Dental Research Journal

Original Article

Antimicrobial properties of glass-ionomer cement incorporated with nano-hydroxyapatite against mutans streptococci and lactobacilli under orthodontic bands: An *in vivo* split-mouth study

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ABSTRACT

Background: Fixed orthodontic appliances enhance dental plaque accumulation. Glass ionomer (GI) is among the most popular orthodontic cement. It possesses antibacterial properties; however, its antibacterial activity may not be sufficient for caries prevention. Although evidence shows that the addition of 8wt% nano-hydroxyapatite (nHA) may enhance the antibacterial properties of GI, no clinical study has been conducted in this respect. Thus, this study aimed to assess the subgingival accumulation of *Streptococcus mutans* (S. mutans) and *Lactobacillus acidophilus* (*L. acidophilus*) around orthodontic bands cemented with conventional GI and GI reinforced with 8wt% nHA.

Materials and Methods: This split-mouth clinical trial was conducted on 20 patients requiring a lingual arch. The patients were randomly assigned to two groups. In group 1, the right molar band was cemented with pure Fuji I (GC), and the left was cemented with Fuji I containing 8wt% nHA. In group 2, the right molar band was cemented with Fuji I containing 8wt% nHA, and the left was cemented with Fuji I. After 3 months, subgingival sampling was performed by sterile paper points. *S. mutans* and *L. acidophilus* were cultured on MSB and MRS agar, and colonies were counted by a colony counter. Data were analyzed by independent samples *t*-test using SPSS 25 at a 0.05 level of significance.

Results: The mean counts of *S. mutans*, aerobic and anaerobic lactobacilli, and total bacterial around orthodontic bands cemented with Fuji I containing 8wt% nHA were significantly lower than those around orthodontic bands cemented with pure Fuji I ($P < 0.05$).

Conclusion: The addition of 8wt% nHA to GI cement can enhance its antibacterial properties for the cementation of orthodontic bands, decrease the accumulation of cariogenic bacteria, and probably decrease the incidence of caries in orthodontic patients.

Key Words: Glass ionomer cement, hydroxyapatite, *Lactobacillus*, *Streptococcus mutans*

Received: 12-Apr-2023
Revised: 19-Oct-2023
Accepted: 30-Oct-2023
Published: 25-Jan-2024

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INTRODUCTION

Orthodontic treatment is performed aiming to improve dental and skeletal relationships. Some patients require fixed orthodontic treatment. Due to more efficient anchorage and elimination of the confounding effect of patient cooperation on the treatment progress,

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Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Shirazi M, Pirzeh A, Atashgaran M. Antimicrobial properties of glass-ionomer cement incorporated with nano-hydroxyapatite against mutans streptococci and lactobacilli under orthodontic bands: An *in vivo* split-mouth study. Dent Res J 2024;21:1.

fixed orthodontic appliances often bring about more favorable results than removable appliances.[1] Despite such advantages, fixed orthodontic appliances installed in the oral cavity enhance food impaction due to their irregular surface. Furthermore, they interfere with effective oral hygiene practice and prevent self-cleaning by the action of saliva and muscles of mastication. Resultantly, the ecology of oral biofilm changes, leading to colonization and proliferation of aciduric and acidogenic bacteria such as *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*) in the oral environment.[2] Thus, although orthodontic treatment can decrease the incidence of caries in the long term, it makes the teeth more susceptible to caries during the treatment course, especially with fixed appliances. Therefore, it is highly important to decrease the risk of caries in orthodontic patients.[2,3]

Although effective oral hygiene practice, cutting down the consumption of fermentable carbohydrates, and regular dental visits can effectively decrease the incidence of caries, all these measures require the cooperation of patients and parents (in pediatric dental patients), and thus, they are not highly reliable in orthodontic patients, particularly in children and adolescents. Therefore, the application of cement with antibacterial properties is recommended for the cementation of fixed orthodontic appliances for caries prevention since they do not require patient cooperation.[4-6] A wise approach toward this problem is incorporation of antimicrobial agents into orthodontic materials. Several studies have been conducted to tackle this problem by adding various nanoparticles (NPs) with antimicrobial properties into orthodontic cement and adhesives.[7]

Glass ionomer (GI) cement is the most commonly used cement for the cementation of orthodontic bands followed by zinc phosphate cement. Resin-modified GI and resin cement are less commonly used for this purpose. Optimal biocompatibility, having a coefficient of thermal expansion close to that of tooth structure, chemical adhesion of GI to tooth structure and metals, and more importantly, fluoride release potential are the main factors responsible for the popularity of GI cement. Fluoride ions interfere with the synthesis of bacterial glycosyltransferase and prevent bacterial adhesion. They also interfere with bacterial metabolism and prevent bacterial colonization and subsequent caries development. They improve the resistance of enamel and dentin to demineralization and enhance their remineralization. All these factors are responsible for the selection of GI as the cement of choice for the cementation of orthodontic bands.[5,8,9] Nonetheless, enhancement of the antibacterial properties of GI can help minimize the risk of caries in orthodontic patients.

With the advances in nanotechnology, researchers have focused on the improvement of the properties of dental materials by using NPs. Hydroxyapatite (HA) is a commonly used biomaterial in medicine and dentistry. HA has significant biological activity, degradability, and osteoconductivity. In recent decades, it has been widely used in dentistry, antitumor drug carriers, and orthopedic repair.[10] It is a crystalline bioceramic containing calcium and phosphate with the formulation of Ca10(PO4) 6(OH) 2, which comprises the mineral phase of the enamel, dentin, bone, and cementum. Studies also found that nHA comes with various unique kind of properties like it does not induce any inflammation or toxicity.[11] Evidence shows that *in vivo* application of synthetic forms of HA crystals does not elicit any inflammation. Due to its optimal biocompatibility and biological activity, nano-HA (nHA) is currently the most commonly used NP in medicine and dentistry. HA is also used in the pharmaceutical industry for the treatment of tumors, reduction of dentin hypersensitivity, bone regeneration, and implant coating. The biological apatite has a nanoscale size. Thus, the size of synthetic nHA is close to the size of biological apatite and is more biocompatible than HA. Moreover, nHA has higher surface energy and higher solubility than larger HA particles due to having a larger surface/volume ratio. It is also more active and more efficient in lower concentrations. Recent *in vitro* studies have shown that the addition of nHA to GI cement enhances its antibacterial properties. Several studies assessed the effect of the addition of HA to GI and showed that it not only enhanced the antibacterial properties of GI but also decreased microleakage, increased its bond strength to enamel and dentin, improved its wear resistance, shear strength, flexural strength, and compressive strength, and even increased fluoride release from GI.[12,13]

HA, as a rich source of calcium and phosphate, has been used for enamel remineralization in several studies, and it has been demonstrated that its oral application in the composition of toothpaste, mouthwash, etc., can efficiently enhance enamel remineralization and increase surface hardness.[14-16]

Although the application of fixed orthodontic appliances increases the load of mutans streptococci and lactobacilli and can lead to enamel demineralization and caries, the efficacy of cement used for cementation of orthodontic bands for the reduction of cariogenic bacteria has not been well investigated. Thus, this study aimed to compare the effects of conventional and nHA-reinforced GI cement on subgingival accumulation of *S. mutans* and *L. acidophilus* adjacent to orthodontic bands.

MATERIALS AND METHODS

This split-mouth clinical trial study was conducted on 20 patients, according to similar studies and using the following formula (20 samples in each group were calculated).

$$n = (Z1\text{-}\alpha/2 + Z1\text{-}\beta)^2\ (S12 + S22)/(\mu1 - \mu2)^2$$

Patients aged 7–10 years (8 girls and 12 boys), who needed mandibular lingual holding arch based on their pediatric and orthodontic treatment plan. This study was approved by the Ethical Committee of Qazvin University of Medical Sciences with an ethical number of IR.QUMS.REC.1398.386. There is no conflict with ethical considerations.

Patients were selected based on the following inclusion criteria: (1) guardians' consent for participation in the study, (2) good oral hygiene (plaque index <10%), (3) absence of active dental caries or periodontal disease, (4) equal conditions in the right and left quadrants concerning the presence of Es (primary second molars), (5) no systemic disease, and (6) no use of chlorhexidine mouthwash or antibiotics in the past 3 weeks. During the study, patients who reported using chlorhexidine mouthwash or antibiotics were planned to be excluded. Moreover, cement washout and subsequent loosening of the band would have resulted in exclusion from the study.[17]

The parents or legal guardians of children were briefed about the study and signed informed consent forms for the participation of their children in the study. All patients underwent a primary clinical examination with a dental probe and an explorer to assess caries and periodontal disease. Eligible patients were enrolled after being informed about the possible advantages of materials that decrease bacterial accumulation, ensuring the parents about the confidentiality of patient information, and obtaining their informed consent.[16]

In the first treatment session, the patients underwent clinical oral examination by a dental mirror and a Williams periodontal probe for the presence of supragingival calculus or periodontitis. In the case of the presence of supragingival calculus, it was removed by a scaler. All patients received dental prophylaxis and oral hygiene instructions. The modified Bass toothbrushing technique and correct use of dental floss were instructed to patients as well.[18] Furthermore, they were asked to brush their teeth and floss twice daily under the supervision of their parents during the study period.

For standardization of the two quadrants, similar bands (3M Unitek, USA) were used for orthodontic treatment of the mandibular arch with a lingual arch in all patients. An experienced and skillful orthodontist selected the bands with maximum adaptation and also made the impressions. The selected band followed the tooth contour, and its inferior margin was located subgingivally to prevent plaque accumulation between the band and the gingival margin. Alginate was used for impressions. Alginate impressions were rinsed with water and immersed in 0.05% sodium hypochlorite for 10 min. After rinsing the alginate impression, the bands were fixed in place, and the impressions were poured with dental stone within 20 min.[19] Dental casts were transferred to a dental laboratory for the fabrication of lingual arch. After fabrication and intraoral assessment of its adaptation, the lingual arches were autoclave-sterilized at 121°C and 15 pounds/inch2 pressure for 15 min before cementation.

The n-HA (NanoSany Corporation, Iran) was sterilized by gamma radiation.[20] To obtain 8wt% nHA, a digital scale (Sartorius, Germany) with 0.000010 g accuracy was used. After calculating the amount of nHA required for 8wt% concentration, it was mixed with GI cement powder (Fuji I, GC Corporation, Japan) on a glass slab with a spatula. To ensure optimal homogenization, the mixture was placed in an amalgam capsule and mixed in an amalgamator for 20 s.[21]

To prepare the cement, one scoop of powder was mixed with two drops of liquid in a 1:8–1 ratio by weight using a plastic spatula on a cold glass slab as instructed by the manufacturer. Mixing took 20 s until a creamy consistency was achieved, such that a 1-inch string was formed when the spatula was pulled away from the glass slab.[22] In both the groups, the

ratio of powder to liquid is the same as instructed by the manufacturer, but in the nHA group, the powder contains 8wt% nHA.

The respective areas in the oral cavity were completely isolated, the cement were applied inside the inferior border of the bands by a spatula, and the bands were placed and adapted to the teeth by an appropriate instrument. Excess cement was removed. The entire process (from initiation of cement mixing to completion of cementation of bands) took 2 min.

Cementation of the lingual arch was performed by an experienced clinician for all patients. Simple random allocation of patients to the two groups and cement preparation were performed by another operator. The clinician who cemented the lingual arches was blinded to the type of cement and group allocation of patients.

Patients were divided into two groups: group 1; right molar bands were cemented with a conventional GIC (Fuji II SC, GC Corp., Tokyo, Japan), while left molar bands were cemented using the same GIC containing 8 wt% nHA. The opposite was performed for group 2.

The patients were recalled after a 3-month interval for clinical examination and sampling. This time interval allowed for colonization and proliferation of bacteria, and was not long enough to allow cement washout and loosening of bands.[23]

The patients were asked to brush their teeth 12 h before sampling, and the parents were requested to supervise.[24] For sampling, the area was isolated with cotton rolls, visible plaque on the surface of bands was removed from the buccal surface with a sterile gauze, and a sterile paper point was used to collect subgingival plaque from the mid-buccal area for 15 s. The collected samples were transferred to microtubes containing 1 mL of sterile transfer liquid and were sent to a laboratory in a cold box.[17,25,26]

For microbiological assessments, the samples were vortexed in a mixer at 1500 rpm for 15 s and were diluted by 10 folds to 10-2.[24]

To isolate and count *S. mutans* colonies, 0.1 mL of the primary sample and each dilution was lawn-cultured on MSB agar (Mitis salivarius bacitracin [Liofilchem, Italy]) containing 0.001% telorite, 15% sucrose, and 0.2 U/mL bacitracin and incubated at 37°C in an anaerobic jar (Anoxomat, Germany) for 48 h.

To isolate and count *L. acidophilus* colonies, 0.1 mL of the primary sample and each dilution were lawn-cultured on two MRS agar plates (DeMan, Rogosa, and Sharpe [Liofilchem, Italy]). One plate was incubated (Binder, USA) under aerobic conditions and another one was incubated at 37°C under anaerobic conditions in an anaerobic jar (Anoxomat, Germany) for 48 h. To increase accuracy, all procedures were repeated in triplicate. Suspected colonies were further assessed morphologically and also by conduction of biochemical tests. Next, the number of colonies was counted and reported as colony-forming units (CFUs) per milliliter.

CFU = number of counted colonies' 1/dilution factor[17]

Data were analyzed using SPSS version 25(.IBM SPSS, Inc. in Chicago, Illinois) Independent samples *t*-test was used to compare the count of aerobic and anaerobic lactobacilli and *S. mutans* in the two groups at a 0.05 level of significance.

RESULTS

Table 1 shows the descriptive data regarding bacterial count and the comparison of bacterial count in two cement groups.

The mean number of total bacteria, aerobic *Lactobacillus*, anaerobic *Lactobacillus*, and mutans streptococci in the Fuji II SC cement containing nHA group was significantly lower than these values in the plain Fuji II SC cement group ($P < 0.05$).

DISCUSSION

Dental caries around orthodontic bands have always been one of the most important complications of orthodontic treatment. It has been well confirmed that initiation of orthodontic treatment and installation of fixed orthodontic appliances enhance the accumulation

Table 1: Comparison of the mean total and separate bacterial counts in the cement groups (colony-forming units per mL) (the number of samples 20 in each group)

Bacteria type	Cement type		Significance
	Fuji II SC with nHA	Fuji II SC	
Total bacteria	9.77×10^5	28.02×10^5	<0.001
Anaerobic lactobacilli	1.66×10^5	2.62×10^5	<0.001
Aerobic lactobacilli	1.02×10^5	2.34×10^4	<0.001
Streptococcus mutans	0.81×10^5	1.87×10^5	<0.001

of dental plaque and microbial species such as *S. mutans* and *L. acidophilus* in the oral environment. These bacteria produce organic acids and cause enamel demineralization and incipient enamel lesions on the tooth surface within a short period.[5] Increased enamel susceptibility to caries upon initiation of orthodontic treatment highlights the need for measures to reduce the count of cariogenic microorganisms.[27] Since the addition of nHA particles to GI enhances its antibacterial properties *in vitro*, this study aimed to assess the effect of the addition of nHA to GI cement on subgingival accumulation of *S. mutans* and *L. acidophilus* adjacent to orthodontic bands under *in vivo* conditions.[12,13]

In the present study, an 8wt% concentration of nHA was used since previous studies tested different concentrations of nHA and found that the addition of 8wt% nHA to GI cement conferred the highest antibacterial activity to the cement.[12,13]

This study had a split-mouth design, allowing the application of both cement in the oral cavity of the same patients. Accordingly, the confounding effect of different intraoral conditions of patients on the results was eliminated. Furthermore, the patients received similar oral hygiene instructions to minimize the effect of different levels of oral hygiene practice on the results.

In the present study, the mean anaerobic *Lactobacillus* count was 1.66' 105 ± 0.11' 105 CFUs/mL^{-1} in the nHA group and 2.62' 105 ± 0.08' 105 CFUs/mL^{-1} in the GI group without nHA. The mean aerobic *Lactobacillus* count was 1.02' 105 ± 0.14' 105 CFUs/mL^{-1} in the nHA group and 2.34' 105 ± 0.13' 105 CFUs/mL^{-1} in the GI group without nHA. The mean *S. mutans* count was 0.81' 105 ± 0.09' 105 CFUs/mL^{-1} in the nHA group and 1.87' 105 ± 0.13' 105 CFUs/mL^{-1} in the GI group without nHA. The mean count of aerobic and anaerobic lactobacilli and *S. mutans* in the Fuji I group containing nHA was significantly lower than that in the Fuji I group. Thus, the addition of nHA improved the antibacterial activity of GI cement against the tested microorganisms.

Batra *et al*., in a review study, stated that nHA can be used as an antibacterial agent that can be applied as a coating on orthodontic brackets and dental implants or can be incorporated in the composition of composite resins, resin-modified GI, acrylic resins, and elastomeric ligatures. They also reported that it can be used for bone regeneration, and also enamel remineralization, and improvement of surface hardness by incorporation in the composition of toothpaste.[28]

Hilal *et al*. compared the antibacterial properties of HA, GI cement, gutta-percha, and amalgam for application as root-filling material. They confirmed the antibacterial activity of HA, which was in line with the present findings.[29] Tin-Oo *et al*., in their *in vitro* study, assessed the antibacterial properties of different concentrations of HA against *S. mutans*. They found that it had antibacterial activity even in the lowest tested concentration, i.e., 50 mg/mL. The highest antibacterial activity was noted in 200 mg/mL concentration. Further increase in concentration had no further inhibitory effect on the bacteria. Their results confirmed the inhibitory effect of HA on *S. mutans*, which was in agreement with the present results.[30]

Owadally *et al*. assessed the biological properties of some dental materials as root-filling material. They assessed and compared the antibacterial properties of zinc oxide eugenol containing 10% HA, zinc oxide eugenol containing 20% HA, pure zinc oxide eugenol, and amalgam. They found that zinc oxide eugenol containing 10% HA and 20% HA and also a pure form of zinc oxide eugenol had similar antibacterial activity and were superior to amalgam. In the present study, nanometer-scale HA particles were used which have greater effects than larger particles due to a very high surface-to-volume ratio. Controversy in the results can be due to differences in the type of nHA particles and the base material. Furthermore, since the antibacterial effects of HA have been confirmed, zinc oxide eugenol may prevent the antibacterial effects of nHA. Furthermore, the structural details of HA particles and their method of synthesis can affect their antibacterial properties. Therefore, such details should be investigated in future studies.[31]

Several other studies assessed the antibacterial activity of GI cement containing nHA. Raedah *et al*., in 2018, evaluated the effect of nHA on GI cement *in vitro*. They compared GI cement containing 1, 3, 5, 7, and 10% HA. They showed that pure GI had no inhibitory effect on bacterial culture but GI containing 1wt% HA (the lowest concentration tested) had some degrees of antibacterial activity. Between tested concentrations, GI containing 8wt% HA caused the largest bacterial growth inhibition zone in the culture medium containing *S. mutans*.

Thus, this concentration was also selected for use in the present study. Moreover, the results revealed that the addition of nHA to GI significantly increased the release of fluoride ions, which may be one reason for the increased antibacterial activity of this cement, confirming the present results.[12]

Bali *et al*., in 2015, compared the antibacterial activity and compressive strength of conventional GI and GI containing 8wt% HA against *S. mutans* under *in vitro* conditions. They found that the addition of 8wt% HA to GI cement significantly increased its antibacterial activity, which was in line with the present findings.[13]

In general, since the antibacterial properties of nHA have not yet been fully understood, some possible mechanisms have been proposed which will be discussed below. The surface roughness of dental materials has a direct correlation with bacterial adhesion.[9,32] The addition of nHA to GI cement decreases the mean size of GI particles, their surface roughness, and the adhesion of bacteria.

According to the literature, the incorporation of nHA in the structure of GI cement increases the fluoride ion release.[12,33] The released fluoride ions interfere with the synthesis of bacterial metabolic enzymes, and impair energy production and synthesis of polysaccharides, which are involved in bacterial attachment to surfaces.[5] Accordingly, the addition of nHA to GI cement can enhance its antibacterial properties. The addition of nHA as a rich source of calcium and phosphate can increase the concentration of ions in the oral environment around orthodontic bands, which can change the bacterial membrane structure, increase membrane permeability, and cause bacterial cell death.[34,35]

Incipient enamel caries have been reported in 12.6%–50% of patients under treatment with fixed orthodontic appliances.[36] These lesions may have a rough surface and enhance bacterial adhesion as such.[5,32] Considering the optimal efficacy of HA in the remineralization of incipient enamel caries, it is possible that the incorporation of nHA in orthodontic band cement decreases the surface roughness of these lesions and prevents bacterial adhesion as such.[37]

Andreas *et al.* showed that the amount of released calcium ions from calcium phosphate compounds has a direct correlation with their antibacterial activity against *S. mutans* and *Lactobacillus casei*.[38] These findings may indicate that calcium ions released from HA, which is a calcium phosphate compound, can have antibacterial effects on these bacteria.

CONCLUSION

The results of the present clinical study showed that the addition of 8wt% nHA to GI significantly improved its antibacterial activity, decreased the accumulation of *S. mutans* and lactobacilli around orthodontic bands, and may decrease the risk of caries in orthodontic patients.

Limitations

This study had the following limitations:

- Difficulty in finding eligible patients for study inclusion
- The difficulty of the conduction of microbiological tests and the possibility of errors
- Split mouth study design's limitations such as difficulty in finding similar comparison sites and the requirement for more complex data analysis
- Unavailability of spiral plater for automatic bacterial culture.

To overcome these limitations, several orthodontists were asked to introduce eligible patients for possible study inclusion. Furthermore, in microbiological assessments, several culture media were prepared for each sample to decrease errors.

Suggestions

Future long-term clinical studies with a larger sample size are required on the effects of different GI cement containing nHA for the prevention of caries around orthodontic appliances and space maintainers. Further studies are also recommended on the application of GI cement containing nHA for the prevention of recurrent caries under crowns and restorations. The antibacterial activity of GI cement containing nHA should also be investigated against different strains of lactobacilli and mutans streptococci. Molecular cellular assessments are required on the mechanisms of antibacterial properties of nHA as well.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Hugo T, Reginaldo C, Trevisi Z. State-of-the-Art Orthodontics. 1st ed. Elsevier Health Sciences; 2011.
2. Mummolo S, Tieri M, Nota A, Caruso S, Darvizeh A, Albani F, *et al.* Salivary concentrations of *Streptococcus mutans* and lactobacilli during an orthodontic treatment. An observational study comparing fixed and removable orthodontic appliances. Clin Exp Dent Res 2020;6:181-7.
3. Chen W, Zhou Y. Caries outcomes after orthodontic treatment with fixed appliances: A longitudinal prospective study. Int J Clin Exp Med 2015;8:2815-22.
4. Cozzani M, Ragazzini G, Delucchi A, Mutinelli S, Barreca C, Rinchuse DJ, *et al*. Oral hygiene compliance in orthodontic patients: A randomized controlled study on the effects of a post-treatment communication. Prog Orthod 2016;17:41.
5. Ritter AV, Boushell LW, Walter R. Sturdevant's Art and Science of Operative Dentistry. 7th ed. St. Louis, Missouri: Elsevier; 2019.
6. Fallahinejad Ghajari M, Eslamian L, Naji Rad A, Morovati SP. Efficacy of glass ionomer cements for prevention of white spot lesions during orthodontic banding: A randomized clinical trial. J Dent (Tehran) 2015;12:913-20.
7. Shirazi M, Qazvini FF, Mohamadrezaie S. Antimicrobial properties of glass-ionomer cement incorporated with zinc oxide nanoparticles against mutans streptococci and lactobacilli under orthodontic bands: An *in vivo* split-mouth study. Dent Res J (Isfahan) 2023;20:45.
8. Powers JM, Sakaguchi RL, Craig RG. Craig's Restorative Dental Materials. 12th ed. St Louis: Mosby Elsevier; 2006.
9. Richard VN. Introduction to Dental Materials. 4th ed. Mosby Ltd; 2013.
10. Du M, Chen J, Liu K, Xing H, Song C. Recent advances in biomedical engineering of nano-hydroxyapatite including dentistry, cancer treatment and bone repair. Compos Part B Eng 2021; 215,:108790.
11. Abere D, Ojo S, Oyatogun G, Paredes-Epinosa M, Niluxsshun M, Hakami A. Mechanical and morphological characterization of nano-hydroxyapatite (nHA) for bone regeneration: A mini-review. Biomed Eng Adv 2022; 4:100056.
12. Alatawi RA, Elsayeda NH, Mohamed WS. Influence of hydroxyapatite nanoparticles on the properties of glass ionomer cement. J Mater Res Technol 2019;8:344-9.
13. Bali P, Prabhakar AR, Basappa N. An *in vitro* comparative evaluation of compressive strength and antibacterial activity of conventional GIC and hydroxyapatite reinforced GIC in different storage media. J Clin Diagn Res 2015;9:C51-5.
14. Liu Q, Huang S, Matinlinna JP, Chen Z, Pan H. Insight into biological apatite: Physiochemical properties and preparation approaches. Biomed Res Int 2013;2013:929748.
15. Al-Sanabani JS, Madfa AA, Al-Sanabani FA. Application of calcium phosphate materials in dentistry. Int J Biomater 2013;2013:876132.
16. Priyadarsini S, Mukherjee S, Mishra M. Nanoparticles used in dentistry: A review. J Oral Biol Craniofac Res 2018;8:58-67.
17. Shirazi M, Tamadon M, Izadi M. Effect of addition of bioactive glass to glass ionomer cement on accumulation of *Streptococcus mutans* and lactobacilli around orthodontic band. OHDM 2017; 11 (6): e521–e526.
18. Sargolzaie N, Amel-Jamedar S, Mokhtari M, Arab H, Piroozi S. Evaluation of subgingival dental plaque microbiota changes in fixed orthodontic patients with syber green real-time PCR. J Dent Mater Tech 2014;3:123-7.
19. Patil SP, Patil PB, Kashetty MV. Effectiveness of different tooth brushing techniques on the removal of dental plaque in 6-8 year old children of Gulbarga. J Int Soc Prev Community Dent 2014;4:113-6.
20. John P, John W. Dental Materials Foundations and Applications. 11th ed. St. Louis, Missouri: Elsevier; 2013.
21. Colaço R, Pires E, Costa T, Serro AP. Influence of sterilization with y-irradiation in the degradation of plasma sprayed hydroxyapatite coatings. Mater Sci Forum 2006; 514–516 (2):1054-1058.
22. Sharafeddin F, Feizi N. Evaluation of the effect of adding micro-hydroxyapatite and nano-hydroxyapatite on the microleakage of conventional and resin-modified glass-ionomer Cl V restorations. J Clin Exp Dent 2017;9:e242-8.
23. Stewart M, Bagby M. Clinical Aspects of Dental Materials. 5th ed. Jones and Bartlett Learning; 2020.
24. Haraszthy VI, Sreenivasan PK. Microbiological and clinical effects of an oral hygiene regimen. Contemp Clin Trials Commun 2017;8:85-9.
25. Zhou X, Li Y. Atlas of Oral Microbiology. 4th ed. Academic Press; 2015.
26. Ronald MA. Handbook of Microbiological Media. 4th ed. Washington D. C.: CRC Press; 2010.
27. Avery DR, McDonald RE. Dentistry for the Child and Adolescent. 10th ed. St. Louis, Missouri: Elsevier; 2016.
28. Batra P, Mushtaq A, Mazumder J, Rizvi M, Miglani R. Nanoparticles and their applications in orthodontics. Adv Dent Oral Health 2016; 2 (2): 555584.
29. Hilal MK. The antibacterial property of hydroxyapatite compared to glass ionomer cement and amalgam. Int J Sci Res 2012;3:402-4.
30. Tin-Oo MM, Gopalakrishnan V, Samsuddin AR, Al Salihi KA, Hamsuria O. Antibacterial property of locally produced hydroxyapatite. Arch Orofac Sci 2007;2:41-4.
31. Owadally ID, Chong BS, Pitt Ford TR, Wilson RF. Biological properties of IRM with the addition of hydroxyapatite as a retrograde root filling material. Endod Dent Traumatol 1994;10:228-32.
32. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Newman, and Carranza's Clinical Periodontology. 13th ed. Philadelphia, PA: Elsevier; 2019.
33. Arita K, Yamamoto A, Shinonaga Y, Harada K, Abe Y, Nakagawa K, *et al*. Hydroxyapatite particle characteristics influence the enhancement of the mechanical and chemical properties of conventional restorative glass ionomer cement. Dent Mater J 2011;30:672-83.
34. Halder S, Yadav KK, Sarkar R, Mukherjee S, Saha P, Haldar S, *et al.* Alteration of zeta potential and membrane permeability in bacteria: A study with cationic agents. Springerplus 2015;4:672.
35. Cieplik F, Rupp CM, Hirsch S, Muehler D, Enax J, Meyer F, *et al.* Ca (2)(+) release and buffering effects of synthetic hydroxyapatite following bacterial acid challenge. BMC Oral Health 2020;20:85.

36. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod 1986;8:229-34.
37. Reis PQ, da Silva EM, Calazans FS, Lopes LS, Poubel LA, Alves WV, *et al.* Effect of a dentifrice containing nanohydroxyapatite on the roughness, color, lightness, and brightness of dental enamel subjected to a demineralization challenge. Gen Dent 2018;66:66-70.
38. Koutroulis A, Kuehne SA, Cooper PR, Camilleri J. The role of calcium ion release on biocompatibility and antimicrobial properties of hydraulic cements. Sci Rep 2019;9:19019.