Titanium Dioxide Nanoparticles and Cetylpyridinium Chloride Enriched Glass-Ionomer Restorative Cement: A Comparative Study Assessing Compressive Strength and Antibacterial Activity

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Objective: *To evaluate the addition of titanium dioxide (TiO_2) nanoparticles and cetylpyridinium chloride (CPC) on the compressive strength and antibacterial activity of conventional glass-ionomer cement (GIC).* ***Study design:*** *TiO_2 nanoparticles enriched GIC was prepared by adding 3% TiO_2 nanoparticles (w/w) into the powder component of conventional GIC. CPC containing GIC was developed by incorporating 1% CPC (w/w) into conventional GIC powder. Samples were segregated into three groups: GIC with 3% TiO_2 nanoparticles, GIC with 1% CPC and unmodified conventional GIC. Compressive strength was assessed using the universal testing machine on cylindrical specimens made from each material. Antibacterial activity was assessed by measuring inhibition zones on Mitis Salivarius Bacitracin (MSB) agar inoculated with pure strain of Streptococcus mutans (S. mutans).* ***Results:*** *GIC containing TiO_2 nanoparticles exhibited significantly greater compressive strength as compared with CPC and conventional GIC groups ($P < 0.01$). However, there was no significant difference between the compressive strengths of CPC and conventional GIC group ($P > 0.05$). Antibacterial activity was significantly greater for TiO_2 group than conventional GIC ($P < 0.05$). CPC increased the antibacterial activity of conventional GIC, though not significantly.* ***Conclusion:*** *The addition of 3% TiO_2 nanoparticles improves the compressive strength of GIC as well as its antibacterial activity against S. mutans.*

Keywords: *Glass ionomer, TiO_2, nanoparticles, cetylpyridinium chloride, CPC, compressive strength, antibacterial activity.*

INTRODUCTION

Glass-ionomer cement has invariably been an indispensable part of restorative dentistry since its inception, chiefly owing to its substantial use in pediatric restorations and atraumatic restorative procedures (ART). Glass-ionomer cement has a number of distinctive properties like biocompatibility, chemical bonding to moist enamel and dentin surfaces, and anticariogenic effect due to fluoride release.[1] Regardless of these favorable attributes GICs possess certain limitations as inferior physical properties including low compressive strength. Several modifications have been made in GI materials since their development to enhance their physical properties.[2]Several materials like hydroxyapatite, silver powder, zirconia, bioactive glass particles, casein phosphopeptide-amorphous calcium phosphate have been proposed to be added in the GI material to enhance its physical properties.

The occurrence of recurrent caries after restorations has also increased over the decade which presents a potential risk of development of new carious lesions.[3] Glass-ionomer cements possess an inherent anticariogenic effect, which according to most authors is due to their fluoride leaching property whereas others believe it to be due to the low pH of GICs whilst setting. Nevertheless, addition

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of antibacterial agents may augment the anticaries effect of GI materials. For this reason various antimicrobial agents like chlorhexidine and its derivatives and many antibiotics have been recommended to be added in GI materials so that reduction in the total number of viable bacteria may be achieved in conjunction with preservation of pulpal vitality. However, incorporation of these antimicrobial agents imperiled the basic mechanical properties of GICs.[2] Cationic salts formed by chlorhexidine may impede the reaction of glasses and polyacrylic acid by intruding upon setting mechanisms like proton attack and ion leaching from glass particles.[4,5]The addition of increasing concentrations of antibiotic powders may also lead to decrease in the reaction between polyacrylic acid and glass particles. This can in turn lead to an increase in the number of unreacted glass particles. Antibiotics also have a tendency to absorb water causing decrease in the compressive strength of GIC.[6] Therefore, the specific antibacterial agent being used as well its quantity is of prime concern for incorporation into the GI materials.

Cetylpyridinium chloride is being used quite frequently as an active ingredient in mouthwashes, toothpastes, varnishes, orthodontic adhesives as well as liners for GIC. CPC has high antimicrobial efficacy along with low toxicity which makes it a good choice for the control of plaque as well as caries.[7] Nevertheless, it is essential to understand that all the formulations of CPC do not provide equal advantages.[8] Therefore, it is crucial to find out the right concentration of CPC for incorporation with GIC at which maximum antimicrobial efficacy can be achieved with minimum limitations.

Nanotechnology is gaining a lot of attention in the field of dental research lately because of the bactericidal and bacteriostatic effects of nanoparticles.[9-11] In dentistry, nanoparticles have been integrated into composite resins[12,13] and disinfection solutions.[14] The nanomaterials containing metal ions may be present in various different forms like metal oxides (e.g., ZnO nanoparticles, TiO_2 nanoparticles), solid metal nanoparticles (e.g. Ag nanoparticles) or as composite materials with layers of different metals (e.g., Cd-Se quantum dots).[15] The use of TiO_2 nanoparticles as reinforcing fillers to dental resin composites and epoxy has been proposed since TiO_2 possesses several favorable properties like chemical stability, biocompatibility and low toxicity to human tissues,[16,17] but only little data is available in the literature pertaining to the effect of addition of TiO_2 nanoparticles to conventional glass-ionomer cement.

This article, therefore, aims at evaluating the addition of titanium dioxide nanoparticles and cetylpyridinium chloride on the compressive strength and antibacterial activity of conventional glass-ionomer restorative cement.

MATERIALS AND METHOD

This *in vitro* study was performed to assess the effectiveness of glass-ionomer restorative cement enriched with antibacterial agents, viz. titanium dioxide (TiO_2) nanoparticles and cetylpyridinium chloride monohydrate USP (CPC). The study samples were segregated into three groups: GIC with 3% TiO_2 nanoparticles w/w with particle size ranging from 10-20 nm (Anatase, Batch number: NSNL2812/2015, Nano Labs, India), GIC with 1% CPC w/w (Batch number: SLS/15/CPC/017, Suyog Life Sciences Pvt. Ltd., India), Unmodified conventional restorative GIC (GC Fuji IX Gold Label, GC India).

For compressive strength determination twelve cylindrical specimens from each material, 6 mm in height and 4mm in diameter (in accordance with ISO specification, 7489:1986),[18] were prepared. These specimens were stored in distilled water in sterile containers at 37º C in an incubator for 24 hours prior to compressive strength determination. Compressive strength (MPa), of the specimens was determined using the universal testing machine at a crosshead speed of 0.5 mm/min and was calculated using the following equation.[19]

$$C_s = \frac{4P_f}{\pi D^2}$$

Where C_s is the Compressive strength, P_f is the load (N) at fracture and D is the diameter of specimen (mm).

For antibacterial activity assessment, *Mitis Salivarius* Bacitracin agar base (M259, HiMedia Laboratories, India) was used as culture medium and pure bacterial strains of Streptococcus mutans were obtained from MTCC, India (S. mutans, MTCC 890) and reactivated on brain heart infusion agar plate. The antibacterial activity of the samples was assessed by agar well diffusion method. A sterile punch was used for making 5 wells at equal distance to each other on twelve_*Mitis Salivarius* Bacitracin agar plates inoculated with the bacterial strain. GICs with 3% TiO2, 1% CPC and conventional GIC were mixed according to the manufacturer's instructions and introduced into three of the wells. Ampicillin was introduced in the fourth well as positive control and distilled water was placed in the fifth well as negative control. The plates were incubated at 37º C for 48 hours. Antibacterial activity was assessed by measuring the diameters of the zones of inhibition (mm) around the wells using inhibition zone measuring scale (HiMedia Laboratories, India). Tests were performed in triplicate.

Statistical Analysis

The results for compressive strength and inhibition zone measurement were analyzed using one-way analysis of variance (ANOVA) and Post-hoc-Tukey honest significant difference (HSD) test to determine if there were significant differences between the sample groups. Statistical significance was set at the 0.05 probability level.

RESULTS

It was found that GIC containing 3% (w/w) TiO_2 nanoparticles had significantly increased compressive strength as compared with GIC containing 1% CPC and the control group ($P < 0.01$). However, there was no significant difference between the compressive strengths of the CPC and the control groups ($P>0.05$) (Table 1).

Table 1- Mean (standard deviation) of the compressive strength of GIC incorporated with TiO_2 nanoparticles, CPC and conventional GIC, and Tukey's analysis.

Group	Compressive Strength (MPa)
GIC + 3% (w/w) TiO_2 nanoparticles	172.5483 (± 14.8844)[a]
GIC + 1% (w/w) CPC	130.1285 (± 9.46317)[b]
Conventional GIC (Control)	140.0287 (± 9.07569)[b]

Mean values for compressive strength represented with the same superscript letter are not significantly different ($P > 0.05$), whilst the mean values with different letters are significantly different ($P < 0.05$).

The antibacterial activity measured in terms of inhibition zones (mm) was found to be the greatest for ampicillin followed by GIC containing TiO_2 nanoparticles. The antibacterial activity of GIC containing TiO_2 nanoparticles was significantly greater than that of conventional GIC. ($P < 0.05$).The addition of 1% CPC also increased the antibacterial activity of conventional GIC but the results were not statistically significant (Table 2).

Table 2- Mean (standard deviation) of the growth inhibition zones of GIC incorporated with TiO2 nanoparticles, CPC and conventional GIC, and Tukey's analysis.

Group	Inhibition zone (mm)
Ampicillin (Positive Control)	31.16667 (± 2.979729)[a]
Distilled water (Negative control)	0 (± 0)[b]
GIC + 3% (w/w) TiO_2 nanoparticles	21.16667 (± 3.563281)[c]
GIC + 1% (w/w) CPC	18.25 (± 2.527126)[c, d]
Conventional GIC	15.75 (± 2.301185)[d]

Mean values for growth inhibition zones represented with the same superscript letter are not significantly different ($P > 0.05$), whilst the mean values with different letters are significantly different ($P < 0.05$).

DISCUSSION

The favorable adhesive properties, fluoride releasing ability, coefficient of thermal expansion which is close to that of tooth structure, biocompatibility and low toxicity have led to the widespread use of glass-ionomer cements as luting materials, cavity liners and bases, and restorative materials. [20-22] Due to the rising occurrence of recurrent caries following restorative treatments and the vast load of microorganisms in the oral cavity which may lead to further spread of caries affecting other teeth and creating newer carious lesions,[3] antibacterial agents like ciprofloxacin, metronidazole, minocycline, chlorhexidine and cetrimide have been proposed to be added in GICs which could reduce the total number of viable bacteria contemporaneously preserving dentinal tissue as well as pulp vitality.[23] Such GICs could potentially be used as a medium for slow release of antibacterial components.[3] However, these antibacterial agents affected the physical properties of GIC. One possible explanation for the reduction in physical strength of GICs may be that antibiotic powders may decrease the reaction between the glass particles and liquid cement, thereby increasing the number of unreacted particles in the structure. Also, the antibiotic particles added to GIC, easily absorb water resulting in the decrease in mechanical strength.[10] Since, GIC is the essence of the ART approach in dentistry which mainly includes restoration of posterior teeth; it is of utmost importance for the restorative material to exhibit favorable mechanical properties to withstand the occlusal forces.[3] Therefore, the definite antibacterial agent selected as well as its quantity play a critical role.[5] To understand the physical properties of GICs, compressive strength testing is, besides other methods, the most commonly employed method.[3]

Cetylpyridinium chloride (CPC) is a quaternary ammonium salt with both hydrophilic and lipophilic affinities. It is an effective antiplaque agent regulated by the Food and Drug Administration (FDA) as an over-the-counter drug[24] and an active ingredient of antiseptic oral mouth rinses. It is a broad spectrum antimicrobial agent having a rapid bactericidal effect on gram-positive pathogens[24] including oral streptococci. Nanostructured materials primarily metal oxide nanoparticles are also gaining recognition owing to their potential and selective toxicity, particularly in biological and pharmaceutical applications (Wu *et al* 2003; Fortner *et al* 2005; Li *et al* 2005).[25] Besides, Titanium dioxide nanoparticles have also been proposed for use as reinforcing fillers to dental resin composites and epoxy.[26,27] Thomas *et al* (2014)[28] reported that TiO2 nanoparticles were effective in inhibiting oral bacteria.

In this study GIC containing 3% TiO_2 nanoparticles appeared to exhibit significantly greater compressive strength than the CPC and conventional GIC groups. This can be attributed to the small sizes of the TiO_2 particles which get incorporated into the glass powder. The nanoparticles fill the voids between the larger GIC glass particles and at the same time act as additional bonding sites for the polyacrylic polymer.[29] Thus, the TiO_2 nanoparticles act as a filler between the GIC powder particles.[1] The mean values of compressive strength for GIC containing 3% TiO_2 nanoparticles were 172.5483 (±14.88441) MPa, which are in conformity with those of Elsaka *et al* (176.27 MPa)[2], El-Negoly *et al* (170 MPa)[1] and Contreras *et al* [29] Addition of 1% CPC reduced the compressive strength of GIC but within the acceptable limits, the result being statistically insignificant ($P > 0.05$).

The present study also revealed the antibacterial activity against Streptococcus mutans to be the highest for ampicillin (positive control) which is a known antibiotic followed by TiO_2 nanoparticles enriched GIC. The difference between the antibacterial susceptibility of GIC incorporated with 3% TiO_2 nanoparticles and conventional GIC was found to be statistically significant (p value <0.01). The findings are in accordance with that of Ahrari *et al* (2015).[30] The accurate mechanism(s) for bacterial toxicity of nanometals is still a matter of exploration, but the possibilities include free metal ion toxicity arising from the dissolution of metals from the surface of the NPs (e.g., Ag+ from Ag NPs, Kim et al. 2007) or oxidative stress via the generation of reactive oxygen species (ROS) on crystal surfaces of some NPs. The latter may be particularly important for anatase forms of TiO2, where the TiO2 surface reacts by photocatalysis with water to release the hydroxyl radical with subsequent formation of superoxide (Linsebigler et al. 1995). The ROS can then synergistically act by attacking polyunsaturated phospholipids in bacteria (Wong *et al* 2006) and cause site-specific DNA damage (Hirakawa *et al* 2004).[15] As previously mentioned, the form of the TiO_2 nanoparticles used in the current study was anatase phase, this explains the greater antibacterial activity of GIC incorporated with TiO_2 nanoparticles. Though statistically insignificant, CPC also increased the antibacterial activity of conventional GIC to some extent. CPC has antimicrobial activity apparently through multiple mechanisms. One mechanism is thought to be due to disruption of intermolecular interactions, causing a dissociation of cellular membrane lipid bilayers, compromising cellular permeability controls, and inducing leakage of cellular contents.[31] In addition, CPC has an inhibitory action against fructosyltransferases, the extracellular enzymes which synthesize fructans from sucrose, which play an important role in the progression of dental caries by serving as an extracellular nutrition reservoir for bacteria.[32] Rodriguez *et al* in 1994 investigated the growth inhibition of glass-ionomer cements on mutans streptococci and concluded that GIC restorative materials

have antimicrobial activity against all strains of oral streptococci. They concluded that inhibition activity of GIC was associated with fluoride release when pH was adjusted to neutrality.[33] The present study confirms the presence of an inherent antibacterial activity against S. mutans in conventional GIC.

Limitations

The current study measures only the compressive strength of the aforementioned materials; other attributes like tensile strength and wear resistance also need to be measured to appreciate the strength of the modified materials. For antibacterial activity testing freshly mixed unset cement was used, which is known to produce greater inhibition zones as compared to set cement specimens. Antibacterial activity of the aged set GIC was also not assessed in this study.

CONCLUSION

Considering the results obtained in this study, it can be concluded that addition of 3% TiO_2 nanoparticles improved the compressive strength of GIC as well as its antibacterial activity against S. mutans as compared to unmodified conventional restorative GIC. Further research should be carried out regarding any delayed undesirable systemic effects of TiO_2 nanoparticles and their different concentrations. Long term success of the modified glass-ionomer cement as a restorative material should also be analyzed, so that TiO_2 enriched GIC may develop as the material of choice in restorative dentistry with extended indications and clinical applications.

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