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ORIGINAL ARTICLE

The Effect of Chlorhexidine on Bacterial Contamination of Hall Technique Elastomeric Orthodontic Separators and Gingival Health: A Pilot Study

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ABSTRACT

Objective: To study the effect of chlorhexidine on elastomeric orthodontic separators (EOS) bacterial-colonisation and gingival-health in Hall technique (HT) patients. **Material and Methods:** Prospective *in-vivo* pilot clinical study of EOS bacterial colonisation and primary-molar gingival health assessment in 20 patients (mean age 5.45±1.27 years) requiring bilateral HT crowns (40 teeth). One side received 1-minute 0.12% chlorhexidine-soaked-EOSs (Chx-EOSs), and the other side dry-EOSs (NoChx-EOSs). The EOSs were removed five-days later and underwent a bacterial enumeration technique. Plaque (PI) and Gingival (GI) indices were assessed pre-, five-days and three-months post-treatment. Wilcoxon-Signed-Rank/McNemar-Chi-square statistics were used ($p<0.05$). **Results:** Baseline unused/packaged EOSs' sterility check yielded zero colony-forming-units (CFU) per millilitre, but 100% of the used EOSs became colonised by oral-microorganisms. An overall trend of lower mean CFU count in Chx-EOSs ($3.415\pm 0.78 \times 10^5$ CFU/ml) compared to NoChx-EOSs ($6.157\pm1.48 \times 10^5$ CFU/ml) was observed ($p=0.009$). Both NoChx-EOSs and Chx-EOSs insertion sites showed evidence of gingivitis with no difference between PI and GI indices by site over time. **Conclusion:** There was a lower trend of bacterial colonization in chlorhexidine treated EOSs and an occurrence of gingivitis pre/post HT-treatment regardless of EOS type. The lack of difference in the gingival health may be inconclusive due to this pilot's low power suggesting the need for robust large scale studies.

Keywords: Orthodontics; Microbiology; Chlorhexidine.

Introduction

Asymptomatic, non-pulpally involved multi-surface dental caries of the primary molar can be treated restoratively either by conventional restorative means or by using the evidence-based [1] but controversial Hall technique (HT) [2]. The HT uses a preformed metal crown (PMC) and cement to arrest the carious lesion and prevent dental pulp inflammation. In the HT, the tooth receiving the crown does not require any drilling or any local analgesia [1]. The only preparation that may be required is the placement of standard elastomeric orthodontic separators (EOSs) three to five days before the procedure [3] to allow sufficient interproximal spaces to insert the crown. The use of EOSs, no doubt, has an effect on the gingiva [4] with potential rare but harmful effects such as abscess formation [5].

EOSs are small elastic rings of polyurethane used for tooth separation [6]. EOSs have historically been used as a standard procedure to help create interdental space for orthodontics [6] and interproximal caries diagnosis [7]. They can cause iatrogenic induced side effects ranging from periodontal problems [8] to bacteremia [9]. EOSs, usually produced in packets and reels, are among the different materials that highly favour the occurrence of cross-infection [10]. If the reels of EOSs are mishandled prior to their clinical use, they might get contaminated [10].

For the EOSs to be employed in the HT, two separate visits are usually needed to fit the crown [1,2]. The separators are usually threaded by dental floss and then placed into the contact point by stretching and flossing, allowing the separators to pop into the contact area. Separators may cause discomfort due to the pressure needed to allow the rubber band to squeeze between the contacts of the teeth. Three to five days later the separators are removed and spaces will be present for the HT crown placement. This required timeframe may be sufficient for the aggregation of oral microorganisms and debris around the EOS and gingivae. This could potentially cause gingival inflammation even if the EOSs are sterile, although there is little evidence that orthodontic elastomeric materials are either sterile or disinfected [11]. Careful manipulation is necessary to avoid colonisation of pathogenic microorganisms since the composition of the elastomeric materials used do not include antimicrobial agents [11]. Attempts have been made to disinfect and sterilize EOSs [10]; however, they did not use one of the most common disinfecting antiseptics used in dentistry, namely chlorhexidine gluconate [12].

Chlorhexidine has been used to decontaminate the oral cavity and sanitize dental products and appliances [13]; thus could be used to disinfect EOSs. Also, chlorhexidine has been proven to reduce gingivitis and evidence has shown that a 30 seconds [14] to 1 minute [15] of chlorhexidine application disinfects and inhibits plaque [16]. The effectiveness of chlorhexidine (0.12% to 0.2%) is related to its substantivity, which is chlorhexidine's intrinsic ability to be retained by oral hard and soft surfaces, and gradually released into oral fluids over many hours [17]. Because of this feature and to reduce contamination, chlorhexidine use has been recommended prior to orthodontics procedures [9]. Nevertheless, it has been shown that antiseptic mouthwashes can weaken EOSs over time, but chlorhexidine brings the least detriment as it has no significant influence on the force degradation of the elastics [18]. A recent study showed that as EOS placement induced high levels of bacteraemia (of oral origin), they suggested the use of a 0.2% chlorhexidine mouth rinse before separator placement in orthodontic patients [9]. This practice is not recommended in the HT context.

The mouth is colonized by many bacteria that contribute to dental disease, namely caries and gingivitis. The gingival margin contains the greatest growth of plaque where it accumulates and is usually visible after a

few days causing inflammation and bleeding. Oral bacteria easily colonises dental hard and soft tissues in addition to foreign bodies in the mouth. One of the units of measuring oral bacterial contamination load is the colony forming unit or CFU [10,19]. It has been used in paediatric dentistry [19], in orthodontic research [20] and also to assess EOSs sterilization [10].

To the best of the authors' knowledge, and despite studies assessing EOSs in orthodontics, their effects in the context of the HT have not been studied, especially from the microbiological and gingival point of view, hence this pilot study. The aim of this research was to study the bacterial colonisation of dry and chlorhexidine-soaked EOSs used for creating interdental spaces in the HT and their effect on gingival health around the HT PMC.

Material and Methods

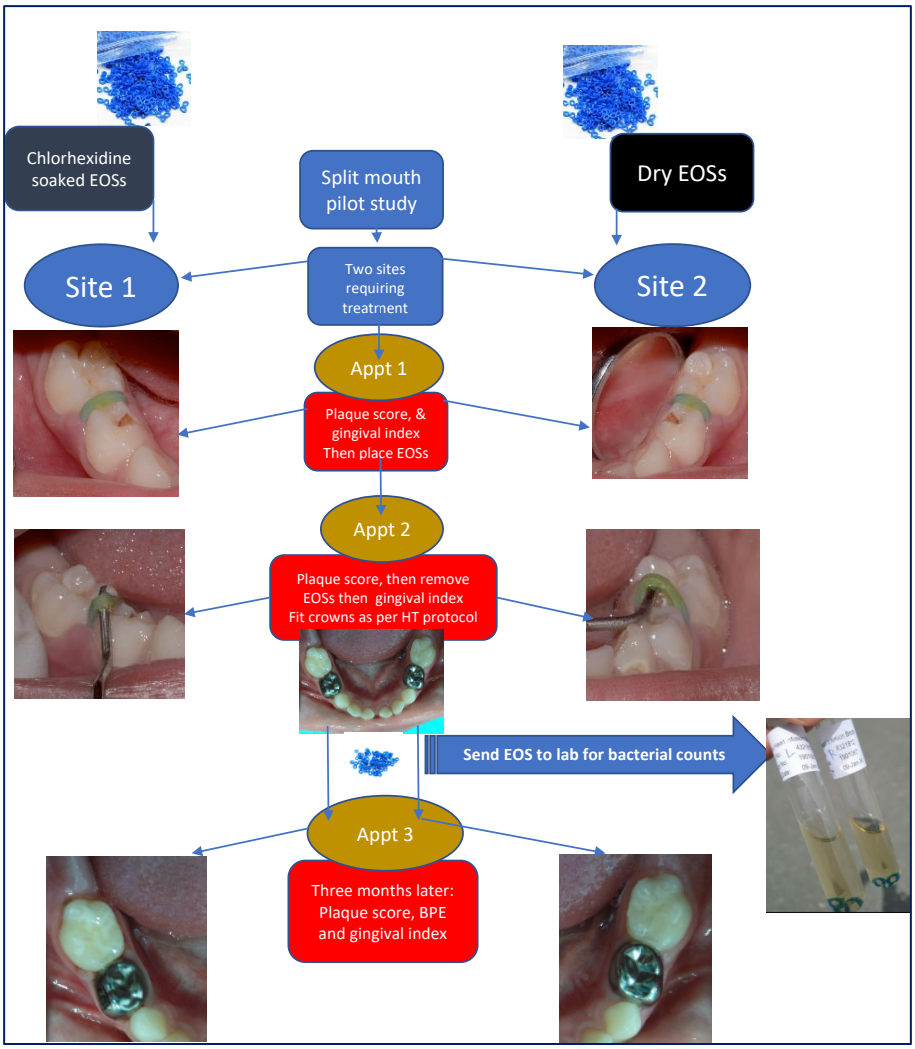
Trial Design

This project was a prospective *in-vivo* split-mouth pilot clinical study. It assessed gingival health and bacterial counts of EOSs (HSI Quick Ring Separators Blue Latex-Free 15/Pk 1067206 | Henry Schein Inc®) used in the context of patients already indicated for treatment using the HT. It was conducted in the paediatric dentistry department of Dubai Dental Hospital (DDH) at Hamdan Bin Mohammed College of Dental Medicine (HBMCDM) in the Mohammed bin Rashid University of Medicine and Health Sciences (MBRU) in Dubai, the United Arab Emirates (UAE). This study was conducted in full conformance with principles of the "Declaration of Helsinki", Good Clinical Practice (GCP), and within the laws and regulations of the UAE/DHCC. The ethical approval was obtained from the Research Ethics Review Committee at HBMCDM and the MBRU-IRB under the number MBRU-IRB-2018-024.

Participants

Fit and healthy patients, between the age of three to nine years of age attending DDH between January to July 2019 and for whom HT crowns were already indicated, were involved in this pilot study, subject to parental consent. The inclusion criteria were: two matched carious lesions in two sites of the same jaw affecting the first or second primary molars. The exclusion criteria were: medically compromised patients; uncooperative patients; patients with primary molars that are not indicated for the HT; patients whose molars are indicated for the HT but had open interdental spaces negating the need for EOSs; children of guardians/carers who refuse to sign the consent form and participate in the study; where the adjacent tooth is loose/exfoliating; any allergy to chlorhexidine and finally patients with oral ulcers.

The patients, indicated for the HT as above, who received parental consent by their guardians to participate in the study, were given three different appointments. At the assessment visit, the parents/guardians of the children indicated for the HT were informed about the study and shown an explanatory infographic sheet based on Figure 1. They were given an information sheet and consent form to sign and return at the following visit. The parents had the full choice to refuse enrolment into the study without affecting their child's planned HT treatment. If the parents accepted, their child was enrolled into the study and given an identifying number. If they did not enroll, they were treated by the HT as originally planned.



HT: Hall technique.

Figure 1. Flow Chart of the pilot study. EOS: elastomeric orthodontic separators.

The Intervention

At the first appointment (appt1), the age of the patient and the position/number of the two teeth requiring HT crowns were recorded. In addition, plaque index scores [3] [PI: 10/10=Perfectly clean tooth, 8/10=Line of plaque around the cervical margin, 6/10 Cervical third of the crown is covered, 4/10= Middle third covered] and the gingival health index [21] [GI: 0 = No inflammation. 1 = Mild inflammation, slight change in colour, slight edema, no bleeding on probing. 2 = Moderate inflammation, moderate glazing, redness, bleeding on probing (BoP). 3= Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding] of both sides (right and left) were examined prior to placing the EOSs. The principal investigator (NA), a paediatric dentistry resident, was calibrated by an experienced clinical supervisor (IH). Using ten clinical scenarios, the intra- and inter-examiner correlations were recorded for the indices above and there was 100% concordance; therefore, no Kappa statistical analysis was conducted. The GI and PI were recorded for assessing the gingival health (mesial or distal where the separators were placed). The worst score was taken in all the indices to reflect the gingival health of that tooth. For instance, if a lower molar preoperatively had the PI of 8 mesially and 6 distally, a score 6 was recorded. This was the same for the GI. The bilateral matched carious lesions' locations (whether mesial/distal or occlusal) were also recorded.

One side received two EOSs that were previously dipped in 0.12% alcohol-free chlorhexidine mouthwash (Perioaid©) for one minute (from now onwards Chx-EOSs) and the other side received an equal number of dry untreated EOSs (from now onwards No-Chx-EOSs). The sites (left or right) receiving either dry or pre-soaked with chlorhexidine were selected randomly by a toss of a coin. The EOSs were picked up using a sterile tweezer sourced from one box; the separators were placed into the mesial and distal contact points using dental floss. The information was documented and linked it to the patient's ID number. The data was pseudo-anonymized. All patients and carers received standardized oral hygiene instructions with equal preventive measures as per the current guidelines [3].

At the second appointment ($appt_2$), five days later, the sites were assessed for PI scores visually so as not to disturb the tooth. The EOSs were retrieved aseptically with sterile dental excavator instruments. Separators were collected into prelabeled serial tubes containing broth media. Samples were processed blindly for bacterial quantification; i.e., the microbiologist did not know which tubes had Chx-EOSs or No-Chx-EOSs. After sending the samples to the laboratory, bilateral GI were assessed again by the principal investigator and recorded using a WHO 621 periodontal probe. After that, the HT crowns were delivered as per the standard HT protocol and using the standard and widely used stainless steel crowns/PMCs [22], the patient was discharged. At the third visit (appt3), which took place three months later, the HT PMCs were assessed clinically and the GI and PI readings were taken.

The bacterial counts were determined using a standard bacterial counting technique. Briefly, the removed separators were immediately transferred to the microbiology laboratory in sterile tubes containing brain-heart infusion (BHI) broth and were processed within one hour. Samples were vortexed for a few minutes and 10-fold serial dilutions were prepared for each sample using phosphate buffer saline (PBS) so that CFU/ml could be determined. One hundred µl of each dilution was spread in duplicates on blood agar using a spread plate technique. The plates were incubated under aerobic conditions at 37°C for 24 hours. After incubation, the bacterial colonies on the plates were counted to calculate CFU/ml of original sample. Colonies were counted using an automated digital colony counter (Synbiosis©, Protocol-3, Biopharm GmbH., Eppelheim, Germany) and reported as ~x10^5 CFU/ml. A baseline *in vitro* bacterial study was conducted to check the sterility of EOSs (dry or previously dipped in 0.12% chlorhexidine solution for 1 minute) prior to use. This was also done to pilot the sampling bacterial culture method described above prior to the commencement of the clinical part of the study and throughout. The CFU is the number of bacteria in 1 ml of the original sample and was calculated mathematically using this equation: CFU/ml = number of colonies x dilution factor / volume of culture plate.

Randomization

This was a pilot study of a convenience sample of consecutive cases of patients already undergoing bilateral HT treatment. As this was a split-mouth pilot study, there was no patient-level randomization. The only randomization was when the primary investigator decided upon which side received the Chx-EOSs Versus No-Chx-EOSs at $Appt_1$ (flip of a coin). In addition, there was no power calculation for this pilot study's sample size due to a lack of similar comparative studies of HT and EOSs in relation to CFUs and the gingivae. However, as each patient was acting as his or her own control, we used other remotely comparable studies [23]. A sample size of 20 patients (40 teeth) was deemed to be adequate for this research.

Blinding

There was no participant or dentist blinding. However, there was blinding for the microbiological laboratory personnel. The EOSs were sent to the laboratory without indication of which sample was chlorhexidine treated. Hence the CFU counts were obtained without knowing the type of EOSs (Chx-EOS or NoChx-EOS).

Objectives

The specific primary objective was to assess bacterial colonisation of chlorhexidine-soaked EOSs (Chx-EOSs) and dry ones (No-Chx-EOSs) used in the context of the HT and their effect on gingival health around the HT PMC. Specifically:

- Were the EOSs used for the HT contaminated prior to/after use?
- Do EOSs used for the HT exacerbate gingival inflammation?
- Was there a difference in gingival health of primary molars treated with HT when Chx-EOSs were used compared to No-Chx-EOSs?
- Was there a difference between the bacterial counts of used Chx-EOSs and bacterial counts of No-Chx-EOSs?

Outcomes and Outcome Measures

This study had two outcomes: a microbiological outcome (bacterial load) and a gingival outcome (health/disease). The microbiological outcome measure was the bacterial counts, while the gingival outcome measure was the GI. Plaque scores were recorded as an indicator for oral hygiene.

Harm and Risk Assessment

Although the use of EOSs in the context of HT is standard practice in paediatric dentistry, it is important that no harm was done to patients. Any reported side effects were recorded at all visits as standard in the patient's clinical notes (like a gingival abscess, a periodontal abscess or severe pain).

Statistical Analysis

Data was entered into a computer using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL). Descriptive statistics was used to describe categorical and continuous variables. The CFU data was tested for normality using Kolmogorov-Smirnov/Shapiro-Wilk test. For the non-normative data, of which CFU data are known to belong to [24], the non-parametric Wilcoxon Signed ranked test was used for the bacterial counts (microbiological outcomes) as they were dependent samples [24]. McNemar chi-square was used to test the association between the two dependent categorical variables (gingival outcomes, PI and GI). A P-value of less than 0.05 was considered significant in all statistical analyses.

Results

A total of 20 patients (mean age 5.45±1.27 years) participated. From these 20 patients, 13 were female (65%). All 20 patients attended the 1st and 2nd appointments. There were no reported acute side effects reported in any of the 20 patients at any of the two visits. Only 13 patients attended the three-month follow-up appointment. Table 1 shows the basic demographic features of the 20 participants and the sites that had the matched carious lesions, that were recipient of the EOSs, and their appointment attendance.

Table 1. The characteristics and attendance of the patients and the characteristics of teeth included in the study.

Patient	Gender	Age (Years)	Sites of EOSs with CHX	SITEs of EOSs with No-CHX	APPT$_1$	APPT$_2$	APPT$_3$
1	F	4	74 M*D*	84 M^D^	√	√	√
2	F	7	74 OD*M*	84 OD^M^	√	√	√
3	M	6	55 OM*D*	65 OM^D^	√	√	√
4	M	7	54 OD*M*	64 OD^M^	√	√	√
5	F	5	55 OD*M*	65 OD^M^	√	√	√
6	F	8	55 OM*D*	65 M^D^	√	√	-
7	F	4	85 D*M*	75 D^M^	√	√	√
8	M	5	85 OD*M*	75 OD^M^	√	√	√
9	M	4	84 OD*M*	74 OD^M^	√	√	√
10	F	6	85 OM*D*	75 OM^D^	√	√	-
11	F	4	85 OM*D*	75 OM^D^	√	√	√
12	F	7	84 M*D*	74 M^D^	√	√	-
13	M	5	55 OM*D*	65 OM^D^	√	√	-
14	M	5	54 OD*M*	64 OD^M^	√	√	√
15	F	4	85 OD*M*	75 OD^M^	√	√	√
16	F	7	85 OD*M*	75 OD^M^	√	√	-
17	F	4	84 OD*M*	74 OD^M^	√	√	√
18	M	6	54 OD*M*	64 OD^M^	√	√	-
19	F	6	85 OD*M*	75 OD^M^	√	√	-
20	F	5	54 D*M*	64 D^M^	√	√	√

Letters" in bold represent caries site; *Refers to the site that received EOSs with Chlorhexidine; ^Refers to the sites that received dry EOSs.

Outcomes

The baseline *in vitro* bacterial study conducted to check the EOSs sterility (dry or previously dipped in 0.12% chlorhexidine solution for 1 minute) prior to use repeatedly showed no growth (zero CFU/ml) repetitively. While all clinically used EOSs results showed bacterial growth expressed in CFUs/ml. These results were obtained in Appt$_2$.

The overall CFU counts in chlorhexidine soaked EOSs were lower compared to those of dry EOSs (p=0.009, Wilcoxon Signed Rank test). The mean CFU count of No-Chx-EOSs was 6.157± Standard Error (SE) 1.419 x10^5 CFU/ml with 95% Confidence Intervals (95% CI:[9.127–3.187]) and a median value of 4.15 x10^5 CFU/ml. While the mean CFU count of Chx-EOSs was 3.415 x10^5 ±7.86 x10^5 CFU/ml (95% CI: [5.061-1.769]) with a median value of 2.30 x10^5 CFU/ml (Figures 2 and 3 and Table 2).

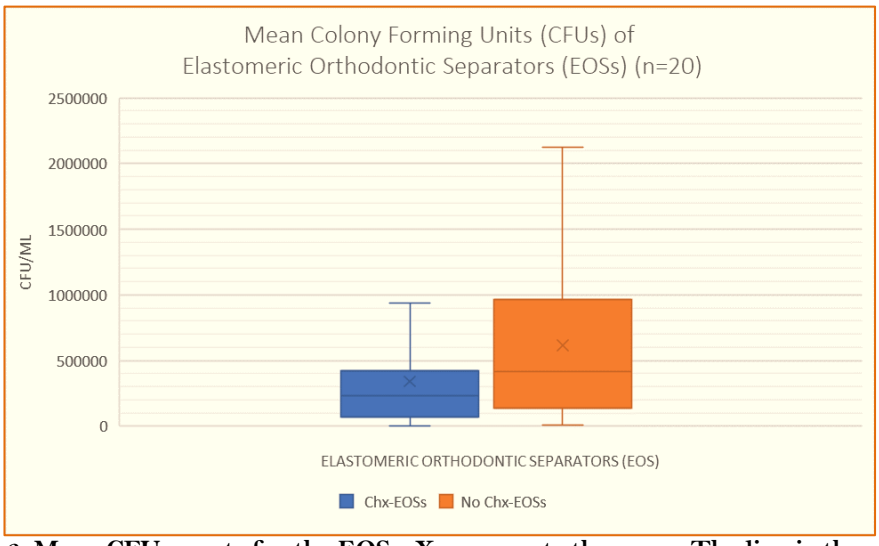
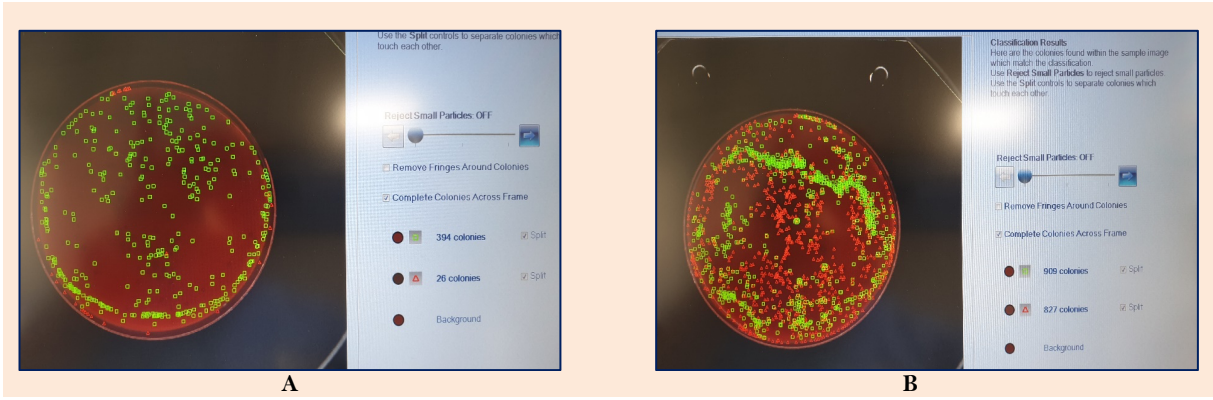


Figure 2. Mean CFU counts for the EOSs. X represents the mean. The line is the median.



A B

Figure 3: Examples of the digital CFU counter display. (A): Chlorhexidine-EOS CFU count shown 4.20x10⁵ CFU/ml; (B): Dry EOS CFU count shown 17.36x10⁵ CFU/ml.

Table 2. The mean colony forming unit (CFU) readings of chlorhexidine and non-chlorhexidine elastomeric orthodontic separators (EOSs).

CFU	Mean#	Std. Error#	95% CI		Median#	Std. Deviation#	p-value*	
			Upper Bound	Lower Bound			Chx-EOSs	No-Chx-EOSs
Chx-EOSs	3.415475	0.78663675	5.06192465	1.76902535	2.30	±3.51794651	_	0.009
NoChx-EOSs	6.15750	1.4190987	9.12770771	3.18729229	4.15	±6.34640232	0.009	_

#(x10⁵ CFU/ml); *Wilcoxon Signed Rank Test (2-tailed).

The GI and PI scores were assessed in all three appointments for each patients' chlorhexidine and no-chlorhexidine sides. The majority had plaque deposits at the gingival margins with moderate inflammation, moderate glazing, redness, and bleeding on probing in all the appointments (Table 3).

Table 3. PI and GI scores of the study's subjects throughout the appointments.

Variables	Score	APPT₁		APPT₂		APPT₃	
		Chlx N (%)	No-Chlx N (%)	Chlx N (%)	No-Chlx N (%)	Chlx N (%)	No-Chlx N (%)
PI	4	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (7.7)	0 (0.0)
	6	7 (35.0)	7 (35.0)	7 (35.0)	7 (35.0)	2 (15.4)	3 (23.1)
	8	10 (50.0)	10 (50.0)	12 (60.0)	12 (60.0)	8 (61.5)	8 (61.5)
	10	3 (15.0)	3 (15.0)	1 (5.0)	0 (0.0)	2 (15.4)	2(15.4)
GI	0	4 (20.0)	4 (20.0)	2 (10.0)	1 (5.0)	2 (15.4)	1 (7.7)
	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	2	16 (80.0)	16 (80.0)	18 (90.0)	19 (95.0)	11 (84.6)	12 (92.3)
	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

APPT₁: 20 Patients; APPT₂: 20 Patients; APPT₃: 13 Patients.

There were no differences in the gingival health and plaque scores between the chlorhexidine side and non-chlorhexidine side within and across the appointments (p>0.05) (Table 4). The above results demonstrated that most of the sites, whether chlorhexidine or no- chlorhexidine sides, had inflamed gingiva related to plaque deposits without spontaneous bleeding.

Because four codes were recorded (4, 6, 8 and 10), Wilcoxon Signed Rank Test was used for all three appointments when comparing plaque scores results within and across the appointments. Within the appointments, there was no significant difference in plaque scores between chlorhexidine side and no-chlorhexidine side in all patients; In Appt$_1$, Appt$_2$,, and Appt$_3$ there was no significant difference between the chlorhexidine side plaque scores when compared to the no-chlorhexidine side (p>0.05) (Table 4).

Table 4. The gingival and plaque scores analysis.

Appointment		Index				Comparison of PI scores (across appointments)#				Comparison of GI scores (across appointments)##			
Index		GI		PI		Chlorhexidine side			Non-Chlorhexidine side				
Side		Chlx	No-Chlx	Chlx	No-Chlx	0.564	0.414	0.366 (between appt 1 and 3)	0.166	0.739	0.206 (between appt 1 and appt 3)	0.625	0.423	1.00 (between appt 1 and 3)	0.250	0.45	0.50 (between appt 1 and 3)
Mean	Appt 1	1.6	1.6	7.6	7.6												
STDev		0.82	0.82	1.39	1.39												
p-value within appointment		1.00*		1.00**													
Side	Appt2	Chlx	No-Chlx	Chlx	No-Chlx												
Mean		1.8	1.9	7.4	7.1												
STDev		0.61	0.44	1.14	1.20												
p-value within appointment		0.746*		0.083**													
Side	Appt3	Chlx	No-Chlx	Chlx	No-Chlx												
Mean		1.69	1.84	7.69	7.84												
STDev		0.75	0.55	1.60	1.28												
p-value within appointment		0.94*		0.705**													

*/## p-value (McNemar Chi Square two tailed);**/# p-value (Wilcoxon Signed Rank Test-two tailed).

The plaque levels were almost the same between chlorhexidine and no-chlorhexidine sides within the appointments. Also, across appointments, for both chlorhexidine and no chlorhexidine sides, there was no statistically significant difference ($p>0.05$). The plaque scores were almost the same between chlorhexidine and no- chlorhexidine side across appointments. Because only two codes were recorded (0 and 2) out of the 4 GI codes, McNemar Chi-Square test was used for all three appointments when comparing GI results within and across the appointments. Within the appointments, there was no significant difference in GI scores between chlorhexidine and no-chlorhexidine sides ($p>0.05$) This was similar across the appointments too for both the chlorhexidine side and no-chlorhexidine sides as no statistically significant difference was found ($p>0.05$) (Table 4). The GI was almost the same across appointments.

Discussion

This pilot study is part of a continuum of studies that have assessed the various aspects of the HT [25]. It studied the microbiological and gingival aspects related to the use of EOSs employed in HT context. The findings demonstrated that all HT EOSs became colonised with bacteria after use, with lower bacterial counts observed in EOSs that had been soaked in chlorhexidine prior to use. The presence of the EOSs appeared to contribute towards the continuation of localized gingivitis around the treated carious primary molars and the use of chlorhexidine treatment appeared to have no effect on the improvement of gingival health clinically. This absence of effect and lack of

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difference between the gingival health in No-Chx-EOSs versus Chx-EOSs treatment sites is inconclusive. This may be due to the low sample size in this pilot study sample, coupled with a loss of some patients at follow-up. This study had no reported acute adverse rare side effects (like gingival abscesses).

In general, and besides creating interdental spaces for the HT (as in this study), EOSs cause plaque accumulation and contribute to periodontal problems [8]. To help counter these potential effects, we used chlorhexidine, known to reduce bacteremia and bacterial contamination [9,26]. The importance of this work lies in being the first study in which the sterility of EOSs was studied, as well as the possible effect of chlorhexidine on EOSs' bacterial colonisation when used for creating interdental spaces for the HT

Reassuringly, the EOSs used in this study for the HT were sterile prior to use in this study's patients. They were removed from the manufacturer's sealed pack (with a single-use only symbol) prior to use clinically and repetitive microbiological testing of the control EOS revealed zero CFU/ml. Attempts had been made to disinfect and sterilise EOSs by Pithon et al. [10] due to concerns that dentists manipulating them may indirectly contaminate them. As found in this study, *in vitro* tests of EOSs had shown no microorganisms' growth occurred after incubation periods [11]. Despite that, little evidence was found regarding whether EOSs were industry-provided sterile or disinfected [11] as manufacturers provide EOSs as single use only 'clean' but non-sterile [27]. In a paper published by Barker et al. [26], the microbial contamination of "as received" and "clinic exposed" orthodontic materials (including EOSs) was assessed. They found that both of those received and exposed to the clinical environment were not free from bacteria contamination before their use in patients; however, this contamination was found to be insignificant [26]. They suggested that as orthodontic brackets are contaminated, they may be disinfected with chlorhexidine before use. This is what was used in the present study. Since the composition of EOSs/elastomeric chains does not include antimicrobial agents, careful manipulation is important to avoid colonization of pathogenic microorganisms [11]. In our study, the EOSs were carefully manipulated when used to avoid cross-contamination (sterile gloves, instruments, cotton wool rolls).

As mentioned above, it is known that EOSs may accumulate dental plaque, which may contribute to and exacerbate gingival inflammation. This is why we used, in addition to dry EOSs, chlorhexidine dipped EOSs. They were dipped in the antiseptic for one minute because the evidence shows that chlorhexidine application disinfects and inhibits plaque with substantivity between 12 [16] to 48 hours [28].

The EOSs used in this study were used to actively open up the interdental spaces for five days using their recoil properties and were not expected to be affected by chlorhexidine use. Generally speaking, elastomeric materials used in orthodontics are still able to perform after being soaked in mouthwashes, despite some force decay [29]. For example, Omidkhoda et al. [29] evaluated the effect of chlorhexidine on the force decay of elastomeric orthodontic chains. They found that about 20% of the force decay happened during the first 24 hours, but they remained active for up to four weeks [29].

Bacterial count expressed in CFU is a commonly used oral bacteriological parameter widely reported in the literature [10,19,20]. All the EOSs used in the study became colonized by oral bacteria, as demonstrated in the bacterial load counting. Our findings showed that all the EOSs used in the study became colonized by oral bacteria, as demonstrated in the mean CFU. Previous *in vitro* work by Vivek Aithal et al. [30] investigated microbial contamination of orthodontic brackets and reported complete decontamination with the elimination of Gram-negative and Gram-positive after treatment with 2% chlorhexidine. They strongly recommended chlorhexidine in clinical practice for the disinfection of orthodontic brackets before placement in the oral cavity. This strategy was adopted in our study, and as a result, the observed mean CFU counts were different in the Chx-EOSs compared to the NoChx-EOSs in most patients. Whilst the characteristic 48 hours substantivity of

chlorhexidine could be a contributory factor for this difference in bacterial load, it is unlikely that the potency of the chlorhexidine will be effective for the five days in which the EOSs remain *in situ*. In these patients, the possible discomfort and avoidance of brushing in the EOS areas is likely to promote the accumulation of food debris in the irregular areas and gaps interdentally created by the EOSs, which generates a conducive environment for microbial propagation. Thus the EOSs potentially remain a source of microbial gingival irritation which could explain the similar gingivitis profiles observed with Chlx-EOSs and NoChx-EOSs treatment sites. One of the aims of this study was to identify if EOSs used for the HT aggravated gingival inflammation and aimed at assessing gingival health around the HT crown. To our knowledge, no studies had assessed gingival health in relation to HT at the time of the start of this project. However, a recent 24-month long randomized control trial (RCT) [31] comparing HT to conventional PMCs reported an improvement in the gingival health of both methods at the end of the two-year period. As our study was only for three months, direct comparisons were not possible.

The gingival health of the study's population primary molars was inadequate before and after treatment. The high level of inadequate oral hygiene and gingival inflammation at baseline might be due to the children's/carers' initial ignorance of oral health [31]. This could also be explained by the fact that they had dental caries in the first place (necessitating the HT). Dental caries is strongly associated with poor plaque control [32], which is usually subsequently associated with poor gingival health (especially plaque/biofilm-induced gingivitis). It would have been ideal if all the patients had very healthy gingiva and plaque control prior to treatment, but this would have been difficult to find in child patients who had dental caries in the first place. This is even more so in our region. Children in the UAE, where this study was conducted, have extremely high caries and gingivitis experience [33].

Our study examined the gingival health by assessing PI and GI scores for all the patients throughout the three appointments. The indices as mentioned earlier are routinely used to screen and assess gingival status. In this study, and at the site of the treated teeth, all 20 patients had poor gingival health and plaque control at $Appts_1$ and $Appt_2$, and 13 at $Appt_3$. There were no improvements between appointments nor sides (Chx and No-Chx sides) over time. In addition, the gingival health did not worsen; it remained in *status quo*. This is despite providing routinely, as part of our pediatric dentistry practice, standardised verbal and oral hygiene instructions. This suggests that the patients in this study did not adhere to the proper oral hygiene instructions and continued their old habits of not brushing adequately to remove the plaque away from their teeth. However, an additional possible reason for that could be related to the physical retention by elastomeric separators used [8], subsequently causing periodontal problems. Indeed, most patients in the current study had localized visible gingival inflammation (edema), coupled with BoP but no abscess formation was noted in any of the sites.

In our study, the GI index did not show any statistically significant difference throughout and within appointments and between sides, despite the relatively lower mean CFU counts in the Chx-EOSs compared to the NoChx-EOSs treatment sites. This, too, indicated that the oral hygiene instructions were not followed promptly and or patients had avoided brushing the areas with separators placed in them because of potential discomfort. Unfortunately, we did not carry out a gingival and plaque evaluation for the whole mouth to see if other areas were different. This may be a limitation in this study and the low sample size.

One interesting finding was that there was BoP around the HT crowns at three months in 13 patients seen at follow-up. Moreover, the recent RCT [31] comparing the HT PMC and conventional prepared PMCs showed that gingival and plaque indices decreased over time (24 months) and that they were similar between the two groups, thus periodontal health improved following treatment [31], a finding not shared by this study.

However, we had not assessed this aspect beyond three months, and our sample size was smaller, so it would be difficult to compare further, and it warrants further investigation. This may be due to residue subgingival cement, larger than normal PMC and PMC margin impingement on the gingiva, and poor plaque control in such high caries-risk patients.

Therefore, in the current study, the plaque control and gingival health at the sites examined were inadequate before starting treatment and remained adequate throughout. Whether the EOSs had chlorhexidine or not did not affect the gingival health. Also, the gingival margins around the HT remained inflamed (due to localized gingivitis) at three months for those patients who showed up at follow-up. Although it appears that gingival health was poor on both sides of the mouth, whether Chx-EOSs or No-Chx-EOSs were used, caution has to be exercised when interpreting these results. The lack of difference may be inconclusive due to the low power of this pilot study and the loss of some patients at the 3rd appointment. This could be considered a limitation of this study, and this aspect warrants further research.

The use of EOSs will remain integral to orthodontics and many HT cases [2], but they are generally associated with plaque accumulation, reversible gingival inflammation and potential but rare abscess formation [5]. Reducing bacterial colonization of the EOSs may be possible by using chlorhexidine. Dipping EOSs in a stronger concentration of chlorhexidine or extended period may yield different results and warrants further research. However, maintaining an adequate level of oral hygiene is of paramount importance when the EOSs are *in situ*. Therefore, it is crucial to advise parents and patients about the importance of brushing gently to avoid dislodgement around the EOSs and that reversible gingivitis may occur. In addition, as EOSs in the context of the HT are contaminated with microorganisms, the HT should not be used in patients at risk of infective endocarditis, as clearly stated in the HT protocol [2].

Some limitations can be observed in this study, such as the study contained a small, convenience sample size, and some participants were lost for the three months follow-up. A reduction in the EOS placement time (between Appt1 and Appt2) may have shown different results, as even 30 minutes to one day of EOSs placement may suffice to create space for the HT [34]. In addition, we did not use chlorhexidine 0.2% in our study (as 0.12% was the only one available). While the latter is an effective antimicrobial, evidence suggests a higher substantivity with increased concentrations [35].

Conclusion

A sample of the stored elastomeric orthodontic separators yielded no bacterial growth, indicating they were not contaminated before use. All elastomeric orthodontic separators used in patients before fitting the Hall technique crowns became colonised with oral microorganisms with relatively lower mean CFU counts in the EOSs treated with chlorhexidine. There was no apparent differential improvement in gingival health with chlorhexidine soaked EOSs compared to dry EOSs. The lack of difference between the sides of gingival health may be inconclusive due to the low power of this pilot study. No abscess formation was noted but localised primary molar plaque-induced gingivitis was present pre- and post-treatment with the Hall technique.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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