Antimicrobial Activity of Chlorhexidine Hexametaphosphate Nanoparticles against *Streptococcus mutans* and *Lactobacillus acidophilus* as a Novel Orthodontic Treatment

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Abstract

Background: Antimicrobial nanoparticles can be an effective strategy to decrease demineralization and white spot lesions associated with orthodontic treatment by coating the surfaces of the used appliances. **Objectives:** As an antimicrobial agent, chlorhexidine hexametaphosphate nanoparticles can be used to coat orthodontic brackets to measure the release of chlorhexidine and assess its antibacterial activity. **Materials and Methods:** The nanoparticles were produced by mixing aqueous solutions of sodium hexametaphosphate and chlorhexidine digluconate. A release test was conducted using artificial saliva for 60 days. Antibacterial assays were performed against *Streptococcus mutans* and *Lactobacillus acidophilus*. This experimental study was conducted on 360 orthodontic brackets. Stainless steel and ceramic brackets were coated with 5 mM chlorhexidine hexametaphosphate nanoparticles. **Results:** Chlorhexidine hexametaphosphate nanoparticles-coated brackets showed continued release of chlorhexidine over 60 days. The release rates were significantly different between coated stainless steel and ceramic brackets on day 1 ($P = 0.002$). More chlorhexidine was released from coated stainless steel than coated ceramic brackets in the next few days, although not significantly. In the antibacterial assay, bacteria were inhibited by coated brackets. Inhibitory zones around coated stainless steel brackets were significantly larger than those around coated ceramic brackets. **Conclusion:** Chlorhexidine hexametaphosphate as antimicrobial nanoparticles is a promising coating for orthodontic brackets, providing sustained chlorhexidine release and bacterial growth inhibition.

Keywords: Antimicrobial, brackets coating, chlorhexidine hexametaphosphate, nanoparticles, orthodontics

INTRODUCTION

The concentration of cariogenic bacteria in saliva and dental plaque, including *lactobacilli* and *Streptococcus mutans*, raised with fixed orthodontic appliances increase and decrease the tooth surface's ability to self-clean. Therefore, the possibility of demineralization or the production of white spot lesions is raised with the use of fixed orthodontic appliances.[1-3] Common side effects of orthodontic therapy include increased microbial biofilm formation on orthodontic appliances and auxiliaries, including caries from white spot lesions as well as eventual tooth cavities and periodontitis.[4] White spot lesions are a term used to describe the demineralization stage of dental caries, which is typically initiated on dental enamel surfaces that exhibit opaque, white tints. Long-term dental plaque collection on the tooth surfaces is typically linked to the development of white spot lesions. Certain medical and dental conditions, as well as hereditary traits, may also play a role.[4-6] The frequency of white spot lesions in orthodontic treatments is still much higher, even with

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hygiene therapies for the control of tooth caries/white spot lesions. According to multiple studies, 2% to 96% of patients may experience white spot lesions associated with orthodontic treatments.[6,7]

Thus, in clinical practice, strategies with the fewest negative impacts and the greatest positive effects are favored. These techniques have been used to reduce or stop bacterial aggregation around the teeth. They involve treating the metal appliance's surface, such as coating it with nanoparticles.[8]

The nanoparticles offer numerous antibacterial qualities due to their small size, high surface-to-volume ratio, and extensive contact with the external environment. A number of methods have been tried to prevent the growth of biofilm on orthodontic appliances and auxiliaries, and one of the best approaches is to incorporate antimicrobial compounds into these appliances.[9] The transformation of certain particles into nanometer size has been found to exhibit exceptional antibacterial properties against both gram-positive and gram-negative bacteria.[10-12] These nano-sized antibacterial agents are favored for inclusion in dental materials because of their higher surface-to-volume ratio. Nanoparticles form close relationships with microbial membranes and offer a significantly larger surface area for antibacterial activity. Therefore, one way to lower the incidence of white spot lesions during fixed orthodontic treatment is to coat orthodontic attachments or add antibacterial nanoparticles to adhesives.[13,14]

The nanoparticles less than 100 nm in size allow for better contact with microorganisms and give them significant antimicrobial characteristics. According to research, coated orthodontic brackets exhibit antibacterial and anti-adhesive features against frequent oral pathogenic microbes.[15-17]

To inhibit microbial adherence and prevent caries, nanoparticles can be employed in dental materials by one of two mechanisms: combining them with the materials or generating surface coatings with nanoparticles.[18-20] Chlorhexidine is currently considered the best disinfectant because of its broad-spectrum bactericidal effect against both gram-positive and gram-negative bacteria.[21,22]

Chlorhexidine hexametaphosphate has offered persistent, slow release of active chlorhexidine over time and hence a good anti-microbial agent. Chlorhexidine has a broad-spectrum antimicrobial activity as well as plaque removal by its cationic action that makes it effective against a wide variety of bacteria and yeasts while not encouraging the development of bacterial resistance.[18,23] Several studies have shown that chlorhexidine hexametaphosphate nanoparticles (CHX-HMP NPs) can be attached to materials and release chlorhexidine as an antibacterial agent. Since chlorhexidine and sodium hexametaphosphate are commonly used in dentistry as antibacterial mouthwash and anticalculus agents and have been demonstrated to be efficient against oral microbes that cause white spot lesions, coating orthodontic materials with antimicrobial CHX-HMP NPs could offer a way to reduce white spot lesions by preventing the microbes that cause the white spot lesions from forming.[24]

To the best of the investigators' knowledge, no prior study was performed to coat orthodontic brackets with CHX-HMP NPs. This *in vitro* study aims to assess (1) the viability of coating orthodontic brackets with CHX-HMP NPs, (2) the release of chlorhexidine from the coated SS and Ceramic brackets over 60 days, and (3) the antimicrobial activity of the released chlorhexidine from the coated brackets.

The null hypothesis of this study was that CHX-HMP NPs were not expected to coat orthodontic brackets for long-term chlorhexidine release or possess antimicrobial ability.

MATERIALS AND METHODS

Specimens and chemicals

The study sample includes stainless steel and ceramic brackets (Dentaurum GmbH & Co. KG, Ispringen, Germany) with MBT prescription and a slot size of 0.022." A chlorhexidine hexametaphosphate solution as a bracket coating agent (Sigma Aldrich, Germany).

Preparation of nanoparticles

At room temperature and ambient conditions, 100 mL of 10 mM aqueous sodium hexametaphosphate and 100 mL of 10 mM aqueous chlorhexidine digluconate were combined while being constantly stirred. This resulted in a CHX-HMP NPs solution of 5 mM. Instantaneously, a colloidal suspension was created by mixing the two chemicals. This colloidal suspension was prepared following the manufacturer's instructions, as outlined in previous research studies.[25,26]

Sample distribution and preparation

The overall sample size of this study was 360 brackets of orthodontic stainless steel brackets (SB) and ceramic brackets (CB). The sample of the present study of the brackets consisted of four groups: two experimental groups from each SB and CB coated with the CHX-HMP NPs, and the other two groups were control groups. Each group (experimental and control) includes 10 brackets from each type of bracket. The complete timetable of the study was 9 weeks. Alcohol was used to sonically clean each bracket for a duration of 15 min. Then, exposed to ultraviolet (UV) radiation for 30 min using a sterilization cabinet (Scie-plas GLE-UVSC UV, England).[16] Each bracket from the experimental groups was coated with 10 mL of the initially made colloidal suspension of nanoparticles by rapidly stirring it within a laboratory tube for 30 s with rapid stirrer equipment (Nanolab-Scientific, Germany) in order to coat the sample. Following that, the coated bracket was gripped gently using a sterile tweezer and dipped in deionized water for 10 s to eliminate all extraneous material and left to dry for a minimum of 60 min. These operations were carried out in a sterile hood. The sample was stored in a sealed, properly labeled test container for each group.

Artificial saliva preparation

The procedure consisted of the following steps:

A sensitive electronic balance was used to measure the components. In a glass cylinder, 500 mL of deionized water and 500 mL of distilled water were filled with the measured materials, and the mixture was swirled until all of the ingredients were dissolved. After that, by using a pH meter and either lactic acid or NaOH, the solution's pH was adjusted to 6.75 (±0.015) to mimic the mean value of human saliva.[27]

The chemical elements with their concentrations in the prepared artificial saliva were as follows: urea, 1000 mg/L; $CaCl_2·2H_2O$, 960 mg/L; distilled water, 500 mL; deionized water, 500 mL; NaCl, 400 mg/L; KCl, 400 mg/L; $NaH_2PO_4·2H_2O$, 690 mg/L; and $Na_2S·9H_2O$, 5 mg/L.

Release test of nanoparticles

The amounts of the soluble CHX that was liberated from the coated orthodontic brackets were measured for a duration of 60 days (nearly 9 weeks, including time for preparation and coating). 140 SB and 140 CB were employed for each of them (10 coated, 10 control from each kind for each study period).

Every bracket from the (SB and CB) experimental and control groups was submerged in a test tube filled with five milliliters of artificial saliva, covered tightly with a lid, and the joint was covered with parafilm. The material was then put in an orbital shaker incubator at 37°C till the end of the experimental days. Subsequently, the material was put in an orbital shaker set to 150 rpm and 37°C. The chlorhexidine quantities were then determined by measuring the absorbance at 255 nm using a UV spectrophotometer (UV-1900i, Shimadzu, Kyoto, Japan) after the set duration for each sample was finished (at 1, 10, 20, 30, 40, 50, and 60 days). A standard solution of 5-50 µM of chlorhexidine was used to guide the calibration of chlorhexidine concentrations.[28]

Antibacterial assay

Bacterial culture

The AL-Almani Hospital's Microbiology Laboratory provided the bacterial strains, including *Streptococcus mutans* and *Lactobacillus acidophilus*. All bacterial strains satisfied the 0.5 McFarland Nephelometer Standard, which was achieved by adding 99.5 mL of 0.18 mol/liter H_2SO_4 (1% vol/vol) to 0.5 mL aliquot of 0.048 mol/liter $BaCl_2$ (1.175% wt/vol $BaCl_2·2H_20$). A spectrophotometer was utilized to determine absorbance with a matching cuvette and a 1-cm light path to confirm that the turbidity standard was the correct density. The 0.5 McFarland standard requires an absorbance of 0.08 to 0.13 at 625 nm. The barium sulfate suspension should be added in 4- to 6-ml aliquots to screw-cap tubes of the same size as those used to standardize the bacterial inoculums. Keep the tubes at room temperature in the dark with a tight-fitting cap.

Specimens cultivation

Streptococcus mutans in brain heart infusion medium and Mueller Hinton broth was used to culture *L. acidophilus*. The separated bacterial suspensions were transferred by a micropipette to the petri dishes and gently splattered throughout the plate in all directions with a sterile cotton swap. A sterile tweezer was used to place the coated and control SB and CB directly on the agar, where they quickly adapted and then incubated for 24 h at 37 °C with 5% CO_2 incubator.

Measuring the diameter of the inhibition zone

The procedure of determining the diameter of this zone of inhibition was automated with image processing. In this work, a computer vision method that can identify the bacteria's zones of inhibition is built. This study presents a practical method for assessing the zone of inhibition by determining the zone's radius using contour drawing and threshold setting. In addition, this work determines whether a certain bacterium is susceptible to or resistant to the supplied antibiotic by applying the computed zone of inhibition and the recommended standard values.[29]

Statistical analysis

The statistical analysis was conducted using SPSS version 26 (SPSS, IBM Company, Chicago, IL 60606, USA). Descriptive statistics, including the Shapiro–Wilk test, mean, standard deviation, standard error, and minimum and maximum, were utilized to describe the dataset. In terms of inferential statistics, a 95% confidence interval served as the benchmark. Statistical significance was declared when the P value fell below 0.05. The independent t test was employed to assess and compare if there were significant differences between independent groups (SB and CB).

RESULTS

Release test of nanoparticles

The coated orthodontic brackets (of both stainless steel and ceramic) with the CHX-HMP NPs constantly released soluble chlorhexidine during the experiment that lasted for 60 days, as seen in Figure 1. The uncoated brackets, namely stainless steel and ceramic, demonstrated a minimal and transient release of a substance with absorbance at 255 nm. Notably, this substance could not

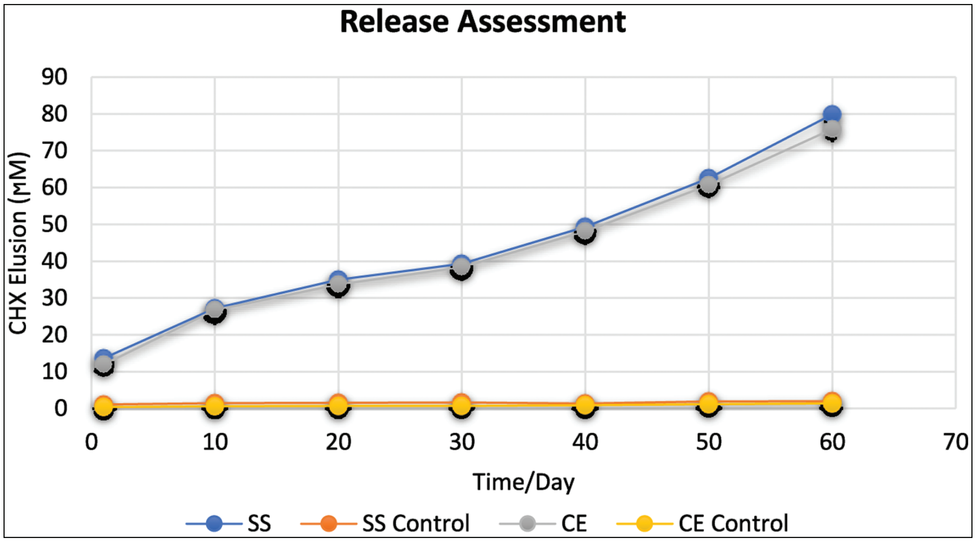

Figure 1: Release of accumulative chlorhexidine from coated stainless steel and ceramic brackets over 60 days compared with the control specimens.

Table 1: Chlorhexidine release (μM) of coated stainless steel and ceramic brackets

Duration	Groups	N	Min	Max	Mean	SD	SE	Shapiro–Wilk test (*P* value)	Independent Samples *t* test (*P* value)
1 day	SB	10	11.7	15.8	13.65	1.19	0.37	0.938	0.002*
	CB	10	10.9	13.2	11.97	0.80	0.25	0.615	
10 days	SB	10	25.6	30.7	27.24	1.57	0.49	0.184	0.605
	CB	10	23.4	31.4	26.74	2.55	0.80	0.633	
20 days	SB	10	28.5	40.0	34.98	3.50	1.10	0.524	0.414
	CB	10	30.0	41.4	33.71	3.28	1.04	0.129	
30 days	SB	10	35.2	42.1	39.27	2.44	0.77	0.205	0.293
	CB	10	35.9	39.7	38.32	1.30	0.41	0.195	
40 days	SB	10	45.3	52.1	49.34	1.77	0.56	0.170	0.140
	CB	10	45.6	50.2	48.15	1.67	0.52	0.363	
50 days	SB	10	56.3	69.5	62.57	3.73	1.18	0.633	0.237
	CB	10	55.2	64.8	60.67	3.18	1.00	0.764	
60 days	SB	10	68.4	87.2	79.86	5.02	1.59	0.176	0.135
	CB	10	65.0	84.3	75.87	6.30	1.99	0.524	

be chlorhexidine as these brackets were not exposed to chlorhexidine salts during the study.

On the first day, the coated SB released 13.65 μM of chlorhexidine, whereas the nanoparticle-coated CB released 11.97 μM. Following that, the release readings of coated SB were 27.24, 34.98, 39.27, 49.34, 62.57, and 79.86 μM on days 10, 20, 30, 40, 50, and 60, respectively.

Simultaneously, during the studies, the chlorhexidine release values from coated CB varied from 26.74 to 33.71 to 38.32 to 48.15 to 60.67 to 75.87 μM. The data above indicates that there were more releases from coated SB than from CB.

An independent *t* test showed that on the first day, the release of chlorhexidine from coated SB was significantly higher than that from CB, whereas on subsequent days, there were no significant differences [Tables 1–3].

Table 2: Raw data of chlorhexidine release of stainless-steel brackets

1 Day	10 Days	20 Days	30 Days	40 Days	50 Days	60 Days
13.3	26.7	28.5	42.1	48.7	63.1	68.4
15.8	25.6	30.4	39.8	49.8	56.3	79.7
13.5	30.7	34.8	40.5	50	69.5	78.2
11.7	28.2	40	36	52.1	65	85.3
12.5	25.7	39.2	40.6	48.5	57.6	80.5
13.8	28.3	36	41.1	50.5	63.3	78.9
14	27	33.8	41.6	48.6	63	80.3
13.9	27.9	35.8	35.2	45.3	64.6	87.2
15.1	26.3	36.2	36.8	49.9	61	82.1
12.9	26	35.1	39	50	62.3	78

Antibacterial assay

The descriptive statistics results for the inhibition zones of the studied bacterial species, including *Streptococcus*

Table 3: Raw data of chlorhexidine release of ceramic brackets

1 Day	10 Days	20 Days	30 Days	40 Days	50 Days	60 Days
11.3	28.5	32.1	39.2	50.2	62.6	68.4
10.9	23.4	32.2	38.5	48.1	57.4	69.7
12.1	25.8	30.5	37.2	48.5	60.7	78.2
13.2	29.6	36	36.7	49.5	64.8	75.3
11.5	24.2	41.4	39.6	46.5	55.2	81.1
12.1	25.4	32.9	38.2	50	60.5	75.9
11	26	34.4	39.7	49.6	63.2	81
12	31.4	30	35.9	45.6	64.6	84.3
13	28.1	35	39.2	46.3	59.7	79.8
12.6	25	32.6	39	47.2	58	65

Table 4: Inhibition zones (mm) for CHX-HMP NPs coated stainless steel and ceramic brackets

Bacterial Species	Groups	N	Min	Max	Mean	SD	SE	Shapiro–Wilk test (*P* value)
Streptococcus mutans	SB	10	8.0	11.0	9.50	0.91	0.28	0.982
	PO. C.	10	12.5	16.0	14.3	1.18	0.37	0.539
	CB	10	6.0	10.0	8.15	1.20	0.38	0.986
	PO. C.	10	13.5	16.5	14.9	0.96	0.30	0.814
Lactobacillus acidophilus	SB	10	6.3	9.8	8.22	1.07	0.33	0.967
	PO. C.	10	12.0	14.0	13.05	0.64	0.20	0.392
	CB	10	5.7	9.0	7.16	1.07	0.34	0.769
	PO. C.	10	11.5	14.0	13.1	0.87	0.27	0.232

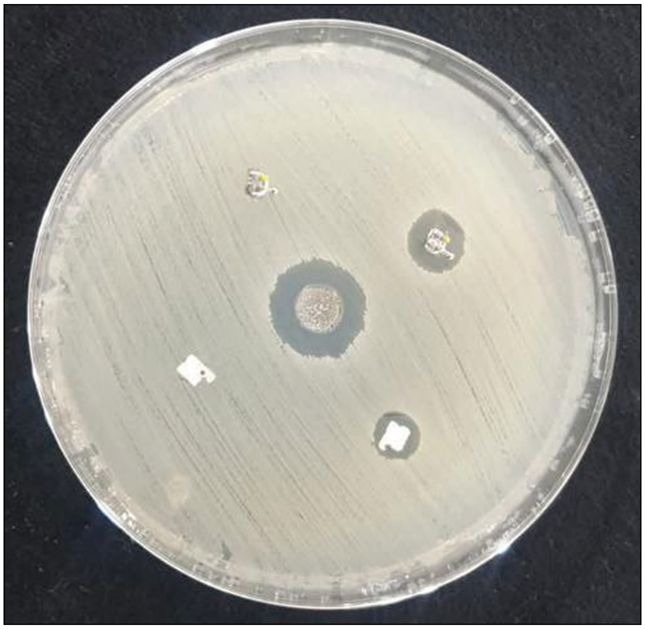

Figure 2: Inhibition zone around stainless steel and ceramic brackets against *Streptococcus mutans*

mutans and *Lactobacillus acidophilus*, are presented in Table 4. The statistical measures included minimum (min), maximum (max), mean (M), standard deviation (SD), standard error (SE), and the Shapiro–Wilk test values.

The antibacterial efficacy of the coated brackets was evidenced by the creation of distinct inhibitory zones around each sample against the tested bacteria. Figure 2 shows the zones of inhibition observed for bacterial species employed in the assessment. The SB and CB control groups showed no bacterial growth suppression.

The results of the present study showed that the inhibition zones around the coated SB were larger for both kinds of bacteria assessed than those around the coated CB.

Considering that the cork borer tool had a diameter of 7 mm, the diameter of the positive control groups (PO. C.), which consisted of drops containing 100 µL of CHX-HMP NPs solution, varied from 11.5 to 16.5 mm for evaluated bacterial species.

According to the Shapiro–Wilk test, the *P* value for all types of groups was more than the significance level of 0.05; consequently, the data were deemed to satisfy the normality requirement within the 5% significance level.

The results of an independent samples *t* test are presented in Table 5, which indicates that for both *Streptococcus mutans* (*P* value = 0.041) and *Lactobacillus acidophilus* (*P* value = 0.011), the inhibitory zones around coated SB groups are significantly higher than those surrounding the coated CB group [Tables 6 and 7].

Table 5: The coated stainless steel and ceramic brackets for each type of bacteria

Bacterial Species	Compared Groups	Independent Samples *t* test (*P* value)
Streptococcus mutans	SB CB	0.041*
Lactobacillus acidophilus	SB CB	0.011*

Table 6: Inhibition zone of stainless-steel brackets (in mm)

Streptococcus mutans	*Lactobacillus acidophilus*
11.0	7.5
8.5	8.2
10.0	9.0
10.5	6.3
10.0	8.5
9.0	7.0
9.5	8.5
8.0	9.4
9.0	8.0
9.5	9.8

Table 7: Inhibition zone of ceramic brackets (in mm)

Streptococcus mutans	*Lactobacillus acidophilus*
9.0	6.9
8.5	7.0
7.5	7.5
8.5	6.0
6.0	6.5
9.5	8.5
10.0	9.0
7.0	5.7
8.0	6.5
7.5	8.0

DISCUSSION

The introduction of nanotechnology in dentistry has opened up a promising avenue for combating microbial plaque formation during orthodontic treatments, especially with the integration of antimicrobial nanoparticles onto orthodontic appliance components such as brackets, archwires, and elastics.[11]

The brackets, commonly used in orthodontic treatments as an essential component of fixed appliances, are susceptible sites for microbial adhesion due to their surfaces. Numerous studies have highlighted the significance of surface roughness in promoting biofilm adherence to orthodontic appliances.[30] To counter this challenge and minimize the development of microbial plaque, researchers are exploring the application of various antimicrobial agents, such as silver (Ag),[31] titanium dioxide (TiO_2),[32] and zinc oxide (ZnO)[15] as coatings on these biomaterials. In recent investigations, researchers have turned their attention to coating stainless steel and ceramic brackets with CHX-HMP NPs due to their demonstrated ability to adsorb onto surfaces and achieve sustained release of chlorhexidine for an extended period.[25]

The findings of this study suggest that during the duration of the observation period, the accumulative concentration of chlorhexidine released from stainless steel and ceramic brackets coated with CHX-HMP NPs increased steadily. Therefore, this may indicate that the incorporation of hexametaphosphate nanoparticles can be effective in allowing chlorhexidine release in a gradual and continuous manner, which would have the advantage of raising the antibacterial effect. These findings are consistent with earlier studies highlighting the role of nanoparticles as effective chlorhexidine transporters that allow for a gradual and prolonged release of this agent.[25,26,33]

Due to its high water solubility, chlorhexidine digluconate salt is a frequently used ingredient in a variety of commercial CHX products, including toothpaste, rinses, gels, lozenges, sprays, and varnishes.[34] However, due to its solubility, chlorhexidine salt immediately elutes upon contact with fluids, which may result in an early burst release rather than a continuous, sustained release.[25] This feature might make it less suitable as a biomaterial for coatings.

Antibiotics' release kinetics are important because they determine how effective they are. Without a drug delivery system, about 65% of the antibiotic substrate is released in the first minute and 95% of it in the next five. Although this rapid-release pattern might be useful in lab settings, it might not be as useful in clinical settings, especially in light of the short half-lives of antibiotics, which might restrict their ability to kill microorganisms.[35] This becomes more important during orthodontic treatments, as patients may be more susceptible to gingivitis, mouth infections, and white spot lesions due to the longer treatment periods.[36]

In the orthodontic field, it is clear that sustained and regulated release mechanisms of antimicrobial agents are essential to solve the problems associated with extended treatment durations. One of the approaches that appears to be promising for creating drug delivery platforms is the use of nanoparticles. Several types of nanoparticles that can be utilized as drug carriers and enable controlled release over a long period of time have been studied.[37] This approach is specifically helpful in orthodontics as it is important to extend therapeutic medication levels for a longer time so it can deal with any oral health problems that appear during treatment.

The investigation results demonstrated that chlorhexidine was released in a flash on the initial day of the test. This release continued for the next sixty days. This may indicate the possibility of sustained release. This is agreed with

Subramani *et al.* (2020) study, who found that coating orthodontic elastomeric chains with chlorhexidine nanoparticles resulted in a slower release of the agent and constant leakage of it over a 28-day period.[24] For more than 8 weeks similar results were found by Kamarudin *et al.* who observed that coated orthodontic elastomeric ligatures in contact with an aqueous environment give sustained chlorhexidine.[18] Al-Fadhily and Abdul-Hadi coated orthodontic archwires and found throughout the study that continued for 28 days, both stainless steel and NiTi arch wires illustrated an ongoing discharge of soluble chlorhexidine in artificial saliva.[33]

The first day of the release test exhibited a significant difference in the amounts of chlorhexidine released from coated SB and CB. The SB showed the greater release. For the following days, there was more release from coated SB than from CB, but it did not reach significant levels. This variation may be attributed to the greater attachment of nanoparticles to the SB surface compared to CB.

This experimental study determined that the CHX-HMP NPs can significantly inhibit the bacterial growth of *Streptococcus mutans* and *Lactobacillus acidophilus* on the orthodontic coated stainless steel and ceramic brackets. This was evidenced by the creation of distinct inhibitory zones around each sample against the tested bacteria in the antibacterial assay. These findings suggest the substantial release of active chlorhexidine from the coated bracket surface.

These results matched with what was found by several previous studies performed on the same nanoparticles but coated on different biomaterials like CHX-HMP NPs coated elastomeric chains capable of preventing the growth of *Streptococcus mutans* and *Lactobacillus rhamnosus* in the laboratory. Also, coating orthodontic mini-screws with CHX-HMP NPs exhibits effective antibacterial and antibiofilm properties against *Streptococcus mutans*, *Streptococcus gordonii*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Candida albicans*.[21]

The application of CHX-HMP NPs as a coating to stainless steel and NiTi orthodontic archwires demonstrated clear antimicrobial effects against various evaluated microorganisms, encompassing *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Aggregatibacter actinomycetemcomitans*, and *Candida albicans*.[33]

The outcomes of an independent samples *t* test revealed notable differences, with *P* value of 0.041 for *Streptococcus mutans* and 0.011 for *Lactobacillus acidophilus*, indicating that the inhibitory zones around the coated stainless steel bracket groups were significantly larger than those surrounding the coated ceramic bracket group. This may indicate increased attachment of nanoparticles on stainless steel brackets, as evident in the release test.

This pioneering approach of employing CHX-HMP NPs as coatings for orthodontic brackets signifies a promising step towards minimizing microbial plaque formation, thereby potentially reducing the risk of secondary oral health issues during orthodontic treatments. However, further investigations are warranted to explore the clinical implications of these coated brackets, including their long-term antimicrobial effects, impact on biofilm reduction, and biocompatibility within the oral environment.

CONCLUSION

The CHX-HMP NPs employed in this study were shown to provide a steady release of chlorhexidine over the duration of the study extended for 60 days. At the same time, it inhibits the bacterial growth ability of *Streptococcus mutans*, and *Lactobacillus acidophilus*. This study implies that CHX-HMP NPs exhibit significant potential for biomedical applications, particularly in preventing dental caries among individuals undergoing orthodontic treatments.

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Nil.

Conflicts of interest

The authors state that there are no conflicts of interest.

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