times a day, with any toothpaste containing 1400 ppm fluoride. Also, they were instructed to correctly use dental floss once a day. It is noteworthy that parents were responsible for performing or supervising the brushing of teeth and cleaning of the appliance, in case a child was not able to follow the instructions.

To instruct the patients and their parents on the correct technique of cleaning of the appliance, the clinician first demonstrated by cleaning the maxillary appliance with a medium toothbrush. To assess the cooperation level of patients, the parents were provided with a questionnaire to record the duration of usage of the appliance over 24 hours. They were requested to fill out the questionnaire and bring it back at the following session. The questionnaire was used for patients' screening; therefore, patients with inadequate appliance wear time were excluded from the study.

In the brushing group, the patients were instructed to brush their orthodontic appliance with a toothbrush.

Patients in the intervention group 1 were provided with 1 pack of Dentipur tablets and a screw-top container to place the appliance and the tablet in. The composition of these tablets includes VP|VA copolymer, sodium lauryl sulfate, sodium lauryl sulfoacetate, aroma, CI 73015, potassium caroate, sodium carbonate, citric acid, and glucose. The patients were instructed to brush their teeth with a toothbrush and toothpaste every night, immerse their orthodontic appliance in a slurry prepared by dissolving a denture cleansing tablet in water for 3 minutes, thoroughly brush the appliance with a clean toothbrush and rinse it under running water.

In the intervention group 2, the patients were provided with a bottle of propolis mouthwash (propolis mouthwash, Soren Tech Toos®, Mashhad, Iran) containing 30% ethanolic extract of propolis. The patients were requested to brush their appliance with a toothbrush and immerse their appliance in the mouthwash for 3 minutes every night.

In the present study, the patients practiced all 3 methods in a consecutive, random fashion. At the 1-month recall session, patient cooperation (using the appliance for a minimum of 10 hours in every 24 hours, continuous use of the appliance, and correct hygiene measures) was evaluated. Next, a plaque disclosing agent (Lactona®, Bergen op Zoom, Netherlands) was

applied on the internal surface of the appliance (the surface in contact with the tissue) by a cotton swab before the commencement of the study and after each follow-up. The appliances were then rinsed, dried, and photographed in a vertical position using a camera (Canon® 40-D, Tokyo, Japan) with a macro100 lens at every appointment, including the baseline. All photographs were captured at a 50-cm distance perpendicular to the appliance at 6.5 fps speed with a 20-mm diaphragm using a 20-megapixel CCD. Afterward, the maxillary appliance was cleaned with a toothbrush, disinfected, and delivered to the patient. The patients were then assigned to another intervention group for the next month. This process was repeated at the end of the second month and the third month as well, such that all patients had practiced all 3 interventions at the end of the third month.

The photographs were analyzed by ImageJ software (ImageJ, LOCI, University of Wisconsin, USA). ImageJ software is open-source JAVA-based software for image processing, manufactured by the National Institute of Health (Figure 1). It is of note that the evaluator was blinded for the calculation of the surface area occupied by the residual plaque on the appliance (in mm²).

The surface area of the new microbial plaques on the appliance, which was pink, was calculated by the software. The ratio of the surface area of the microbial plaque to the entire surface area of the appliance was also calculated and analyzed.

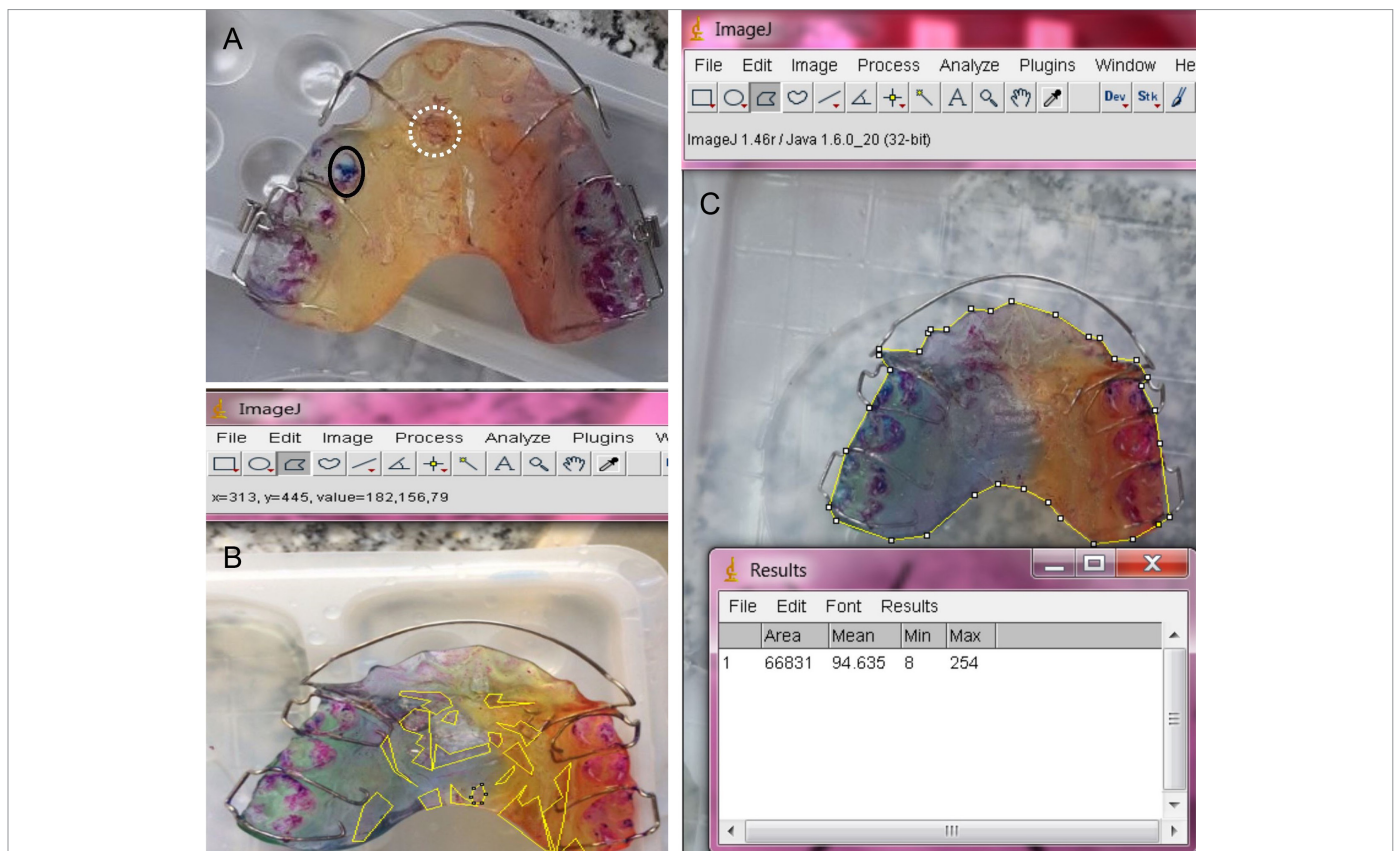


Figure 1. Plaque's Image analysis by the Image J software: (a) Dotted white line: new plaque, continues black line: old plaque

Table 1. The mean ratio of biofilm surface area to the entire surface area of the orthodontic appliance in the 3 groups (n = 32)

Group	Mean	Standard Deviation	Minimum	Maximum
Brushing	0.3209	0.20	0.03	0.69
Brushing +denture cleansing tablets	0.0678	0.05	0.01	0.20
Brushing + propolis mouthwash	0.2441	0.15	0.18	0.55

Table 2. Pairwise comparisons of the groups regarding the amount of biofilm on the surface of orthodontic appliances

Cleaning Method 1	Cleaning Method 2	Mean Difference	P*
Brushing	Brushing + denture cleansing tablets	.2531	<.001
	Brushing + propolis	.0767	.105
Brushing + denture cleansing tablets	Brushing + propolis	-.1763	<.001

*Tukey's test.

Table 3. The effect of age or duration of usage of orthodontic appliance during the day or at night, on the amount of biofilm

Variable	F	P*
Gender	0.04	0.833
Age	0.31	0.578
Duration of usage during the day	1.72	0.198
Duration of usage at night	1.11	0.298

*mixed-model analysis.

All statistical analyses were carried out using SPSS Version 21 (SPSS Inc., IL, USA). The data of different groups and all of the follow-ups were merged as one.

Normal distribution of data was evaluated using the Kolmogorov-Smirnov test. Considering the normal distribution of data, one-way ANOVA followed by the Tukey's test was applied to compare the groups regarding the ratio of biofilm surface area to the entire surface area. The effect of age, gender, and duration of usage of the orthodontic appliance on the results was also analyzed using the mixed-model analysis. To assess the intra-observer reliability, 13 photographs (20%) were analyzed again after 2 weeks by the same experienced observer. Based on these measurements, the intra-observer correlation coefficient was calculated to be 0.81.

RESULTS

At the beginning of this study, 51 patients were assessed for eligibility, and 37 of them were included in the study. Among these 37 participants, 3 were excluded due to poor cooperation in using the appliance and 2 were excluded due to their absence at the recall session (Figure 2). The number of

evaluated photographs was 37, 32, and 34 in the 3 groups of denture cleansing tablets+brushing, propolis mouthwash+brushing, and brushing, respectively. It is of note that only 32 patients completed the study and were allocated to each of these 3 groups. (Table1)

As shown in Table 2, 16 males and 16 females with a mean age of 11.22 ± 1.91 years remained in the study. The initial plaque index of all patients was 27%. Also, the duration of appliance usage by the patients was 6.61 ± 2.24 and 7.02 ± 1.84 hours, during the day and at night, respectively.

The mean ratio of the biofilm surface area to the entire surface area of the orthodontic appliance was 0.3209 ± 0.20 , 0.0678 ± 0.05 , and 0.2441 ± 0.15 in the brushing, brushing + denture cleansing tablets, and brushing + propolis groups, respectively.

One-way ANOVA showed a statistically significant difference in the biofilm surface area between the 3 groups ($P < .001$). Thus, pairwise comparisons were carried out using the Tukey's test. As shown in Table 2, the amount of biofilm on orthodontic appliances was significantly lower in the group that followed brushing + denture cleansing tablets compared with the other 2 groups ($P < .001$).

Although the amount of biofilm on orthodontic appliances was lower in the brushing + propolis mouthwash group compared with the brushing group, this difference did not reach statistical significance ($P = .105$).

As shown in Table 3, according to the mixed-model analysis, the age, gender or duration of usage of the orthodontic appliance during the day or at night had no significant effect on the results.

DISCUSSION

ROAs often interfere with the natural cleaning of the oral cavity. The clasps, retainers, and other components of the appliance cause food impaction and microbial plaque accumulation and lead to dental caries and periodontal disease. A study demonstrated greater adhesion of *Streptococcus mutans* to surfaces in children with ROAs, compared with those without an orthodontic appliance.¹⁰

In the present study, a plaque disclosing agent, which was an active solution lacking any erythrosine, was used to assess the efficacy of cleaning methods. This solution stains the old plaque dark blue and the new plaque pink. The use of a plaque disclosing agent is the most common method applied for research purposes, because its accuracy has been previously confirmed.⁷ ImageJ software was used in this study for the accurate calculation of the plaque surface area stained by the disclosing agent.⁸

In this study, the old plaque was only detected in concave areas of the appliance. According to Madl  na,¹¹ 2-3% of all deposits remained on the orthodontic appliances after cleaning with cleansing tablets. Normally, these tablets have optimal efficacy when used from the first day. It appears that the old plaque

remains in depressions due if the cleanser tablets are not used from the first day of using the appliance.

One major concern in the use of cleansing tablets is the corrosion of soldered areas. Nonetheless, it seems that the susceptibility to corrosion mainly depends on problems during soldering. In this study, none of the orthodontic appliances had soldered areas, and no change occurred in the appearance of the appliances.³

The current results are in line with those of Diedrich et al.¹⁰ They compared the efficacy of 3 cleaning methods for ROAs, namely brushing with toothpaste, the use of denture cleaning tablets, and the use of an ultrasonic device. They reported that a toothbrush and toothpaste adequately cleaned the accessible surfaces. According to their study, denture cleaning tablets and the ultrasonic device had higher efficacy for cleaning the hard-to-reach areas. Nonetheless, none of the 3 methods could completely eliminate the microbial plaque.⁴ It appears that the clasps, expansion screws, marginal borders, and surface irregularities are inaccessible with the toothbrush. Moreover, rough acrylic surfaces would enhance plaque accumulation such that the presence of porosities deeper than 0.2 μm would cause microbial adhesion.¹² The microporosities of the material can serve as a microbial source, and microorganisms mainly spread in the acrylic base. Moreover, a combination of toothbrush and toothpaste would cause further wear of the appliance surface compared with water or self-acting tablets.¹³ Diedrich et al.¹⁰ reported that brushing alone was not acceptable, and denture cleaning tablets should be used along with brushing. They showed that the tablets released oxygen and cleaned the appliance in the sensitive and hard-to-reach areas by enzymatic proteolysis. Organic residues are oxidized in an alkaline solution and thus the surface of the appliance is disinfected. According to Moore et al.,¹⁴ Miller's and Kleenite were more effective cleaning solutions. Brushing and immersion in a cleaning solution containing potassium dichloroisocyanurate, trisodium phosphate, and sodium lauryl sulfate (with the commercial name of Mersene) was less effective. Dentipur tablets, similar to Mersene, contain sodium lauryl sulfate; however, it is present in the form of copolymer. Sodium lauryl sulfate is a detergent utilized for solubilizing proteins in microbiological laboratories.¹⁴

The current results were in contrast to those of Tarbet et al.,¹⁵ regarding dentures. They compared the cleaning efficacy of precise brushing with toothpaste and immersion in solutions of denture cleansing, and concluded that precise brushing with toothpaste was more effective for plaque removal from the denture surface. In this study, the patients cleaned the entire surface of the appliance with a toothbrush after immersion in the slurry of denture cleansing tablets; however, in the study by Tarbet et al.,¹⁵ patients only used the tablets. The absence of mechanical load for cleaning of the appliance may explain the inefficacy of tablets in their study. Moreover, the differences in the structure and composition of the acrylic denture and ROAs as well as the differences in the composition of tablets, may explain the controversy in the results. Dodwad et al.¹⁶ evaluated the efficacy of propolis as an oral irrigating solution for the prevention of plaque formation and the promotion of gingival health. As they

have mentioned in their study, the exact mechanism of the antimicrobial action of propolis is not known exactly; though flavonoids and cinnamic acids seem to be the main compounds responsible. They evaluated 30 patients who were randomly assigned to 3 groups of 10, namely the propolis mouthwash, negative control, and saline groups. The positive control group used 0.2% chlorhexidine. The results revealed that chlorhexidine was more effective than propolis and saline for the prevention of plaque formation. Propolis was found to be slightly superior to chlorhexidine in promoting the gingival score. Their results were similar to our findings regarding the lower efficacy of propolis than other antibacterial agents for the prevention of plaque formation. It seems that the lower efficacy of propolis mouthwash may be due to the lack of sodium lauryl sulfate as a component, and the dependency of its plaque-inhibiting action on natural components.¹⁷

To the best of the authors' knowledge, the cleaning effect of propolis and its derivatives on ROAs has not been evaluated before. Thus, a precise comparison of the current results with other studies regarding this topic is not possible.

The mixed-model analysis showed that age, gender, and duration of usage of the appliance had no significant effect on the cleaning efficacy of the 3 interventions. This finding maybe explained by the fact that parents were responsible for the cleaning of the orthodontic appliance of their children.

Not being able to precisely monitor patient cooperation was a limitation of this study. To minimize this problem, a questionnaire was designed to assess the cooperation of patients in using the appliance. Another limitation of this study was the inter-individual differences in the composition of biofilm, personal hygiene, and the dimensions and morphology of the orthodontic appliances. To overcome this problem, the study had a crossover design and all patients alternatively practiced all the cleaning methods.

CONCLUSION

According to the results of this study, the simultaneous use of brushing and denture cleaning tablets compared with brushing alone decreased the biofilm on the surface of ROAs. Thus, it appears that the use of denture cleaning tablets may be suitable for effective cleaning of orthodontic appliances.

Ethics Committee Approval: This study was approved by Ethics committee of Hamadan University, (Approval No: IR.UMSHA.REC.1397.513) and registered in the Iranian Registry of Clinical Trials (IRCT20190106042253N2).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

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REFERENCES

- Levrini L, Novara F, Margherini S, Tenconi C, Raspanti M. Scanning electron microscopy analysis of the growth of dental plaque on the surfaces of removable orthodontic aligners after the use of different cleaning methods. *Clin Cosmet Investig Dent*. 2015;7:125-131. [\[CrossRef\]](#)
- Littlewood SJ, Tait AG, Mandall NA, Lewis DH. The role of removable appliances in contemporary orthodontics. *Br Dent J*. 2001;191(6):304-309. [\[CrossRef\]](#)
- Eichenauer J, Serbesi C, Ruf S. Cleaning removable orthodontic appliances: A survey. *J Orofac Orthop*. 2011;72(5):389-395. [\[CrossRef\]](#)
- Ahuja V, Ahuja A. Apitherapy-a sweet approach to dental diseases. Part II: Propolis. *JoAOR*. 2011;2(2):1-8. [\[CrossRef\]](#)
- Rahman MM, Richardson A, Sofian-Azirun M. Antibacterial activity of propolis and honey against staphylococcus aureus and Escherichia coli. *Afr J Microbiol Res*. 2010;4(18):1872-1878.
- Batoni G, Pardini M, Giannotti A, et al. Effect of removable orthodontic appliances on oral colonisation by mutans streptococci in children. *Eur J Oral Sci*. 2001;109(6):388-392. [\[CrossRef\]](#)
- da Silva CH, Paranhos Hde F, Silva CHLd, Paranhos HdFO. Efficacy of biofilm disclosing agent and of three brushes in the control of complete denture cleansing. *J Appl Oral Sci*. 2006;14(6):454-459. [\[CrossRef\]](#)
- Paranhos Hde F, da Silva CH. Comparative study of methods for the quantification of biofilm on complete dentures. *Braz Oral Res*. 2004;18(3):215-223. [\[CrossRef\]](#)
- Giraudeau B, Ravaud P, Donner A. Sample size calculation for cluster randomized cross-over trials. *Stat Med*. 2008;27(27):5578-5585. [\[CrossRef\]](#)
- Diedrich P. Microbial colonization and various cleaning procedures for orthodontic appliances. *Fortschr Kieferorthop*. 1989;50(3):231-239. [\[CrossRef\]](#)
- Madléna M. The importance and possibilities of proper oral hygiene in orthodontic patients. In: *Orthodontic: Basic Aspects and Clinical Consideration*. Rijeka: INTECH; 2012:69-110.
- Lima EM, Moura JS, Del Bel Cury AA, Garcia RC, Cury JA. Effect of enzymatic and naocl treatments on acrylic roughness and on biofilm accumulation. *J Oral Rehabil*. 2006;33(5):356-362. [\[CrossRef\]](#)
- Gonser F, Kiesow A, Sarembe S, Petzold M. Bewertung durch zahnburstenreinigung hervorgerufener oberflächenveränderungen bei prothesenkunststoffen. *Dtsch Zahnärztl Hypn*. 2008;63(10):689.
- Moore TC, Smith DE, Kenny GE. Sanitization of dentures by several denture hygiene methods. *J Prosthet Dent*. 1984;52(2):158-163. [\[CrossRef\]](#)
- Tarbet WJ, Axelrod S, Minkoff S, Fratarcangelo PA. Denture cleansing: a comparison of two methods. *J Prosthet Dent*. 1984;51(3):322-325. [\[CrossRef\]](#)
- Dodwad V, Kukreja BJ. Propolis mouthwash: a new beginning. *J Indian Soc Periodontol*. 2011;15(2):121-125. [\[CrossRef\]](#)
- Murray MC, Worthington HV, Blinkhorn AS. A study to investigate the effect of a propolis-containing mouthrinse on the inhibition of de novo plaque formation. *J Clin Periodontol*. 1997;24(11):796-798. [\[CrossRef\]](#)