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Effects of Zinc Oxide Nanoparticles in Combination with Conventional Glass Ionomer Cement: In vitro Study

Pranav Vanajassun P[1*] MS Nivedhitha[2] NT Nishad[3] Dona Soman[4]

[1]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha University, Chennai, India.
[2]Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha University, Chennai, India.
[3]Post Graduate Student, Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha University, Chennai, India.
[4]Post Graduate Student, Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha University, Chennai, India.

ABSTRACT

Aim: The aim of this study was to evaluate the effect of addition of Zinc oxide nanoparticles (ZnO) to the conventional Glass-Ionomer Cement (GIC) on its antibacterial and selective mechanical properties.

Materials and Methods: Conventional posterior glass ionomer restorative cement- Fuji IX was used as the control group (control-GIC). Experimental group was prepared by incorporating zinc oxide nano particles of size <50nm into the powder of Fuji IX at 3% W/W concentration with P/L ratio 3.6:1. The antibacterial activity of the set material against *Streptococcus mutans* was assessed using the agar diffusion test. The compressive strength and shear bond strength were measured using the Universal testing machine. Data was analyzed using Independent T test.

Results: Experimental group showed significant increase in antibacterial properties for both set and unset specimens. Incorporation of ZnO nanoparticles has no significant difference over the mechanical properties of set Glass Ionomer Cement.

Conclusion: The study showed significant increase in antibacterial property of set GIC with ZnO nanoparticles without modifying the mechanical properties. Thus incorporation of ZnO nanoparticles in glass ionomer cement can be considered as a better alternative to conventional GIC.

Keywords: Antibacterial agent, Glass ionomer cement, Zinc oxide.

INTRODUCTION

Secondary caries is one of the primary causes for failure of dental restorations. The recognition of fewer incidences of recurrent caries around the silicate restorations and its attribution to fluoride led to the incorporation of fluoride into a number of dental restorative materials. Glass-ionomer cement (GIC) was invented by Wilson and Kent at the Laboratory of the Government Chemist in 1972. These materials are water-based cements known as polyalkenoate cements. Their generic name is based on the reaction between silicate glass and polyacrylic acid, and the formation arises from an acid/base reaction between the components. These cements are translucent and adhesive to tooth structure.

Glass-ionomer cements have unique properties such as biocompatibility, anticariogenic action and adhesion to moist tooth structure. In addition, the coefficient of thermal expansion for glass ionomers is low and close to the values of tooth structure[1]. Although fluoride has been known for many decades to be antimicrobial, there has

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***Correspondence**: **Dr. Pranav Vanajassun P.**
Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha University, Chennai, India.
E-mail: docpranav@gmail.com

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been little understanding of its precise effects on cells or of the mechanisms that might be used by organisms to overcome this toxicity. Few bacteria have evolved numerous strategies to alleviate the toxic effects of fluoride ions [2].

Several attempts in developing GIC with enhanced antibacterial effects by addition of agents, such as, chlorhexidine hydrochloride[3], cetyl pyridinium chloride, cetrimide, benzalkonium chloride and titanium oxide nano particles have been reported in the literature[1, 4]. The most appropriate choice of antibacterial agents to combine with GIC would be nanoparticles that have proven to be useful in clinical dentistry, and are the ones that do not disturb the mechanical properties. Cationic disinfectants have been investigated both in-vitro and in-vivo for their antibacterial effects against various microorganisms. Literature reveals that titanium oxide is the only nanoparticle that has been widely incorporated in GIC and studies have shown an increase in the antibacterial effects in in-vitro study [4].

In recent years nanotechnology has permitted the development of new properties of materials. Zinc oxide nanoparticle has been effective against wide range of gram negative and gram positive bacteria [5]. Recent studies have shown that these nano particles have selective toxicity to bacteria but exhibit minimal effects on human cell [6]. However there is lack of studies on antimicrobial behavior of zinc oxide nanoparticles incorporated into glass ionomer cement. In an approach to enhance the antibacterial potential of conventional glass ionomer cement, zinc oxide nanoparticles were incorporated into conventional GIC and its antibacterial properties, compressive and shear bond strength were investigated their. Hence, the aim of the study was to evaluate the antibacterial and selective mechanical properties of glass ionomer cements containing zinc oxide nanoparticles.

MATERIALS AND METHOD

A conventional powder and liquid of Glass ionomer cement (Fuji IX, GC, Tokyo Japan) was used as a control. Experimental group was prepared by incorporating Zinc Oxide nanoparticles (Sigma-Aldrich Chemicals, USA) of mean particle size <50nm into the powder of Fuji IX Glass ionomer cement at 3% W/W concentration with P/L ratio 3.6:1(one scoop powder and one drop of liquid).

In order to obtain two groups of 3% concentration of zinc oxide nanoparticles in the GIC experimental formulation, 1000 mg of Fuji IX Glass ionomer cement was proportioned on a mixing pad and weighed, to which 30 mg (3%) of zinc oxide nano particles were added.

Agar diffusion test

Streptococcus mutans bacteria which are commonly associated with active caries were used for the study. The antibacterial activity of the set and unset materials against *Streptococcus mutants* (HF1037 a local strain obtained from Basic Medical Research Centre, Chennai, Tamil Nadu, India) were assessed using the agar diffusion test. All procedures were carried out under an aseptic condition.

Strains of bacteria were stored at -20^{0}C and cultured on blood agar (Merck, Darmstadt, Germany) for 24 hours. Single colonies from plates were transferred to BHI broth and incubated at 37^{0}C for 24 hours. Suspensions of strains prepared in PBS at ca 1.5x 10^6 organisms /ml using Mc Farland 0.5 turbidity tube were flood-inoculated onto surface of BHI agar plates.[3]

The set disc-shaped specimens (10 mm in diameters, 2 mm thick) were prepared by mixing powder and liquid from each group (P/L ratio: 3.6/1). After setting at room temperature for 30 minutes, the specimens were placed onto BHI agar plates. All the specimens were then sterilized with UV before the experiment. For unset specimens, 10 mm-diameter wells were cut from the agar by using sterile glass-made pipettes attached to a vacuum pump and filled with GIC paste using a syringe. After incubation at 37°C for 48 hours, inhibition zones around the specimens were measured. The sizes of the inhibition zones were calculated by subtracting 10 mm (diameter of wells) from the average diameter of the zones for each specimen and control. Five specimens were tested for each group.

Compressive strength

The compressive strength was measured using the Universal testing machine (Lloyd

Instruments Ltd, Hampshire, England). Five cylindrical specimens per group were prepared using a plastic mold with an inner diameter of 4 mm and 6 mm height. The inner surfaces of the molds were coated with a thin layer of petroleum jelly and the experimental groups were hand-mixed and loaded into the mold with the help of a sterile dental instrument and stored at room temperature for 24 hours. Prior to testing, the molds were removed and the diameter of each specimen was determined using a micrometer gauge. The specimens were then placed between the plates of the universal testing machine. A compressive load along the long axis was applied using a crosshead speed of 1 mm/minute. The maximum force when the specimen fractured was recorded.

Shear bond strength

Occlusal dentin specimens were obtained from 12 human premolars, then the dentin surface was polished flat with 200-, 400- and 600-grit silicon carbide papers to expose the flat surface. The dentin surface was conditioned with a polyacrylic acid (Cavity Conditioner) for 10 seconds followed by rinsing of the conditioner with air-water spray for 10 sec. The powder and liquid of each cement was mixed (P/L ratio : 3.6/1) and placed into the center of the prepared dentin surface by packing the material into cylindrically-shaped plastic tubes with an internal diameter of 3 mm and a height of 4mm. After storing the specimens at 37°C for 24 hours, the shear bond strength was measured using an Instron universal testing machine (Lloyd Instruments Lt, Hampshire, England) at a crosshead speed of 0.5mm/minute. Six specimens were tested for each group.

RESULTS

Antibacterial activity

The mean values (mm) of the growth inhibition zones for the control and experimental groups are shown in Table1A and 1B. Minimal inhibition zone existed for bacterial species tested in the set specimens of the control groups, while unset specimens of the control groups exhibited zones of growth inhibition. However, a large inhibition zone was produced when tested against bacteria with set or unset specimens of the experimental group. In set specimens, the size of the inhibition zones was significantly smaller than in the unset specimens against *Streptococcus mutants*. Significant differences existed in the size of the inhibition zones produced among the control and experimental groups in the set ($p \leq 0.015$) as well as unset ($p \leq 0.0014$) specimens.

Compressive strength

The mean compressive strength of the control and experimental groups after 24 hours of storage in water is shown in Table 2. No significant differences existed between the control and experimental group at 24 hours ($p \leq 0.951$).

Shear bond strength

The shear bonding strengths for the control and experimental groups are shown in Table 3. No statistically significant difference in shear bond strength existed between the control and experimental group ($p \leq 0.164$).

Table 1a: Mean inhibition zone for unset specimen

Groups	N	Mean (mm)	Std. dev	p Value
Control group	5	14.40	1.475	0.015
Test group	5	20.60	3.595	

Table 1b: Mean inhibition zone for set specimen.

Groups	N	Mean (mm)	Std. dev	p Value
Control group	5	1.828	1.394	0.0014
Test group	5	8.728	2.929	

Table 2: Compressive strength.

Group	N	Mean (M Pa)	Std dev	p Value
Control	5	84.096	8.969	0.951
Test	5	84.462	9.221	

Table 3: Shear bond strength.

Group	N	Mean (M Pa)	Std dev	p Value
Control	6	4.98	2.111	0.164
Test	6	6.80	2.078	

DISCUSSION

Glass-ionomer cements have unique properties such as biocompatibility, anticariogenic action and adhesion to moist tooth structure. Initially the anticariogenic property was attributed to the fluoride releasing property of GIC[7,8], but this information is not reliable. The primary mechanism of action of fluoride in caries prevention is widely believed to be its ability to bond with enamel of teeth to form fluorhydroxyapatite[9]. However, fluoride is strongly antimicrobial, and this property has been proposed to contribute to the anticariogenic activity by reducing metabolism and growth of organisms such as *Streptococcus mutans* [10,11]. Although fluoride has been known for many decades to be antimicrobial[12,13], there has been little understanding of its precise effects on cells or of the mechanisms that might be used by organisms to overcome this toxicity[14]. Fluoride more efficiently enters bacterial cells at acidic pH values as HF and dissociates when exposed to the more neutral intracellular pH[16]. Few bacteria have evolved numerous strategies to alleviate the toxic effects of fluoride ions. *crcB* and *eriC*[F] are the fluoride resistance genes present in E.coli which can effectively transport the fluoride ion out of the bacteria cell or simply rejects absorption of fluoride into the cell[15]. According to Vermeersch et al, the low pH of GIC while setting, may contribute more to their antibacterial properties than their fluoride-leaching capabilities[17]. Additionally, Yap and others reported that there was no antibacterial activity despite the presence of fluoride in the agar around the set materials[18].

According to the results of the current study, for pure GICs (control group), the set specimens produced negligible bacterial inhibition, although the unset specimens demonstrated antibacterial activity. These results supported previous studies[19]. The antibacterial effects of unset control specimens may be correlated with decreasing pH during the setting reaction. Therefore, the influence of fluoride on the antimicrobial properties of GICs may be limited, especially after the setting reaction is completed.

Zinc oxide nanoparticles have shown to be effective against wide range of gram negative and gram positive bacteria [5]. Smaller particles of ZnO nanoparticles have been found to be more effective than larger particles [20]. Kuhn et al in 2003 proved antibacterial property of ZnO nano particles against E coli [21]. ZnO nanoparticles (average particles diameter 12 nm) are able to slow down the bacterial growth (100% with 3mM) as a result it increases the membrane permeability leading to the accumulation of nanoparticles in the bacterial membrane and cytoplasm regions of the cells[22]. More over Zhang et al in 2006 proved that antibacterial effect of zinc oxide nanoparticles increases with increase in nanoparticle concentration and decrease in size of nanoparticles[23]. *Nagarajan et al* showed the bioactivity property of ZnO nanoparticles which depicts that ZnO nano particles suspension with a concentration 5 to 100 mM effectively inhibits bacterial growth[24].

One mechanism proposed to explain the antimicrobial property of ZnO nanoparticles is that they generate active oxygen species such as H_2O_2 which inhibit growth of planktonic microbes[25]. ZnO nanoparticles can be photo catalytic under ultraviolet light leading to the production of antimicrobial active oxygen species[26]. An alternate mechanism for the antimicrobial activity of ZnO nanoparticles (even in the dark) has been proposed in which halogens adsorbed on the nanoparticle surfaces behave as oxidizers, imparting bactericidal activity[27]. Such a mechanism would be enhanced in an aerobic environment. Another potential antimicrobial mechanism of ZnO nanoparticles occurs via the leaching of Zn^{2+} into the growth media. Toxicological mechanisms of zinc ions play an important role in biofilm inhibition by inhibiting the active transport and metabolism of sugars[28] as well as disrupting enzyme systems of dental biofilms by displacing magnesium ions essential for enzymatic activity of the plaque[29]. Zinc can also reduce acid production by *S.mutans* biofilms, which includes both *S. mutans* and *S. sobrinus*, due to its ability to inhibit glucosyltransferase activity, thus impeding decalcification[30,31]. Thus incorporation of ZnO nanoparticles in glass ionomer cement leads to increase in antibacterial property without changing the mechanical properties of the set cement. Even though samples with Zinc oxide nanoparticles showed high antibacterial results without altering the selective mechanical properties, further studies are required to evaluate other properties.

CONCLUSION

Within the limitations of this in-vitro study, it can be concluded that GIC incorporated with 3% w/w concentration of <50nm size Zinc Oxide Nanoparticles can be used as a better alternative to conventional GIC for restorative purposes which provides greater antibacterial property and comparable mechanical properties with respect to conventional GIC.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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