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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

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ABSTRACT

Background: The aim of this study was to assess the effect of incorporation of zinc oxide nanoparticles (ZnO-NPs) to glass-ionomer cement (GIC) (Fuji II SC, GC Corp., Tokyo, Japan) on subgingival accumulation of mutans streptococci and lactobacilli under orthodontic bands.

Materials and Methods: In order to conduct this *in vivo* split-mouth study, 20 patients aged between 7 and 10 years who required lingual holding arch on their mandibular first molars were divided into two groups. In one group, Fuji II SC GIC was used for cementation of the right molar band, and the same cement containing 2 wt% ZnO-NPs was used for the left one. The opposite was performed for the second group while the operator was blinded to the cement types. Subgingival microbial sampling was performed 16 weeks after cementation of lingual arch. Mutans streptococci and lactobacilli colony counts were compared. Paired *t*-test was used to compare the two cement groups. Data were analyzed using SPSS version 21, and $P \leq 0.05$ was considered statistically significant.

Results: The mean colony counts of mutans streptococci, lactobacilli, and total bacterial count in Fuji II SC containing ZnO-NPs were significantly lower than the corresponding values in plain Fuji II SC group.

Conclusion: Incorporation of ZnO-NPs into GIC reveals antimicrobial features against mutans streptococci and lactobacilli under orthodontic bands.

Key Words: Antimicrobial agents, glass-ionomer cement, nanoparticle

INTRODUCTION

Fixed orthodontic treatment makes profound changes in oral microenvironment by facilitating plaque accumulation around brackets and margin of bands. It also increases the number of *Streptococcus mutans* which is the main primary cariogenic pathogen, and *Lactobacillus acidophilus* which plays a crucial role in progression of severe dental caries.[1,2]

Since fixed orthodontic treatment usually takes long enough to cause serious caries, patients undergoing this treatment are more susceptible to both white spot lesions – the very first manifestations of dental caries – and the consequent more destructive cavitated caries.[2-4]

Traditional oral hygiene maintenance methods are not reliable enough due to their extreme dependence on

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the patient's compliance, and might be impaired in noncompliant, pediatric, and disabled individuals.[5] Complete removal of dental plaque around brackets and bands only by brushing is not readily feasible. Therefore, demineralization would be more efficiently managed by changing the oral condition around the bands and brackets.[6,7] In addition, high frequency of white spot lesions in orthodontic patients implies that routine dental care approaches have failed to hamper the initiation of dental caries in these patients.[8] Application of glass-ionomer cement (GIC) has been a milestone in this regard due to its gradual fluoride release as well as the reasonable strength and high biocompatibility.[9] Although GIC is the most popular material for band cementation based on its invaluable properties, conventional GI has limited antibacterial potential which makes it some what vulnerable in terms of caries prevention.[10,11]

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.[4,5,7,12-17]

In recent years, zinc oxide NPs (ZnO-NPs) with verified antimicrobial features have been widely used in the composition of different dental materials such as cement, composites, acrylic resins, and bonding agents due to their antibacterial properties and biocompatibility.[12,13,18-22]

Jatania and Shivalinga conducted an *in vitro* study to evaluate the effects of addition of ZnO to an orthodontic bonding agent. They concluded that incorporation of ZnO into a resin-modified light-cure GIC added antimicrobial property to the original compound.[18] Vanajassun *et al*. also evaluated the effects of ZnO-NPs in combination with conventional GIC; there was a significant increase in antimicrobial property of set GIC with ZnO-NPs without modifying the mechanical properties.[23]

However, most of these precious researches have been performed *in vitro*, and lack of *in vivo* data in literature is conspicuous. Due to the dynamic and complex physiology of oral cavity, results of *in vitro* studies cannot be authoritatively generalized to *in vivo* conditions. Therefore, the aim of the present study was to evaluate the antimicrobial properties of GIC incorporated with ZnO-NPs through an *in vivo* split-mouth study.

MATERIALS AND METHODS

According to similar studies and using the following formula, the number of samples 20 in each group (totally 20 patients right and left side) was calculated

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

This *in vivo* split-mouth study was conducted on 20 patients aged 7 to 10 years (8 girls and 12 boys), who needed mandibular lingual holding arch (LHA) based on their pediatric and orthodontic treatment plan that referred to Qazvin Dental Faculty. This study was approved by the Ethical Committee of Qazvin University of Medical Sciences with ethical number of IR.QUMS.REC.1397.256. There is no conflict with ethical considerations.

The aim and process of the study were fully explained to the patients and their parents, and informed consent was obtained.

Patients were selected based on the following inclusion criteria: (1) guardians' consent for participation in the study; (2) good oral hygiene; (3) absence of active dental caries or periodontal disease; (4) no systemic disease; and (5) 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 band would have resulted in exclusion from the study.

Patients were divided into one of the study 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 2 wt% ZnO-NPs.[24] The opposite was performed for group 2.

The ZnO-NPs used purity 99+% and particle size 10–30 nm (Nanosany, Mashhad, Iran; Figures 1 and 2 show the scanning electron microscopy and X-ray diffraction of the ZnO NP that were sent by the manufacturer). The required amount of powder according to the standard mixing protocol of GIC was weighed by a digital scale (FX300L) with 0.001 g accuracy. Next, 2 wt% of it was removed and replaced with the same amount of ZnO-NPs weighed by the digital scale. The added ZnO-NPs were mixed with the remaining 98% powder. To ensure the complete mixing of the two powders, the final compound was placed in an amalgam capsule and

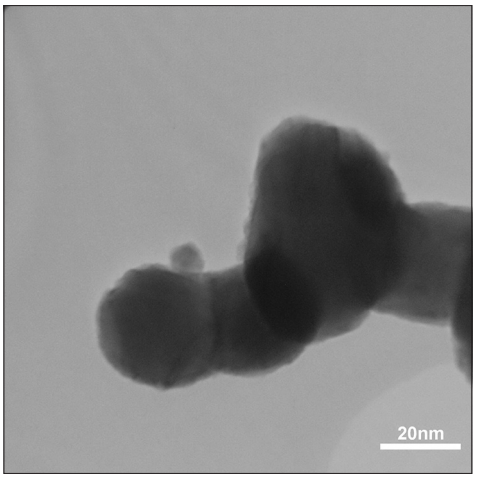

Figure 1: SEM results of ZnO-NPs. SEM: Scanning electron microscopy, ZnO-NPs: Zinc oxide nanoparticles.

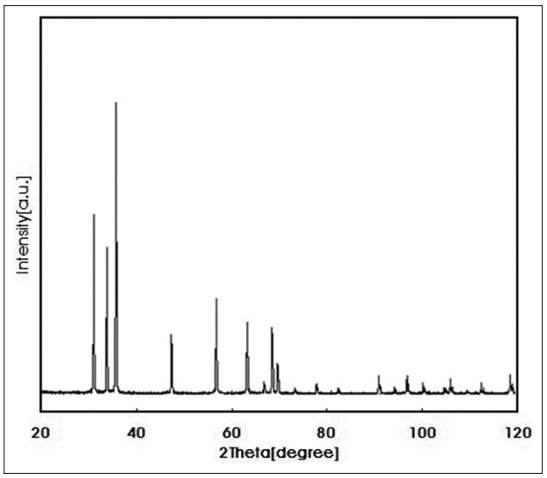

Figure 2: XRD results of ZnO-NPs. XRD: X-ray diffraction, ZnO-NPs: Zinc oxide nanoparticles.

mixed in an amalgamator for 20 s (Ultramat 2, SDI Limited, Bayswater, Victoria, Australia).

Patients were thoroughly examined and oral hygiene instructions including appropriate brushing technique and flossing were given to them.

Orthodontic bands (American Orthodontics, Sheboygan, WI, US) were selected, fitted, and adapted for the patients. Finally, impressions were taken and sent to dental laboratory for fabrication of LHAs. Two days later, when appliances were ready, patients were recruited for cementation. All lingual holding arches were autoclave-sterilized before insertion, and teeth were cleaned with pumice and isolated via cotton rolls. Cement were prepared based on the manufacturer's instruction and cementation was performed by a single clinician blinded to the type of cement for this aim the process was done by two persons, one person prepared the material and put it inside the lingual arch, and the other cemented lingual arch in mouth without knowing the type of cement in each side also the sample was collected by a person who was unaware of the type of cement. All the cement remnants have been cleaned from the band edges.

After placement of lingual arches, the patients were recruited again and clinically examined. The 16 weeks was selected to allow adequate proliferation of bacteria without washout of cement or loosening of bands. The cement had not been washed away, and the bands were not loose in any of the patients. Using sterile gloves, isolation was performed with cotton rolls, and visible dental plaque was removed from the buccal tooth surface using a sterile gauze. Afterward, subgingival sampling in the mid-buccal region of all subjects was performed for 15 s using sterile paper points. The process was performed by a clinician blinded to the cement types. The samples were placed in 4 ml of Stuart's transport medium and sent to the microbiology laboratory.

In order to isolate and count mutans streptococci, samples were diluted, and 0.1 ml of each dilute was transferred to Mitis Salivarius Agar culture medium (including 0.001% tellurite solution, 15% sucrose, and 0.2 U/ml bacitracin) and incubated in anaerobic conditions at 37°C for 48 h. Investigation of lactobacilli started with adding 0.1 ml of each dilute to two plates containing MRS agar. The plates were then incubated at 37°C for 48 h, one in aerobic and the other in anaerobic conditions. The suspected colonies were evaluated by inspecting the macroscopic properties of colonies (large and white colonies) and conduction of biochemical tests (microscopic assessment after Gram staining, evaluation of motility, and use of catalase and glucose tests).

To determine the total count of bacteria, 0.1 ml of each dilute was transferred to blood agar culture and the samples were incubated in aerobic conditions at 37°C for 48 h. Furthermore, the number of colony-forming units per ml (cfu/ml) for each microorganism was calculated on the incubation at 37°C.[25]

Statistical analysis

Paired *t*-test was used to compare the two cement groups. Data were analyzed using SPSS version 21, and $P \leq 0.05$ was considered statistically significant.

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 Fuji II SC cement containing ZnO-NPs group was significantly lower than these values in plain Fuji II SC cement group ($P < 0.05$).

DISCUSSION

Orthodontic banding increases susceptibility to dental caries by creating barely cleansable plaque accumulation sites.[26] This study tried to overcome this problem by inducing antibacterial activity in GIC by adding ZnO-NPs to this cement.

Results of the present study revealed that containing ZnO-NPs compared to GIC decreases the microbial count under the orthodontic bands significantly. The mean counts of total bacteria, aerobic *Lactobacillus,* anaerobic *Lactobacillus,* and *S. mutans* in Fuji II SC cement containing ZnO-NPs compared to Fuji II cement were decreased by 2.87, 2.15, 1.7, and 1. 56 times, respectively.

Various NPs such as silver, ZnO, TiO_2, and ZrO_2 have been successfully incorporated into dental materials in order to induce antibacterial activity.[27] Selection of ZnO-NPs for this study was firstly based on their exceptional ability to bond to the poly-acrylic liquid of GI which could improve the flexural bond strength. In addition, Jones *et al*. showed that antibacterial effect of ZnO-NPs against *Staphylococcus aureus* was remarkably higher than five other metal oxide NPs.[28] Other studies showed incorporation of 2 wt% ZnO NPs into the RMGI cement adds antimicrobial activity to the cement without sacrificing FS and fluoride release properties, while decreased μSBS.[24] Otherwise, incorporation of nanospherical and nanoflower ZnO to glass ionomer decreased their surface hardness, without any changes on their flexural strength. Incorporation of nanorod ZnO particles caused no effect on the mechanical properties.[29]

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 ZnO-NPs	Fuji II SC	
Total bacteria	9.77×10^4	28.02×10^4	0.034*
Anaerobic lactobacilli	18.04×10^4	30.68×10^4	0.029*
Aerobic lactobacilli	4.39×10^4	9.45×10^4	0.029*
Streptococcus mutans	1.90×10^4	2.96×10^4	0.05*

*: $P \leq 0.05$ was considered statistically significant

Antimicrobial properties of ZnO-NPs have been confirmed by a number of researches.[13,18-20] Different mechanisms such as generation of hydrogen peroxide which prevents microbial growth and adhesion of NPs to the bacterial surface serve responsible for its antimicrobial activity.[30] However, health concerns about widespread use of ZnO-NPs have been expressed. Using ZnO-NPs in low doses is quite safe, as Wang *et al*. showed that even long-term exposure to 50 and 500 mg/kg ZnO-NPs diets has minimal toxicity in mice.[31] In humans, application of nanomaterials such as ZnO-NPs and TiO_2 in sunscreens and cosmetics does not result in clinical toxicity. Likewise, inhalation of relatively high dose (500 mg/mm_3 of ultrafine ZnO-NPs) for 2 h does not induce acute systemic effects.[32]

Although the results of the present study support adding ZnO-NPs to GIC for induction of antimicrobial effects, this could only be clinically applicable if it does not undermine the mechanical properties of the cement. Earlier researches have evaluated the effects of ZnO-NPs on mechanical features and handling properties of GIC and have not reported any adverse effects.[19,23] Furthermore, Andrade *et al*. resulted that adding ZnO-NPs to interim cement increases its diametral tensile strength.[13]

Previously, some studies have evaluated the antimicrobial effects of ZnO-NPs incorporated in cement and reached somewhat controversial results; antibacterial effects of interim cement containing ZnO-NPs against *S. mutans* have been reported.[13] Likewise, Vanajassun *et al*. proved the antibacterial effect of ZnO-NPs incorporated in GIC.[23] On the other hand, Garcia *et al*. added 1–2 wt% ZnO-NPs to GIC and did not find any significant antibacterial features against *S. mutans*.[22] However, the results of the previous studies are not fully comparable to ours since they were mainly *in vitro* experiments. The *in vivo* and split-mouth design of the present study takes the advantage of investigation inside the complex intraoral environment as well as maximum matching of the study groups.

Results of the present study support adding 2 wt% ZnO-NPs to glass ionomer in order to prevent caries in banded molars. However, further long-term studies might be necessary to achieve more applicable results.

About the limitations of this study can be mentioned its cost and difficulty in preparing materials. Limitation in finding patient with lingual arch treatment plan that had acceptable hygiene.An other limitation is inability to mix two solid powders completely and uniformly.

CONCLUSION

Cementation of bands with GIC containing 2 wt% ZnO-NPs decreases the accumulation of mutans streptococci and lactobacilli under orthodontic bands, which could decrease the risk of dental caries under bands.

Regulatory statement

This study was approved by the Ethical Committee of Qazvin University of Medical Sciences with ethical number of IR.QUMS.REC.1397.256. There is no conflict with ethical considerations.

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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.

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