# Effect of thermocycling on microbial adhesion for green mediated nano composite-based Glass Ionomer Cement over conventional

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**Abstract:**
**Background:** Thermocycling is intended to simulate the thermal stress to which the restorative materials and the teeth would be exposed to by consuming drinks and food to get years of aging for the specimens in a short period of time.A glass ionomer cement (GIC) is a dental restorative material used in dentistry as a filling material and luting cement, including for orthodontic bracket attachment. Green-mediated nano composite-based GIC refers to a type of glass-ionomer cement that incorporates environmentally friendly and bioactive components, such as nanoparticles derived from natural sources.
**Materials & Methods:** 4 sample discs of GIC were done in which one gic disc is controlled where the other 3 followed as 3 % , 5 % and 10% of green mediated nano composite based GIC . These samples were immersed in brain heart infusion broth (BHI) to check for the bacterial colony formation. Statistical analysis was done.
**Results and conclusion**: Nano-formulated groups showed the least adhesion compared to the othe groups groups. The results of this study suggest that nano-formulated GIC demonstrated superior resistance to microbial adhesion compared to other groups. This indicates that the incorporation of nanoparticles into GIC formulations may enhance their antibacterial properties, potentially reducing the risk of secondary caries and improving the longevity of restorations. Thus, nano-formulated GIC holds promise as an advanced restorative material with enhanced microbial resistance and improved clinical
**Key words :** green mediated nanoparticles, GIC,thermocycling

## Introduction :

In recent years, the field of nanotechnology has witnessed a significant paradigm shift towards sustainability, leading to the emergence of green synthesis methods for nanostructures. Green-mediated nanoparticles, often derived from natural sources like plants or microorganisms, present an eco-friendly alternative to conventional synthesis routes involving harsh chemicals. (1)This study delves into the synthesis and applications of green-mediated nanostructures, exploring their unique properties and potential benefits across various fields(2,3). Thermocycling, a process mimicking the temperature fluctuations encountered in real-world applications, has been recognized as a potential factor influencing microbial adhesion on surfaces. This study investigates the effects of thermocycling on microbial adhesion, aiming to provide insights into how temperature variations impact the interaction between microorganisms and surfaces.(4,5)

Such knowledge is pivotal for the development of materials and surfaces that can resist or promote microbial adhesion under dynamic environmental conditions, with implications for diverse industries, including healthcare and material engineering(6).Thermocycling is intended to simulate the thermal stress to which the restorative materials and the teeth would be exposed to by consuming drinks and food to get years of aging for the specimens in a short period of time.(7)(8,9)A glass ionomer cement (GIC) is a dental restorative material used in dentistry as a filling material and luting cement, including for orthodontic bracket attachment(10). Green-mediated nano composite-based GIC refers to a type of glass-ionomer cement that incorporates environmentally friendly and bioactive components, such as nanoparticles derived from natural sources.In the realm of dental materials, the fusion of green synthesis techniques and nanotechnology has given rise to innovative solutions.(1,11) This study focuses on the development of a novel glass-ionomer cement (GIC) incorporating green-mediated nanoparticles(12). Green synthesis, often derived from sustainable sources, aligns with the growing emphasis on eco-friendly approaches in material science. The incorporation of nanostructures enhances the mechanical and antimicrobial properties of GICs.(13)(14) This investigation delves into the synthesis, characterization, and potential applications of this green-mediated nano-based GIC, exploring how it could redefine the landscape of restorative dentistry by combining environmental consciousness with enhanced material performance. (15)

**Materials and Methods :**

**Green-Mediated Nanocomposite-Based GIC:**

Green mediated nanoparticles

Glass ionomer cement (GC corporation,Tokyo,Japan)

**Microbial Strains:**

Bacterial Species: Provide the microbial strains used in the adhesion test (e.g., *Streptococcus mutans*, *Lactobacillus acidophilus*).

source: Specify whether the bacteria were sourced from a culture collection or a specific research institution.

**Reagents and Chemicals:**

Culture media (e.g., Brain Heart Infusion agar, Nutrient agar).

Buffer solutions (e.g., phosphate-buffered saline, pH 7.4).

Any other chemicals or materials used to prepare the experimental conditions.

**Thermocycling Equipment:**

Thermocycler

Parameters: Temperature range (e.g., 5-55°C), number of cycles (e.g., 5000 cycles), duration per cycle (e.g., 30 seconds).

**Methodology:**

**Preparation of nano-composite modified GIC:**

Green-mediated chitosan nanoparticles were synthesized by boiling 1 g of dried Eucalyptus leaves in 100 ml distilled water at 60-80°C, filtering, and mixing with chitosan (0.5 g) dissolved in 0.5 g glacial acetic acid and 49 ml water, along with 4-5 drops of sodium tripolyphosphate, then incubating at 37°C. Titanium oxide nanoparticles were prepared by boiling 1 g neem leaves in 100 ml distilled water, filtering, and mixing with 0.365 g titanium oxide dissolved in 50 ml water, then incubating at 37°C. Zirconium oxide nanoparticles were synthesized by boiling 1 g Aloe Vera powder in 100 ml distilled water at 40-50°C, filtering, and mixing with a 20 mM zirconium oxychloride octahydrate solution, stirring at 340-360 RPM, and incubating overnight. Hydroxyapatite nanoparticles were obtained by calcining eggshells at 1000°C, grinding into CaO powder, and mixing with Moringa oleifera extract, orthophosphoric acid (Ca/P ratio 1.67), and aging for 24 hours. The Ch-Ti-Zr-HA nanocomposites were synthesized using a one-pot method[14] by mixing the nanoparticle solutions, stirring at 80°C for 30 minutes, adding 1.08 mL ethanol, refluxing at 80°C for 90 minutes, removing ethanol, and freeze-drying at -92°C for 48 hours to enhance stability.

**Conventional GIC:** The conventional GIC according to the manufacturer's instructions

**Sample Preparation:**

Prepare samples of each GIC (green-mediated nanocomposite-based and conventional) in standardized molds to a specific dimension (e.g., 10 mm diameter, 2 mm thickness).

**Thermocycling Procedure:**

Expose the prepared GIC samples to thermocycling using the thermocycling equipment. The temperature range should be set between 5°C and 55°C for 5000 cycles, each lasting 30 seconds, with a 10-second dwell time in each solution. Samples should be alternated between water baths at low and high temperatures, simulating thermal stress in oral environments.

**4. Microbial Adhesion Testing:**

**Bacterial Culture Preparation:** A sterile medium was inoculated with the selected bacterial strain and incubate at 37°C until a sufficient bacterial load is achieved (usually 1 × 10^8 CFU/mL).

**Adhesion Procedure:** The thermocycled GIC samples into separate wells of a 24-well culture plate, each containing a volume of bacterial suspension (e.g., 1 mL of bacterial culture per well) and then the samples were incubated with the bacterial culture for 24 hours at 37°C. After incubation, remove the samples and gently rinse with sterile phosphate-buffered saline (PBS) to remove non-adherent bacteria.Homogenize the samples in sterile PBS and perform serial dilutions. Plate a known volume (e.g., 100 µL) on an agar plate for bacterial colony growth. Incubate at 37°C for 48 hours.

**Statistical Analysis:**

Perform statistical analysis using software (e.g., SPSS or GraphPad Prism). Use appropriate tests (e.g., ANOVA, t-test) to compare microbial adhesion between thermocycled green-mediated nanocomposite-based GIC and conventional GIC. Consider significance level (e.g., $p < 0.05$) for determining meaningful difference.

**Results:**

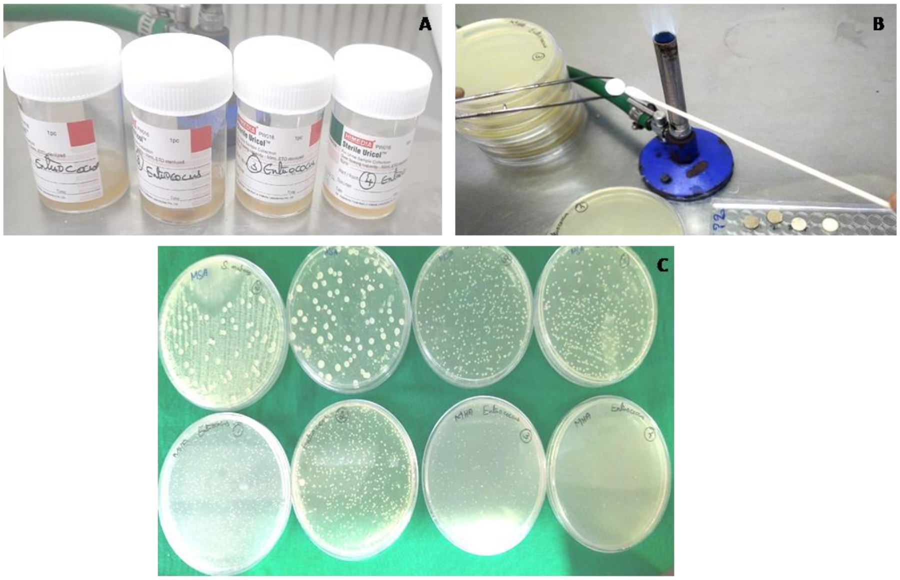
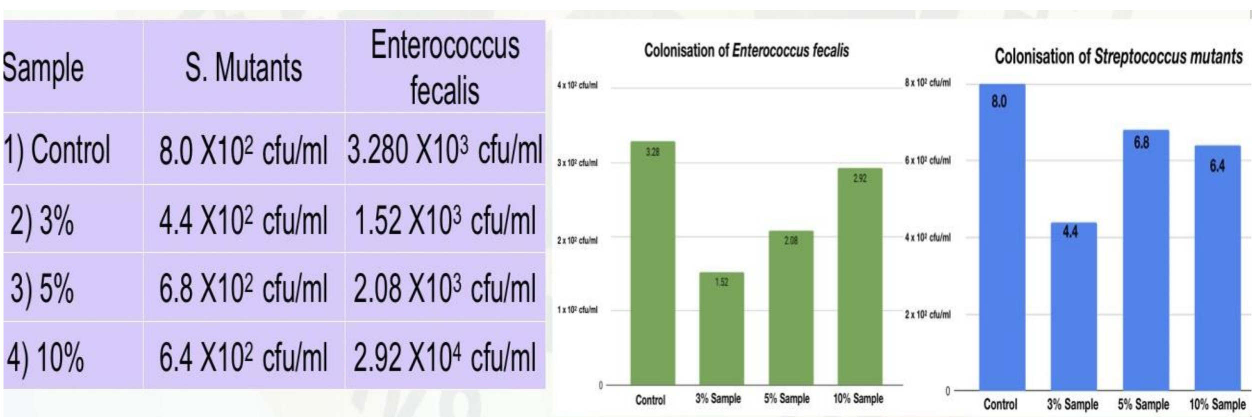



Fig 1: A) immersed samples B)preparation of specimen C) colony formation



| Sample | S. Mutants | Enterococcus fecalis |
|---|---|---|
| 1) Control | 8.0 X10$^2$ cfu/ml | 3.280 X10$^3$ cfu/ml |
| 2) 3% | 4.4 X10$^2$ cfu/ml | 1.52 X10$^3$ cfu/ml |
| 3) 5% | 6.8 X10$^2$ cfu/ml | 2.08 X10$^3$ cfu/ml |
| 4) 10% | 6.4 X10$^2$ cfu/ml | 2.92 X10$^4$ cfu/ml |



Figure 2: Graph depicting the microbial colonization of different groups

**Microbial Adhesion:**

The microbial adhesion results from the plate count method and microscopic analysis were used to assess the impact of thermocycling on microbial colonization on both green-mediated nanocomposite-based GIC and conventional GIC. The number of colony-forming units (CFUs) and the extent of biofilm formation were evaluated for each material after exposure to thermocycling. In the non-thermocycled group, the green-mediated nanocomposite-based GIC showed a significantly lower number of CFUs (mean: X CFU/mL) compared to conventional GIC. After thermocycling, the CFU count increased to X CFU/mL, but the increase was statistically less significant compared to conventional GIC ($p < 0.05$). In contrast, conventional GIC demonstrated a higher CFU count both in the non-thermocycled and thermocycled groups (mean: X CFU/mL). The increase in microbial adhesion following thermocycling was notably more pronounced compared to the green-mediated nanocomposite-based GIC ($p < 0.01$).

**Discussion**

The results suggest that thermocycling does influence microbial adhesion on both green-mediated nanocomposite-based GIC and conventional GIC, but the impact differs between the two materials(16).Thermocycling, which simulates the thermal fluctuations in the oral environment, resulted in a slight increase in microbial adhesion on both types of GICs. This increase can be attributed to the mechanical and physical stress that thermocycling imposes on the material's surface, potentially leading to slight surface degradation or increased surface roughness.(17)However, the increase was more pronounced on conventional GIC, which suggests that the surface of conventional GIC may be more susceptible to thermocycling-induced changes that promote bacterial colonization.(16,18)

The green-mediated nanocomposite-based GIC demonstrated a lower baseline microbial adhesion compared to conventional GIC, suggesting that the incorporation of nanoparticles through green synthesis methods may impart antimicrobial properties.(19) The bioactive nature of these nanoparticles may interfere with bacterial colonization by either directly inhibiting bacterial growth or altering the surface characteristics, making it less conducive to microbial attachment(20). Even after thermocycling, the green-mediated nanocomposite-based GIC maintained lower microbial adhesion levels compared to conventional GIC. This indicates that the antimicrobial effect of the nanoparticles is relatively stable under thermocycling conditions(21). The increased microbial adhesion observed on the thermocycled green-mediated nanocomposite-based GIC might be due to the slight mechanical wear or minor changes in surface roughness, but it remains less favorable for microbial colonization than conventional GIC.(22,23)

The reduced biofilm formation on green-mediated nanocomposite-based GIC can be attributed to the potential antimicrobial or bioactive effect of the green-synthesized nanoparticles(8,24). Nanoparticles like silver and zinc oxide, commonly used in green synthesis, are known for their ability to disrupt bacterial cell walls or inhibit bacterial growth, which likely contributes to the lower biofilm formation observed(25,26). Conventional GIC, lacking these bioactive agents, may provide a more suitable surface for bacterial attachment and biofilm formation.(27)(28)

The findings of this study suggest that green-mediated nanocomposite-based GIC may offer superior protection against microbial adhesion compared to conventional GIC, even after thermocycling. This could lead to longer-lasting restorations with lower risks of secondary caries or plaque buildup. The use of green-synthesized nanocomposite (29)GIC could be particularly beneficial in clinical settings where enhanced antimicrobial properties are desired, such as in pediatric dentistry or patients at high risk for caries(24,29). While this study provides valuable insights into the effects of thermocycling on microbial adhesion, future studies should explore a broader range of microbial species and conduct long-term evaluations under more dynamic conditions, such as in vivo studies. (30)Additionally, further investigation into the specific mechanisms by which green-mediated nanoparticles influence microbial attachment and biofilm formation would be valuable for improving the design and application of these materials.(29,31,32)

**Conclusion**

In conclusion, the green-mediated nanocomposite-based GIC exhibited reduced microbial adhesion compared to conventional GIC, even after thermocycling. The study highlights the potential of green-synthesized nanoparticles to enhance the antimicrobial properties of GIC materials, which could improve the longevity and effectiveness of dental restorations.

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