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Effect of Incorporating Various Nanoparticles on the Flexural Strength, Compressive Strength, and Antimicrobial Activity of Conventional Glass Ionomer Cement: An in Vitro Study

[1]Dr. Priti Maurya, Postgraduate Student, Department of Conservative Dentistry and Endodontics, Post Graduate Student, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

[2]Dr. Aisha Habeeb, Department of Conservative Dentistry and Endodontics, Reader, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

[3]Dr. Shekhar Kamishetty, Department of Conservative Dentistry and Endodontics, HOD and Professor, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

[4]Dr. Smitha Reddy, Department of Conservative Dentistry and Endodontics, Professor, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

[5]Dr. Pooja Srivastava, Department of Conservative Dentistry and Endodontics, Post Graduate Student, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

[6]Dr. K. Ravalika, Department of Conservative Dentistry and Endodontics, Post Graduate Student, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

Corresponding Author: Dr. Priti Maurya, Postgraduate Student, Department of Conservative Dentistry and Endodontics, Post Graduate Student, Sri Sai College of Dental Surgery, Kothrepally, Vikarabad, Telangana

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Abstract

Objective: The purpose of this study is to evaluate the effect of the incorporation of Graphene nanoparticles (GNP), Titanium dioxide nanoparticles (TiO_2NP), and Chitosan nanoparticles (CNP) on the flexural strength, compressive strength, and antimicrobial properties of conventional glass ionomer cement(CGIC) [GC Gold Label 9 high strength posterior restorative, GC Corporation, Tokyo, Japan]

Methods: CGIC was mixed with different nanoparticles to obtain forty-eight samples in the form of blocks (25x2x2 mm) for flexural(n=6) and compressive tests(n=6) and twenty four samples in the form of discs (3x1 mm) for antimicrobial property(n=6) according to the following groups: CGIC (group -1), CGIC +

GNP(group-2), CGIC + TiO_2NP(group-3), and CGIC + CNP(group-4). The samples were subjected to a universal testing machine individually for flexural strength and compressive strength analysis. Antibacterial activity was tested against Streptococcus mutans using the Kirby-Bauer agar disc diffusion assay. Data was analyzed using two-way ANOVA and post hoc Tukey test ($p<0.001$).

Results: There was a significant difference ($p < 0.001$) between the groups for compressive strength, flexural strength, and antimicrobial properties. Both compressive and flexural strength were highest for CGIC + TiO2NP, followed by CGIC + GNP > CGIC + CNP > CGIC. The zone of inhibition was highest for CGIC + TiO2NP, followed by CGIC + CNP > CGIC + GNP > CGIC. The CGIC + TiO_2NP group demonstrated better compressive strength, flexural strength, and antibacterial properties in comparison to the other tested groups.

Conclusion: The incorporation of nanoparticles into CGIC enhanced the mechanical properties while also providing improved antibacterial properties.

Keywords: Chitosan nanoparticles, conventional glass ionomer cement, Graphene nanoparticles, Steptococcus mutans, Titanium dioxide nanoparticles.

Introduction

Cervical lesions are commonly seen and are challenging to restore due to certain drawbacks, such as contamination from blood and gingival crevicular fluid due to their close proximity to the gingiva, and less or no enamel at the cervical margin[1]. Heymann et al. suggested the tooth flexure theory in 1991, which explains the increased risk of failure of class V lesions[2]. Gabel focused on the disruption of class V restorations caused by biomechanical forces and Hood suggested that excursive mandibular movements will place the buccal cusp in tension and compression[3].When a restoration is placed on the tension side, the interface between the tooth and restoration at the occlusal margin tends to open up, creating a wedge-shaped defect. Conversely, when the restoration is placed on the compression side, it may move out of the cavity due to the pressure applied to it [4,5]. A high C-factor leads to internal bond disruption, eventually leading to microleakage, which causes the seepage of fluids, ions, and bacteria, resulting in secondary caries, hypersensitivity, and pulpal infection [6]. Restorative materials can induce a variable amount of bacterial retention responsible for secondary caries formation. Streptococcus mutans (S. mutans) is one of the most common causes of secondary caries. S. mutans is a gram-positive facultative anaerobic bacterium. In dental restorations, bacterial adhesion occurs primarily at material margins. This interaction involves reversible processes between bacterial cell surfaces and material surfaces, governed by electrostatic forces, van der Waals forces, hydrophobic effects, acid–base pairs, and contact interactions [7].

Glass ionomer cement (GIC) is formed through the reaction between an aqueous solution of polyalkenoic acids and an ion-leachable fluoro-aluminosilicate glass powder. GIC exhibits biocompatibility, fluoride release, good bonding properties, and a coefficient of thermal expansion closer to that of natural tooth structure[8]. Numerous researchers have incorporated antibacterial agents (such as chlorhexidine and antibiotics) into GIC to increase its antibacterial effects, but this has decreased the physical properties of GIC due to water sorption and increased unreacted particles in the structure. GIC often fails due to voids and cracks and therefore needs to be reinforced with fillers [9,10].

Advancements in nanotechnology have broadened its therapeutic applications in dentistry. Nanoparticles are submicroscopic in size, and their unique properties,

including antibacterial action, and physical, mechanical, and biological characteristics, have rendered them effective vehicles for dental applications.

Graphene nanoparticles (GNP), a promising two-dimensional (2D) carbon-based nanomaterial, possess oxygen-containing functional groups and are the thinnest and strongest lamellar materials. Due to the numerous advantages of graphene-based materials, they have been utilized to enhance the properties of adhesive materials[11]. Titanium dioxide nanoparticles (TiO2NP) are inorganic metal oxides that release metal ions and act as crosslinking species[12]. Chitosan, a cationic polymer made of N-acetylglucosamine and D-glucosamine, is a chitin derivative. Chitosan nanoparticles (CNP) have demonstrated better biological effects and have gained recognition as a useful biopolymer. Nanosized chitosan formulations have found extensive applications in pharmacology as drug delivery agents due to their known properties, including anti-fungal, anti-microbial, anti-protozoal, anti-cancer, anti-plaque and tartar, and hemostatic effect [13].

The objective of this study is to evaluate the effect of the incorporation of

GNP, TiO_2NP, and CNP on the flexural strength, compressive strength, and antimicrobial properties of conventional GIC (CGIC). The null hypothesis states that there is no effect of incorporation of GNP, TiO_2NP, and CNP on the flexural strength, compressive strength, and antimicrobial properties of CGIC.

Materials and Methods

Preparation of Nanoparticle /GIC Mixture

The concentration of nanoparticles (Nano Research Lab, Nanotechnology Product) to be incorporated into CGIC was derived from studies done by Zhou J et al. for chitosan (cell viability of 2% CNP was acceptable), Liu R et al. for graphene (cell viability with 2% GNP was acceptable), and Daniela Dellosso et al. for titanium dioxide (cell viability of 5% TiO2NP was acceptable) based on MTT assay of these materials against human gingival fibroblasts[14-16]. To determine the desired weight percentage, a scale with an accuracy of ±0.0001 grams was used. Employing this apparatus at ambient temperature, the intended quantity of nanoparticles according to the groups was mixed with GIC powder [GC Gold Label 9 high strength posterior restorative, GC Corporation, Tokyo, Japan]. After adding the nanoparticles to the GIC powder, the mixture was manually mixed.

Sample Preparation

Forty-eight samples in the form of blocks (25x2x2 mm) and twenty-four discs (diameter 3 mm and thickness 1 mm) were prepared according to the following groups: (table 1)

According to the manufacturer's instructions, using an agate spatula, a scoop of CGIC powder containing the nanoparticles was mixed with a drop of CGIC liquid on a paper pad for 25 seconds. The samples were then positioned within a mold made of heavy body addition silicone (Zhermack Elite HD+ Putty Soft Normal Set, United Kingdom) for 5 minutes. Meanwhile, a transparent celluloid strip covered the top surface of the samples.

Compressive strength testing was performed using two flat metal discs, and three-point flexural strength analysis was done using a universal testing machine (Shenzhen Sans Testing Machine Co., Ltd., China) at a crosshead speed of 1 mm/min [17].

Flexural strength value was calculated by the equation

$\sigma = 3FL/2WD^2$

F: force maximum applied (Newtons),

L: distance of the specimen between the support (mm).

W: specimen width (mm),

D: specimen thickness (mm)

σ: Flexural strength (MPa) (σ - sigma)

Antimicrobial Property

Isolation and identification of bacteria

Shamsulddin ZD et al.'s methodology for collecting S. mutans from deep dentinal caries was followed [18]. Dentinal shavings from deep dental carious teeth were collected using a spoon excavator from a freshly extracted grossly decayed tooth. These shavings were transferred into brain heart infusion (BHI) broth and vortexed. A loopful of the resultant broth was streaked on BHI agar and incubated for 48 hours. S. mutans colonies were picked, cultured, and reconfirmed using Gram staining under 100x oil immersion microscopy. The isolated S. mutans was then inoculated in peptone water.

Kirby-Bauer method agar disc diffusion test

The isolated S. mutans culture was then streaked onto six Muller Hinton agar plates. Each agar plate was divided into four parts. Four punch cuts were made in each agar plate, and one disc from each group was placed into the agar plate and incubated for 48 hours. The antimicrobial activity of the disc was evaluated by determining the diameter of the clear zone of inhibition around the disc [19].

Statistical Analysis

The gathered data was inputted into SPSS version 20.

One-way ANOVA analysis was used to compare the means of compressive strength and flexural strength and zone of inhibition in between groups. Tukey hsd post-hoc analysis was done to confirm where the differences occurred between groups. P value was kept<0.001.

Results

One-way ANOVA analysis and Tukey hsd post-hoc analysis showed significant difference ($p < 0.001$) between the groups for compressive strength, flexural strength, and antimicrobial properties. (Table 2, Figures 1 and 2)

Compressive and Flexural Strength-The highest values for both compressive and flexural strength were observed in the CGIC + TiO_2NP. The next best-performing group was CGIC + GNP followed by CGIC + CNP. The unmodified CGIC exhibited the lowest compressive and flexural strength.

Antimicrobial Properties (Zone of Inhibition) -largest zone of inhibition was observed in CGIC + TiO_2NP followed by CGIC + CNP followed by CGIC + GNP group. Plain CGIC exhibited the lowest antimicrobial activity.

Discussion

GIC is commonly used for restoring cervical lesions due to its chemical bonding (adhesive properties), thermal compatibility with teeth, fluoride release, and mild pulpal response. The low susceptibility to fracture of glass ionomers is often attributed to the presence of porosity in the cement matrix. Once the material sets, these voids become confined within the cement, acting as stress concentrations and points of mechanical weakness [20].

During chewing, teeth and restorations undergo constant flexural and compressive forces. Additionally, these stresses are often heightened during parafunctional habits such as bruxism and clenching. The flexural strength of a material, also known as bend strength, is a crucial property that gauges its resistance to bending or breaking when subjected to stress. Compressive strength evaluates the masticatory stress on a restoration. Compressive and flexural strength are used for laboratory simulation of stresses associated with loads applied clinically to restorative materials and therefore are used in this study [21].

TiO_2NP showed improved mechanical properties compared to other groups, possibly because the small-sized nanoparticles are said to fill the voids between the larger GIC particles and act as additional bonding sites for polyacrylic polymer. When metallic nanoparticles are mixed with GIC powder containing acid, metal ions are released, serving as crosslinking species, thereby yielding stable cement [22].

Contrary to our results, a study by Ula A. Fathi et al. concluded that the incorporation of TiO_2NP in CGIC did not show any significant difference in the flexural strength of CGIC due to the insufficient amount of polyacrylic ionomer for efficient bonding [23].

Due to its crack bridging, pulling-out, crack deflection, and protection of crack tips, GNP exhibited superior results. As a matrix crack initiates and spreads, the load shifts from the matrix to graphene owing to their distinct elastic moduli. The textured surface of graphene with wrinkles facilitates effective mechanical interlocking and transfer of loads within the matrix [24].

CNP carries numerous acetamide and hydroxyl groups, with the ability to form bonds with both the hydroxyl groups of the GIC particles and the carboxyl groups of polyacrylic acid (PAA) through hydrogen bonding. The framework established by CNP and PAA surrounding the inorganic GIC particles could decrease the interfacial tension between the components of GIC, thereby enhancing mechanical performance [25].

GIC exhibits antimicrobial activity through its property of fluoride release, which inhibits glycolytic enolase and proton-extruding ATPase. TiO_2NPs have been documented to induce oxidative stress by producing reactive oxygen species (ROS). These ROS target polyunsaturated phospholipids within bacteria, leading to enzyme inactivation, site-specific DNA damage, and protein damage [26,27].

CNP functions by altering the electrical potential of cellular membranes. The protonated amino groups of CNP adhere to the anionic groups found in microbes, leading to agglutination. This process facilitates the displacement of calcium ions from the anionic sites of the membrane, ultimately causing cellular harm. Additionally, CNPs with low molecular weight possess the capability to penetrate bacterial cells and bind to bacterial DNA, thereby inhibiting transcription and translation [28].

GNP exhibits antimicrobial properties, likely due to its layered arrangement and well-defined edges, which cause physical damage to bacterial cell membranes. GNP also induces oxidative stress reactions that weaken bacterial resistance and lead to disruption [29]. Deokar et al. proposed that carbonaceous nanomaterials interact with the teichoic acid wall of Gram-positive bacteria via electrostatic or hydrogen bonding.

The interaction of GNP with teichoic acid, lipids, and amino acids results in morphological deformations observed in bacteria, including homogeneous thickening of the peptidoglycan cell wall, enlargement of cell size, delays in cell growth, and agglomeration of cells in solution [30].

The present study's limitations include the lack of cyclic loading and thermal cycling, which are crucial for evaluating material durability and temperature resilience. Additionally, the absence of saliva storage fails to simulate real clinical conditions, potentially affecting the material's performance and biocompatibility. These factors may limit the study's applicability to practical, real-world scenarios.

Conclusion

Given the constraints of the study, it is possible to infer that 5% w/w TiO2NP and 2% w/w CNP can be incorporated into CGIC for improved mechanical

properties and antibacterial properties. Although 2% w/w GNP improved the mechanical properties, there was not much significant improvement in antibacterial properties and it also led to blackening of the cement. Thus, TiO2NP and CNP are preferable for achieving better overall performance without adverse effects.

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Legend Tables and Figures

Table 1: shows various study groups considered during this study

Groups	Sample distribution (n=6)	Sample dimension
Group I CGIC (GC Gold label type 9) (Control)	Compressive Strength	25x2x2 mm blocks
	Flexural Strength	
	Antibacterial properties	3x1 mm discs
Group II CGIC + 2% w/w GNP (3-6nm size)	Compressive Strength	25x2x2mm Blocks
	Flexural Strength	
	Antibacterial properties	3x1 mm discs
Group III CGIC +5% w/w TiO_2NP (30-50nm size)	Compressive Strength	25x2x2mm Blocks
	Flexural Strength	
	Antibacterial properties	3x1 mm discs
Group IV CGIC + 2% w/w CNP (50-100nm)	Compressive Strength	25x2x2mm Blocks
	Flexural Strength	
	Antibacterial properties	3x1 mm discs

CGIC - Conventional glass ionomer cement, GNP - Graphene nanoparticles, TiO_2NP - Titanium dioxide nanoparticles, CNP - Chitosan nanoparticles

Table 2: Comparison of mean Compressive Strength, Flexural Strength, and Zone of Inhibition

Group (n=6)	Compressive Strength (Mpa) Mean±SD	Flexural Strength (Mpa) Mean±SD	Inhibition Zones Mean±SD (mm)
CGIC	3.15±0.22	2.0±0.15	12.33±1.751
CGIC+GNP	5.02±0.13	8.5±0.2	15.50±1.517
CGIC+TiO2NP	5.18±0.24	9.4±2.9	25.33±1.751
CGIC+CNP	4.8±0.10	6.17±0.18	23±0.577
P-VALUE	0.001*	0.001*	0.001*

Figure 1: shows the mean Compressive and Flexural Strength between groups

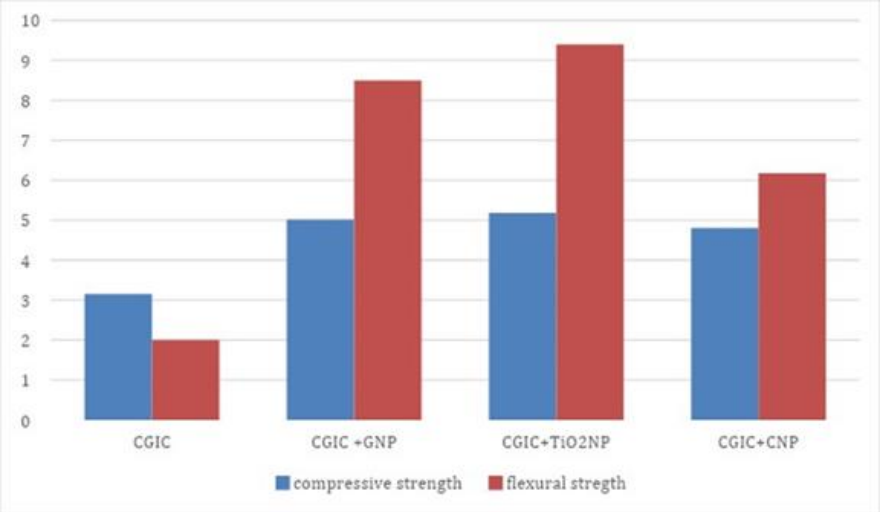

Figure 2: shows the mean Zone of Inhibition between the groups