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SYNTHESIS OF REINFORCED CONVENTIONAL GLASS IONOMER CEMENT WITH NANO-HYDROXYAPATITE-SILICA, TITANIUM DI OXIDE AND COMPARATIVE EVALUATION OF MICROLEAKAGE, SHEAR BOND STRENGTH AND ANTIMICROBIAL ACTIVITY :-AN IN VITRO STUDY

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Abstract

Aims and Objectives: To assess and contrast reinforced glass ionomer cement with nano-hydroxyapatite-silica, titanium di oxide, and ordinary glass ionomer cement in terms of microleakage, shear bond strength, and antibacterial activity.

Materials and Methods: 45 removed premolar teeth had class v cavities for the examination of microleakage. Three groups were created from the samples. Regular GIC (GP 1), Nano hydroxyapatite GIC (GP 11), and GIC with TiO2 incorporation are all examples of GIC. The samples were maintained in a thermocycling machine after polishing. After that, nail polish was used to cover the teeth everywhere but the two millimeter boundary around the restoration margins. After soaking for 24 hours at room temperature in methylene blue solution (2%), they were examined under a microscope. The samples were washed in regular salt water and then dried naturally. After the teeth were anchored with acrylic resin, the samples were prepared for sectioning. The specimens were divided in half along their buccal and lingual surfaces. The stereomicroscope, set at 40x magnification, was used for the examination. Dye penetration into dentin and enamel was measured using a dye penetration scale.

For the purpose of measuring shear bond strength, 30 teeth were selected and cut in half mesiodistally along the tooth's long axis. Each piece was positioned so that the bonding surfaces faced each other in an acrylic block. After the block was polymerized, it was smoothed out on both the buccal and lingual sides to create a uniform dentinal layer. The samples were then randomly split into three groups, each of which represented a different GIC. The materials were then stored in a thermocycling machine for 24 hours. After 24 hours at a cross speed of 1mm/min, the test was evaluated using universal testing equipment.

In order to conduct the antimicrobial test, sixty GIC pellets were manufactured and the specimen was split into three groups of twenty. After being held for 24 hours at 37°C and 100% humidity, the samples were maintained in a thermocycling machine. Then, ethylene oxide gas was used to sanitize the samples. A single colony of streptococcus mutans was cultured in 3ml of brain heart infusion broth & grows over night at 37°c for 4 hrs & transfer on to a sterile brain heart agar plate. Wells of diameter 6mm was made in the agar medium using sterile templates. GIC was incorporated into the wells & ensured uniform contact & incubation will be done for 24hrs. The zone of inhibition growth of bacteria was measured using a vernier caliper.

Result: Between Gr1 and Gr2, a sizable difference in mean shear bond strength was observed, Gr2 & Gr3 (table 2,3 & 4 graph 1). Highest mean mean value for shear bond strength was found of Gr 3(2.2733) followed by Gr 2(1.2067) and the lowest mean value was found of Gr 1(0.6133). The microleakage scores was found to be maximum among Gr 1(2.2667), followed by Gr 3(1.9333) & Gr 2(1.8667) in decreasing order. The mean zone of inhibition between the two groups showed a significant difference. Gr 1, Gr 2 & Gr 3 (table 8, 9 & graph 4). The highest mean value for zone of inhibition was found of Gr 2(15.6745) followed by Gr 3(14.6685) and the lowest mean value was found of Gr 1(6.649).

Conclusion: By adding nano-sized fillers of HAP silica and TiO2 to traditional glass ionomer cement, it is possible to nano-modify the material, which will improve both its mechanical and antibacterial properties by increasing the release of fluoride.

Keywords: antimicrobial activity, Nano-modification, Microleakage, Shear bond strength.

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Introduction

Caries is one of the most prevalent diseases that affects people of different ages. The best course of treatment to maintain oral health status is early restoration. With the development of the diagnostic system and the revolution in adhesion technology, restorative dentistry has now switched from an intrusive approach to a minimally invasive method.1 Glass ionomer cement (GIC), which has revolutionized restorative techniques, particularly in minimally invasive dentistry, is an excellent example of contemporary dental materials.[2]

Wilson and Kent first presented glass-ionomers cement to the dentistry industry in 1972.[3] Positive clinical features of GICs include their chemical bonding to healthy tooth structure, the release of fluoride, the elimination of bacteria, the prevention of caries, and the promotion of remineralization of carious lesions.[.4] Despite this, GIC typically cannot sustain the forces produced in the posterior region of the mouth due to their poor mechanical characteristics, particularly their poor fracture toughness.[5] GIC's mechanical characteristics are still inferior to those of other restorative materials.

Because of its clinical significance, GIC continues to undergo constant development, upgrading, and modification. Mostly change its composition to enhance the cement's chemical and physical qualities and clinical handling.[6]

Nanoparticles or "nanoclusters" can be added to tooth restorative material to enhance its mechanical qualities.[7] Systems, changes, or materials of a size between 1 and 100 nm are used in nanotechnology. Top-down and bottom-up are the two methods used to create nano-sized particles. To produce nano-sized particles for integration into GICs, bulk materials such apatites, silicate glasses, and certain metal oxides are typically nanofabricated top-down.[8]

One of the most significant calcium phosphate bioceramics, hydroxyapatite (HA) has a crystal structure and chemical makeup that is identical to apatite found in bone tissue and human tooth structures (Ca/P=1.67; chemical formula: Ca10(PO4)6(OH)2.[9] Numerous studies have attempted to determine whether adding nanoparticles or "nanoclusters" may enhance the mechanical properties of Cgic[10-12] in recent years. The addition of nano HA silica to cGIC was reported to boost its hardness by 73 percent when compared to cGIC made using only traditional GIC.[10]

Due to its chemical stability, biocompatibility, and antibacterial characteristics, titanium dioxide is a viable inorganic addition. Prior research looked into using titanium dioxide Nano powders (TiO2 NP) A composite resin used for dental restorations as strengthening fillers.[13] TiO2 NP was included into the powder component of GICs in a study by Alaska et al. (3% w/w), and this led to a considerable improvement in the material's physical and antibacterial properties.[14]

Numerous research have been conducted up to this point, but no information is known on the comparison and incorporation of both nanoparticles at 6% weight in the GIC Fuji IX. The goal of this research was to compare and evaluate the microleakage, shear bond strength, and antibacterial properties of titanium dioxide, nano hydroxyapatite silica, and ordinary glass ionomer cement.

Materials and Methods

Materials for synthesis of Nano Hydroxy apatite silica

Powdered and liquid forms of commercial GIC (Fuji IX GP; GC International, Japan) were used in this study. CaO was utilized as a chemical in the study (99%, Sigma Aldrich, Germany). (Sigma Aldrich, Germany, 99% purity) 99% phosphoric acid from Germany's Sigma-Aldrich Orthosilicate tetraethyl Sigma Aldrich, a

German chemical company, sells both ammonia and ethanol. TISAB III (total ionic strength buffer; Sigma-Aldrich, Germany).

Materials for synthesis of Titanium di oxide

All of the Sigma Aldrich, Germany, materials used in this experiment are of the highest quality and purity (99% ethanol, 99% distilled water, 99% liquid ammonia, and 99% double-distilled water).

Construction of Nano-Silica-Hydroxyapatite

The sol-gel technique allowed the synthesis to be performed in a single vessel. We were able to dissolve 7.408g of calcium hydroxide in 100mL of pure water. This mixture was stirred for 30 minutes with a magnetic stirrer. The calcium hydroxide solution was slowly infused with phosphoric acid until 4.104 milliliters had been added. This suspension was shaken for a full day. Liquid ammonia was used to maintain a pH of between 11 and 12 in the suspension. After 12 hours, 20 mL of TEOS was dissolved in 10 mL of 100% ethanol and added to the calcium hydroxide suspension in a slow, steady stream. After 48 hours, we centrifuged the produced sol (Eppendorf Centrifuge 5804, Germany), freeze-dried it (ScanVac CoolSafe, Denmark), and calcined it (WiseTherm, Germany) at 600°C. The calcined powder was pounded by hand with a mortar and pestle for 10 minutes.

Synthesis of Titanium di oxide

TTIP (20ml) + absolute ethanol (50 ml) + distilled water (100 ml). Stir for 20min. Add liquid ammonia drop by drop till PH of solution becomes 10. Leave the solution for 2hr. Centrifuge for 7-8 mins 3-4 times by double distilled water and ethanol and drying it for 12hrs at 60°C. Calcined in furnance at 500°C for 2 hrs and left for day to cool down. The sample is then crushed and collected.

Incorporation of the synthesized material into the GIC

Both the synthesized material was added at 6% into the GIC (fuji IX) after weighing on an balance accurate. Using a mortar and pestle, the powder combination was gently pounded by hand for 30 minutes. Fig 1.

Fig 1- Synthesized Nano-HA-SI & TiO_2 incorporated into GIC fuji IX

The teeth that were gathered for the experiments weren't particularly extracted for this investigation. Instead, they were gathered from Sharda University's School of Dental Sciences' Oral Surgery department. Specific teeth were chosen from among those extracted in accordance with the inclusion criteria for our study.

Inclusion/ Exclusion criteria

Freshly extracted Sound Premolars and teeth having Caries, Cracks, fracture Developmental defects, Wasting diseases and restored teeth were excluded from the study. Freshly prepared GIC pellets were included and Distorted or Porosity incorporated GIC pellets were excluded from the study.

For Microleakage Testing

45 excised premolars were removed, cleaned for one week in a 0.5 chloramine solution, and then stored in a 10% formalin solution. Each tooth's buccal surface has a conventional box-shaped cavity created there(fig. 10). Each cavity was restored and measured at 3 by 0.3 and

Score	Dye Penetration Scale
0	No dye penetration
1	Dye penetration involving half the occlusal or gingival wall
2	Dye penetration involving more than half the occlusal or gingival wall
3	Dye penetration involving the axial wall

1.5 millimeters in depth. Three groups of 15 samples each were created from the repaired teeth. Groups 1 and 2 are conventional GIC, and Group 3 is titanium dioxide reinforced GIC. Group 1 is conventional GIC. A very fine diamond bur was used for polishing, and cocoa butter was then applied to the restoration's surface. The samples were then subjected to 500 cycles of 4° to 60°C with a 15s dwell time in a water bath maintained at 37°C and 100% relative humidity. After 24 hours at room temperature, the teeth were dipped into a 2% solution of methylene blue and then coated with nail polish, leaving a 2 mm margin. In order to prepare the samples for sectioning with a diamond saw in a buccolingual direction, storage teeth were cleaned with ordinary saline, allowed to air dry, and then embedded in acrylic resin. To assess microleakage, tooth slices were produced (Fig. 2) and examined under a stereomicroscope at a 40x magnification. According to the dye penetration scale, the depth of penetration will be measured (Table 1).[15]

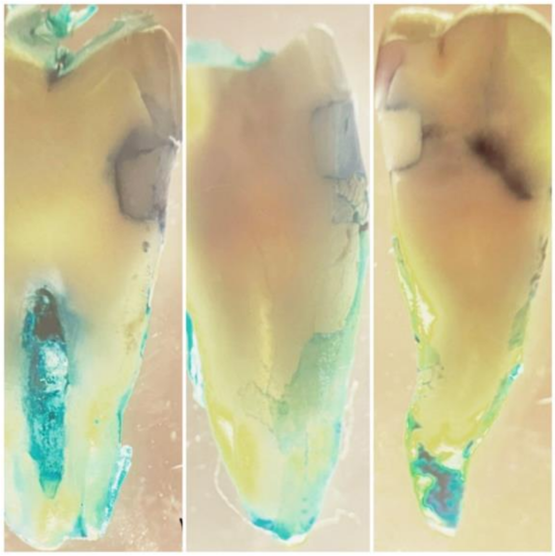


Fig 2- Depth of dye penetration of Conventional, Nanohydroxyapatite silica and Titanium di oxide GIC

Table 1 - Dye penetration scale (UMER et al)[15]

For Shear Bond Strength

30 teeth were chosen. The teeth were split into two parts mesiodistally, perpendicular to the tooth's long axis. Each section's buccal and lingual surfaces were placed for bonding while it was immersed in an acrylic block. The dentinal layer was made uniform by polishing the buccal and lingual sides of the block using 600 grit silicon carbide paper after polymerization. Under a stereomicroscope, the exposed dentinal surface was inspected to ensure that no enamel remained and that pulp exposure had not occurred. The samples were then randomly split into three groups, each of which represented a different GIC. Group 1 consists of regular GIC, Group 2 of GIC reinforced with nano hydroxyapatite silica, and Group 3 of GIC reinforced with titanium di oxide. Attaching the restoration to the teeth required the use of a bonding jig and a Teflon bonding template (diameter: 2 mm; height: 2 mm). After 24 hours in a 37°C water bath, samples were subjected to 500 cycles of 4° and 60°C with a 15-second dwell time at each temperature. After 24 hours, the test was evaluated using a

universal testing machine at a cross speed of 1mm/min. (Fig 3).

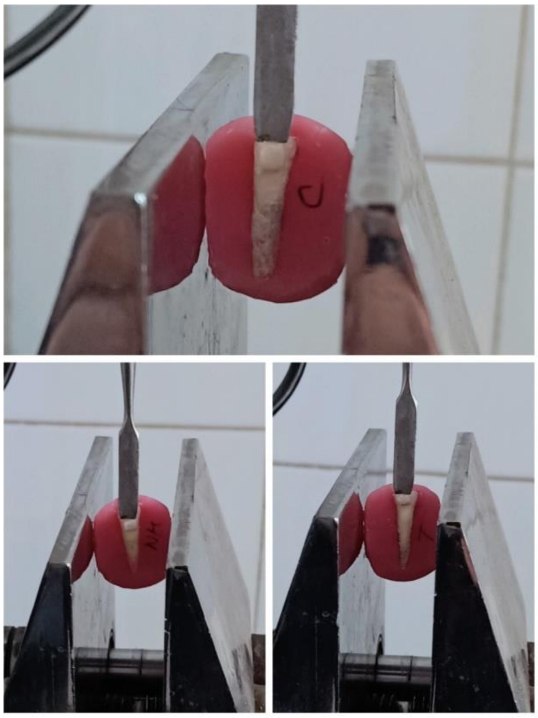


Fig 3- Testing of the samples at a pace of 1mm/min in a universal testing equipment

For Antimicrobial Testing

A split Teflon mold was used to create 60 GIC pellets. The specimen was divided into three groups, each with 20 samples, and was then restored. Groups 1 and 2 are conventional GIC, and Group 3 is titanium dioxide reinforced GIC. Group 1 is conventional GIC. The samples were kept for 24 hours at 37 degrees Celsius and 100% humidity before being put through 500 cycles of 4 and 60 degrees with a dwell period of 15 seconds. Then, ethylene oxide gas was used to sterilize the specimens. A single colony of streptococcus mutans was cultured in 3ml of brain heart infusion broth & grows over night at 37°c for 4 hrs & transfer on to a sterile brain heart agar plate. Wells of diameter 6mm was made in the agar medium using sterile templates. GIC was incorporated into the wells & ensured uniform contact & incubation will be done for 24hrs. The zone of inhibition growth of bacteria was measured using a vernier caliper. Fig 4

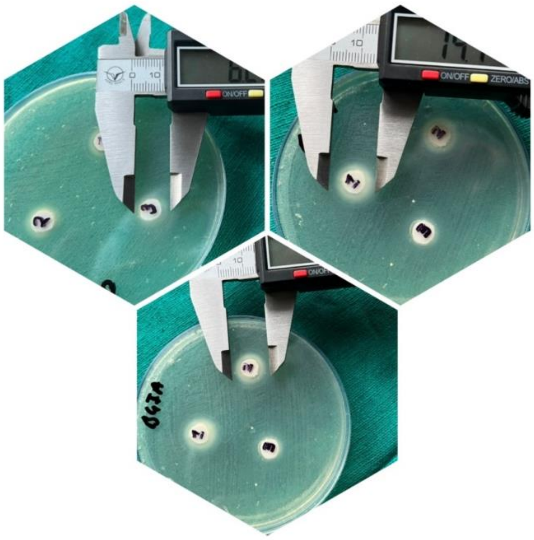
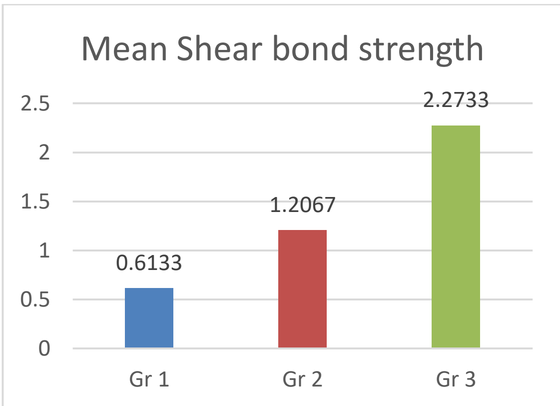


Fig 4- Zone of inhibition around conventional GI

Results

Micro leakage, shear bond strength, and their respective means were compared statistically using a one-way ANOVA test, and antimicrobial assessment between Nano hydroxyapatite silica and titanium di oxide, with traditional glass ionomer cement (GIC fuji IX) serving as the control. Means were compared using the Post hoc pairwise and Kruskal Wallis tests. A significance level of 0.01 was established for this study.

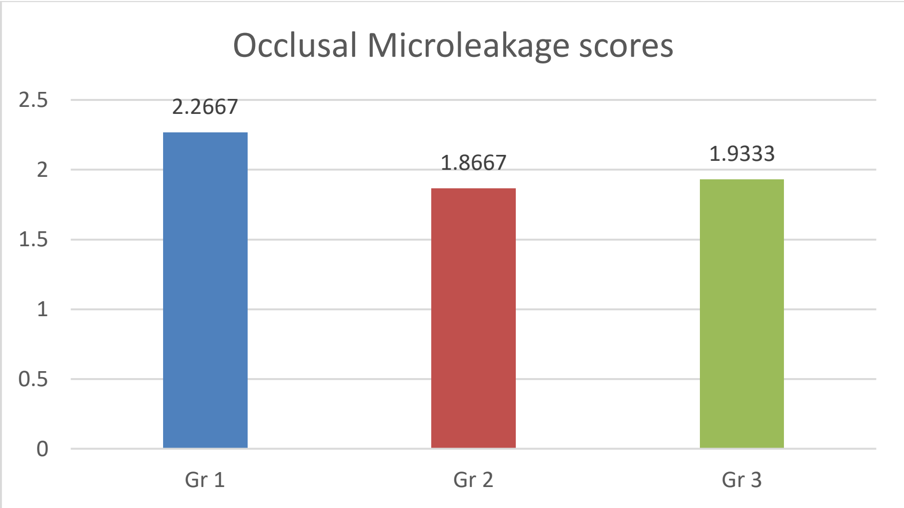


Graph 1- Shear bond strength descriptions for several groups

Table 2- Strength of the shear bond described

Shear bond strength					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Gr 1	10	.6133	.13020	.5412	.6854
Gr 2	10	1.2067	.19445	1.0990	1.3143
Gr 3	10	2.2733	.18696	2.1698	2.3769

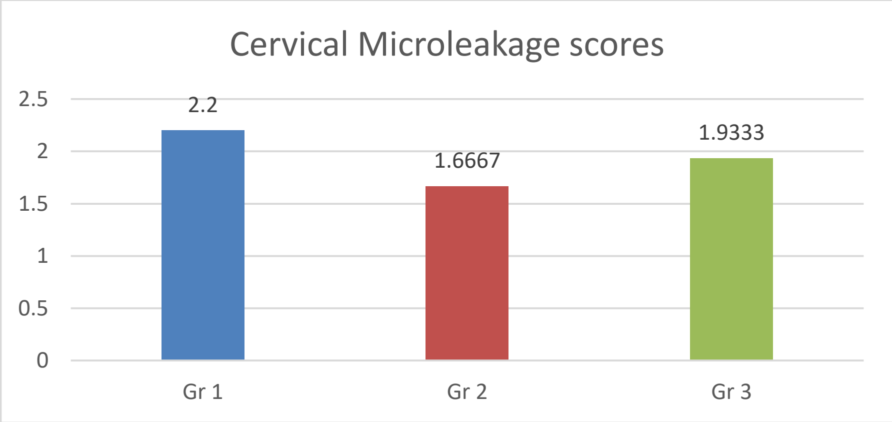
The mean shear bond strength varied significantly between Gr1, Gr2 & Gr3 (table2 & graph 1). Highest mean mean value for shear bond strength was found of Gr 3(2.2733) followed by Gr 2(1.2067) and the lowest mean value was found of Gr 1(0.6133) using one way ANOVA test ($p \leq 0.05$). For pairwise comparison, it is found to be statistically significant using Post hoc pairwise test.



Graph 2- Intergroup comparison of Occlusal Microleakage scores

Table 3- Descriptives of occlusal microleakage scores

Occlusal Microleakage scores					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Gr 1	15	2.2667	.79881	1.8243	2.7090
Gr 2	15	1.8667	.83381	1.4049	2.3284
Gr 3	15	1.9333	.70373	1.5436	2.3230
P value		0.323			

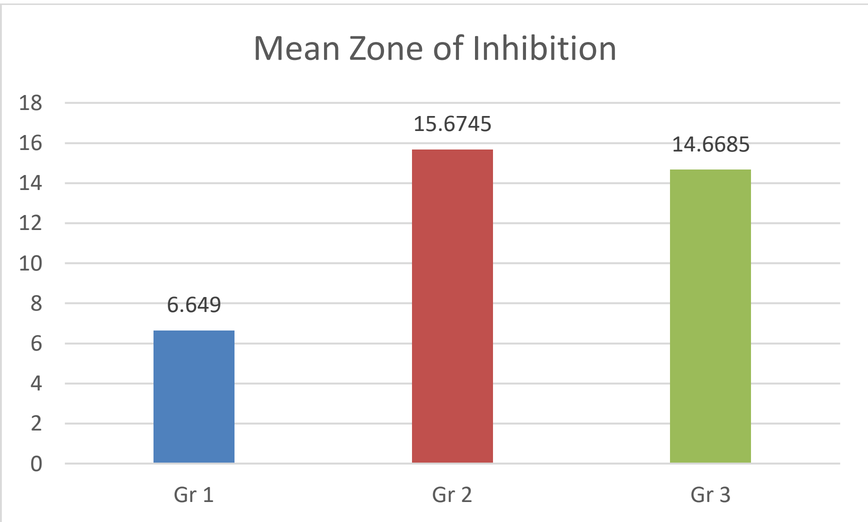


Graph 3- Intergroup comparison of Cervical Microleakage scores

Table 4- Descriptives of cervical microleakage scores

Cervical Microleakage scores					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Gr 1	15	2.2000	.77460	1.7710	2.6290
Gr 2	15	1.6667	.61721	1.3249	2.0085
Gr 3	15	1.9333	.79881	1.4910	2.3757
P value		0.159			

The dye penetration of the cervical and occlusal groups differed significantly (tables 3 and 4 and graphs 2 and 3). Overall, the microleakage scores was found to be maximum among Gr 1(2.2667), followed by Gr 3(1.9333) & Gr 2(1.8667) in decreasing order using Kruskal Wallis test.



Graph 4- Descriptions of each group's Zone of Inhibition

Table 5 - Zones of Inhibition for various groups described

Zone of Inhibition					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Gr 1	20	6.6490	.44299	6.4417	6.8563
Gr 2	20	15.6745	.44830	15.4647	15.8843
Gr 3	20	14.6685	.54997	14.4111	14.9259

Between Gr 1, Gr 2, and Gr 3, there was a discernible variation in the mean zone of inhibition (table 5 and graph 4). The highest mean value for zone of inhibition was found of Gr 2(15.6745) followed by Gr 3(14.6685) and the lowest mean value was found of Gr 1(6.649) using one way ANOVA test ($p \leq 0.05$). For pairwise comparison, it is found to be statistically significant using Post hoc pairwise test.

Discussion

Glass ionomer cement (GIC) is one of the most used restorative materials in dentistry. The acid phase of GIC is commonly a polymer liquid, and the basic glass phase is an acid-degradable fluoro-aluminosilicate powder. Positive clinical features of GICs include their ability to chemically bond to healthy tooth structure, release fluoride, fight germs, prevent caries, and remineralize decayed areas.[3]

Numerous investigations have shown that typical GICs have antimicrobial properties. The most widely accepted theory is that the bacterial inhibitory effect is caused by fluoride ions that leach from GICs.[16] Additionally; Polyacrylic acid's (PAA) acidity has been shown to have antibacterial properties in the past. Another explanation highlighted the GICs' zinc component's antimicrobial properties.[17]

Traditional GICs have been shown to be effective against many other types of bacteria, but their ability to kill S. mutans, a key factor in the development of dental caries, is still up for debate.

A good adhesion between restorative material and enamel and dentin is also very important. There should be a complete and perfect seal between the restoration and tooth cavity walls. A measure of the materials ability to adhere with tooth structure is assessed through microleakage evaluation.[18]

Because it is a major cause of secondary caries and pulpal irritation, microleakage is one of the most frequent reasons for the failure of different restorative materials. Consequently, there is a growing concern in finding a dental restorative material, which has good bonding characteristics, thus reducing microleakage and minimizing the potential for caries development and pulpal irritation. Conventional GIC is one dental material that bonds chemically to tooth structure. However because of low mechanical properties, the risk of fracture exists for larger restorations. GIC is still inferior to other restorative materials when it comes to mechanical properties. Hence, there has been an ongoing pursuit for further improvement in the properties of GIC.[18]

Thermo-light polymerization and the addition of reinforcement phases like metal oxides like ZrO_2 or minerals like hydroxyapatite or polymeric substances like N-vinyl pyrrolidone or fibers or ceramic additives were both considered to enhance the mechanical properties of GICs. Although medicinal uses of these discoveries have not yet been developed, the mechanical properties were improved by all methods to varied degrees.[19]

Nanotechnology can be used to a material to improve some flaws (like weak mechanical properties) or increase other qualities (like antibacterial properties). Nanoparticles' (NPs) ability to kill bacteria is contingent on both the physicochemical qualities of the NPs themselves (such as their size, shape, and the amount of silver ions they release) and the specific bacteria they are up against (in terms of species, cell structure, and susceptibility).

Both hydroxyapatite (Ca10(PO4)6(OH)2, HAP) and titanium dioxide (TiO2 NP) Nano powder were synthesized in this study by sol-gel synthesis. Later on after the synthesis of the material it was added to the conventional GIC fuji IX at 0.6% to access the microleakage, shear bond strength and antimicrobial activity between Nanohydroxyapatite incorporated GIC, Titanium di oxide incorporated GIC and conventional GIC.

Results showed that the microleakage scores was found to be maximum among Gr1(specimens restored with conventional GIC, followed by Gr3(specimens restored with titanium di oxide incorporated GIC) & Gr2(specimens restored with nano hydroxyapatite silica incorporated GIC)

Due to their function in the contact of GIC's powder and liquid, HAP particles may release ions that take part in the acid-base process. More hydrogen and ionic bonds may occur if ions are present at the interface between the tooth structure and the restoration material. Lower micro leakage appears to be the result of better bonding.

The greater filler loading in both Nano HAGIC and Tio2 GIC may also be the cause, since this may have reduced heat expansion and polymerization shrinkage, increasing long-term bonding to tooth structure.

Titanium di oxide enhanced GIC had the strongest bond strength, followed by Nano-HA GIC and regular GIC, according to the shear bond strength test.

The ultimate mechanical properties of cements are strongly influenced by the cross-linking formation during setting, in addition to particle size and particle size distribution.

The salt hydrogel formed during the setting process acts as both the binding component of the matrix and the reinforcing component of the glass. Metal ions can be liberated from the particles by treating them with acid. These free metal ions serve as cross-linking species, allowing for the development of robust cement. Therefore, it appears that the tio2 nano particles and the cement matrix may cross-link and interfacially connect effectively.

Also when nano particles are added into the glass ionomer cement powder, as the size of nano particles is very small so they try to fill all the empty spaces generated among unreacted and large particles in cement from glass ionomer powder quite easily and it became an extra site of linkage for the poly acrylic polymer, hence strengthening the cement of glass ionomer.

Cement samples' antibacterial efficacy is measured by the diameter of the inhibitory zone around them. According to this study, when compared to standard glass ionomer cement, the antibacterial activity of GIC is improved when restored with nano hydroxyapatite silica and titanium di oxide. But when compared to Nano HA GIC and Tio2 GIC, the ZOI in Nano HA silica integrated GIC was higher.

Because the nanoparticles enter the bacterial cell, create oxidative stress, and enhance the release of fluoride ions, the formation of inhibitory zones around the cement samples, which reduces bacterial growth and kills the cells it contains. This implies that boosting GIC's fluoride ion release and preventing lingering bacteria in dentine can be accomplished by adding Nano HA or Tio2 particles.

Conclusion

Because of its numerous desirable properties, including as its biocompatibility, fluoride ion release, chemical adhesion to the tooth surface, and low coefficient of thermal expansion, GIC is widely used as a restorative material in clinical dentistry. Despite its advantages, GIC is not frequently used in clinical dentistry due to a number of serious disadvantages, especially when applied to stress-bearing areas. Some of the issues with GIC can be mitigated by adding certain chemicals.

Based on the findings of the current study, conventional glass ionomer cement can be nano-modified to improve its mechanical properties as well as its fluoride release, which will increase its antimicrobial properties. This can be done by adding nano-sized fillers of HAP silica and Tio2 to conventional glass ionomer cement. The filler particle can increase the stability of the set cement and strengthen the bond with the tooth structure. Fluoride release can be increased to assist prevent secondary caries around restorations. The use of nano-modified glass ionomers in routine clinical dentistry is still constrained, nonetheless, due to a dearth of long-term clinical research. It is anticipated that in the future, high-quality dental restorative materials will be generated in conjunction with the use of nanotechnology, material understanding, and advancements in biomaterials.

Then again More randomized clinical trials and in vivo research are needed to support the usage of these promising materials.

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