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Comparative Evaluation of Antibacterial and Cytotoxic Effects of Strontium-Doped Bioactive Glass Nanoparticle-Incorporated Orthodontic Primer: An In Vitro Study

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ABSTRACT

Orthodontic treatment is primarily aimed at improving occlusion, function, and esthetics; however, it is frequently associated with certain challenges and risks. Among these, the development of white spot lesions (WSLs) remains one of the most prevalent and esthetically concerning complications of orthodontic therapy. Within the limitations of this in vitro study, orthodontic primers modified with bioactive glass nanoparticles showed effective antibacterial activity against Streptococcus mutans while remaining biocompatible with periodontal ligament cells. The strontium-doped bioactive glass primer demonstrated antibacterial performance comparable to zinc- and silver-doped formulations without showing any cytotoxic effects. These findings suggest that incorporating strontium-doped bioactive glass into orthodontic primers may provide a safe and effective approach to reduce bacterial activity at the enamel–adhesive interface. However, further in vivo and clinical studies are required to confirm its long-term effectiveness in preventing white spot lesions during orthodontic treatment.

KEYWORDS: Orthodontic treatment, bioactive glass primer, cytotoxic effects, WSLs

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INTRODUCTION

Orthodontic treatment is primarily aimed at improving occlusion, function, and esthetics; however, it is frequently associated with certain challenges and risks. Among these, the development of white spot lesions (WSLs) remains one of the most prevalent and esthetically concerning complications of orthodontic therapy[1]. White spot lesions are defined as subsurface enamel porosities resulting from carious demineralization and clinically present as milky white opacities on smooth tooth surfaces .These lesions represent the earliest non-cavitated stage of dental caries and arise when the rate of enamel demineralization exceeds the natural remineralization capacity of saliva and fluoride. The pathogenesis of WSLs is driven by a localized acidic microenvironment created by the metabolic activity of cariogenic bacteria, predominantly *Streptococcus mutans* and *Lactobacillus* species, which metabolize fermentable carbohydrates to produce organic acids, particularly lactic acid.[2] These acids diffuse into the enamel, resulting in the dissolution of calcium and phosphate ions and the formation of a subsurface lesion beneath an intact enamel surface, producing the characteristic white spot appearance[3,4]

White spot lesions are of particular concern in orthodontic patients, especially those undergoing treatment with fixed appliances. Orthodontic brackets, bands, and archwires significantly alter the oral environment by creating additional plaque-retentive sites that facilitate bacterial accumulation and hinder effective mechanical plaque removal[1]. Even patients who maintain good oral hygiene practices often find it difficult to adequately clean around these appliances, thereby increasing the risk of enamel demineralization [5].

Given the esthetic implications and the potential progression of white spot lesions into cavitated carious lesions, effective prevention and management of these early enamel changes are of paramount importance[6]. Several remineralization strategies have been proposed for the management of non-cavitated enamel lesions, including topical fluoride agents, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), nano-hydroxyapatite, bioactive glasses, and resin infiltration techniques[6]. Although topical fluoride applications remain the most commonly used approach, their effectiveness is highly

dependent on patient compliance and may show limited long-term benefits in orthodontic patients [7]. Consequently, there has been increasing interest in incorporating antibacterial and remineralizing agents directly into orthodontic bonding materials to provide continuous protection at the enamel–adhesive interface.

Bioactive glass (BAG) is a silica-based biomaterial capable of releasing calcium and phosphate ions, increasing local pH, and promoting enamel remineralization [6]. Doping bioactive glass with therapeutic ions such as silver and zinc has been shown to enhance antibacterial activity; however, concerns regarding cytotoxicity, enamel discoloration, and potential compromise of bond strength have been reported [8–10]. Therefore, the search for bioactive additives that combine effective remineralization with antibacterial properties while maintaining favorable mechanical performance remains ongoing.

Strontium-doped bioactive glass (Sr-BaG) has emerged as a promising alternative and recent in vitro studies have demonstrated that orthodontic adhesives modified with strontium-doped bioactive glass nanoparticles can induce calcium phosphate precipitation at the enamel surface, indicating active remineralization rather than passive bonding behavior. Despite growing evidence supporting the remineralizing and antibacterial potential of strontium-doped bioactive glass, limited literature exists regarding its incorporation specifically into orthodontic primers, particularly with respect to its combined biological and mechanical performance. Since any modification of orthodontic bonding materials must preserve essential properties such as shear bond strength and adhesive integrity, comprehensive evaluation is necessary prior to clinical application. Therefore, the present *in vitro* study aimed to evaluate the antibacterial activity and cytocompatibility of an orthodontic primer incorporated with strontium-doped bioactive glass nanoparticles and to compare its performance with zinc- and silver-doped bioactive glass-modified primers and a conventional unmodified primer.

MATERIALS AND METHODS:

Study Design

This in vitro experimental study evaluated the antibacterial activity, cytotoxicity, and shear bond strength of an orthodontic primer modified with strontium-doped bioactive glass (Sr-BAG) nanoparticles, compared with zinc- and silver-doped bioactive glass–modified primers and an unmodified control primer.

Synthesis of Strontium-Doped Bioactive Glass Nanoparticles

Strontium-doped bioactive glass nanoparticles were synthesized using the sol–gel method based on the conventional 45S5 bioactive glass composition, comprising SiO_2 (45%), P_2O_5 (6%), CaO (24.5%), and Na_2O (24.5%). A single Sr-BAG formulation was prepared by partially substituting Na_2O with 1.5 mol% SrO, yielding the experimental Sr-BAG used throughout the study.

Briefly, tetraethyl orthosilicate and orthophosphoric acid were dissolved in a mixture of ethanol, nitric acid, and double-distilled water under continuous stirring to obtain a homogeneous sol. Calcium nitrate and sodium hydroxide were subsequently added, followed by the addition of strontium nitrate as a sodium-substituting precursor. All precursor solutions were prepared separately and added sequentially to the reaction mixture.

The resulting gel was dried overnight at 80 °C and further dried at 100 °C for 24 h to remove residual moisture. The dried samples were then heat-treated at 600 °C for 3 h to obtain amorphous Sr-BAG nanoparticles. Zinc-doped and silver-doped bioactive glass nanoparticles were synthesized following the same sol–gel protocol, with partial substitution of Na_2O by ZnO or Ag_2O, respectively.This synthesis protocol was adapted from previously established sol–gel BAG preparation methods for orthodontic applications[11].

The reaction was carried out at room temperature until complete gelation occurred. The formed gel was dried overnight at 80 °C and further dried at 100 °C for 24 h to remove residual moisture. The dried samples were then heat-treated at 600 °C for 3 h to obtain amorphous Sr-BAG nanoparticles

Incorporation of Nanoparticles into Orthodontic Primer

The synthesized Sr-BAG nanoparticles were incorporated into a commercially available orthodontic primer (Transbond™ XT Primer, 3M Unitek). The nanoparticles were mixed into the primer at concentrations of 0.5%, 1.0%, and 1.5% (wt%) using a vortex mixer at 3400 rpm for 2 min in a dark environment to ensure homogeneous dispersion.

Zinc-doped and silver-doped bioactive glass nanoparticles were incorporated into the primer using the same protocol for comparative evaluation. The unmodified primer served as the control group

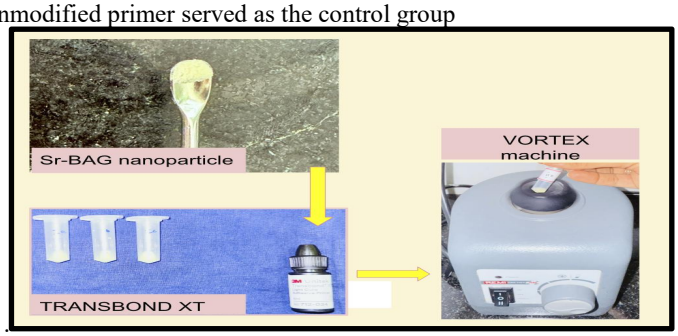

Fig 1- Preparation of strontium-doped bioactive glass (Sr-BAG) nanoparticle–incorporated orthodontic primer using a

vortex mixing technique.

Antibacterial Assessment

The antibacterial activity of the experimental primers was evaluated using the agar well diffusion method against *Streptococcus mutans*. The bacterial strain was cultured in brain–heart infusion broth and incubated at 37 °C for 24 h. Mueller–Hinton agar plates were uniformly inoculated with the bacterial suspension.

Standardized wells were prepared in the agar plates, and primer samples modified with Sr-BAG, Zn-BAG, and Ag-BAG at 0.5%, 1.0%, and 1.5% concentrations, along with the control primer, were placed into the wells. The plates were incubated at 37 °C for 24 h. Antibacterial efficacy was determined by measuring the zone of inhibition (ZOI) in millimeters using a digital caliper. All tests were performed in triplicate. The methodology followed previously validated protocols for BAG-modified orthodontic primers

Cytotoxicity Assessment

Cytotoxicity of the experimental primers was evaluated using the MTT assay on human periodontal ligament (PDL) cells. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and antibiotics and maintained at 37 °C in a humidified 5% CO_2 atmosphere.

Cells were seeded into 96-well plates and allowed to attach for 24 h. Subsequently, cells were exposed to extracts of primer samples modified with Sr-BAG, Zn-BAG, and Ag-BAG at concentrations of 0.5%, 1.0%, and 1.5%, along with the control primer. Cell viability was assessed at 24, 48, and 72 h by adding MTT reagent and incubating for 4 h in a dark environment. The resulting formazan crystals were dissolved using dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader.

Cell viability was expressed as a percentage relative to the control group. A viability value exceeding 80% was considered indicative of acceptable cytocompatibility.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics software. Antimicrobial activity, expressed as zone of inhibition values, and cytotoxicity results, expressed as percentage cell viability, were presented as mean ± standard deviation. Data normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were carried out using one-way analysis of variance (ANOVA). For cytotoxicity evaluation, comparisons across different incubation periods were also analyzed using ANOVA. A p-value < 0.05 was considered statistically significant.

RESULTS

Antibacterial Activity

All bioactive glass nanoparticle–incorporated orthodontic primers exhibited antibacterial activity against *Streptococcus mutans*, whereas the unmodified primer showed no detectable inhibitory effect. An increase in the zone of inhibition (ZOI) was observed with increasing nanoparticle concentration across all experimental groups, indicating a concentration-dependent antibacterial response.

At the highest tested concentration (1.5%), the Zn-BAG–modified primer demonstrated the greatest mean ZOI, followed by the Sr-BAG and Ag-BAG groups. Although numerical differences in antibacterial activity were evident among the nanoparticle-modified primers, statistical analysis revealed that these intergroup differences were not statistically significant ($p > 0.05$). In contrast, all BAG-modified primers showed significantly greater antibacterial activity compared with the unmodified control primer.(Fig 2)

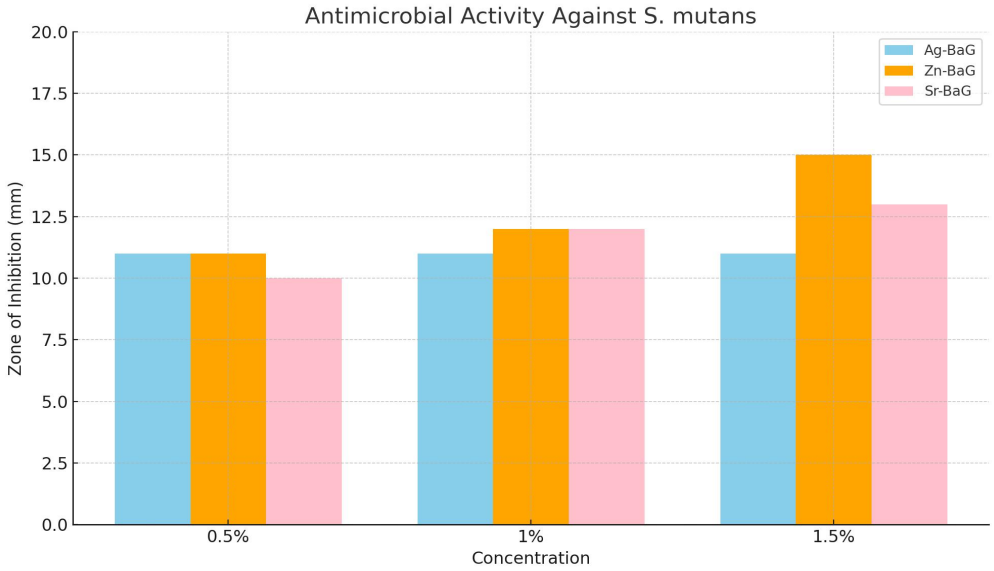

Fig 2 - Antibacterial activity of Ag-, Zn-, and Sr-doped bioactive glass–modified orthodontic primers against *Streptococcus mutans* at different concentrations.

Cytotoxicity Evaluation

Cytotoxicity assessment using the MTT assay on human periodontal ligament (PDL) cells revealed that all nanoparticle-modified orthodontic primers exhibited acceptable cytocompatibility, with mean cell viability values exceeding 80% at all evaluated time points (24, 48, and 72 h).

Among the experimental groups, Zn-BAG–modified primers demonstrated higher mean cell viability values, followed by Sr-BAG and Ag-BAG groups. Although variations in cell viability were observed across different concentrations and time points, these differences were not statistically significant ($p > 0.05$). A mild dose- and time-dependent increase in cell viability was noted in all nanoparticle-incorporated groups. Morphological examination confirmed that PDL cells retained their characteristic spindle-shaped morphology, with no evidence of cytotoxic or degenerative changes. (Fig 3,4,5)

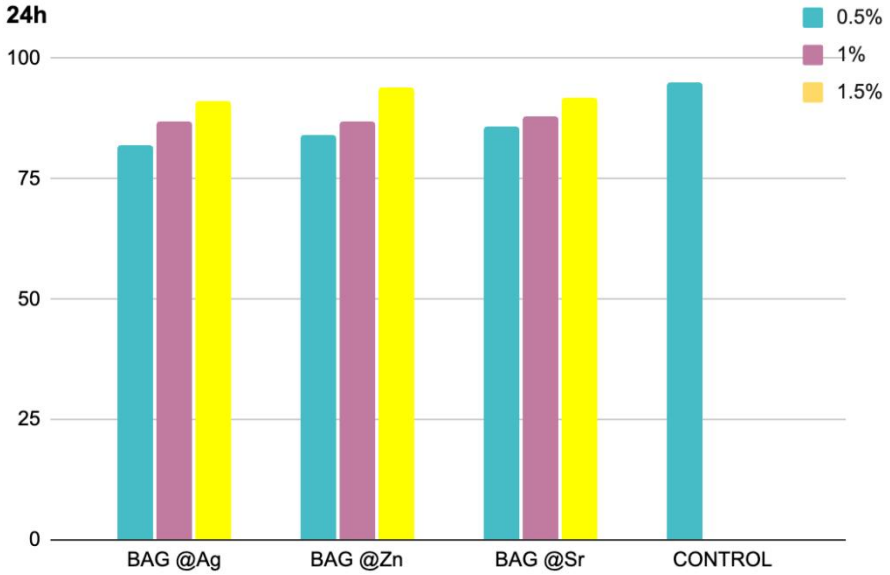

Fig 3 - Cytotoxicity evaluation of Ag-, Zn-, and Sr-doped bioactive glass–modified orthodontic primers showing mean cell viability (%) of human periodontal ligament cells at 24 hours across different concentrations

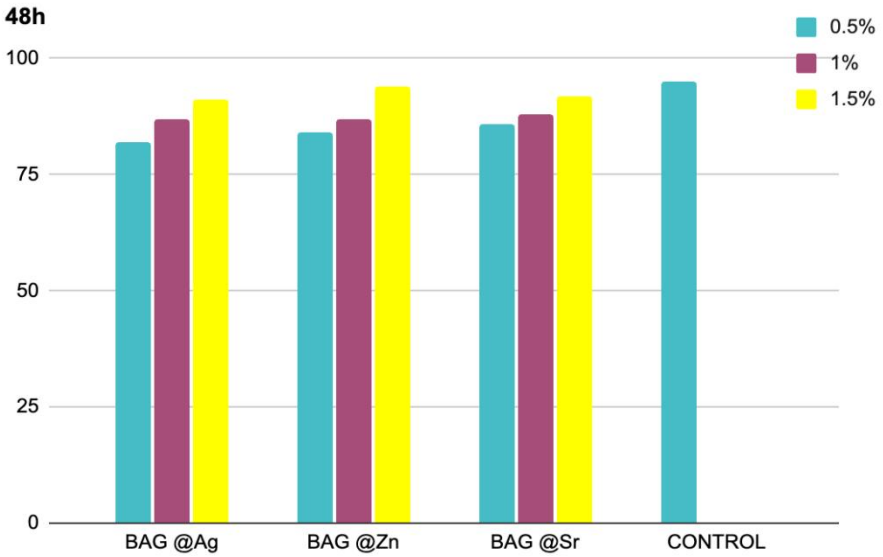

Fig 4 - Cytotoxicity evaluation of Ag-, Zn-, and Sr-doped bioactive glass–modified orthodontic primers showing mean cell viability (%) of human periodontal ligament cells at 48hours across different concentrations

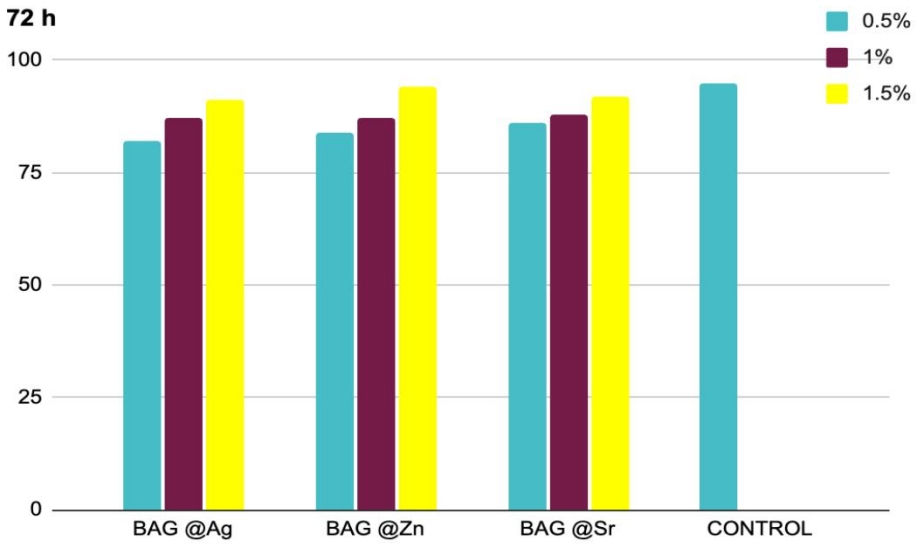

Fig 5 - Cytotoxicity evaluation of Ag-, Zn-, and Sr-doped bioactive glass–modified orthodontic primers showing mean cell viability (%) of human periodontal ligament cells at 72 hours across different concentrations

DISCUSSION

White spot lesions remain a common adverse effect of fixed orthodontic therapy due to sustained plaque retention and prolonged acidic challenges at the enamel–adhesive interface[2]. Conventional preventive measures such as fluoride application provide only intermittent protection and rely heavily on patient compliance[6]. In this context, modifying orthodontic bonding materials with bioactive agents offers a biologically driven approach to provide continuous antibacterial activity at vulnerable sites[1,2].

In the present study, all bioactive glass–modified primers demonstrated antibacterial activity against *Streptococcus mutans*, while the unmodified primer showed no inhibitory effect. This finding confirms that incorporation of bioactive glass nanoparticles can impart intrinsic antibacterial properties to orthodontic primers. The concentration-dependent increase in antibacterial activity observed across all experimental groups is consistent with previous reports on ion-doped bioactive glass systems. Although Zn-BAG showed the highest mean antibacterial activity, followed by Sr-BAG and Ag-BAG, the differences among groups were not statistically significant, indicating comparable antibacterial efficacy.

The antibacterial effect of bioactive glass is largely attributed to ion release and local pH elevation, which disrupt bacterial metabolism and biofilm viability[12]. Strontium ions, in particular, have been shown to interfere with bacterial enzymatic activity while maintaining favorable biological compatibility[13]. Unlike silver-based systems, which may raise concerns regarding cytotoxicity and discoloration, strontium-doped bioactive glass offers a balanced antibacterial effect with improved safety[14,15]. Cytotoxicity evaluation demonstrated that all nanoparticle-modified primers exhibited acceptable biocompatibility, with cell viability exceeding 80% at all evaluated time points. These findings are consistent with previous studies reporting favorable cytocompatibility of strontium-containing bioactive glass formulations[13,14]. Preservation of normal periodontal ligament cell morphology further supports the biological safety of these materials for orthodontic use.

The antibacterial efficacy observed in the present study aligns with previous research indicating that bioactive glass nanoparticles exhibit substantial antimicrobial effects due to their increased surface area and resulting alkalinity [16]. The release of ions such as strontium, zinc, and silver from the glass matrix further contributes to bacterial membrane disruption and inhibition of enzymatic activity [14]. Specifically, strontium ions have been reported to enhance remineralization potential while maintaining favorable cytocompatibility with human dental pulp stem cells and periodontal ligament fibroblasts[14,16,17].This dual functionality of promoting mineralization and inhibiting bacterial growth makes strontium-doped bioactive glass a particularly attractive candidate for orthodontic applications where both enamel protection and soft tissue biocompatibility are essential[18,19]. The comparable antibacterial performance of Sr-BAG to Ag-BAG and Zn-BAG in this study is consistent with literature showing that ion-doped bioactive glasses can significantly reduce Streptococcus mutans growth, with antibacterial activity often increasing with higher nanoparticle concentrations[20,21]. However, higher nanoparticle concentrations may lead to particle agglomeration, which can increase surface roughness and potentially facilitate bacterial adhesion, necessitating optimization of dispersion techniques and filler content [22]. Furthermore, the long-term stability of antibacterial properties under hydrothermal fatigue conditions has been confirmed in composite resins containing strontium-modified phosphate-based glass microfillers, suggesting that such modifications can maintain efficacy over extended periods[23].

Despite these promising in vitro findings, the present study did not simulate the complex oral environment, including salivary dynamics, dietary variations, and long-term biofilm maturation. Therefore, well-designed in vivo and clinical studies are required to confirm the durability and clinical effectiveness of Sr-BAG–modified orthodontic primers in preventing white spot lesion development during fixed appliance therapy. In addition, further investigations evaluating critical mechanical and

physicochemical properties—such as shear bond strength, adhesive remnant index, microhardness, degree of conversion of the resin matrix, and long-term interfacial stability—are essential to ensure that incorporation of bioactive glass nanoparticles does not adversely affect polymerization efficiency or compromise bonding performance, which could otherwise increase the risk of bracket failure during treatment. From a clinical perspective, incorporation of strontium-doped bioactive glass into orthodontic primers may offer a meaningful advantage over conventional bonding agents by providing continuous, material-driven antibacterial activity with favorable biocompatibility, thereby enabling simultaneous bracket bonding and sustained enamel protection at high-risk sites without relying on patient compliance.

CONCLUSION

Within the limitations of this *in vitro* study, orthodontic primers modified with bioactive glass nanoparticles showed effective antibacterial activity against *Streptococcus mutans* while remaining biocompatible with periodontal ligament cells. The strontium-doped bioactive glass primer demonstrated antibacterial performance comparable to zinc- and silver-doped formulations without showing any cytotoxic effects. These findings suggest that incorporating strontium-doped bioactive glass into orthodontic primers may provide a safe and effective approach to reduce bacterial activity at the enamel–adhesive interface. However, further *in vivo* and clinical studies are required to confirm its long-term effectiveness in preventing white spot lesions during orthodontic treatment.

Conflict of Interest

The authors declare that they have no conflict of interest related to this study.

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