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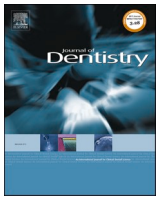

Effect of carbamide peroxide on biomechanical properties of vacuum-formed retainers: A split-mouth randomized controlled trial

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ABSTRACT

Objective: To investigate the effect of tooth whitening on biomechanical properties of vacuum-formed retainers (VFRs).
Methods: Using a split-mouth, randomised controlled trial design, thirty participants were randomly allocated to receive whitening on either the upper or the lower arch, using 10 % carbamide peroxide for two weeks. Biomechanical properties such as hardness, tensile strength, and surface roughness were assessed two weeks after whitening was completed.
Results: Tensile strength of the whitening arch (mean ± SD: 40.93 ± 3.96 MPa) was significantly lower than that of the control (47.40 ± 5.03 MPa) (difference 6.47 MPa, 95 % CI 4.51 – 8.42, $p < 0.001$). Hardness and internal roughness of the whitening arch (VHN = 14.63 ± 2.29 N/mm^2 and Ra = 1.33 ± 0.35 μm, respectively) were significantly greater than those of the control (12.22 ± 1.86 N/mm^2 and 0.96 ± 0.29 μm, respectively) (differences 2.41 N/mm^2, 95 % CI 1.56 – 3.25, $p < 0.001$ and 0.37 μm, 95 % CI 0.23 – 0.51, $p < 0.001$, respectively). The whitening arch showed greater tooth colour change (ΔE = 6.00 ± 3.32) than the control (ΔE = 2.50 ± 1.70) (difference = 3.50, 95 % CI 2.43 – 4.56, $p < 0.001$).
Conclusions: Based on this short-term study, marked tooth colour change was achieved by whitening with VFRs as the whitening trays, but this changed the VFRs' biomechanical properties, including a decrease in tensile strength and an increase in hardness and internal roughness.
Clinical significance: The application of carbamide peroxide in VFRs may compromise their mechanical properties.

1. Introduction

Orthodontic retainers are routinely used after orthodontic treatment to preserve the alignment of the teeth [1]. Vacuum formed retainers (VFRs) are the preferred removable retainers due to their advantages of aesthetics, cost-effectiveness, and patient acceptance [2,3]. VFRs can provide good retention while allowing for occlusal settlement [4], with a clinically satisfactory survival time [5].

Some patients try to use VFRs as whitening trays for tooth whitening during the supervised retention period [6,7]. About 90 % of the orthodontists in the United States and over 99 % of the orthodontists in Colombia have reported that patients requested tooth whitening procedure following appliances removal [8]. Tooth whitening is often performed after braces are removed to improve dental aesthetics, and it is also a conservative treatment approach for enamel conditions associated with discolouration and white spot lesions [9]. The antiseptic effect generated by the whitening ingredient carbamide peroxide can also provide improvement in plaque index and gingival index in younger patients with poor oral hygiene [10]. The most commonly used at-home whitening material is 10 % carbamide peroxide gel. With 10 % carbamide peroxide, the reservoirs, spacers, and scalloping are not necessary; any tray that fully covers the teeth can be used for bleaching [9]. It has been reported that patients often request a tooth whitening procedure following the removal of orthodontic appliances [6–9].

Despite widespread use, the use of VFRs as tooth whitening trays is still controversial. By doing so, patients can benefit from an immediate

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start on tooth whitening, reducing time and cost for fabricating whitening trays, and the use of VFRs will minimise the risk of dental relapse during the whitening phase. On the other hand, concerns have been raised as the peroxide-based whitening material may compromise the dimensional stability and longevity of the VFRs [2]. Some *in vitro* studies have reported that the exposure of VFRs material to hydrogen peroxide increased the material stiffness [11] and surface roughness [12]. To date, there is no direct clinical evidence about the use of VFRs as tooth whitening trays.

The aims of this clinical trial were: (1) To investigate the effect of 10 % carbamide peroxide on biomechanical properties of VFRs, and (2) To evaluate tooth colour change when using VFRs as the whitening trays.

2. Materials and methods

2.1. Ethics approval

This research was approved by the University of Otago Human Ethics Committee (H22/030) and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12622000644763). Written informed consent was obtained from each participant/parent before the study.

2.2. Research design

This study was a randomised, assessor-blinded, split-mouth clinical trial (Fig. 1), in which the primary investigator (C.J.) and biostatistician (A.G.) were blinded to the treatment assignment. The investigator (L.M.) who performed randomisation was not involved in the outcome measurements nor statistical analyses. Participants were given randomly generated intervention allocations (see below for details) in sealed opaque envelopes to achieve allocation concealment. The study was performed between 3 May 2022 and 22 December 2022 in the University of Otago, Faculty of Dentistry Orthodontic Clinic, Dunedin, New Zealand.

2.3. Participants

Inclusion criteria: Patients who finished the fixed orthodontic treatment and had twelve vital anterior teeth from canine to canine with no direct or indirect restorations, no need for attachments or divots on the labial surfaces of the maxillary canines or incisors, no history of dentine hypersensitivity, and no history of tooth whitening during the previous three years.

Exclusion criteria: patients who had caries or periodontal disease, severe internal tooth discolouration, such as fluorosis, tetracycline stain, or discoloured endodontically treated teeth. Participants who were smokers, pregnant, breastfeeding, with known allergic reactions to tooth whitening materials were also excluded.

2.4. Sample size calculation

In order to provide 80 % power to detect 0.8 standard deviation differences in outcomes ($d = 0.8$ being a "large" effect size) when using a paired *t*-test at the two-sided 0.05 level, without assuming anything about the size of the positive correlation between the treated and comparison retainers, and allowing for up to 10 % drop-out, missing, or unusable data, 30 participants were recruited [13].

2.5. Randomisation

Allocations were produced using blockrand 1.5 in R 4.0.5 using blocks of 2 or 4, with equal probability, and with equal probability of bleaching upper and bleaching lower arch. The allocation slips were sealed in opaque envelopes; and participants only opened the envelopes after being recruited into the study. The allocation was not revealed to those involved in data analysis until all the data collection and statistical analyses were completed. Fifteen participants were randomly allocated to having their upper arch whitened and fifteen to having their lower arch whitened, with the opposite dental arch serving as the control arch in each case.

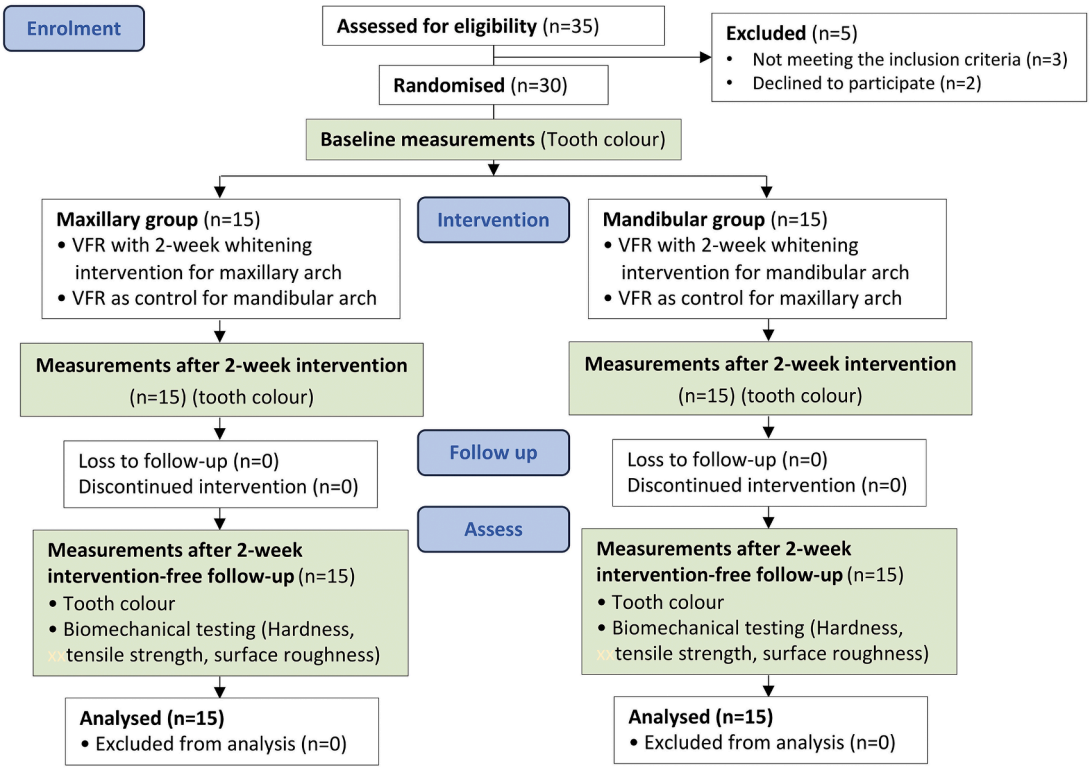

Fig. 1. Flow diagram of study design.

2.6. Vacuum formed retainers (VFRs)

The VFRs were made from 0.78 mm Zendura (Zendura Dental, USA) using a Biostar VI vacuum forming machine (Scheu-Dental GmbH, Germany) following the manufacturer's instructions. Each VFR was trimmed 2–3 mm extending over the gingival margin, and all occlusal surfaces were covered including the most distal tooth in the arch.

2.7. Tooth whitening intervention

Participants were asked to apply whitening gel containing 10 % carbamide peroxide (Pola Night, SDI Ltd., Bayswater, Australia) according to the manufacturer instruction after oral hygiene procedures at night time. The VFRs were worn for eight hours daily overnight for 2 weeks, followed by 2-week intervention-free follow-up. Patients were instructed to apply one drop of whitening gel at the buccal surfaces of each tooth inside the VFRs in the intervention arch (either maxillary arch or mandibular arch, depending on the treatment allocation); patients wore the VFRs without whitening gel in the opposite arch (control).

All patients received a standard hygiene procedure at the debonding appointment, *e.g.* calculus or plaque, if any, was removed using an ultrasonic scaler; the buccal and labial surfaces of teeth were polished using prophylactic paste.

All patients were instructed to brush teeth with a provided manual toothbrush (Slimsoft, Colgate, New York, US) and fluoridated toothpaste (Total Advanced Clean, Colgate, New York, US). Eating and drinking beverages were not allowed during VFRs wear. The VFRs were cleaned with a toothbrush under cold running water without using disinfectant.

After the 2-week intervention, patients were instructed to continue wearing VFRs without any whitening gel 8 h daily overnight for another 2-week intervention-free period as follow-up. At the end of the trial, new maxillary and mandibular VFRs were provided to patients for retention purposes. For ethical reasons and to encourage compliance, upon study completion, participants were provided with additional whitening material to whiten the untreated arch.

2.8. Outcome measurements

The worn VFRs from both arches were retrieved from patients for the measurement of biomechanical properties, including tensile strength, hardness, and surface roughness. All the biomechanical tests were conducted at a room temperature of 25 °C. Each test was repeated three times and a mean value calculated. All tests were measured by a blinded dental investigator (C.J.). The primary outcome was the biomechanical properties of VFRs, while the secondary outcome was the change in tooth colour.

2.9. Tensile strength

The VFRs specimens were prepared according to the ISO standard ASTM D882–18 for tensile strength. The test was performed using a universal testing machine (Model 3367, Instron Co., Norwood, USA) with a loadcell of 1 kN. Maximum tensile load with a loading rate of 1.5 mm/min was measured. All data were collected and analysed using Bluehill software (Instron Co., Norwood, USA).

2.10. Hardness

Hardness was evaluated using the instrumented indentation test with the Instron testing machine with a loadcell and the Vickers indenter. The indentation was made by applying a force of 10 N for 10 s. The dimensions of the indentation were measured with light microscope (Nikon SMZ800N, Tokyo, Japan). Vickers hardness was calculated with the following formula [14]: $VHN = \frac{1.8544F}{d^2}$ (N/mm^2), where F is the force applied on the surface, and d is the mean diameter of the indentations.

2.11. Surface roughness

Surface roughness was measured using a profilometer (TopMap Micro.View optical surface profiler, Polytec GmbH, Germany) with a field of view of 782.08 μm by 578.61 μm. The mean surface roughness value (Ra) was calculated using the TMS 4.1 surface metrology software (Polytec GmbH, Germany).

2.12. Tooth colour

Tooth colour was evaluated using the spectrophotometer VITA EasyShade V (VITA Zahnfabrik, BadSa ̈ckingen, Germany) and VITA Classical guide (VITA Zahnfabrik, BadSa ̈ckingen, Germany) at baseline (T_0, just after debonding), after the two-week intervention (T_1), and after the 2-week intervention-free follow-up (T_2). The tooth colour was measured at the middle third of the labial surface of the anterior teeth at the same clinical environment setting.

Tooth colour change (ΔE, representing the colour difference) was calculated according to the Commission Internationale de l'Eclairage (CIE) L*a*b* system, where the L*, a* and b* correspond to the luminosity, the measurement along red-green axis, and the measurement along yellow-blue axis respectively [15]: $\Delta E^* = \sqrt[2]{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$

The National Bureau of Standards (NBS) system was used to assess the magnitude of colour variation by using the equation [15]: NBS = $\Delta E^* \times 0.92$

2.13. Statistical analysis

Data were analysed using Stata 17.0 (StataCorp 2021). For each outcome, a linear mixed model was used due to the split mouth design, specifically with a random participant effect included to allow for the repeated measurements. Restricted (or residual) Maximum Likelihood (REML) was used to estimate fixed effects and variance components. Model diagnostics were performed including looking at histograms of the residuals, scatter plots of residuals against fitted values, and histograms of best linear unbiased predictions (BLUPs) of random effects. Two-sided p-values less than 0.05 were considered statistically significant and 95 % confidence intervals (95 % CI) are presented to assist with interpretation of possible clinical significance. Outcomes were not prespecified in the clinical trial registry entry and no adjustments were made for multiple comparisons given the exploratory nature of this study.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the participants (age, sex, and ethnicity) were similar for those allocated to whitening their upper and those allocated to whitening their lower arch (Table 1). All participants received the intended intervention and completed the trial. There were no discontinuation or dropout. No side-effects were observed throughout the trial. The statistical analysis was carried out by the original treatment assignments (*i.e.*, according to intention to treat principles).

3.2. Tensile strength

The tensile strength (mean ± standard deviation) of VFRs in the whitened arch (40.93 ± 3.96 MPa) was significantly lower than that in the control arch (47.40 ± 5.03 MPa) (difference = 6.47 MPa, 95 % CI 4.51 – 8.42, $p < 0.001$) (Fig. 2A). The tensile strength, maximum load,

Table 1
Baseline demographics of the participants.

Demographics	Overall (n = 30)	Bleach upper (n = 15)	Bleach lower (n = 15)
Age			
Mean ± Standard deviation (Years)	18.6 ± 6.4	17.8 ± 3.9	19.4 ± 8.3
18+ years old (n,%)	8 (27 %)	3 (20 %)	5 (33 %)
Under 18 years old (n, %)	22 (73 %)	12 (80 %)	10 (67 %)
Sex (n,%)			
Male	11 (37 %)	6 (40 %)	5 (33 %)
Female	19 (63 %)	9 (60 %)	10 (67 %)
Prioritised ethnicity (n,%)			
New Zealand European	27 (90 %)	13 (87 %)	14 (93 %)
Asian	2 (7 %)	1 (7 %)	1 (7 %)
Māori	1 (3 %)	1 (7 %)	0 (0 %)

and elastic modulus were summarised in Table 2.

3.3. Hardness

The hardness of VFRs in the whitened arch (14.63 ± 2.29 N/mm^2) was significantly greater than that in the control arch (12.22 ± 1.86 N/mm^2) (difference = 2.41 N/mm^2, 95 % CI 1.56 – 3.25, $p < 0.001$) (Fig. 2B).

3.4. Surface roughness

The internal surface roughness of VFRs in the whitened arch (1.33 ± 0.35 μm) was significantly higher than that in the control arch (0.96 ± 0.29 μm) (difference = 0.37 μm, 95 % CI 0.23 – 0.51, $p < 0.001$) (Fig. 2C and Fig. 3). No statistically significant difference was found in the external surface roughness of VFRs between the whitened arch (0.71 ± 0.36 μm) and the control arch (0.66 ± 0.34 μm) (difference = 0.05 μm, 95 % CI −0.12 – 0.22, $p = 0.554$).

3.5. Tooth colour

After the 2-week intervention (T_0-T_1), the whitened arch had a greater colour change (ΔE = 5.65 ± 3.31) than the control arch (ΔE = 3.21 ± 1.81) (difference = 2.44, 95 % CI 1.39 – 3.49, $p < 0.001$). After the 2-week intervention-free follow-up (T_0-T_2), the whitened arch still had a significantly greater colour change (ΔE = 6.00 ± 3.32) compared with the control arch (ΔE = 2.50 ± 1.70) (difference = 3.49, 95 % CI 2.43 – 4.56, $p < 0.001$) (Fig. 2D).

According to the National Bureau of Standards (NBS), (Table 3), a greater proportion of patients exhibited marked colour change (76 % = 33 % for marked change + 43 % extremely marked change) in the whitening arch compared with the control arch (30 % = 27 % for marked change + 3 % for extremely marked change).

4. Discussion

VFRs are commonly used for orthodontic retention [1–3]. A

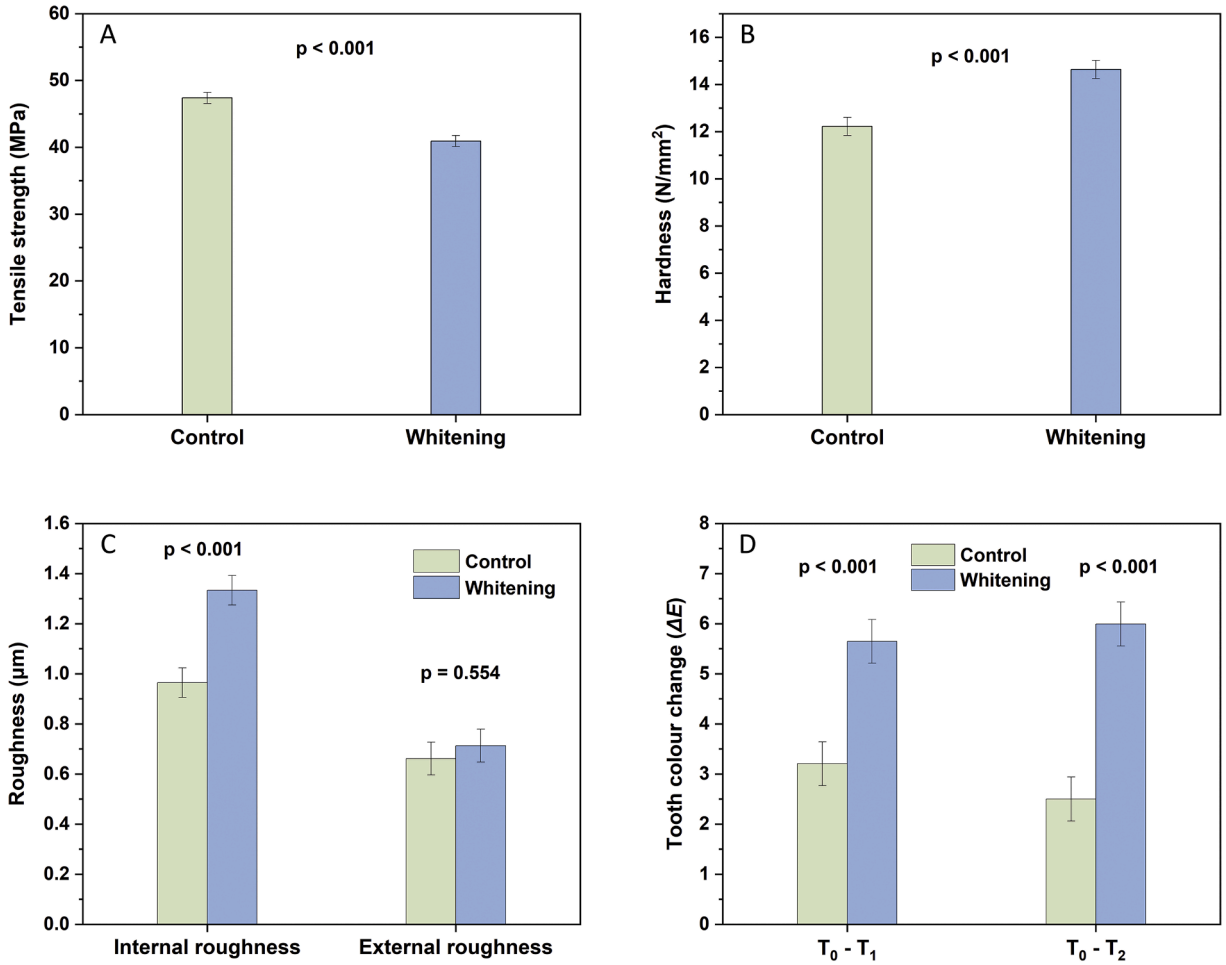

Fig. 2. Tensile strength (A), hardness (B), surface roughness (C) and tooth colour change (D) of the whitened and control arch (showing standard error bars).

Table 2
The mean tensile strength, elastic modulus, and maximum load in the whitening arches were significantly less than these in the control arches.

	Whitened arch ($n = 30$)	Control arch ($n = 30$)	Difference (95 % CI) p-value
Tensile strength (MPa)			
Mean ± Standard deviation	40.93 ± 3.96	47.40 ± 5.03	6.47 (4.51 – 8.42) $p < 0.001$
Minimum – maximum	32.42 – 47.20	33.88 – 55.71	
95 % Confidence interval around mean	39.29 – 42.57	45.76 – 49.04	
Elastic modulus (MPa)			
Mean ± Standard deviation	743.69 ± 160.08	831.65 ± 131.86	87.96 (23.04 – 152.89) $p = 0.008$
Minimum – maximum	407.65 – 978.35	439.32 – 1063.48	
95 % Confidence interval around mean	692.28 – 795.10	780.24 – 883.06	
Maximum load (N)			
Mean ± Standard deviation	124.42 ± 12.03	144.09 ± 15.30	19.66 (13.72 – 25.61) $p < 0.001$
Minimum – maximum	98.57 – 143.48	103.00 – 169.36	
95 % Confidence interval around mean	119.44 – 129.40	139.10 – 149.07	

significant number of patients use VFRs as whitening trays for tooth whitening after orthodontic treatment [6–8], however, the effect of whitening on the biomechanical properties of VFRs is still unclear. This study, to the best of our knowledge, is the first randomised controlled trial investigating the *in vivo* biomechanical changes of VFRs when being used as whitening trays. The findings of this study suggested that marked tooth colour change could be achieved by whitening with VFRs as whitening trays, but the tooth whitening procedure decreased VFRs' tensile strength and increased hardness and internal roughness.

VFRs are usually made from thermoplastic polymer materials, such as polyurethane (PU) and polyethylene terephthalate glycol (PETG) [16]. PU has been most commonly used to fabricate VFRs due to its superior mechanical properties, including abrasion resistance, wear resistance, and dimensional stability with higher temperature [17], hence the usage in the current study.

Biomechanical properties of the thermoplastic polymer materials can be affected by a variety of factors, including moisture, temperature, intraoral aging process, and biological effect of plaque and enzymes [18]. For example, PU is sensitive to moisture resorption, which can lead to the degradation and swelling of the material, causing deterioration of the biomechanical properties [19,20]. Other biomechanical property changes such as surface roughness and colour stability can also be induced in the routine use of VFR by patients due to the cleaning methods or the chemical agents used by patients [15].

Carbamide peroxide can release hydrogen peroxide, which is the main ingredient for tooth whitening. The 10 % carbamide peroxide used in the present study is the standard at-home tooth whitening gel [9]. Free oxygen radicals released from hydrogen peroxide can result in an oxidation reaction of the thermoplastic materials accompanied by the aging process [21]. The tensile strength of VFRs significantly decreased after the whitening procedure in the current study. This is in agreement with the previous *in vitro* study on Zendura PU in the simulated oral environment [22]. The tensile strength of the PU after whitening in the current study was still higher than the PETG material after intraoral aging [23], therefore the impact on clinical retention effect of VFRs may be limited.

A few *in vitro* studies reported that the use of hydrogen peroxide

Table 3
Tooth colour changes in the whitening and control arches based on the National Bureau of Standards (NBS).

NBS Units	Colour changes	Whitened arch (n, %)	Control arch (n, %)
0.0–0.5	Extremely slight change	0 (0 %)	1 (3 %)
0.5–1.5	Slight change	2 (7 %)	11 (37 %)
1.5–3.0	Perceivable change	4 (13 %)	9 (30 %)
3.0–6.0	Marked change	10 (33 %)	8 (27 %)
6.0–12.0	Extremely marked change	13 (43 %)	1 (3 %)
> 12.0	Change to another colour	0 (0 %)	0 (0 %)

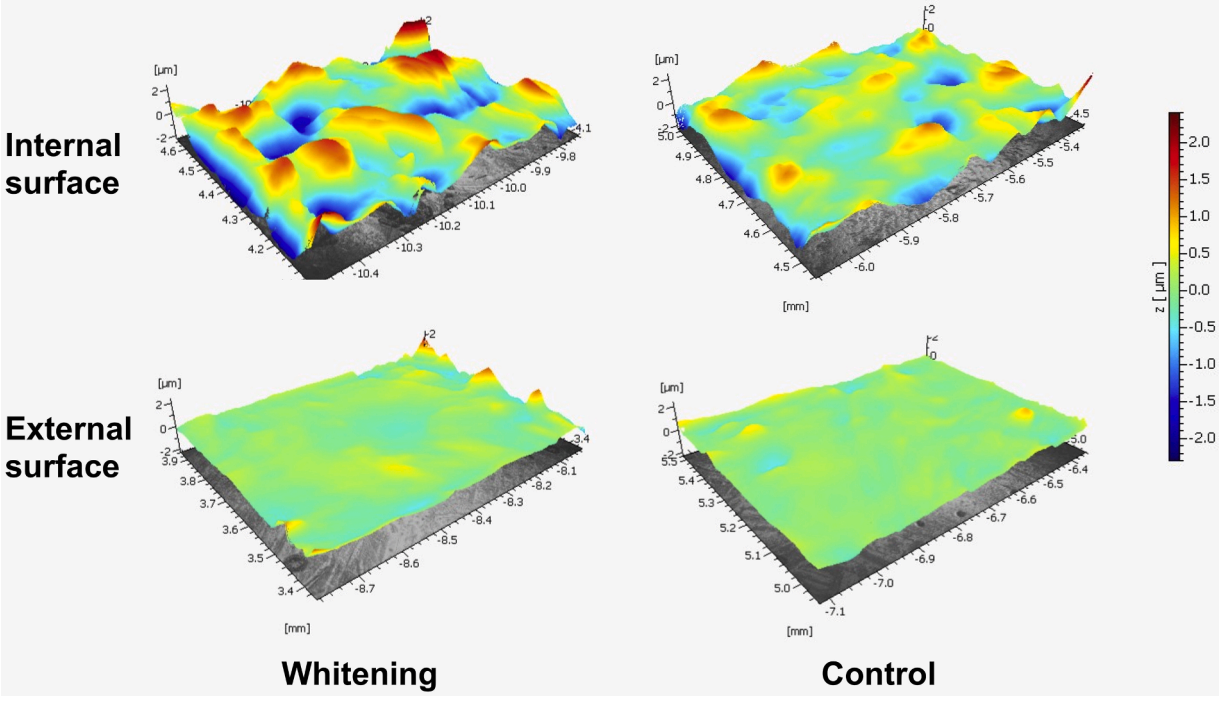

Fig. 3. A representative example of the internal and external surface roughness of the whitened and control arches.

could compromise the flexibilities of VFRs and make it stiffer. Two studies reported a decrease in flexural modulus of the thermoplastic materials after being treated with hydrogen peroxide [11,24]. Other studies reported a reduction of force generated by three-point bending test [12], and less energy was required for fracture to propagate after immersion in hydrogen peroxide solution [25]. Only one study reported no significant change in flexural modulus after treated with hydrogen peroxide, which may be due to the difference in the thermoplastic polymer materials [17].

Hardness of the VFRs increased after the whitening process in the current study, which may be due to the alteration of crystal and amorphous structure of the thermoplastic material or the release of plasticiser [26]. An *in vitro* study reported a decrease in hardness of VFRs material after being treated with 15 % hydrogen peroxide [12]. This may be due to the different thermoplastic material used in that study: the *in vitro* study used PETG while PU was used in the current study. The increase in hardness of VFRs after whitening could lead to an increase in the wear resistance against occlusal force and a decrease in shear strength but could also increase the risk of microcracks and breakages [27,28]. It has been found that the material hardness is related with the delivery of forces of orthodontic aligners to produce tooth movements [29,30]. It is unclear whether this hardness change affect the clinical effectiveness of retention [31], suggesting a need for further trial to investigate the clinical significance.

In the current study, internal surface roughness of the VFR was increased after whitening. This is in agreement with another *in vitro* study [12]. An increase of surface roughness more than 0.2 μm has been found to promote the biofilm formation on restorative materials [32,33], however, this may not be critical for VFRs because unlike restorations that remains intraorally permanently, patients often remove VFRs to mechanically or chemically clean.

Tooth colour was improved after whitening using VFRs as the whitening trays in the current study; and the colour change was similar with the studies in which the normal whitening trays were used [34,35]. This suggests that, from a tooth whitening effectiveness perspective, VFRs can provide similar effect as whitening trays. It is interesting to note that the control arch in the current study also showed slight colour change ($\Delta E = 2.50 \pm 1.70$). This tooth colour change post orthodontic treatment was observed in other studies [36–38]. For example, an *in vivo* split-mouth study found that the colour of natural teeth after the first-year retention showed changes of ΔE from 1.4 to 2.1 units [37]; another study reported a ΔE from 1.5 to 3.6 units one year after debonding [38]. The post debonding tooth colour change may be associated with the alteration of surface morphology, structure of the enamel, and increased convenience of tooth cleansing. Procedures such as acid etching or polishing residual adhesives could cause changes in the roughness of sound enamel causing scattering of light [39]. Furthermore, the remineralisation effect from saliva may also contribute to the colour change [36].

In the current study, the changes of biomechanical properties of VFRs were statistically significant. However, the clinical significance of these changes still requires further studies. The lifespan and survival time of the VFRs can be affected by multiple factors, such as patient factors and daily wear and tear. It has been reported that VFRs can experience perforation and cracks 6–18 months after insertion [40]. One study found that the median survival time of VFRs to be 105 days [5]. The biomechanical properties changes caused by whitening could affect the survival time of VFRs and this remains to be investigated.

There are limitations of this study. The VFRs were made from only one type of thermoplastic material PU (Zendura). As the thermoplastic material varies significantly among different types and commercial brands, the results may not be generalised to other VFR materials. In addition, only one type of whitening agent (10 % carbamide peroxide – the most commonly used standard at-home whitening gel) was used in this study. It is possible for different thermoplastic material react differently with different concentrations of the whitening agent. Different types and concentrations of whitening materials, as well as different regimens of home whitening including wearing time and number of days, can affect the whitening outcome. The present study was specifically focused on assessing the impact of whitening on the biomechanical properties of VFRs. Future studies should explore the effect of different concentrations and regimens of whitening. It is also important to note that the present study did not assess inter-rater and intra-rater reliability; all measurements were performed by a single investigator who was blinded. We do not know that all participants followed instructions although they did not describe any departures from the study protocol when asked. Although the change of VFRs biomechanical properties was statistically significant, the clinical consequence of this changes on the survival or retention effectiveness is unknown. The results were based on a short term, and it is likely that some patients may re-use these VFRs for other cycles of whitening. The long-term effect of the biomechanical properties was not evaluated due to the feasibility of the study. Patients were followed up for four weeks, leaving the long -term effects on VFRs unknown. Since the typical survival times of VFRs are about 105 days [5], further studies with longer follow up would be recommended. Future study could also consider investigating the clinical retention effectiveness of VFRs after being used as tooth whitening trays.

5. Conclusion

Marked tooth colour change was achieved by using VFRs as tooth whitening trays. However, the whitening process affected the biomechanical properties of the VFRs, including a decrease in tensile strength and an increase in surface hardness and internal roughness. While statistically significant, it is still unclear whether these biomechanical changes will meaningfully affect the VFRs' clinical effectiveness for retention. This requires further studies and long-term evaluation.

CRediT authorship contribution statement

Carrol Jin: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Andrew R. Gray:** Formal analysis, Methodology, Software, Supervision, Writing – review & editing. **Paul A. Brunton:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Mauro Farella:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Li Mei:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, 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.

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