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EVALUATION OF ANTIMICROBIAL EFFECTS OF ORTHODONTIC BAND CEMENT INCORPORATED WITH ZIRCONIA, GOLD, COPPER BIOSYNTHESIZED NANOPARTICLES - AN IN-VITRO STUDY

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ABSTRACT :

OBJECTIVE: To evaluate the antimicrobial effects of orthodontic band cement incorporated with various biosynthesized nanoparticles**.**

MATERIALS AND METHODS: Zirconia, gold, copper Nanoparticles were green synthesized using white tea extract and dry ginger which were incorporated into orthodontic band cement, characterized using FTIR analysis. Antimicrobial activity against Streptococcus mutans and Lactobacillus acidophilus was tested in vitro by disc diffusion and Time Kill Curve Assay.

RESULTS: Zirconia nanoparticles incorporated GIC exhibited better antimicrobial activity against Streptococcus mutans, and gold nanoparticles incorporated GIC against lactobacillus acidophilus at varying concentrations at different time periods.

CONCLUSION: The antibacterial effect against streptococcus mutans and lactobacillus acidophilus were distinctly noted in Zirconia nanoparticles, gold nanoparticles followed by copper nanoparticles. Zirconia and gold nanoparticles produced more efficient antibacterial property and eventually would be effective in curtailing White Spot Lesions.

KEY WORDS: Orthodontics, Band Cement, Banding, White Spot Lesions, Dental materials, Nanoparticles; Gold; Copper; Zirconia, Glass ionomer cement, antimicrobial activity.

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INTRODUCTION:

Orthodontics and Dentofacial Orthopaedics focused on correcting the malalignment of teeth and jaws. Fixed orthodontic treatment involved the use of brackets and wires to align teeth and correct bite issues. [1] Banding and bonding were two primary techniques [2] used in fixed orthodontic treatment. Orthodontic banding involved placing metal bands around the molars to provide anchorage. Orthodontic band cement is a crucial component in orthodontic banding. [3]

However, if not adequately maintained, this contributed to the development of white spot lesions (WSLs) on the tooth enamel. White spots occurred if there was an imbalance between the pathogenic bacteria and the commensal inhabitants of the plaque biofilm. Lesions were known to occur in the first four weeks of orthodontic treatment. [4] For prevention of white spot lesions, various methods such as fluoride-containing products like varnishes, mouthwashes, gels, chlorhexidine mouthwashs,[5] diet modifications, and calcium-containing remineralization products were employed. These were limited by several challenges and depended on patients' maintenance, which was unreliable, especially in children and teenage patients. Therefore, the development of new approaches towards the prevention of white spot lesions was required. [4,5]

Nanoparticles had significant applications in dentistry. They were used in dental materials such as composites and cements to improve strength and antimicrobial properties.[6]Their tiny size allowed precise control and manipulation. Nanoparticles were synthesized using a top-down, bottom-up approach; each method had its own set of advantages and was chosen according to its nano properties and applications. [7] In recent years, biosynthesized nanoparticles had emerged as a promising solution to enhance the properties of orthodontic band cement. These nanoparticles were synthesized using biological agents like plant extract, which were eco-friendly and cost-effective. [6]

The incorporation of biosynthesized nanoparticles into orthodontic band cement improved its mechanical properties, biocompatibility, and antimicrobial activity. [9] For example, zirconium nanoparticles were shown to enhance the mechanical properties of orthodontic band cement by increasing its microhardness and compressive strength. [10] Similarly, copper nanoparticles demonstrated excellent antimicrobial activity against Streptococcus mutans, a common oral pathogen. [11,12] Gold nanoparticles, on the other hand, were shown to enhance the biocompatibility of orthodontic band cement by improving its cell adhesion and proliferation. [13]

Despite the promising results mentioned above, there remained a limited understanding of the effects of biosynthesized nanoparticles incorporated into orthodontic band cement. Therefore, this study aimed to evaluate the antimicrobial activity of orthodontic band cement incorporated with various biosynthesized nanoparticles, such as zirconium, gold, and copper.

MATERIALS AND METHODOLOGY

STUDY DESIGN

This was an in-vitro Nano-laboratory-based study that evaluated the antimicrobial activity of orthodontic band cement incorporated with biosynthesized zirconium, gold, and copper nanoparticles. Group 1 included Zirconia Nanoparticles (ZrNps) incorporated GIC, Group 2 had Gold Nanoparticles (AuNps) incorporated GIC, Group 3 featured Copper Nanoparticles (CuNps) incorporated GIC, and Group 4 served as the control with GC Fuji GIC Type 1 Luting Cement.

NANOPARTICLE SYNTHESIS

The green synthesis method was followed for the preparation of three types of nanoparticles (Zirconium, Gold, Copper) using dry ginger and white tea extracts. The plant extracts were mixed with respective metal solutions using different dilution protocols. For example, zirconia nanoparticles were prepared by weighing 20mlMol (0.664gms) of zirconium oxychloride octahydrate (Figure 1a) and diluting it in 40ml of distilled water in a beaker (Figure 2a). This resulted in 60ml of the zirconium solution, which was then combined with 40ml of dry ginger and white tea extract in a beaker (Figure 3a). Similarly, gold nanoparticles were prepared by weighing 2ml of gold chlorite (Figure 1b) and diluting it in 88ml of distilled water in a beaker (Figure 2b). This 90ml gold solution was then combined with 10ml of dry ginger and white tea extract in a beaker (Figure 3b). For copper nanoparticles, 30mlMol (0.476gms) of Copper sulphate anhydrous ($CuSO_4$) was weighed (Figure 1c) and diluted in 30ml of distilled water in a beaker (Figure 2c). This 60ml copper solution was then combined with 40ml of dry ginger and white tea extract in a beaker (Figure 3c). The process was followed by centrifugation.

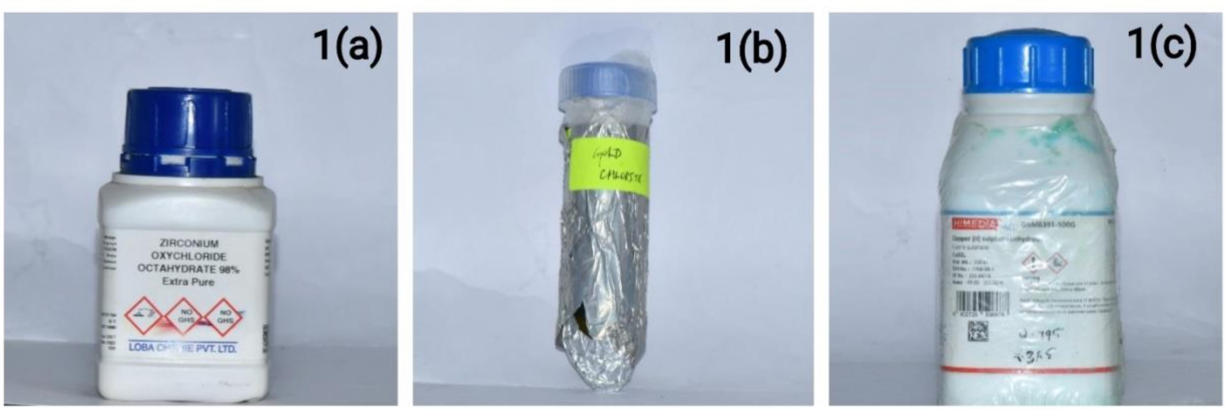


Figure: 1(a) Zirconium oxychloride octahydrate, 1(b) Gold chloride, 1(c) Copper sulphate anhydrouss.

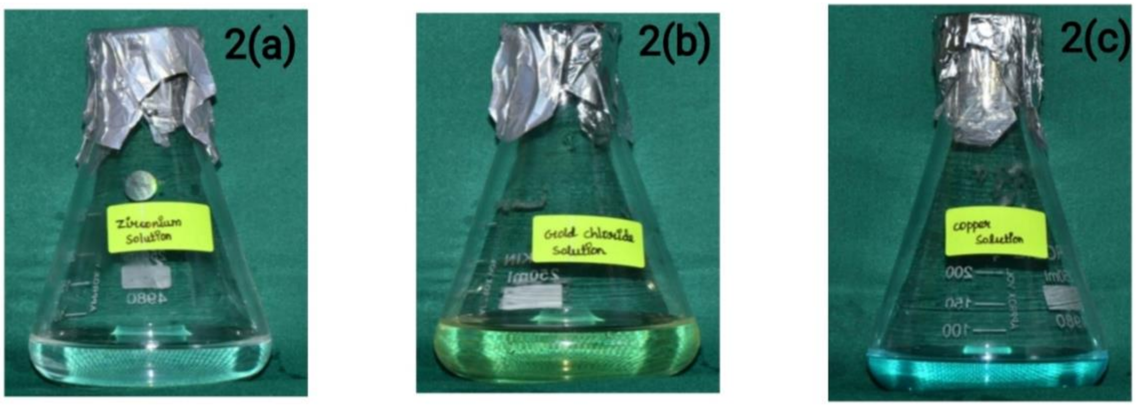


Figure: 2(a) Dilution of Zirconium oxychloride octahydrate, 2(b) Dilution of Gold chlorite with distilled water in a beaker, 2(c) Dilution of Copper sulphate anhydrous.

Figure: 3(a) Dilution of Zirconium nanoparticles, 3(b) Dilution of Gold nanoparticles, 3(c) Dilution of Copper nanoparticles with dry ginger and white tea extract.

The nanoparticles obtained were dried and stored. Subsequently, these nanoparticles were incorporated into orthodontic band cement (GIC type 1 luting cement) for three different groups. The mixing process involved using a Vortex and IKA ® T25 digital ULTRA-TURRAX ® machine at 3400 rpm for 2 minutes in a dark room, employing a rotor-stator mechanism for each group, respectively.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity was assessed through the Time Kill Curve Assay at varying concentrations—specifically, 25 µg/ml, 50 µg/ml, and 100 µg/ml of nanoparticles. The test included a Standard and Control, where the Standard was loaded with antibiotics (Amoxyrite 15µg), and the Control (Negative Control) had no nanoparticles or antibiotics. Test results were regularly measured on an hourly basis over 1, 2, 3, and 4 hours (Graph 4, 5, 6, 7, 8, 9). The decrease in optical density values indicated effective antibacterial activity against Streptococcus mutans and Lactobacillus acidophilus.

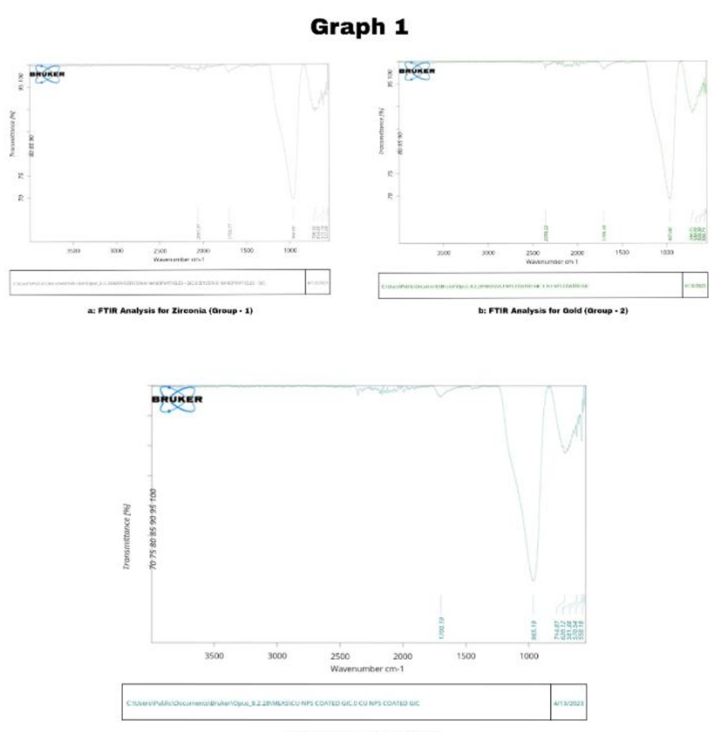
RESULTS

EVALUATION OF FTIR ANALYSIS

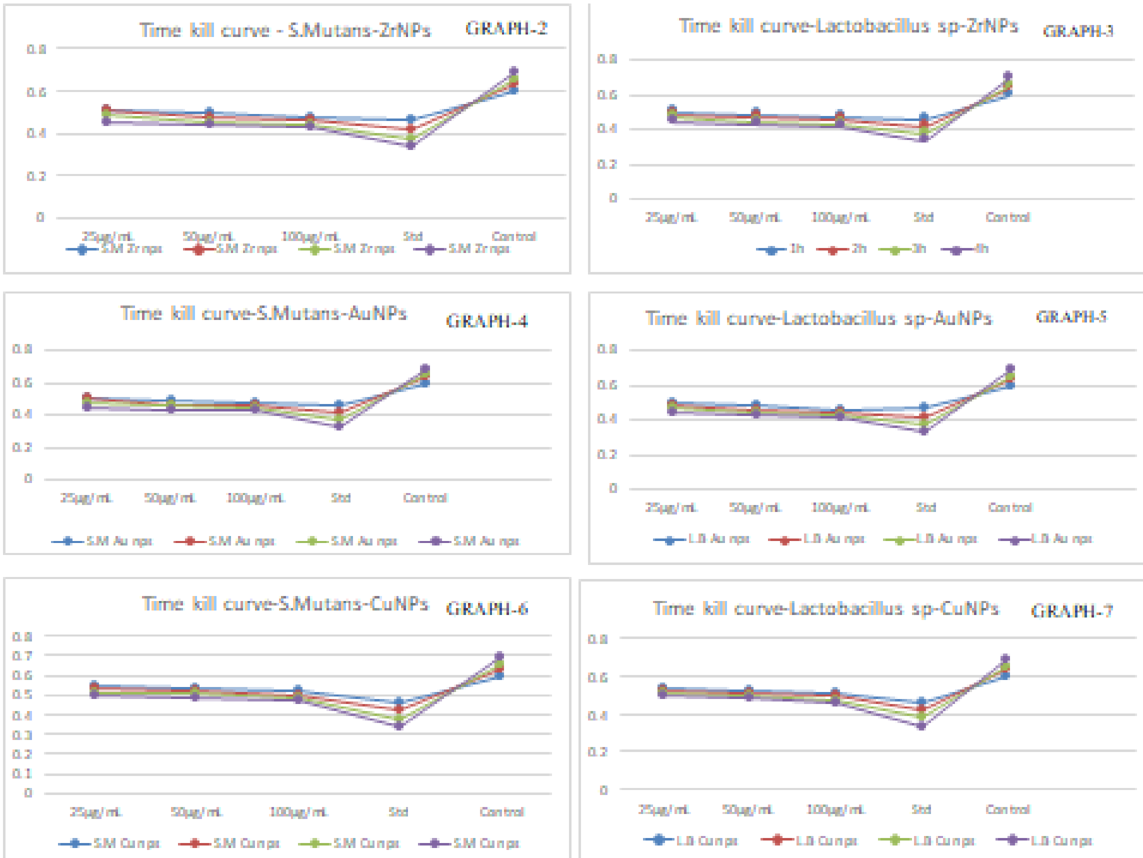
In the FTIR analysis for three different nanoparticles (Zirconia, Gold, Copper), distinctive absorbance bands were observed. Zirconia (Group -1) {Graph 1 (a)} exhibited notable peaks at 2061.97 cm^-1 (indicating isothiocyanate N=C=S stretching), 1700.77 cm^-1 (associated with various functional groups like aldehydes and acids), 966.09 cm^-1 (representing alkene bending), and 709.16 cm^-1 (related to C=C bending and benzene derivatives). These peaks suggested the presence of functional groups contributing to zirconia nanoparticle reduction and stabilization.

Gold (Group -2) {Graph 1 (b)}: A broad peak at 2358.22 cm^-1 (O=C=O stretching) was observed, along with other peaks indicating the presence of aldehydes, acids, and aromatic compounds. These functional groups were likely involved in the reduction and stabilization of gold nanoparticles.

Copper (Group -3) {Graph 1 (c)}: The spectrum showed a broad peak at 1700.19 cm^-1 (C-H bending aromatic compound overtone) and other peaks indicative of aldehydes, acids, and aromatic compounds. These functional groups were likely involved in the reduction and stabilization of copper nanoparticles.



Graph 1: Graphical Representation of FTIR Analysis of Zirconia (a), Gold (b), and Copper (c)



Graphs 2-7 :Graphical representation of Time Kill Curve Assay of (2) Zirconia Nano incorporated GIC against S.mutans, (3) Lactobacillus acidophilus, (4) Gold Nano incorporated GIC against S.mutans, (5) Lactobacillus acidophilus, (6) Copper Nano incorporated GIC against S.mutans, (7) Lactobacillus acidophilus.

EVALUATION OF THE ANTIMICROBIAL ACTIVITY:

		Sum of Squares	df	Mean Square	F	P value
1h	Between Groups	.003	5	.001	.240	.941
	Within Groups	.066	24	.003		
	Total	.069	29			
2h	Between Groups	.004	5	.001	.110	.989
	Within Groups	.154	24	.006		
	Total	.158	29			
3h	Between Groups	.005	5	.001	.086	.994
	Within Groups	.257	24	.011		
	Total	.262	29			
4h	Between Groups	.005	5	.001	.059	.997
	Within Groups	.412	24	.017		
	Total	.417	29			

Table 1: One way ANOVA showing Multi group comparison at each hour in between groups and within the same groups (Zirconia, Gold and Copper)

The Time kill curve assay revealed that Antibacterial Activity against Streptococcus mutans is well exhibited by Zirconia incorporated GIC disc and against Lactobacillus acidophilus it is best shown by Gold incorporated GIC discs at 100µg/ml at 4 hours with the statistical significance (P-value-0 .997)

Table 1 depicted the multiple group comparison of the Time Kill Curve assay between Zirconia, Gold, and Copper nanoparticle incorporated GIC, revealing statistical significance (P Value -0.997). This suggested marked antibacterial activity against Streptococcus mutans, particularly well exhibited by Zirconia incorporated GIC disc. Against Lactobacillus acidophilus, the best result was observed with Gold incorporated GIC discs at 100µg/ml at 4 hours, with statistical significance (P-value-0.997).

DISCUSSION

The value of facial aesthetics in Orthodontics dated back to the early days of dentistry. The fixed appliance was the most preferred form of orthodontic treatment; however, these fixed orthodontic appliances contributed to the adhesion of oral bacteria due to their complex design, preventing proper cleaning around orthodontic brackets and potentially resulting in enamel demineralization. [14] As plaque biofilm development increased and dental caries causing microorganisms proliferated, White Spot Lesions (WSL) formed, becoming a significant area of concern in orthodontic therapy. [14,15] Despite numerous methods to prevent these white spot lesions, such as fluoride varnish, gels, and mouthwashes, they had drawbacks of staining and high solubility in the oral cavity and were nonetheless common in patients receiving orthodontic treatment, resulting in a lack of satisfaction in aesthetics among patients and dentists. [16]

Dental nanotechnology became a new area of interest. Nanoparticles smaller than 100 nm exhibited a higher surface-to-volume ratio, enhancing their ability to interact with microbial membranes. Numerous studies have been carried out to treat these lesions by enhancing antibacterial properties, with some including the usage of Benzalkonium chloride, Zinc oxide [11], Chlorhexidine [5], and Calcium fluoride. [9] However, each of these approaches had its own drawbacks.

In our current study, we employed the use of Glass Ionomer Cement (GIC) GC FUJI, as it is most commonly used for band cementation with fluoride-releasing property [3], less water-soluble, and high strength. There is a need to improve the property of Glass Ionomer Cement since it has antibacterial activity against a small spectrum of microorganisms. We chose Zirconia, Gold, and Copper nanoparticles to be incorporated into the orthodontic band cement (GIC). Copper exhibited good antibacterial properties, destroying the cell membrane of bacteria. [17] Zirconia produced reactive oxygen species leading to cell death, [18] and Gold had inhibitory effects.[19]

These nanoparticles were green-synthesized using White Tea [20], Dry Ginger [21] extract and incorporated into GIC. The nanoparticles were green-synthesized to curtail cytotoxicity and subjected to characterization. Characterization can be done by SEM, TEM, and FTIR. We utilized FTIR (Fourier Transform Infrared) to obtain the structural and compositional characteristics of the nanoparticles incorporated into GIC.[22] The Time Kill Curve Assay was employed to check for the antibacterial effect against Streptococcus mutans and Lactobacillus acidophilus, determining the killing efficiency of microorganisms under a specific time period.

The antibacterial properties of the nanoparticles incorporated in GIC were tested against Streptococcus mutans and Lactobacillus acidophilus, as they are commonly found bacteria in the oral cavity [5], predisposing to White Spot Lesion. For our study, we employed the Time Kill Curve Assay as it provides more precise results at varying concentrations. In a study conducted by Moreiraa et al, the results revealed that silver nanoparticles exhibited better antibacterial properties, but they were proven to be cytotoxic. [8]

In addition, the outcomes demonstrated that Zirconia incorporated GIC discs were more effective against Streptococcus mutans, and Gold incorporated GIC discs were more effective against Lactobacillus acidophilus, with a statistical significance (p value- 0.997) (Table 1). This was compared to the control group, which was uncoated with nanoparticles.

In-vitro activities on Silver Nanoparticles for multidrug resistance were carried out by the Time Kill Curve Assay, showing better results. [23,24] Furthermore, studies conducted on Polygonum cuspidatum showed better antibacterial activity against S. mutans and S. sobrinus, thereby reducing plaque biofilm formation. [25]

LIMITATIONS:

In vitro research is conducted in a controlled laboratory setup that may fail to reflect the complex and dynamic conditions seen in the oral cavity. Thus, the reactivity to nanoparticles may differ in such conditions. Therefore, the use of nanoparticles incorporated in Glass Ionomer Cement can produce variable results and is recommended for clinical trials.

CONCLUSION:

- The antibacterial and time kill curve analyses indicated that Gold and Zirconia exhibited good bacterial growth inhibition properties against Streptococcus mutans and Lactobacillus acidophilus, followed by which copper was also effective.
- These biosynthesized nanoparticles demonstrated comparable antibacterial properties to the control.

RECOMMENDATIONS:

The study can be conducted as a clinical trial (in vivo) to assess the accuracy of the in vitro test results.

CONFLICT OF INTEREST:

None to declare.

SOURCE OF FUNDING:

Nil.

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