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Original Article

Dentistry Section

Antimicrobial and Mechanical Properties of GIC Incorporated with Silver Vanadate Nanoparticles: An In-vitro Study

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ABSTRACT

Introduction: Glass Ionomer Cement (GIC) is a widely used restorative material, but its anti-bacterial property is not sustained overtime. The addition of nanosized silver vanadate (AgVO3) is an attempt to enhance the anti-bacterial property of GIC without affecting its mechanical properties.

Aim: To evaluate the anti-microbial property, Flexural Strength (FS) and Surface Micro Hardness (SMH) of GIC added with silver vanadate (AgVO3) nanoparticles.

Materials and Methods: This in-vitro study was conducted in the Department of Pedodontics, Vishnu Dental College, Bhimavaram, India from June 2020 to July 2021. The AgVO3 nanoparticles were proportionally added to Type II GIC (Group-1) powder at the concentrations of 0.5% (Group-2), 1.0% (Group-3) and 2.0% w/w (Group-4). A total of 56 specimens were prepared to evaluate the test parameters. Anti-microbial property was evaluated using disk diffusion method. FS was determined using a universal testing machine and SMH using Vicker's Microhardness (VHN) tester. The obtained data was analysed using one-way analysis of variance (ANOVA), Tukey's test and unpaired t-test.

Results: The GIC with AgVO3 nanoparticles exhibited higher anti-microbial property compared to un-modified GIC (Group-1). Group-4 exhibited highest anti-microbial property, followed by Group-3 and Group-2. Group-4 exhibited highest FS with a mean value of 26.90 MPa (p=0.002) and SMH with a mean value of 61.29 VHN (p=0.001) than Group-1.

Conclusion: Addition of 2.0% w/w $AgVo_3$ nanoparticles to Type II GIC shown to have higher anti-bacterial property, and also displayed higher FS and SMH.

Keywords: Flexural Strength, Glass ionomer cement, Microhardness, Nanosized

INTRODUCTION

Restorative care in children and adolescents is constantly evolving. In the 1950s and early 1960s, silicate cement was the tooth-coloured restorative material available. It has beneficial properties attributed to the presence of fluoride in silicate cement powder. However, these cements were replaced with GIC over a period of time due to their high acidic nature, high solubility in oral fluids, poor mechanical properties, discolouration with time and no proper adhesive bond between the tooth and restoration [1].

GIC is a widely used tooth coloured restorative material in paediatric dentistry as it chemically bonds to the hard tissues, leaches out fluoride ions and has anti-cariogenic property [1,2]. However, some properties of GIC limit its extensive use as a restorative material. It is shown to have weak mechanical and physical properties including low fracture strength and hardness, reduced wear resistance and opaqueness. In order to improve the mechanical, physical and chemical properties, GICs are subjected to many modifications since it's introduction [1].

The use of nanotechnology has attracted significant attention in recent years in the field of dentistry. The use of nanosized particles in dental materials was found to improve the functional and structural properties; while optimising the clinical and aesthetic attributes of the material [2]. The addition of silver, titanium dioxide [3] and gallocatechin-3-gallate nanoparticles [4] to GIC was shown to have improved anti-microbial properties with varying physical and mechanical properties.

Vanadium is one of the hardest metals and has good resistance to corrosion. Vanadium added to silver is proven to be biocompatible and anti-microbial, which is used in making implantable devices like cardioverter defibrillators, neurotransmitters, Artificial External Defibrillators (AEDs) and drug infusion devices in the medical field. Modification of vanadium oxide nanostructures with silver nanoparticles (AgNPs) has biomedical applications [5]. The incorporation of AgVO3 in acrylic dentures promoted anti-microbial activity without altering mechanical properties [6]. Its addition to endodontic sealers was found to have an increased antimicrobial effect without major changes in physico-chemical properties [7].

Considering its beneficial effects, this study was formulated to incorporate AgVO3 nanoparticles into Type II GIC to improve the anti-microbial property. The literature search did not reveal such efforts being done earlier, and also its influence on the mechanical properties of the cement. Hence, this study was carried out to evaluate the anti-microbial and mechanical properties of GIC added with AgVO3 nanoparticles. The null hypothesis was that there will be no effect on anti-microbial property, FS, and SMH of GIC incorporated with AgVO3 nanoparticles.

MATERIALS AND METHODS

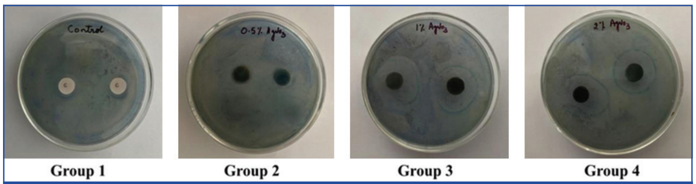
This in-vitro study was performed in the Department of Pedodontics at Vishnu Dental College, Bhimavaram, India during the time period between June 2020 to July 2021. The study was reviewed and approved by the Institutional Ethical Committee (IEVDC/19/PG01/PPD/IVT/39).

Sample size calculation: Considering the methodology and results of the study conducted by Jowkar Z et al., (2019) [8]. Using G*Power 3.1.9.2 software for power analysis indicated that the authors required a total of 56 samples. The number of samples to evaluate anti-microbial property was 28, and 14 samples each to test FS and SMH.

Preparation of samples: Incorporation of AgVO3 into resins in the earlier studies have shown that FS was decreased with increase in the concentration of AgVO3 nanoparticles with no change in SMH. Also, as increase in percentage of AgVO3 cause agglomeration of nanoparticles, the maximum concentration of AgVO3 nanoparticles in the present study was limited to 2% [6]. A 4 mg, 8 mg and 16 mg of AgVO3 nanoparticles (Nano Elements sub-branch of Sigma Aldrich, Germany) was added to 1.8 g of GIC (GC Type II cement, Tokyo, Japan) powder, to obtain 0.5%, 1%, 2% w/w concentration AgVO3 nanoparticles, respectively [6,9]. The modified GIC was manipulated following the manufacturer's instructions and transferred into spherical glass moulds of 10 mm diameter, 2 mm thickness and rectangular glass moulds of 25x2x2 mm dimension, and covered with a thin glass slide on either side. After initial setting of four minutes, specimens were removed from the mould. Finishing and polishing was carried out using 400, 600, 1200 grit sand paper. The specimens were then stored in distilled water at 37°C for 24 hours.

Antimicrobial property: The anti-microbial property was evaluated using disk diffusion in a direct contact method. Un-modified GIC (Type II GIC) served as a control group and considered as

- **Group-1:** Twenty-eight spherical disks of GIC (10 mm diameter and 2 mm thickness),
- **Group-2:** Seven samples of each concentration of AgVO3 nanoparticles were prepared and grouped as Group-2 (GIC with 0.5% w/w AgVO3),
- **Group-3:** GIC with 1.0% w/w AgVO3 and
- **Group-4:** GIC with 2.0% w/w AgVO3. The specimens were placed in a hot air oven at 60°C for one hour to remove moisture. The mutans strain obtained from lawn culture was stored in Phosphate Buffer Solution (PBS) and then transferred to the solidified agar petriplates by pour plate method. The disks were placed in petriplates, incubated at 37°C and examined for the zone of inhibition around the disks after 48 hours [Table/Fig-1]. These zones were measured with digital Vernier callipers (Mitutoyo, absolute company) of 0.001 accuracy. Since addition of 2.0%w/w AgVO3 showed the highest anti-microbial efficiency, only this concentration was used to evaluate the FS and SMH [7,10].



[Table/Fig-1]: Zone of inhibition around the disks after 48 hours.

Flexural Strength (FS): The FS was measured using a 3-point bending test on a computerised universal testing machine (Instron 8801, United Kingdom) at a crosshead speed of 1 mm/minute. A total of 14 rectangular specimens measuring 25x2x2 mm dimension were prepared, 7 samples of GIC with 2.0% w/w AgVO3 nanoparticles (Group-4) and seven samples of unmodified GIC (Group-1). The test specimen was mounted, and the load was applied until the specimen fractured, and the FS was computed in units of Megapascal (MPa) [8].

Surface Micro-Hardness (SMH): The evaluation of SMH was done using Vickers's micro-hardness tester (Daksh Quality Systems Pvt., Ltd., India) with a diamond indenter at 25 gm load for 15 seconds dwelling time. Fourteen spherical specimens of 10 mm diameter and 2 mm thickness, seven made of unmodified GIC (Group-1) and seven from 2.0% w/w AgVO3 nanoparticles (Group-4) were prepared to evaluate SMH. A total of five indentations were made at different points for each specimen, and the mean hardness values of these five indentations were measured as VHN [10].

STATISTICAL ANALYSIS

The obtained data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 21.0. Quantitative variables were compared using ANOVA as the data sets were normally distributed between the groups. An unpaired t-test was used to compare the data between the two groups for FS and SMH. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Antimicrobial property: The mean values of inhibition zones (mm) between the groups are given in [Table/Fig-2]. The modified groups exhibited higher anti-microbial activity compared to the un-modified group. Group-4 (2.0% w/w AgVO3) exhibited the highest anti-microbial activity (27.70±1.14 mm) followed by Group-3 (24.39±0.75 mm) and Group-2 (20.50±1.33 mm). Group-1 (unmodified GIC) exhibited the least anti-microbial property with a mean zone of inhibition of 15.98±1.11 mm. One-way ANOVA showed a significant difference (p=0.001) between the groups [Table/Fig-2]. On pair-wise comparison, a highly significant difference (p<0.001) was observed in the formation of inhibition zones (mm) between all the groups [Table/Fig-3].

Groups	Mean (mm)	SD (mm)	F-value	p-value
Group-1	15.98	1.11	168.238	**0.001***
Group-2	20.50	1.33		
Group-3	24.39	0.75		
Group-4	27.70	1.14		

[Table/Fig-2]: Comparison of mean values of inhibition zones (mm) between the groups.
One-way ANOVA, *Significant

Comparison of groups		Mean difference	Std. Error	p-value
Group-1	Group-2	4.52500*	0.55117	**<0.001****
	Group-3	8.41250*	0.55117	**<0.001****
	Group-4	11.72500*	0.55117	**<0.001****
Group-2	Group-3	4.52500*	0.55117	**<0.001****
	Group-4	3.88750*	0.55117	**<0.001****
Group-3	Group-4	7.20000*	0.55117	**<0.001****

[Table/Fig-3]: Pair wise comparison of zones of inhibition (mm) between the groups.
*One-way ANOVA, **Highly Significant

Flexural Strength (FS): GIC added with 2.0% w/w AgVO3 nanoparticles (Group-4) exhibited higher FS with a mean of 47.72±10.12 MPa than the un-modified GIC (Group-1). A statistically significant difference (p=0.002) in mean FS was observed between the two groups [Table/Fig-4].

Groups	Mean	SD	t-test	p-value
Group-1	26.90	8.99	-4.070	**0.002***
Group-4	47.72	10.12		

[Table/Fig-4]: Comparison of mean Flexural Strength (MPa) between the groups.
Unpaired t-test, *Significant

Surface Microhardness (SMH): GIC added with 2.0% w/w AgVO3 nanoparticles (Group-4) exhibited higher SMH with a mean of 61.29±2.59 VHN than the un-modified GIC (Group-1), and showed a statistically significant difference (p=0.001) in SMH between the two groups [Table/Fig-5].

Groups	Mean	SD	Unpaired t-test	p-value
Group-1	49.48	0.78	-11.539	**0.001***
Group-4	61.29	2.59		

[Table/Fig-5]: Comparison of Surface Micro Hardness (SMH) between the groups.
Unpaired t-test, *Significant

DISCUSSION

In the current study, it was observed that GIC incorporated with AgVO3 nanoparticles exhibited higher anti-microbial property and increased mechanical properties compared to conventional GIC. Ionomer cements are known to have anti-cariogenic activity due to the release of fluoride ions. However, the occurrence of secondary caries has been reported due to their poor physical properties. Further, the fluoride release property is not sustained over time and not potent enough to inhibit bacteria [1].

Hence, various additives were proposed to enhance the anti-bacterial property of GICs. Understanding the effect of anti-bacterial additives on the physical and mechanical properties of GICs is critical. Not all additives to glass ionomer powder have resulted in the desired effects. Addition of theobromine [11], polymers (2-methacryloxytroponones, Epigallocatechin-3-gallate (EGCG), sodium fusiadate, triclosan, furanone, poly quaternary ammonium salts) [3,12], metals (Zinc, strontium, bioglass) [12], natural products (propolis [13], *Salvadora persica* (miswak) [14], trialpha [13], curcuma [15], chitosan [16]), chlorhexidine and its derivatives (CHX acetate, CHX gluconate, CHX hydrochloride) [17] were found to improve anti-bacterial activities of GICs.

Nano-materials involve the use of 1-100 nm size particles [18]. The use of such nanosized particles is found to have beneficial effects on the properties of dental materials due to the increase in surface area and surface energy, along with better particle distribution. Inclusion of TiO_2, ZnO, and hydroxyapatite nanoparticles in the GIC was shown to improve mechanical properties along with anti-cariogenic properties. The addition of stainless-steel nano-powder to GIC showed improved mechanical properties [19]. Although addition of silver nanoparticles (AgNPs) to GIC exhibited improved antimicrobial properties, agglomeration of silver nanoparticles was a common problem. To overcome this, silver vanadate (AgVO3) nanoparticles were developed. AgVO3 functions as a carrier of AgNPs and reduces the loss of AgNPs from leaching [6]. AgVO3 also promotes a high dispersion of silver nanoparticles providing a large surface area to pathogenic microorganisms and thus showing a greater effect and higher duration of anti-microbial action [20].

It was evident from this study that antimicrobial activity was directly proportional to the amount of AgVO3 nanoparticles added. GIC with 2.0% w/w of AgVO3 nanoparticles showed the highest anti-microbial activity followed by 1.0% w/w AgVO3 and the lowest with 0.5%. Since the modified GIC samples with AgVO3 showed the higher anti-bacterial property, it is affirmative to mention that, both fluoride release and silver must have contributed to anti-microbial activity [19]. The release of fluoride ions inhibits plaque formation and inhibits metabolism and microbial growth [21]. The other possible reasons include structural damage to bacteria by oxygen free radicals produced by silver, replication of bacterial DNA by active silver ions and other phosphorus contains compounds [22]. Direct contact with the higher concentration of silver particles may cause lysis of the cell wall [23].

Many modifications in the composition of GIC powder have been done, to improve its mechanical properties. The use of dispersed components such as titanium oxide and zirconium oxide in glass powder, 'miracle mix' produced by adding silver alloy powder to GIC, 'cermet ionomer' produced by sintering metal and glass powder in GIC are some of the modifications [1]. However, not all modifications of GIC produced beneficial results. Inclusion of niobium pentoxide (Nb_2O_5), ytterbium fluoride (YbF_3) and Barium Sulfate ($BaSO_4$) to GIC have shown to reduce the mechanical properties of GIC, while Bioactive Glass (BAG) have shown to improve the mechanical properties of GIC [1]. Resin Modified Glass Ionomer Cement (RMGIC) further had enhanced physical and mechanical properties [24]. No alteration in the mechanical properties of GIC was observed on addition of fillers like hydroxyapatite and zirconia powder, zinc [22,23].

GIC powder particles' size, density and entrapped voids could influence the mechanical properties of the cement. Smaller size filler particles occupy more of the empty spaces between glass ionomer particles resulting in higher mechanical properties [1,25]. However, a reduction in the mechanical properties of the cement was observed due to the addition of a higher concentration of nanoparticles. It could be due to improper wetting of the matrix and filler bond, overcrowding of filler particles as well as a high powder/liquid ratio leading to a dry mix [19]. The samples with a higher concentration of AgVO3 in the current study displayed higher FS and SMH along with greater anti-bacterial activity. Greater packing of particles within the set cement matrix may justify the improvement of the FS. Also, during the gelation state, AgVO3 nanoparticles along with the unreacted glass particles could occupy the voids in polymer matrix and thus enhance the mechanical properties [26].

SMH is the resistance of a material to indentation or penetration. In the earlier study conducted by Castro de DT et al., it was observed that there was no change in SMH of resins observed on addition of AgVO3 nanoparticles in varying concentrations (0.5, 1, 2.5, 5 and 10) [9]. However, FS decreased with addition of greater than 1% of AgVO3. The lower concentration of AgVO3 in acrylic resins may be ideal to obtain the mechanical properties, but not to promote anti-microbial activity. The addition of higher concentrations of AgVO3 was shown to enhance anti-microbial activity, maintaining the same values of SMH [9]. Other studies stated that addition of 0.1% and 0.2% (w/w) Silver nanoparticles [8] and 3% (w/w) TiO_2 nanoparticles [10] to GIC showed a significant increase in the surface hardness.

In the current study, similar to FS, GIC added with 2.0% w/w AgVO3 showed the highest SMH (61VHN) than Type II GIC Group-1 (49VHN). Both the SMH and FS are based on the duration of the setting, which depends on the formation of base/polyacid complexes that block the cross-reactions between cationic ions and polyacrylic chains. The improved microhardness can lead to increased FS, thus improving the mechanical properties of the GIC [26].

Limitation(s)

The proportionate adding of AgVO3 nanoparticles to the GIC powder, handling of GIC, and the preparation of the samples were done under normal room conditions which could be one of the limitations of the present study.

CONCLUSION(S)

Type II GIC added with AgVO3 nanoparticles exhibited superior anti-bacterial and mechanical properties compared to un-modified one. Samples with 2.0% w/w AgVO3 displayed higher anti-bacterial activity, FS and SMH compared to the other concentrations studied. GIC with sustained anti-bacterial activity and improved mechanical and physical properties is desirable in paediatric restorative dental practice. The addition of AgVO3 nanoparticles resulted in improved properties of GIC. However, before its clinical use, such a modification should be tested in-vitro for biocompatibility and other physical and mechanical properties.

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