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Research Article

EVALUATION OF THE EFFECTIVENESS OF DIFFERENT CLEANING METHODS FOR REMOVABLE SPACE MAINTAINERS

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ABSTRACT

Aim: The present study aimed to evaluate the antimicrobial effectiveness of different cleaning methods in reducing microbial colonization on space maintainer appliances.

Materials and Methods: Acrylic blocks (5×5×3 cm) were fabricated for the study. The surfaces of these blocks were sterilized using 70% ethanol and subsequently air-dried. A standardized microorganism suspensions (*Streptococcus mutans* ATCC 10449 and *Candida albicans* ATCC 60193) were prepared at a density of OD600 = 0.01, and 400 µL of the suspension was applied to the surfaces of the acrylic blocks. The blocks were then covered with sterile plastic films (4×4 cm) and incubated at 37°C for 24 hours. After incubation, microbial load was quantified, and five different cleaning protocols were applied to the block surfaces. The groups were as follows; Group1: Control (No treatment), Group2: Toothbrush+liquid soap, Group3: Toothbrush+toothpaste, Group4: Toothbrush+liquid soap+cleanser tablet, Group5: Toothbrush+toothpaste+cleanser tablet, Group6: Cleanser tablet only. All blocks were rinsed in sterile phosphate buffered saline, and the residual microbial load was determined by inoculation on to Brain Heart Infusion Agar (for *S. mutans*) and Sabouraud Dextrose Agar (for *C. albicans*). Data analysis was performed using SPSS 25.0 software program by the chi-square and t-tests.

Results: All cleaning methods significantly reduced the colonization of both *S. mutans* and *C. albicans* (p=0.01). Among the tested methods, Group 5 demonstrated the highest antimicrobial efficacy, followed by Groups 4>6>3>2, respectively.

Conclusion: This study highlights the effectiveness of various cleaning methods in reducing microbial colonization on removable space maintainers. The combination of mechanical cleaning with a toothbrush and the use of cleanser tablets showed superior efficacy.

Keywords: Space maintainer, Streptococcus mutans, Candida albicans

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INTRODUCTION

Early loss of primary teeth can lead to space loss, the mesialization of adjacent teeth, resulting in a decrease in arch length, and malocclusions. Space maintainers have long been used for the management of space loss in primary and mixed dentition, maintain arch length and prevent malocclusion (1–3). Space maintainers can be categorized as a removable and a fixed one; unilateral or bilateral, and functional or nonfunctional (4). The selection of an appropriate space maintainer depends on various factors, including the stage of dental development, the dental arch, the location and size of the missing tooth, the age and the cooperation of the patient, and the condition of the adjacent teeth (1,3–5).

Space maintainers can contribute to plaque accumulation and pose challenges in maintaining proper oral hygiene, which may lead to the development of gingivitis (2,4). Additionally, they come into direct contact with the oral microflora, facilitating the formation of microbial biofilms on their surfaces (2,6). To prevent microbial retention, patients using space maintainers should be more careful to maintain optimal oral hygiene (2,7).

In cases where oral hygiene is insufficiently maintained, orthodontic appliances (e.g., space maintainers) can lead to plaque accumulation (8–10). The use of space maintainers has been associated with an increase in bacterial concentrations within the oral environment, as well as a reduction in buffering capacity, pH levels, and salivary flow rate (8,11). *Streptococcus mutans*, which is strongly associated with early enamel demineralization, exhibits elevated salivary levels in conditions promoting its colonization. While some studies report an increase in *S. mutans* during orthodontic treatment, others do not observe such a trend (8,10,11). Candida species, commonly found in the oral cavity, are known to colonize surfaces such as cement, enamel, and dentin, acting as reservoirs for microbial spread. Despite extensive research on the effects of orthodontic appliances on the oral microbiota, there is limited focus on the impact of removable appliances and fixed space maintainers (8,12). Studies have shown that patients using removable appliances exhibit elevated salivary levels of *Candida albicans*, which increases the likelihood of developing candidiasis and stomatitis (13).

Removable orthodontic appliances that are not effectively cleaned pose a risk of cross-infection for clinicians, technicians, and patients (14). Various chemical and mechanical cleaning methods are employed to eliminate microorganisms on the surface of removable orthodontic appliances (15). While products such as sodium hypochlorite, peroxide and enzymes are used in chemical cleaning methods; toothbrushes and ultrasonic cleaning products are used in mechanical cleaning. Studies also showed that the combination of chemical and mechanical cleaning has a positive effect on cleaning these appliances (15,16).

Despite the widespread use of space maintainers, there is no consensus on the most effective cleaning protocols, particularly for removable appliances. This lack of standardization increases the risk of microbial accumulation and subsequent oral infections, underscoring the need for evidence-based cleaning methods. The aim of the present study is to address this gap by evaluating the antimicrobial efficacy of various cleaning methods for space maintainers, with a specific focus on clinically significant microorganisms such as *Streptococcus mutans* and *Candida albicans*.

MATERIALS AND METHODS

Study Design and Sample Preparation

The present study aimed to evaluate the effectiveness of various cleaning methods and their combinations in reducing microbial retention on acrylic materials used in space maintainers. A total of 30 cold acrylic blocks, each measuring 5x5x3 cm, were prepared by a single operator to ensure consistency in methodology. These blocks were sterilized prior to inoculation with microbial cultures and subjected to various cleaning treatments. The cleaning methods included toothbrush (Colgate Extra Clean soft, ABD), toothpaste (Colgate Anti-caries, ABD), liquid soap (Palmolive, Colgate-Palmolive, ABD), and cleanser tablet (Corega Orthodontics, GlaxoSmithKline, ABD), which were tested in the experimental groups. A detailed description of these groups is presented in Table 1.

Microbiological Procedures

Following the preparation of the cold acrylic materials (n=30) for use in the study, the microbiological procedure was initiated. The microorganisms and culture media utilized are outlined below:

Microorganisms: *Streptococcus mutans* ATCC 10449 and *Candida albicans* ATCC 60193

Culture Media: Brain Heart Infusion Agar (BHIA) (Difco) and Sabouraud Dextrose Agar (SDA) (Difco)

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These media were selected for their proven efficacy in promoting the growth of the target microorganisms, ensuring reliable and reproducible results. The experiments were conducted with two replicates, following a modified JIS Z 2801 standard.

Table 1. Different cleaning methods tested in the study

Groups	Cleaning Methods
Group 1	Control Group-No Treatment
Group 2	Toothbrush-Colgate Extra Clean soft (Colgate-Palmolive, ABD Liquid soap (Palmolive, Colgate-Palmolive, ABD)
Group 3	Toothbrush-Colgate Extra Clean soft (Colgate-Palmolive, ABD) Toothpaste-Colgate Anti-caries (Colgate-Palmolive, ABD)
Group 4	Toothbrush-Colgate Extra Clean soft (Colgate-Palmolive, ABD) Liquid soap (Palmolive, Colgate-Palmolive,ABD) Cleanser tablet-Corega Ortodontics (GlaxoSmithKline, ABD)
Group 5	Toothbrush-Colgate Extra Clean soft (Colgate-Palmolive, ABD) Toothpaste-Colgate Anti-caries (Colgate-Palmolive,ABD) Cleanser tablet-Corega Orthodontics (GlaxoSmithKline, ABD)
Group 6	Cleanser tablet-Corega Orthodontics (GlaxoSmithKline, ABD)

The acrylic block surfaces were sterilized using 70% ethanol (v/v) and subsequently dried. After sterilization, the surfaces were inoculated with the microbial suspension (*Streptococcus mutans* ATCC 10449 and *Candida albicans* ATCC 60193), with the concentration adjusted to OD600=0.01. A total of 400 µl of the microbial suspension was applied to each surface, which was then covered with sterile plastic films measuring 4cm×4cm. The samples were incubated in a humid atmosphere at 37ºC for 24 hours.

Experimental Procedures

After the 24-hour incubation period, the surfaces of the samples were subjected to five different treatment groups, which included cleaning agents and/or their combinations as listed in Table 1. The control group received no treatment. The protocols for using the cleaning methods in each group are presented in Table 2. The selected cleaning methods and combinations were chosen to replicate real-life cleaning practices, ensuring the clinical relevance of the study findings.

Table 2. Clinical procedures for the different cleaning methods

Groups	Clinical procedures
Control Group	No treatment
Toothbrush – liquid soap	A pea-sized amount of liquid soap was applied to the brush, and the surface of the samples was cleaned with three brush strokes. After cleaning, the samples were rinsed with water.
Toothbrush – toothpaste	A pea-sized amount of toothpaste was applied to the brush, and the surface of the samples was cleaned with three brush strokes. The samples were then rinsed with water.
Toothbrush – liquid soap – cleanser tablet	A pea-sized amount of liquid soap was applied to the brush, and the surface of the samples was cleaned with three brush strokes. Afterward, the samples were rinsed with water. An effervescent tablet was dissolved completely in one glass of water, and the samples were immersed in the effervescent solution for three minutes. The samples were then rinsed with water.
Toothbrush – toothpaste – cleanser tablet	A pea-sized amount of toothpaste was applied to the brush, and the surface of the samples was cleaned with three brush strokes. The samples were then rinsed with water. An effervescent tablet was dissolved completely in one glass of water, and the samples were immersed in the effervescent solution for three minutes. The samples were then rinsed with water.
Cleanser tablet	An effervescent tablet was dissolved completely in one glass of water, and the samples were immersed in the effervescent solution for three minutes. The samples were then rinsed with water."

After the cleaning procedures, all samples were rinsed with sterile phosphate-buffered saline (PBS) to recover any residual microorganisms from the surfaces. The recovered microorganisms were then cultured on Brain Heart Infusion Agar (BHIA) for *S. mutans* and Sabouraud Dextrose Agar (SDA) for *C. albicans* to assess the final microbial load. The *S. mutans* plates were incubated in a CO_2 incubator at 5% CO_2. The reduction in microbial load was calculated in logarithmic values, comparing the results to the control group. According to the JIS Z 2801 standard, a reduction of at least 2 log units was considered indicative of antimicrobial efficacy.

Statistical Analysis

Data analysis was performed using SPSS 25.0 software program by the chi-square and t-tests. Results were reported with 95% confidence intervals, and statistical significance was set at $p<0.05$.

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RESULTS

The results of the present study demonstrated that all cleaning methods assessed significantly reduced the colonization of *S. mutans* and *C. albicans* (p=0.01). The most effective cleaning method for reducing both *S. mutans* (Table 3) and *C. albicans* (Table 4) colonization was found to be Group 5, which utilized a combination of toothbrush, toothpaste, and cleanser tablet. The effectiveness of the cleaning methods decreased in the following order: Group 4>Group 6> Group 3>Group 2, showing a gradual decline in antimicrobial efficacy across the different cleaning protocols.

Table 3. The efficacy of different cleaning methods on S. mutans colonization

Groups	Baseline (log)	Post-treatment (log)	Reduction (log)
Group 1	5.42	5.42	-
Group 2	5.42	3.39	2.03
Group 3	5.42	3.32	2.10
Group 4	5.42	2.96	2.46
Group 5	5.42	2.79	2.63
Group 6	5.42	3.02	2.40

Table 4. The efficacy of different cleaning methods on C. albicans colonization

Groups	Baseline (log)	Post-treatment (log)	Reduction (log)
Group 1	5.29	5.29	-
Group 2	5.29	3.29	2.00
Group 3	5.29	3.26	2.03
Group 4	5.29	2.85	2.44
Group 5	5.29	2.65	2.64
Group 6	5.29	2.96	2.33

DISCUSSION

The present study evaluated the effectiveness of various cleaning methods in removing microbial adherence, specifically *S. mutans* and *C. albicans*, on cold acrylic materials commonly used in space maintainers. The cleaning agents selected; liquid soap, toothbrush, toothpaste, cleanser tablets, and/or their combinations were applied, and the subsequent microbial loads were quantified. The results revealed that all cleaning methods effectively reduced microbial colonization on the cold acrylic surfaces, with the combination of a toothbrush with toothpaste and cleanser tablets emerging as the most effective approach.

The use of orthodontic appliances notably disrupts oral hygiene and contributes to the development of retentive areas, which in turn promotes the plaque and biofilm accumulation. Orthodontic treatments, through the incorporation of bands, brackets, wires, and acrylic resins, create environments contribute to the retention of food particles and microorganisms, ultimately influencing the oral microbiota (8–12). Research by Kundu et al. (8) highlighted a significant increase in the levels of *Streptococcus mutans* following the placement of both fixed and removable space maintainers. Similarly, Topaloğlu et al. (11) found that orthodontic appliances, by providing retention sites, facilitate the colonization of Candida species, thereby altering the microbial environment of the oral cavity. Pithon et al. (17) further emphasized that the size and surface roughness of acrylic materials could influence microbial growth by acting as reservoirs for food debris. Additionally, study by Budtz-Jorgensen and Bertram suggested that the increased prevalence of Candida colonization following the use of removable appliances may be attributed to the inherent affinity of *Candida* species for plastic polymers (8,18,19).

The oral cavity harbors a diverse range of microbial species, with *S.mutans* and *C. albicans* being two key microorganisms often implicated in oral health-related issues. *S. mutans* is widely recognized as a primary etiological agent of dental caries and is among the most frequent contaminants of orthodontic appliances. *C. albicans*, commonly present in the oral microbiota, has been linked to various oral pathologies, including candidiasis (18). Rammohan et al. (9) further emphasized the importance of oral hygiene in the context of orthodontic appliances, noting that microbial adhesion, particularly of *S. mutans* and *C. albicans*, is significantly higher on these appliances. Based on these findings, *S. mutans* and *C. albicans* were specifically selected for the present study due to their clinical relevance.

Brushing is widely regarded as the most preferred mechanical method for cleaning removable orthodontic appliances (20,21). Eichenauer et al. (20) reported that many orthodontists in Germany favor brushing with toothpaste for the maintenance of removable appliances. Similarly, in a study by Tsolakis et al. (21), it was emphasized that regular brushing is universally recommended by orthodontists for cleaning orthodontic appliances. Furthermore, a significant proportion of orthodontists also suggest supplementary cleaning methods such as denture cleaners, disinfection solutions, and vinegar in conjunction with brushing. In another study by Duyck et al. (15), which compared the effectiveness of toothbrush cleaning and ultrasonic cleaning with or without effervescent tablets, no

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significant impact of effervescent tablets on biofilm formation was observed. Consistent with these findings, the present study demonstrated that the combined use of a toothbrush with toothpaste and cleanser tablet was more effective than the other cleaning methods.

Charavet et al. observed that commercial cleaning tablets had a notable effect on biofilm removal. Their study further indicated that one of the tablets tested exhibited a bacteriostatic, rather than a bactericidal, effect on Candida species. They also pointed out that manual brushing, as reported in the studies they reviewed, was not directly compared with antimicrobial agents but was primarily used as an initial cleaning step. Charavet et al. emphasized that manual brushing plays a critical role as the foundational cleaning method (22) . Similarly, the present study included brushing as the initial cleaning step and evaluated its effectiveness in combination with other cleaning agents, providing a comparative analysis of various cleaning protocols.

In a clinical study by Farhadifard et al. (23), three different cleaning methods were compared; manual brushing as the control group, a combination of brushing and cleanser tablets in the second group, and brushing combined with a propolis-based mouthwash in the third group. The results indicated that the combination of brushing with cleanser tablets was more effective than the other methods. Similarly, Diedrich et al. (24), in their comparative study evaluating toothbrush and toothpaste, cleanser tablets, and ultrasonic devices, concluded that toothbrush and toothpaste were effective for cleaning accessible surfaces, while cleanser tablets and ultrasonic devices were superior in cleaning hard-to-reach areas. These findings are consistent with the results of the present study, where the combination of brushing and cleanser tablets proved to be the most effective method for significantly reducing microbial colonization.

Despite the increasing use of space maintainers, there is a lack of standardized cleaning protocols. The present study provides critical insights into the comparative efficacy of different cleaning methods, contributing to the development of evidence-based guidelines for oral hygiene maintenance in patients with removable appliances.

CONCLUSION

In conclusion, all cleaning methods evaluated in the present study were effective in reducing microbial colonization on acrylic blocks. Mechanical cleaning with a toothbrush was identified as a crucial component of the cleaning process, with the addition of cleanser tablets further enhancing cleaning efficacy. Notably, no standardized cleaning protocol for space maintainers has been established in the literature, and previous studies have also failed to propose a universally accepted approach.

Future research should focus on exploring the long-term effects of various cleaning protocols, as well as evaluating additional microbial species, to optimize oral hygiene strategies during the use of removable space maintainers. Additionally, further studies are needed to investigate patient adherence to these cleaning methods in daily use and to assess their long-term impact on microbial populations and appliance durability.

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Authorship contributions
Surgical and Medical Practices: CS, AÇ, YY and AU; Concept: DÇ, Design: DÇ and AU; Data Collection or Proccessing: CS, DÇ, AÇ and AU; Analysis or Interpretation: CS, DÇ and AU; Literature Search: CS and DÇ; Writing: CS and DÇ. All authors read and approved the final manuscript.

Data availibity statement
The data that support the findings of this study are available from the corresponding author, [CS], upon reasonable request.

Declaration of competing interest
The authors deny any conflicts of interest related to this study.

Ethics
This study does not require Ethics Committee Approval.

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REFERENCES

1. Ramakrishnan M, Dhanalakshmi R, Subramanian EMG. Survival rate of different fixed posterior space maintainers used

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in Paediatric Dentistry – A systematic review. Saudi Dent J. 2019;31:165-172.
2. Gökcek Taraç M. Are Plaque Disclosing Agents Effective for Improving Self-Performed Dental Hygiene in Patients with Space Maintainers? A Randomized Controlled Clinical Trial. Eur J Ther. 2024;30:616-625.
3. Gurcan AT, Koruyucu M, Kuru S, Sepet E, Seymen F. Effects of Fixed and Removable Space Maintainers on Dental Plaque and DMFT/dft Values. Odovtos - Int J Dent Sci. 2021;23:137-147.
4. Gumber P, Sarawgi A, Dutta S, Goenka P. Simple Fixed Functional Space Maintainer. Int J Clin Pediatr Dent. 2014;7:225-228.
5. Kargul B, Çaglar E, Kabalay U. Glass fiber-reinforced composite resin as fixed space maintainers in children: 12-Month clinical follow-up. J Dent Child. 2005;72:109-112.
6. Kilian M, Chapple ILC, Hannig M, et al. The oral microbiome - An update for oral healthcare professionals. Br Dent J. 2016;221:657-666.
7. Oliveria ML, Pazinatto J, Zanatta FB. Are oral hygiene instructions with aid of plaque ‐ disclosing methods effective in improving self-performed dental plaquecontrol? A systematic review of randomized controlled trials. Int J Dent Hyg. 2021;19:239-254.
8. Kundu R, Tripathi AM, Jaiswal JN, Ghoshal U, Palit M, Khanduja S. Effect of fixed space maintainers and removable appliances on oral microflora in children: An in vivo study. J Indian Soc Pedod Prev Dent. 2016;34:3-9.
9. Rammohan S, Juvvadi S, Gandikota C, Challa P, Manne R, Mathur A. Adherence of Streptococcus mutans and Candida albicans to different bracket materials. J Pharm Bioallied Sci. 2012;4:212.
10. Lara-Carrillo E, Montiel-Bastida NM, Sánchez-Pérez L, Alanís-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of Streptococcus mutans and Lactobacillus. Med Oral Patol Oral Cir Bucal. 2010;15.
11. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota-6 month follow-up. J Clin Pediatr Dent. 2011;35:433-436.
12. Pathak AK, Sharma DS. Biofilm associated microorganisms on removable oral orthodontic appliances in children in the mixed dentition. J Clin Pediatr Dent. 2013;37:335-340.
13. Hibino K, Wong RWK, HÄgg U, Samaranayake LP. The effects of orthodontic appliances on Candida in the human mouth. Int J Paediatr Dent. 2009;19:301-308.
14. Aydoğan Akgün F. Cleaning Methods and Materials for Removable Orthodontic Appliances: A Questionnaire Study. Black Sea J Heal Sci. 2021;4:136-140.
15. Duyck J, Vandamme K, Krausch-Hofmann S, et al. Impact of denture cleaning method and overnight storage condition on denture biofilm mass and composition: A cross-over randomized clinical trial. PLoS One. 2016;11:1-16.
16. Cruz PC, de Andrade IM, Peracini A, et al. The effectiveness of chemical denture cleansers and ultrasonic device in bio film removal from complete dentures. J Appl Oral Sci. 2011;19:668-673.
17. Pithon MM, dos Santos RL, Alviano WS, Ruellas AC de O, Araújo MT de S. Quantitative assessment of S. mutans and C. albicans in patients with Haas and Hyrax expanders. Dental Press J Orthod. 2012;17:1-6.
18. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol. 1985;12:360-368.
19. Budtz-Jorgensen E, Bertram. U. Denture Stomatitis. I. The Etiology in Relation to Trauma and Infection.; 1970.
20. Eichenauer J, Serbesis C, Ruf S. Reinigung herausnehmbarer kieferorthopädischer Apparaturen - eine Umfrage. J Orofac Orthop. 2011;72:389-395.
21. Tsolakis A, Kakali L, Prevezanos P, Bitsanis I, Polyzois G. Use of different cleaning methods for removable orthodontic appliances: A questionnaire study. Oral Heal Prev Dent. 2019;17.
22. Charavet C, Graveline L, Gourdain Z, Lupi L. What are the cleaning and disinfection methods for acrylic orthodontic removable appliance? A systematic review. Children. 2021;8:1-13.
23. Farhadifard H, Sohilifar S, Bakhshaei A. Plaque Removal Efficacy of 3 Cleaning Methods for Removable Orthodontic Appliances: A Crossover Randomized Clinical Trial. Turkish J Orthod. 2021;34:170-175.
24. Diedrich P, Aachen. Keimbesiedlung und verschiedene Reinigungsverfahren kieferorthopädischer Geräte. Fortschr Kieferorthop. 1989;50:231-239.S