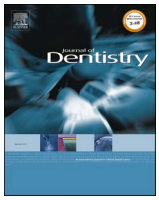
Journal of Dentistry 156 (2025) 105709

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent



A randomised crossover clinical trial of the efficacy of an ultrasonic cleaner combined with a denture cleanser on the microbiome on removable dentures among community-dwelling older adults

Tong Wah Lim [a], Shi Huang [b], Michael Francis Burrow [a], Colman McGrath [b,*]

[a] Division of Restorative Dental Sciences, Faculty of Dentistry, The University of Hong Kong, Sai Ying Pun, Hong Kong SAR
[b] Division of Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR

ARTICLE INFO

Keywords:
Biofilm
Microbiome
Plaque
Removable denture
Ultrasonic

ABSTRACT

Objective: To evaluate and characterise the microbial compositional changes of removable dentures after interventions by comparing the efficacy of the test arm (a portable self-operated ultrasonic cleaner combined with an enzymatic peroxide-based denture cleanser solution) to the control arm (immersion of the denture in the same cleanser solution followed by conventional brushing).

Materials and Methods: A prospective, single-blind, block-randomised, two-period crossover, controlled clinical trial was conducted, involving 56 community-dwelling older adults wearing removable acrylic dentures. They were block-randomized into the test/control or control/test denture cleaning sequence. Type IIB Restriction-site Associated DNA for Microbiome metagenomic sequencing was adopted to characterize the species-resolved microbial composition for denture biofilm.

Results: For the intervention effect, the overall microbial richness in both arms was not significantly different based on the Chao 1 index ($P = 0.343$). However, Beta diversity analysis (Jaccard qualitative distance matrix) demonstrated significant differences in the microbial community structures between the Test and Control arms after interventions, confirmed by the Permanova test ($R^2 = 0.01118$, $P = 0.034$). Among the opportunistic pathogenic bacteria, *Pseudomonas aeruginosa* was detected as one of the top 30 species by relative abundance at the end of the clinical trial, and *Enterobacter kobei* was significantly enriched in the control arm, as determined by LEfSe analysis.

Conclusions: The microbial community of denture biofilm samples after both interventions were significantly 'shifted' and had limited numbers of opportunistic pathogens, suggesting the interventions equally effective in mitigating the overall number of pathogenic bacteria.

Clinical significance: Denture cleaning intervention using ultrasonic cleaner combined with immersion in denture cleanser solution appears to be effective in shifting the denture microbiome with reduced pathogenic bacteria among community-dwelling denture wearers.

1. Introduction

The replacement of missing teeth using removable dentures remains the most common oral rehabilitation treatment for older adults in Hong Kong, either in the form of removable complete dentures, or in cases of substantial tooth loss, extensive partial dentures [1]. However, maintaining or improving denture hygiene has long been a challenge in old age, owing to the physical effort required for denture hygiene care and the systematic approach needed to achieve acceptable levels of plaque control [2–5]. Poor denture hygiene has serious consequences, not only negatively impacting oral health but also posing risks for systemic diseases [5,6]. Denture-related oral and systemic diseases primarily result from denture biofilm.

A Cochrane systematic review recommended using a combination of mechanical and chemical denture cleaning to combat the build-up of denture biofilm [7]. However, the current scientific evidence supporting ultrasonic denture cleaning was limited to four clinical trials [8]. In addition, the effectiveness of personal ultrasonic cleaners when used with or without chemical immersion has yet to be established. Additionally, previous microbiological studies of the denture microbiome

* Corresponding author at: Division of Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR.
E-mail address: mcgrathc@hku.hk (C. McGrath).

https://doi.org/10.1016/j.jdent.2025.105709
Received 27 January 2025; Received in revised form 18 March 2025; Accepted 21 March 2025
Available online 22 March 2025

composition have been limited by culture- and target-based assessment and focussed mainly on presence of the fungi. Due to the high prevalence of respiratory pathogens colonising denture surfaces [9], a recent randomised clinical trial adopting ultrasonic cleaners using liquid soap for denture cleaning was conducted [10]. However, the study was unable to detect a significant reduction in the abundance of *Staphylococcus spp* compared with the control group, highlighting the importance of incorporating chemical intervention. Therefore, an innovative and impactful denture cleaning solution combining ultrasonic and chemical intervention warrants consideration to determine its efficacy in overcoming poor denture hygiene problems among older adults.

To date, microbiome analysis on denture biofilm using culture-independent molecular methods is limited [11]. Although the formation of salivary pellicles on polymethylmethacrylate resins, one of the most common denture materials, differs from that on human enamel [12], the microbial composition of denture biofilm closely resembles that of dental biofilm on natural tooth surfaces, but shows differences in the diversity, type, and proportion of microorganisms [13,14]. Factors that can influence the oral microbiome include the number and type of teeth, the type of denture, dietary changes, denture cleanliness, oral and systemic health factors, denture material, and extent of environmental contamination on the denture [13,15]. For example, microbial diversity was lower in the edentulous subjects compared with dentate subjects [14]. O'Donnell et al. reported that dental plaque and partial denture samples were significantly more diverse and different to complete denture plaque samples [16]. There was a significant difference in the microbial composition as determined by Beta diversity analysis while comparing clean and unclean dentures. In the same study, several pathogenic bacteria were identified at relatively higher abundances in the unclean dentures using LEfSe analysis [17]. In contrast, adopting good denture hygiene practices was found to be insignificant in altering the microbiome, with denture cleansing interventions appearing to have no effect on the *Candida* load [18]. In addition, dysbiosis is characterised by changes in microbial diversity (richness, evenness, and composition) and an increase in pathogenic microorganisms, disrupting the balanced symbiosis within the denture microbiome. Therefore, denture-related diseases, such as denture stomatitis, can be precipitated by dysbiosis in the denture microbiome and disruption of the host-microbial homeostasis by certain pathogenic bacteria [19–22]. As of now, differences in microbial Alpha and Beta diversity analyses between denture stomatitis and healthy mucosa remains uncertain [11,23]. However, Candida albicans, Staphylococcus aureus, and Streptococcus mutans were commonly found on oral mucosa affected by denture stomatitis [24].

It is widely acknowledged today that denture biofilm, which serves as a reservoir for various pathogens, may also be associated with systemic diseases, including respiratory, cardiovascular, and gastrointestinal infections [5,25]. One large retrospective cohort study in the United States identified denture-wearing as a risk predictor for pneumonia incidence among community-dwelling older adults [26]. Additionally, oral mucosal inflammation including denture stomatitis, was found to be associated with cardiovascular diseases in a large cross-sectional study with 17,235 participants [27]. Nakajima et al. also reported a significant relationship between oral candidiasis and bacterial pneumonia [28]. Growing evidence suggested that removable dentures can act as a colonisation surface for opportunistic respiratory pathogens, which may predispose high-risk frail older adults to respiratory diseases [17,29,30]. A recent study revealed that the microbial community diversity and richness of denture microbiome was significantly correlated with pneumonia status. This study also demonstrated that an increase in the overall abundance of opportunistic respiratory pathogens in patients diagnosed with pneumonia [31]. Regrettably, the importance of denture hygiene care is often underestimated by patients [2]. Thus, adopting good denture hygiene practices among denture wearers as a preventative measure against oral and systemic diseases should be promoted.

A prospective crossover clinical trial demonstrated that the combination of ultrasonic cleaner with an enzymatic peroxide-based chemical denture cleanser was significantly more effective than immersion in the same denture cleanser solution followed by conventional denture brushing in reducing percentage plaque area coverage among community-dwelling older adults [32]. However, the impact of this denture hygiene care on the denture microbiome still needs to be better characterised. Therefore, the aim of this clinical trial was to determine the composition of microbial communities on removable dentures before and after the test and control denture cleaning interventions. The research hypothesis was the ultrasonic cleaning combined with a denture cleanser leads to significant microbiome changes compared with the conventional method with better efficacy in removing the pathogenic bacteria associated with removable dentures.

2. Materials and methods

2.1. Trial design and participants

A prospective, open-label, single-blind, block-randomised, two-period crossover, superiority-controlled clinical trial was registered with the Chinese Clinical Trial Registry (ChiCTR2300071365) and published by Lim et al [32]. This clinical trial was reported in accordance with the CONSORT 2010 Statement: extension to randomised crossover trials [33]. The flow diagram of the trial design is presented in Fig. 1 with a pre-intervention (two weeks), intervention period one (three months), washout (two weeks), and intervention period two (three months). The study setting was conducted at the Clinical Research Centre, Faculty of Dentistry, The University of Hong Kong and ethics approval (IRB Reference Number: UW23-037) was obtained. After providing written informed consent, ninety-two prospective removable denture participants were invited for an oral health screening for eligibility from March to June 2023. A total of 56 participants were recruited for this study. The inclusion and exclusion criteria are listed in Table 1.

2.2. Intervention

The denture hygiene care intervention in the Test Arm combined ultrasonic and chemical cleaning. The denture was cleaned daily at night using a portable ultrasonic cleaner (Lohas ultrasonic cleaner GXZ UC01, Lohas Technology (Int.) Ltd., HK) filled with 225 mL of water to which the enzymatic peroxide-based denture cleanser tablet (Polident 3 minTM, GlaxoSmithKline Healthcare, Moon Township, PA) was added and dissolved, followed by ultrasonication at 45 kHz frequency for 15 min [34,35]. The Control Arm denture cleaning intervention used conventional mechanical brushing using a denture brush (GUM® denture toothbrush, Sunstar Singapore Pte. Ltd.) and the chemical cleaning. The denture container was filled with 225 mL tap water and then immersed in the denture cleanser solution for 15 min. Then, participants applied a standardised brushing technique with the denture cleanser solution for 30 s [35].

2.3. Outcome

The outcome measure was the microbiome profile of removable denture biofilm determined at baseline and the endpoint for each period using a high throughput metagenomic sequencing method (2bRAD-M). The recommended denture biofilm sampling method of O'Donnell et al. [30] was followed. Adherent denture biofilm was collected in 50 mL of sterile phosphate-buffered saline after ultrasonication at 45 kHz frequency for 15 mins. Immediately, the denture sonicate was centrifuged at 9880 rpm (14,000 g) for 10 mins. The biofilm pellet was resuspended in 2 mM EDTA, 180 μL of 20 mM Tris-Hcl, and 1.2 % Triton with 20 μL of 20 mg/ml lysozyme, followed by overnight incubation at 37 °C. After adding 20 μL of proteinase K extraction buffer, the mixture was then vortexed and incubated at 56 °C for 2 h and 95 °C for 15 mins. Then, 200

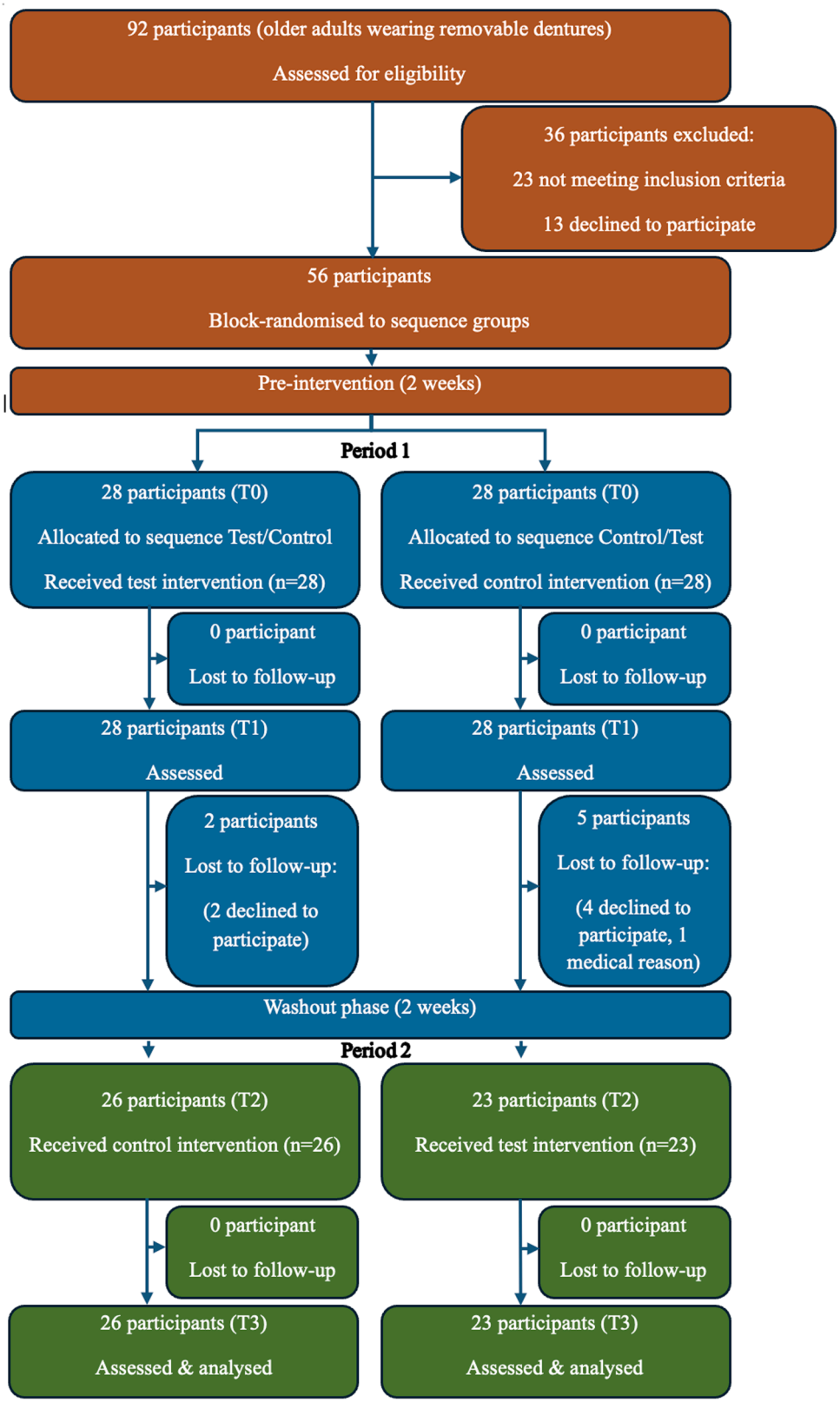


Fig. 1. CONSORT flow diagram for this crossover trial. CONSORT = Consolidated Standards of Reporting Trials.

μL of ethanol was added and underwent 15 s of pulse-vortexing. The DNA was extracted using a standardised protocol with the QIAmp Mini DNA Extraction Kit (Qiagen GmbH, Germany). The DNA concentration was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, California, US) prior to storing at -70 °C. All DNA samples were transferred to the Qingdao OE Biotech Co., Ltd. laboratory under cool conditions, and the sequencing was carried out. The metagenomic sequencing and library construction using the 2bRAD method were performed. A description of the complete protocol has been previously published [17,36]. The extracted DNA underwent a series of reactions,

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Aged 60 years or older	Smokers (including e-cigarettes and tobacco chewing).
Removable complete or extensive partial dentures (≤6 remaining teeth) wearing for at least 6 months.	Diagnosed with acquired immune deficiency syndrome, uncontrolled diabetes mellitus, anaemia, xerostomia, or immunosuppression.
Dentures fabricated with heat-polymerized acrylic resin and biofilm present on denture surfaces (as assessed by the Denture Cleanliness Index)	Use of steroid treatment, antibiotics/ antifungal medication, or undergoing radio- or chemotherapy within the past 4 weeks before recruitment and during follow-up.
Able to provide informed consent (as assessed by the Mini-Mental State Examination with scores ≥18)	Diagnosed with denture stomatitis Type II and III (as assessed by the Newton classification)
Adequate denture retention and stability (as assessed by the Kapur index, retention scores ≥2 and stability scores ≥1)	The denture has been relined, repaired, or retained using denture adhesive.
	Completely or partially ill-fitting/fractured denture.

including digestion with a Type IIB restriction enzyme (BcgI), ligation, enzyme heat inactivation, and Polymerase Chain Reaction amplification. The DNA was then diffused in nuclease-free water, and unique barcodes were incorporated into each sample. The products were purified, then sequenced using the Illumina Novaseq 6000 platform. The raw sequences were processed using the FastQ Quality Control tool and underwent filtration to extract enzyme reads. Clean reads were identified after discarding reads with ≥ 8 % of unknown bases and filtering out low-quality reads. The bioinformatics pipeline (https://github.com/shihuang047/2bRAD-M) then processed the 2bRAD-M sequencing data (32-bp long reads) to create a species-resolved compositional profile for each biofilm sample, identifying microbial species based on a prebuilt 2bRAD species-specific marker database and estimating their abundance based on the sequencing coverage of its species-specific markers.

2.4. Sample size

The sample size was determined based on the effect size difference in the microbial richness (Alpha diversity; Chao 1 index) between the test and control interventions using a software program (G*Power 3.1.9.6). The sample size was calculated based on the mean difference of Chao 1 index between clean and unclean dentures from a previous study carried out by the same research group [17], power of 0.80, and 0.05 significance level. Then, a sample size of 48 participants was required to compare two means (paired T-test). To allow for a potential drop-out rate of 15 % during the crossover randomised controlled clinical trial, a minimum total sample size of 56 was indicated for two intervention arms.

2.5. Randomisation and blinding

Fifty-six participants were block-randomised in groups of four (ABBA) into either the Sequence Test/Control or Sequence Control/Test. A computer random digit generation system generated the randomisation sequence, and the allocation concealment was performed using the 'Sequentially Numbered Opaque Sealed Envelopes' method (SNOSE). An independent technical officer in the Faculty of Dentistry handled both procedures. After baseline assessments, participants were given a SNOSE, and the attending research assistant released the SNOSE code. The same research assistant provided one-on-one training on the test or control arm intervention the participant was assigned for each period and provided them with all required materials and educational leaflets. Participant compliance was measured by completing the daily logbook and counting the remaining denture cleanser tablets. The clinical examiner and data analyst were blinded to the sequence arm allocation.

2.6. Statistical methods

Baseline comparison of sociodemographic and denture-related factors between both sequence groups were performed. The average read coverage of 2bRAD markers for each species, representing the number of microbes belonging to a species in the biofilm sample, was first confirmed. The relative abundance was calculated by dividing this value by the total number of microbes from all detected species in the same sample. A G score of five was also set as a threshold to control false positives. The Alpha diversity index (Chao 1) was calculated based on the taxonomic abundance profiles. These calculations were performed using the R software (version 4.2.1), explicitly leveraging the functionalities provided by the 'vegan' package. The Jaccard distance matrix was obtained for Beta diversity estimation, and the result was visualised using Principal Coordinate Analysis (PCoA). The Alpha and Beta diversity variations between the two groups were determined using the Wilcoxon signed-rank test and permutational multivariate analysis of variance (Permanova test), respectively. P-values of 0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristic comparison

A total of 49 participants completed the whole clinical trial and were considered in the analysis. Seven participants dropped out in period two, resulting in an 87.5 % retention rate. The mean age of participants (29 male and 20 female) was 69.71 ±5.78 years. Whereas the mean denture age was 23.82 ±18.10 months. Twenty-six (53.1 %) wore extensive partial dentures; the remaining 23 (46.9 %) had complete dentures. 53.1 % ($n = 26$) of participants were randomly allocated into the Sequence Test/Control group and 46.9 % ($n = 23$) to the Sequence Control/Test group. Following screening, baseline information for sociodemographic and denture-related factors between sequence groups demonstrated no statistically significant difference (Appendix 1). Additionally, there was no statistically significant difference in the microbial richness according to the Chao 1 index between the sequences at baseline ($P = 0.912$). Beta diversity analysis based on Jaccard distance matrix in both sequence groups also showed no statistically significant difference ($P = 1.000$). There were only three taxa that exhibited significant enrichment upon comparison using LEfSe analysis (Appendix 2).

3.2. Intra-arm comparison (Fig. 2)

There were significant differences in Beta diversity (Test: Jaccard, $R^2 = 0.05235$, $P = 0.001$; Control: Jaccard, $R^2 = 0.05006$, $P = 0.001$) following both the test and control interventions, but no difference in Alpha diversity analysis.

3.3. Inter-arm comparison

For inter-arm comparisons, the intervention, carryover, and period effects associated with the changes in Alpha (microbial richness) and Beta (microbial community) diversity in this two-period crossover clinical trial were assessed.

Upon investigating the intervention effect, the changes in microbial richness and community composition following each intervention were examined. There was no significant difference in the overall microbial richness (Chao 1 index; $P = 0.343$) between Test and Control arms. However, Beta diversity analysis in both arms showed statistically significant difference according to the Jaccard distance matrix, as assessed by the Permanova test ($R^2 = 0.01118$, $P = 0.034$) (Fig. 3). The taxonomic distribution of this clinical trial is shown in Fig. 4. Notably,

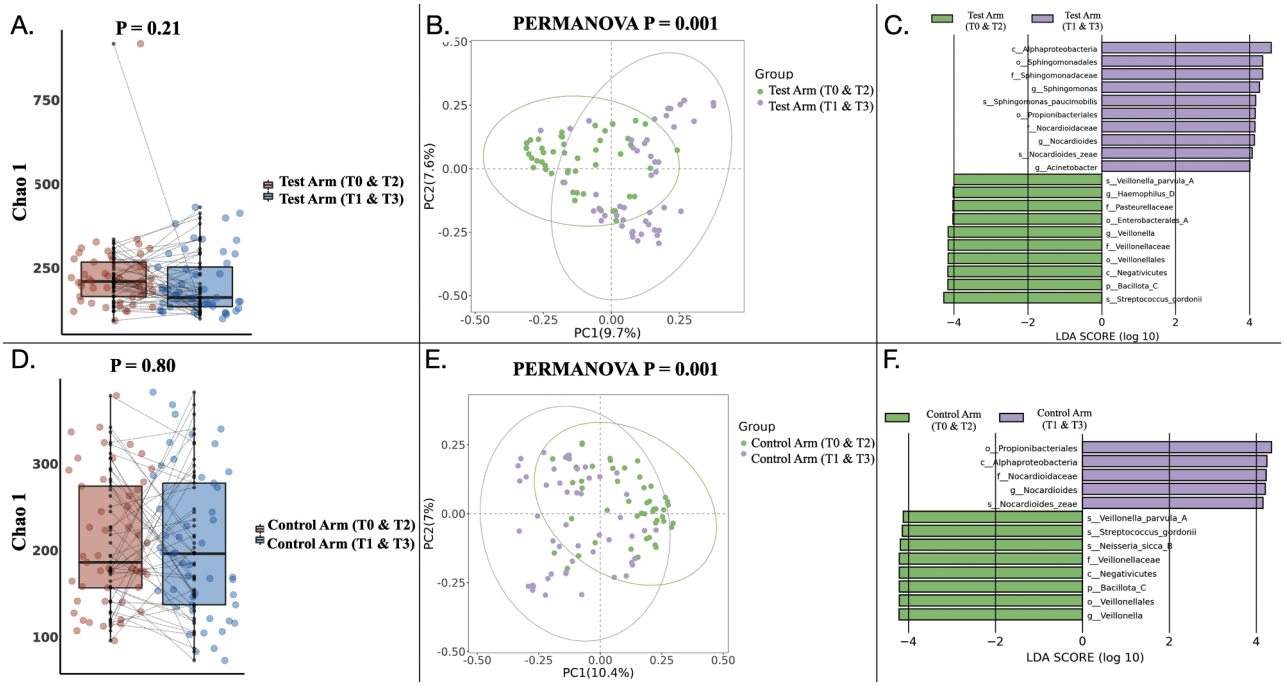


Fig. 2. Comparison of Alpha and Beta diversity, and LEfSe analyses at baseline (T0 and T2) and after 3 months (T1 and T3) in the Test arm (A, B, and C) and Control arm (D, E, and F).

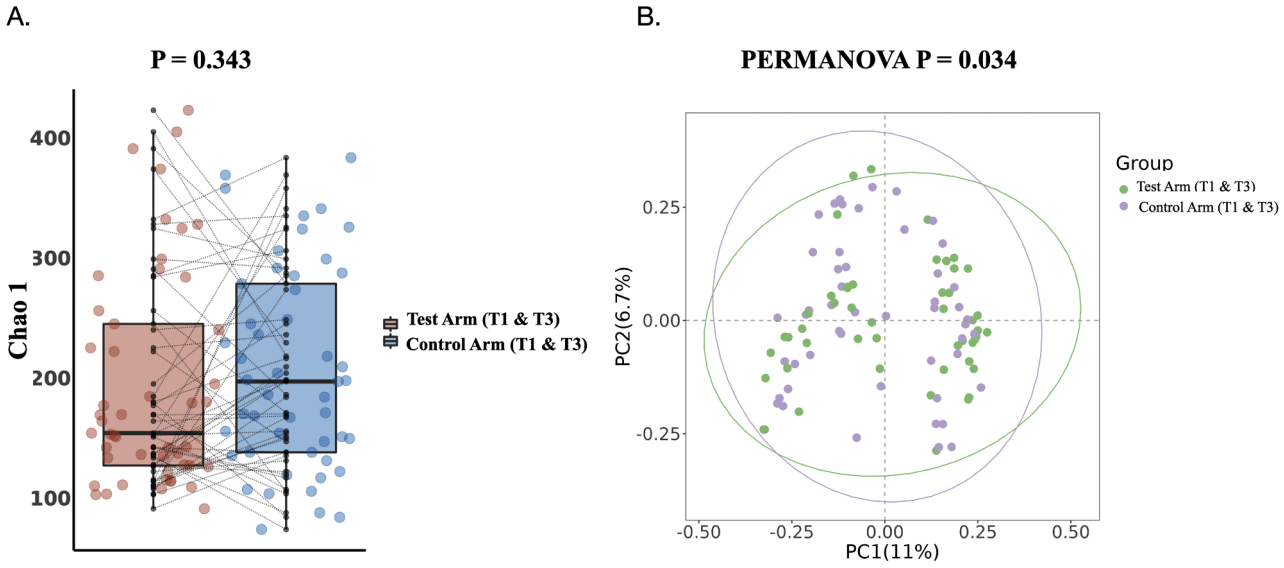


Fig. 3. A. Alpha diversity analysis (Chao 1) indicated no significant difference in microbial richness between the Test and Control arms after interventions. B. Beta diversity analysis, Principal Coordinate analysis based on the Jaccard (PERMANOVA; $R^2 = 0.01118$, $P = 0.034$) distance matrix.

among the top 30 species colonising denture surfaces after intervention, *Pseudomonas aeruginosa* was a recognised respiratory pathogen. Nevertheless, the relative abundance of several pathogenic bacteria including *Mycobacterium tuberculosis* and *Streptococcus sobrinus*, differed significantly between the Test and Control arms. The microbial communities in both arms were also compared using LEfSe analysis [LDA score (log 10) > 3] (Fig. 5). Following both interventions, 19 taxa were found to be significantly more abundant in the Test arm, and 11 taxa were significantly more abundant in the Control arm. Notably, most of them were considered as commensal bacteria.

For the carryover effect investigation, there was no significant difference in Chao 1 index between Sequence Test/Control and Control/Test groups ($P = 0.434$). Similarly, Beta diversity analysis based on the Jaccard distance matrix demonstrated no statistically significant difference between these two groups, Permanova test: $R^2 = 0.01096$, $P = 0.313$ (Fig. 6).

The period effect for the Alpha and Beta diversity in this two-period crossover clinical trial was estimated prior to the interventions. There was no statistically significant difference in Chao 1 index between period one and period two ($P = 0.240$). However, the microbial community (Beta diversity) between periods differed significantly, confirmed by the Permanova test (Jaccard: $R^2 = 0.0442$, $P = 0.001$) (Fig. 6). Due to a significant period effect, inter-arm comparisons during the period one are presented in Fig. 7. There was a significant difference

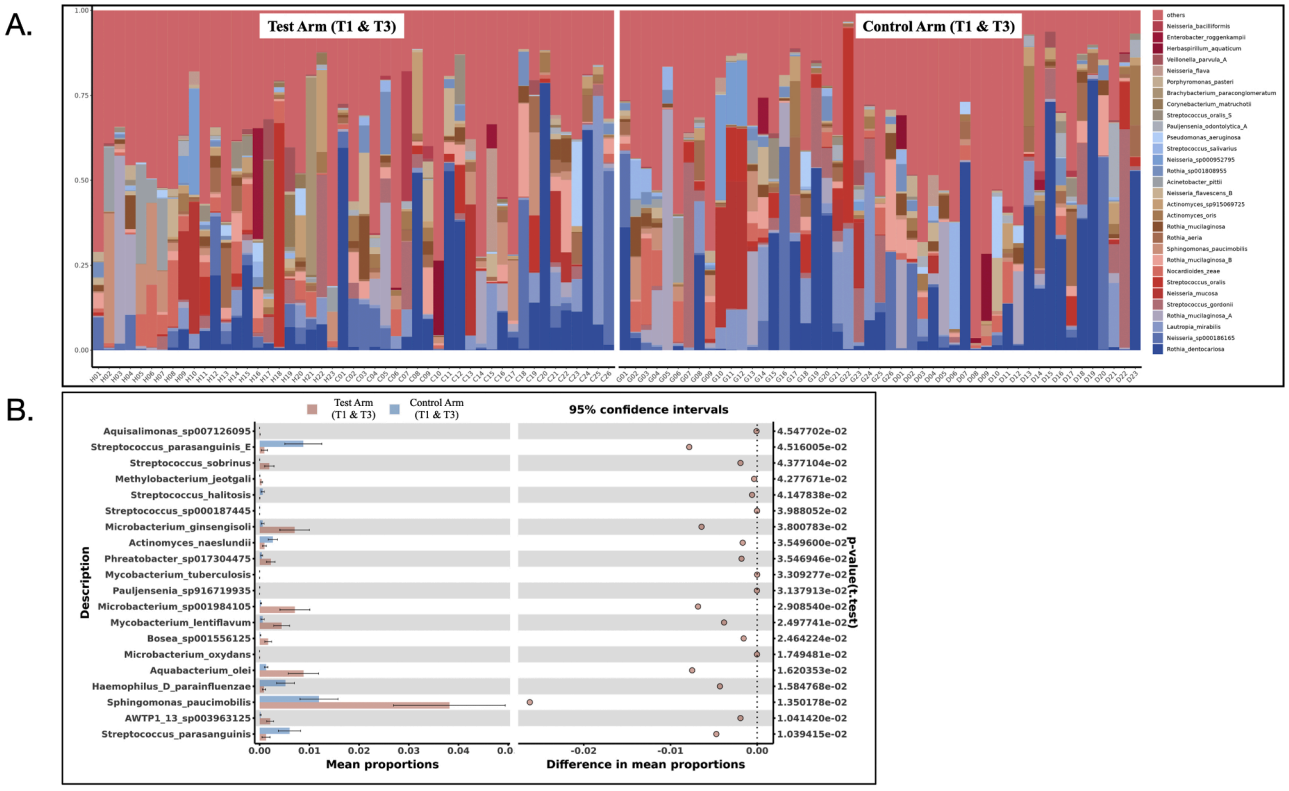


Fig. 4. A. Top 30 most abundant species in the denture biofilm. B. Stamp Plot revealed the top 20 species with significantly different abundance.

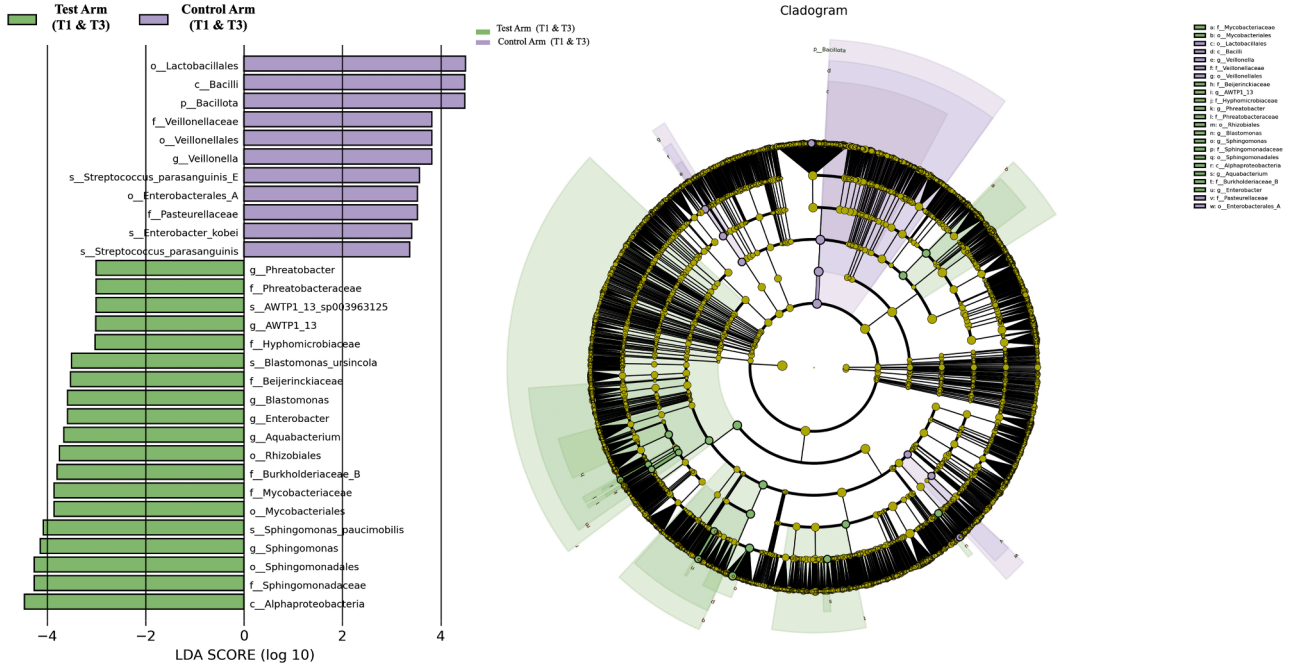


Fig. 5. The LEfSe analysis indicated a higher abundance of 19 and 11 taxa in the Test and Control arms, respectively. The brightness of each point was proportional to the size of its effect.

in Alpha diversity (Chao 1 index) between Test and Control arms ($P = 0.03$), but no difference in Beta diversity analysis based on the Jaccard distance matrix (Permanova test: $P = 0.679$, $R^2 = 0.01678$).

4. Discussion

This randomised crossover clinical trial aimed to characterise and compare the microbiome of removable dentures after a novel denture home-care program of mechanical and chemical cleaning using a portable ultrasonic cleaner with denture cleanser among community-

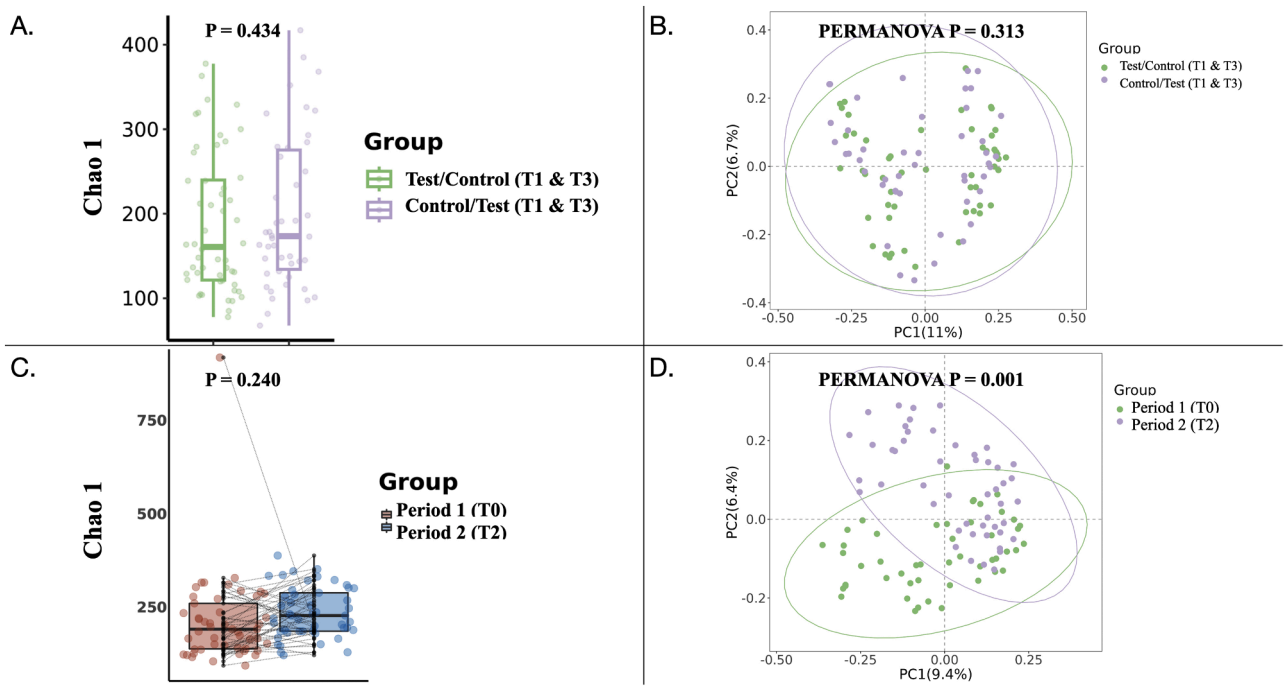


Fig. 6. A & B. For carryover effect, Alpha and Beta diversity analyses showed no statistically significant difference between the two sequences after interventions. C & D. For period effect, Alpha diversity in both periods before intervention showed no significant difference but Beta diversity analysis between periods differed significantly.

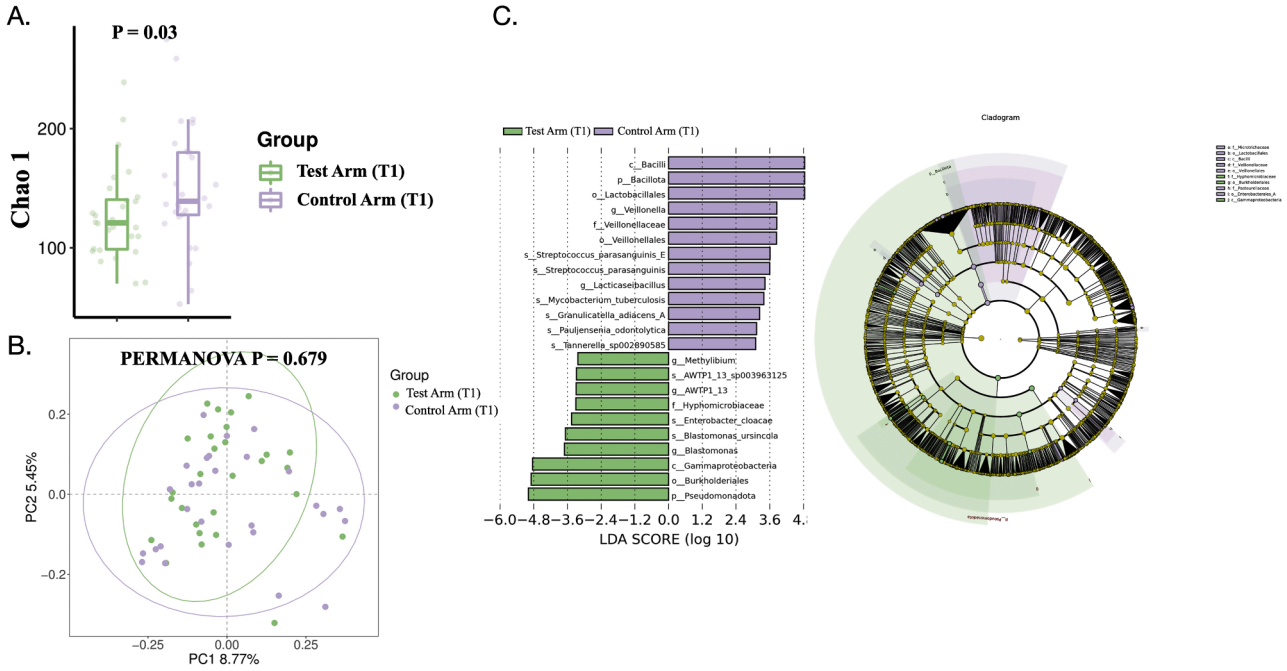


Fig. 7. A. The Test arm showed a significant decrease in Chao 1 index compared to the Control arm after 3 months. B. The beta diversity, based on the Jaccard distance matrix, showed no difference. C. The microbial communities were compared between Test and Control arms after 3 months using LEfSe analysis.

dwelling older adults for a 3-month intervention for each period. The test intervention arm showed a significantly different microbial community in the test arm compared with the control arm, with negligible carryover effect. However, there were no significant changes in the microbial richness between both arms. Thus, the research hypothesis was partially accepted. Notably, the microbial community differed significantly between periods, suggesting a significant period effect. Therefore, the findings should be interpreted in combination with the findings from period one. After both period one and the entire crossover clinical trial, findings revealed a pattern of rising Chao 1 index after the Control arm intervention, thereby supporting the credibility of the outcomes. However, based on the Jaccard distance matrix, the Beta diversity analysis revealed a significant treatment effect at the endpoint of period two but not period one. This discrepancy could be due to the difference in sample size.

The efficacy of both the Test and Control arms in reducing biofilm

coverage and improving patient satisfaction was confirmed in the previous analysis of the same clinical trial [32]. In addition, several reviews and the American College of Prosthodontists strongly recommended combining mechanical and chemical cleaning of removable dentures [7,8,37]. However, due to the unknown effect on denture microbiome, the present study further investigated this research question and reported similar species richness and a distinctly different microbial community for intra-arm and inter-arm comparison. For Alpha diversity analysis, there was no significant difference in Chao 1 index between Test and Control arm interventions. This finding is consistent with several studies of denture microbiome [17,18,20]. The present study revealed a tendency toward an increase in Chao 1 index after the Control arm intervention. Possibly, the formation of denture biofilm colonies was more prevalent in the Control arm compared to the Test arm, contributing to the increased microbial richness [38], although it was not statistically significant. Nevertheless, this finding can be concealed by the resilience, dynamics, and rate of redevelopment of the denture biofilm [18,38]. The Beta-diversity analysis revealed a significant separation difference both before and after interventions within each arm, as well as between arms after the intervention, indicating a clear distinction and unique microbial profiles between the two microbial communities associated with different cleaning interventions. The higher percentage of biofilm area coverage detected on the dentures in the Control arm compared to the Test arm could contribute to the more significant complexities of microbial community structures [17]. The intra-arm microbial community finding observed in this clinical trial is consistent with those of Teles et al. [38]. They also reported that the microbial composition of denture biofilms differed substantially after cleaning. Additionally, the present study demonstrated that the microbial community composition of denture biofilms after the Test and Control interventions differed substantially. Possibly, the bacterial attachment, colonisation pattern, and composition of the microbiota present on the denture surface are affected by the ultrasonic wave exposure in the Test arm [39]. In contrast, Delaney et al. reported that microbial composition and diversity were not influenced by the in-vitro denture cleansing [18]. The result may be explained by the different study design, short study period (7 days), and no ultrasonic cleaning involved in their study.

The relationship of denture biofilm-related oral or systemic diseases and denture hygiene is complex. The microbial community in this ecological niche may be disrupted under suboptimal denture hygiene, causing dysbiosis involving decreased microbial diversity and increased pathogenic microorganisms [19–21]. Furthermore, the increased abundance of certain pathogenic bacteria may also disrupt the host-microbial homeostasis; subsequently, oral or systemic diseases may develop [40,41]. In the present study, very few pathogenic bacteria were present after both test and control interventions. *P. aeruginosa* was detected as one of the top 30 species at the endpoint of the clinical trial, which aligns with a previous study showing a high abundance of this pathogen in the denture group [20]. This gram-negative bacterium is one of the most common pathogens associated with nosocomial infections, most likely antibiotic-resistant, and highly prevalent on denture surfaces [9,42]. Additionally, there was an increased abundance of one type of pathogenic bacteria in each group after the intervention (*M. tuberculosis* and *S. sobrinus*), suggesting that both arms were equally effective in controlling the overall number of pathogenic bacterial species. *S. sobrinus* is one of the common cariogenic bacteria. Whereas *M. tuberculosis* is the aetiological agent responsible for tuberculosis, significantly contributing to high mortality and morbidity rates globally. As reported, it might be latently present in over 2 billion people, a quarter of the world population [43]. Despite its potential threat, the infection only becomes active depending on the complex interplay of bacterial, host, and environmental factors [44]. Furthermore, the LEfSe analysis at the species level showed significant enrichment of *Enterobacter kobei* in the control arm. This type of *Enterobacter cloacae complex* has been frequently reported in nosocomial infections [45]. In general, the presence of pathogenic bacteria in this study may not necessarily indicate their virulence to hosts, as in ecological systems, several oral and systemic pathogens have the ability to exist in a dormant or low abundance state without impacting the ecological balance and causing diseases [46]. Nonetheless, the potential threat of keystone pathogens colonising the denture surface should not be underestimated [47].

The present study can assist in the understanding of the influence of denture cleansing on the ecology of the denture biofilm, highlighting the relevance of ultrasonication in shifting the microbial community structure. This alternative ultrasonic mechanical cleaning has largely been confined to ‘in-clinic settings’ or when shared among patients [34,48]. Therefore, the personal, portable, over-the-counter ultrasonic cleaner used in this study is much more hygienic. It may potentially help to reduce occupational infections among caregivers in hospital or aged care facility settings and prevent cross-contamination among institutionalised residents. Furthermore, the improved denture cleanliness by reducing the pathogenic microbiome is essential to prevent oral and systemic diseases, particularly given that denture biofilm has been implicated in cardiovascular, gastrointestinal, and respiratory diseases [5,49]. It is proposed that denture hygiene care can be incorporated as part of the general supportive care for older adults.

The main limitation of this clinical trial was the observed significant period effect [50]. A reasonable supposition could be a ‘learning effect’, as denture hygiene care interventions are considered cognitive tasks. Participants may have gradually altered their denture hygiene care behaviour and improved their performance over the study period due to practice, and possibly, they also become more familiar with the clinical trial protocol. Therefore, the significant period effect suggests that for future crossover randomised clinical trials, a re-evaluation of the randomisation should be considered, along with the inclusion of a training period prior to the clinical trial and an extension of the washout period [51]. Moreover, the influence of "learning effect" on long-term denture hygiene behaviours can also be investigated in future studies.

5. Conclusion

The microbial community of removable dentures significantly changed after combining ultrasonic and chemical denture cleanser cleaning, highlighting the potential role of ultrasonication in altering the microbial community structure. Very few pathogens were present after the interventions, suggesting that both groups were equally effective in controlling the overall number of pathogenic bacterial species.

Funding

This study was supported by funding from the RGC General Research Fund 2024/25 (Project Number: 17122724).

Availability of data

As of the date of publication, the data from this study are publicly available in the NCBI Short Read Archive (SRA) under BioProject ID number PRJNA1208595.

CRediT authorship contribution statement

Tong Wah Lim: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shi Huang:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis, Conceptualization. **Michael Francis Burrow:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Colman McGrath:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

1. This study was supported by funding from the RGC General Research Fund 2024/25 (Project Number: 17122724).

2. The authors would like to thank research assistants, Centre Research Laboratory, Centre Research Clinic, and patients at the University of Hong Kong and Prince Philip Dental Hospital for participating in this study.

3. The authors would like to thank Qingdao OE Biotechnology Company.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2025.105709.

References

[1] Department of Health, Oral Health Survey 2021, The Government of Hong Kong, Hong Kong Special Administrative Region, 2021.
[2] T.W. Lim, M.F. Burrow, C. McGrath, Evaluating risk factors associated with poor removable prosthesis hygiene in community-dwelling elders: a cross-sectional study, J. Prosthet. Dent. (2023), https://doi.org/10.1016/j.prosdent.2023.09.033. In Press.
[3] T.W. Lim, H. Pan, M. Pan, M.F. Burrow, C. McGrath, Agreement in quantification of removable prosthesis plaque area coverage using a semi-automated planimetric assessment method, J. Dent. 138 (2023) 104721, https://doi.org/10.1016/j.jdent.2023.104721.
[4] J.Y.M. Ng, T.W. Lim, N. Tarib, T.K. Ho, Effect of educational progressive web application on patient's oral and denture knowledge and hygiene: a randomised controlled trial, Health Informatics J 27 (2021), https://doi.org/10.1177/14604582211035821, 14604582211035821.
[5] L. Coulthwaite, J. Verran, Potential pathogenic aspects of denture plaque, Br. J. Biomed. Sci. 64 (2007) 180–189, https://doi.org/10.1080/09674845.2007.11732784.
[6] T.W. Lim, K.Y. Li, M.F. Burrow, C. McGrath, Association between removable prosthesis-wearing and pneumonia: a systematic review and meta-analysis, BMC Oral Health 24 (2024) 1061, https://doi.org/10.1186/s12903-024-04814-5.
[7] R.F. de Souza, H.d.F.O. Paranhos, C.H.L. Da Silva, L. Abu-Naba'a, Z. Fedorowicz, C. A. Gurgan, Interventions for cleaning dentures in adults, Cochrane Database Syst. Rev. 4 (2009) CD007395, https://doi.org/10.1002/14651858.CD007395.pub2.
[8] A. Schmutzler, A. Rauch, I. Nitschke, B. Lethaus, S. Hahnel, Cleaning of removable dental prostheses - a systematic review, J. Evid. Based Dent. Pract. 21 (2021) 101644, https://doi.org/10.1016/j.jebdp.2021.101644.
[9] T.W. Lim, K.Y. Li, M.F. Burrow, C. McGrath, Prevalence of respiratory pathogens colonizing on removable dental prostheses in healthy older adults: a systematic review and meta-analysis, J. Prosthodont. 33 (2024) 417–426, https://doi.org/10.1111/jopr.13802.
[10] R. Chan, J. Zhang, C. McGrath, P. Tsang, O. Lam, A randomized trial of the effectiveness of an ultrasonic denture hygiene intervention program among communitydwelling elders, Eur. Oral. Res. 57 (2023) 83–89, https://doi.org/10.26650/eor.20231025114.
[11] J. Redfern, L. Tosheva, S. Malic, M. Butcher, G. Ramage, J. Verran, The denture microbiome in health and disease: an exploration of a unique community, Lett. Appl. Microbiol. 75 (2022) 195–209, https://doi.org/10.1111/lam.13751.
[12] I. Svendsen, L. Lindh, The composition of enamel salivary films is different from the ones formed on dental materials, Biofouling 25 (2009) 255–261, https://doi.org/10.1080/08927010802712861.
[13] D.R. Monteiro, V.E. de Souza Batista, A.C.M. Caldeirão, R.d.C. Jacinto, J.P. Pessan, Oral prosthetic microbiology: aspects related to the oral microbiome, surface properties, and strategies for controlling biofilms, Biofouling 37 (2021) 353–371, https://doi.org/10.1080/08927014.2021.1912741.
[14] R.K. Gazdeck, S.R. Fruscione, G.R. Adami, Y. Zhou, L.F. Cooper, J.L. Schwartz, Diversity of the oral microbiome between dentate and edentulous individuals, Oral Dis. 25 (2019) 911–918, https://doi.org/10.1111/odi.13039.
[15] F. D'Ambrosio, B. Santella, M.P. Di Palo, F. Giordano, R. Lo Giudice, Characterization of the oral microbiome in wearers of fixed and removable implant or non-implant-supported prostheses in healthy and pathological oral conditions: a narrative review, Microorganisms 11 (2023) 1041, https://doi.org/10.3390/microorganisms11041041.
[16] L.E. O'Donnell, D. Robertson, C.J. Nile, L.J. Cross, M. Riggio, A. Sherriff, D. Bradshaw, M. Lambert, J. Malcolm, M.J. Buijs, The oral microbiome of denture wearers is influenced by levels of natural dentition, PLoS One 10 (2015) e0137717, https://doi.org/10.1371/journal.pone.0137717.
[17] T.W. Lim, S. Huang, Y. Jiang, Y. Zhang, M.F. Burrow, C. McGrath, Characterization of pathogenic microbiome on removable prostheses with different levels of cleanliness using 2bRAD-M metagenomic sequencing, J. Oral Microbiol. 16 (2024) 2317059, https://doi.org/10.1080/20002297.2024.2317059.
[18] C. Delaney, L.E. O'Donnell, R. Kean, L. Sherry, J.L. Brown, G. Calvert, C.J. Nile, L. Cross, D.J. Bradshaw, B.W. Brandt, Interkingdom interactions on the denture surface: implications for oral hygiene, Biofilm 1 (2019) 100002, https://doi.org/10.1016/j.bioflm.2019.100002.
[19] N. Yacob, S.H. Safii, N.A. Ahmad, N. Yunus, F.A. Razak, Denture microbiome shift and changes of salivary inflammatory markers following insertion of 3D printed removable partial PMMA denture: a pilot study, BMC Oral Health 24 (2024) 1216, https://doi.org/10.1186/s12903-024-05012-z.
[20] H.-H. Wong, C.-H. Hung, J. Yip, T.-W. Lim, Metagenomic characterization and comparative analysis of removable denture-wearing and non-denture-wearing individuals in healthy and diseased periodontal conditions, Microorganisms 12 (2024) 1197, https://doi.org/10.3390/microorganisms12061197.
[21] D.J. Morse, A. Smith, M.J. Wilson, L. Marsh, L. White, R. Posso, D.J. Bradshaw, X. Wei, M.A. Lewis, D.W. Williams, Molecular community profiling of the bacterial microbiota associated with denture-related stomatitis, Sci. Rep. 9 (2019) 10228, https://doi.org/10.1038/s41598-019-46494-0.
[22] A. Tada, N. Hanada, Opportunistic respiratory pathogens in the oral cavity of the elderly, FEMS Immunol. Med. Microbiol. 60 (2010) 1–17, https://doi.org/10.1111/j.1574-695X.2010.00709.x.
[23] B. Shi, T. Wu, J. McLean, A. Edlund, Y. Young, X. He, H. Lv, X. Zhou, W. Shi, H. Li, The denture-associated oral microbiome in health and stomatitis, mSphere 1 (2016), https://doi.org/10.1128/mSphere.00215-16 e00215-16.
[24] T. Baena-Monroy, V. Moreno-Maldonado, F. Franco-Martínez, B. Aldape-Barrios, G. Quindós, L.O. Sánchez-Vargas, Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patients wearing dental prosthesis, Med. Oral. Patol. Oral. Cir. Bucal. 10 (2005) E27–E39.
[25] K.H. Neppelenbroek, The importance of daily removal of the denture biofilm for oral and systemic diseases prevention, J. Appl. Oral Sci. 23 (2015) 547–548, https://doi.org/10.1590/1678-77572015ed006.
[26] H. Alzamil, T.T. Wu, E. van Wijngaarden, M. Mendoza, H. Malmstrom, K. Fiscella, D.T. Kopycka-Kedzierawski, R.J. Billings, J. Xiao, Removable denture wearing as a risk predictor for pneumonia incidence and time to event in older adults, JDR Clin. Trans. Res 8 (2021), https://doi.org/10.1177/23800844211049406, 23800844211049406.
[27] S. Fedele, W. Sabbah, N. Donos, S. Porter, F. D'Aiuto, Common oral mucosal diseases, systemic inflammation, and cardiovascular diseases in a large cross-sectional US survey, Am. Heart J. 161 (2011) 344–350, https://doi.org/10.1016/j.ahj.2010.11.009.
[28] M. Nakajima, Y. Umezaki, S. Takeda, M. Yamaguchi, N. Suzuki, M. Yoneda, T. Hirofuji, H. Sekitani, Y. Yamashita, H. Morita, Association between oral candidiasis and bacterial pneumonia: a retrospective study, Oral Dis. 26 (2020) 234–237, https://doi.org/10.1111/odi.13216.
[29] T.W. Lim, S. Huang, Y. Zhang, M.F. Burrow, C. McGrath, A comparison of the prevalence of respiratory pathogens and opportunistic respiratory pathogenic profile of 'clean'and 'unclean'removable dental prostheses, J. Dent. 145 (2024) 104968, https://doi.org/10.1016/j.jdent.2024.104968.
[30] L.E. O'Donnell, K. Smith, C. Williams, C.J. Nile, D.F. Lappin, D. Bradshaw, M. Lambert, D.P. Robertson, J. Bagg, V. Hannah, G. Ramage, Dentures are a reservoir for Respiratory pathogens, J. Prosthodont. 25 (2016) 99–104, https://doi.org/10.1111/jopr.12342.
[31] J.A. Twigg, A. Smith, C. Haury, M.J. Wilson, J. Lees, M. Waters, D.W. Williams, Compositional shifts within the denture-associated bacteriome in pneumonia–an analytical cross-sectional study, J. Med. Microbiol. 72 (2023) 001702, https://doi.org/10.1099/jmm.0.001702.
[32] T.W. Lim, M.F. Burrow, C. McGrath, Efficacy of ultrasonic home-care denture cleaning versus conventional denture cleaning: a randomised crossover clinical trial, J. Dent. 148 (2024) 105215, https://doi.org/10.1016/j.jdent.2024.105215.
[33] K. Dwan, T. Li, D.G. Altman, D. Elbourne, CONSORT 2010 statement: extension to randomised crossover trials, BMJ 366 (2019) l4378, https://doi.org/10.1136/bmj.l4378.
[34] A.P. Sponchiado, M.C.C.L. Giacomel, E.M. de Souza, R.T. Rosa, E.A.R. Rosa, R. N. Rached, Randomized clinical trial of complete denture cleaning methods in residents of care institutions, Braz. J. Oral Sci. 20 (2021), https://doi.org/10.20396/bjos.v20i00.8661060 e211060-e211060.
[35] G. Ramage, L. O'Donnell, L. Sherry, S. Culshaw, J. Bagg, M. Czesnikiewicz-Guzik, C. Brown, D. McKenzie, L. Cross, A. MacInnes, Impact of frequency of denture cleaning on microbial and clinical parameters–a bench to chairside approach, J. Oral Microbiol. 11 (2019) 1538437, https://doi.org/10.1080/20002297.2018.1538437.
[36] Z. Sun, S. Huang, P. Zhu, L. Tzehau, H. Zhao, J. Lv, R. Zhang, L. Zhou, Q. Niu, X. Wang, Species-resolved sequencing of low-biomass or degraded microbiomes using 2bRAD-M, Genome Biol. 23 (2022) 36, https://doi.org/10.1186/s13059-021-02576-9.
[37] D. Felton, L. Cooper, I. Duqum, G. Minsley, A. Guckes, S. Haug, P. Meredith, C. Solie, D. Avery, N. Deal Chandler, Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists, J. Prosthodont. 20 (2011) S1–S12, https://doi.org/10.1111/j.1532-849X.2010.00683.x.
[38] F.R. Teles, R. Teles, A. Sachdeo, N. Uzel, X. Song, G. Torresyap, M. Singh, A. Papas, A. Haffajee, S. Socransky, Comparison of microbial changes in early redeveloping

biofilms on natural teeth and dentures, J. Periodontol. 83 (2012) 1139–1148, https://doi.org/10.1902/jop.2012.110506.
[39] T. Nishikawa, A. Yoshida, A. Khanal, M. Habu, I. Yoshioka, K. Toyoshima, T. Takehara, T. Nishihara, K. Tachibana, K. Tominaga, A study of the efficacy of ultrasonic waves in removing biofilms, Gerodontology 27 (2010) 199–206, https://doi.org/10.1111/j.1741-2358.2009.00325.x.
[40] R. Farina, M. Severi, A. Carrieri, E. Miotto, S. Sabbioni, L. Trombelli, C. Scapoli, Whole metagenomic shotgun sequencing of the subgingival microbiome of diabetics and non-diabetics with different periodontal conditions, Arch. Oral Biol. 104 (2019) 13–23, https://doi.org/10.1016/j.archoralbio.2019.05.025.
[41] G. Hajishengallis, S. Liang, M.A. Payne, A. Hashim, R. Jotwani, M.A. Eskan, M. L. McIntosh, A. Alsam, K.L. Kirkwood, J.D. Lambris, Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement, Cell. Host. Microbe 10 (2011) 497–506, https://doi.org/10.1016/j.chom.2011.10.006.
[42] S. Karakonstantis, M. Rousaki, L. Vassilopoulou, E.I. Kritsotakis, Global prevalence of cefiderocol non-susceptibility in enterobacterales, Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia: a systematic review and meta-analysis, Clin. Microbiol. Infect. 30 (2024) 178–188, https://doi.org/10.1016/j.cmi.2023.08.029.
[43] E.L. Corbett, C.J. Watt, N. Walker, D. Maher, B.G. Williams, M.C. Raviglione, C. Dye, The growing burden of tuberculosis: global trends and interactions with the HIV epidemic, Arch. Intern. Med. 163 (2003) 1009–1021, https://doi.org/10.1001/archinte.163.9.1009.
[44] H. Getahun, A. Matteelli, R.E. Chaisson, M. Raviglione, Latent mycobacterium tuberculosis infection, N. Engl. J. Med. 372 (2015) 2127–2135, https://doi.org/10.1056/NEJMra1405427.
[45] K. Zhou, W. Yu, X. Cao, P. Shen, H. Lu, Q. Luo, J.W. Rossen, Y. Xiao, Characterization of the population structure, drug resistance mechanisms and plasmids of the community-associated Enterobacter cloacae complex in China, J. Antimicrob. Chemother. 73 (2018) 66–76, https://doi.org/10.1093/jac/dkx361.
[46] J. Sun, Q. Tang, S. Yu, M. Xie, Y. Xie, G. Chen, L. Chen, Role of the oral microbiota in cancer evolution and progression, Cancer Med. 9 (2020) 6306–6321, https://doi.org/10.1002/cam4.3206.
[47] S. Banerjee, K. Schlaeppi, M.G. van der Heijden, Keystone taxa as drivers of microbiome structure and functioning, Nat. Rev. Microbiol. 16 (2018) 567–576, https://doi.org/10.1038/s41579-018-0024-1.
[48] Y. Nishi, K. Seto, Y. Kamashita, A. Kaji, A. Kurono, E. Nagaoka, Survival of microorganisms on complete dentures following ultrasonic cleaning combined with immersion in peroxide-based cleanser solution, Gerodontology 31 (2014) 202–209, https://doi.org/10.1111/ger.12027.
[49] R.T. Glass, J.W. Bullard, C. Hadley, E. Mix, R. Conrad, Partial spectrum of microorganisms found in dentures and possible disease implications, J. Am. Osteopath. Assoc. 101 (2001) 92–94.
[50] S. Wellek, M. Blettner, On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications, Dtsch Ärztebl Int 109 (2012) 276, https://doi.org/10.3238/arztebl.2012.0276.
[51] S.S. Senn, Cross-over trials in clinical research, John Wiley & Sons, 2002.