

Invitro Evaluation of an Orthodontic Adhesive Incorporated with Copper-Substituted Hydroxyapatite Nanoparticles: Synthesis and Antibacterial Assessment

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Abstract:

Orthodontic adhesives play a critical role in the successful bonding of fixed appliances; however, their prolonged presence in the oral cavity favors plaque accumulation and subsequent microbial colonization, increasing the risk of enamel demineralization and white spot lesion formation. Incorporation of antibacterial agents into orthodontic adhesives has therefore gained increasing attention as a preventive strategy. This study aimed to synthesize copper-substituted hydroxyapatite (Cu-HAp) nanoparticles using the co-precipitation method and to evaluate their antibacterial efficacy when incorporated into an orthodontic adhesive. The successful synthesis of Cu-HAp nanoparticles was confirmed through Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), which demonstrated characteristic functional groups and uniform nanoscale morphology. Antibacterial activity was assessed by measuring zones of inhibition against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus mutans*. The Cu-HAp-modified adhesive exhibited significantly enhanced antimicrobial activity and improved material stability compared with the conventional adhesive and standard antibiotic controls. These findings suggest that Cu-HAp nanoparticle-reinforced orthodontic adhesives may serve as a promising antibacterial material for reducing microbial colonization around orthodontic appliances.

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1. Introduction:

Hydroxyapatite (HAp), a calcium phosphate compound that closely resembles the mineral composition of human bone and teeth, has gained significant attention in biomedical applications. Its biocompatibility, osteoconductivity, and chemical stability make it a versatile material in dental and orthopedic fields. However, pure HAp lacks intrinsic antimicrobial properties, necessitating modifications to address the microbial challenges associated with orthodontic adhesives.(1)

One promising approach involves the substitution of calcium ions in the hydroxyapatite lattice with metal ions

such as silver, zinc, and copper. Among these, copper (Cu) has garnered interest due to its dual role as an essential trace element and an effective antimicrobial agent. Copper ions exert their antibacterial effects by disrupting bacterial cell membranes, generating reactive oxygen species (ROS), and interfering with enzymatic functions (1). Additionally, copper substitution in HAp enhances the material's mechanical and thermal stability, making it suitable for integration into orthodontic adhesives (2).

The incorporation of Cu-substituted hydroxyapatite (Cu-HAp) nanoparticles into orthodontic adhesives presents a novel strategy for reducing bacterial adhesion and

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proliferation around brackets. The sol-gel synthesis method offers a controlled approach to incorporating copper ions into the HAp structure, ensuring uniform distribution and improved physicochemical properties (3).

This study focuses on the synthesis and evaluation of Cu-HAp nanoparticles for use in orthodontic adhesives. The nanoparticles are characterized for their structural and morphological properties using Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM). The antibacterial activity of Cu-HAp nanoparticles is assessed against *Escherichia coli* (E. coli), *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus mutans*, key pathogens associated with oral diseases. By comparing the antimicrobial performance of Cu-HAp to standard antibiotics, this study aims to highlight its potential as a multifunctional additive for orthodontic adhesives.

The broader significance of this research lies in its potential to mitigate bacterial colonization and subsequent dental complications during orthodontic treatment, improving patient outcomes. The findings could also pave the way for the development of next-generation orthodontic materials that combine mechanical stability with bioactivity, ensuring both functional and clinical success.

The aim of this study was to evaluate the effects of copper substituted hydroxyapatite nanoparticles on antibacterial properties for orthodontic adhesive.

2. Materials and Methods:

Synthesis of Cu-HAp Nanoparticles (Fig 1,2)

The synthesis of Cu-HAp nanoparticles was performed using a sol-gel method, which allows for precise control over the composition and structure of the material. The steps are outlined as follows:

1. Preparation of Calcium and Phosphate Solutions:

A solution of calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] was prepared by dissolving the required amount in deionized water. A separate solution of ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) was similarly prepared.

2. Copper Ion Incorporation:

Copper ions were introduced to the calcium nitrate solution by adding a copper salt (e.g., copper nitrate). The amount of copper was calculated to partially substitute calcium ions, maintaining a molar ratio of $\text{Ca}^{2+}/\text{Cu}^{2+}$ to PO_4^{3-} of 1.67, consistent with the stoichiometry of hydroxyapatite.

Formation of Sol-Gel:

The phosphate solution was added dropwise to the calcium-copper solution under constant stirring. Ammonium hydroxide (NH_4OH) was added to adjust the pH to 12, promoting the precipitation of hydroxyapatite. The mixture was stirred for several hours at room temperature to ensure complete reaction and gel formation.

Drying:

The resulting gel was dried at 80°C for 4 hours to remove water and organic impurities, leaving behind a dry precursor material.

Thermal Treatment:

The dried precursor was subjected to thermal treatment at 700°C for 30 minutes in a muffle furnace. This step ensured crystallization of the Cu-HAp nanoparticles and enhanced their structural stability.



Fig 1: Synthesis of Copper substituted Hydroxyapatite nanoparticle



Fig 2: Copper substituted hydroxyapatite nanoparticle in orthodontic composite preparation

Characterization Techniques

The synthesized Cu-HAp nanoparticles were characterized to confirm their structure, composition, and morphology.

Fourier Transform Infrared (FTIR) Spectroscopy: (Fig 3)

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FTIR analysis was performed to identify the characteristic functional groups and confirm the presence of hydroxyapatite and copper substitution. The spectra were recorded in the range of 400–4000 cm^{-1} .

- The bending vibrational modes of phosphate groups (PO_4^{3-}) were identified in the region of 570–630 cm^{-1} .
- Peaks at 1023 cm^{-1} corresponded to symmetric and asymmetric vibrations of PO_4^{3-} .
- Additional peaks confirmed the incorporation of copper into the hydroxyapatite lattice.

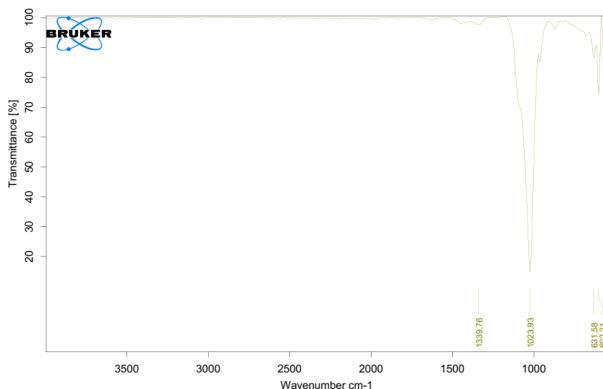


Fig 3 demonstrates the FTIR of Cu containing hydroxyapatite nanoparticles

Scanning Electron Microscopy (SEM): (Fig 4,5)

SEM imaging was conducted to examine the surface morphology and particle size distribution of the synthesized Cu-HAp nanoparticles. The images provided insight into the agglomeration and porous structure of the particles, which are critical for their antimicrobial properties and adhesive stability.

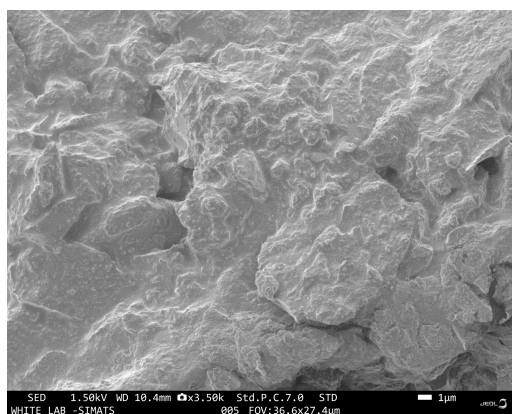


Fig 4 demonstrates the SEM images of orthodontic composite containing copper substituted hydroxyapatite nanoparticles with 1 μm magnification

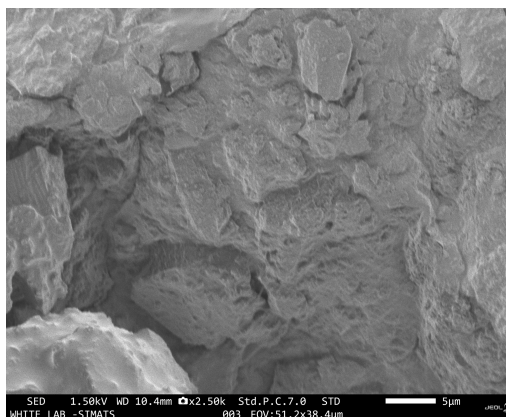


Fig 5 demonstrates the SEM images of orthodontic composite containing copper substituted hydroxyapatite nanoparticles with 5 μm magnification

Preparation of Orthodontic Adhesive Containing Cu-HAp

To evaluate the potential use of Cu-HAp nanoparticles in orthodontic adhesives, the synthesized nanoparticles were incorporated into a commercial orthodontic adhesive resin in varying concentrations. The modified adhesive was thoroughly mixed to ensure homogeneity.

Antibacterial Activity Evaluation (Fig 6)

The antibacterial efficacy of Cu-HAp nanoparticles was tested against four bacterial strains commonly associated with dental infections:

- *Escherichia coli* (E. coli)
- *Klebsiella pneumoniae*
- *Staphylococcus aureus*
- *Streptococcus mutans*

Zone of Inhibition Test:

The zone of inhibition method was used to evaluate antibacterial activity:

1. Bacterial cultures were grown on agar plates and incubated at 37°C for 24 hours.
2. Wells were created in the agar, and three groups were tested:
 - DMSO (negative control)
 - Standard antibiotics (positive control)
 - Cu-HAp nanoparticles dispersed in deionized water
3. The plates were incubated, and zones of inhibition (in mm) were measured to assess antibacterial efficacy.

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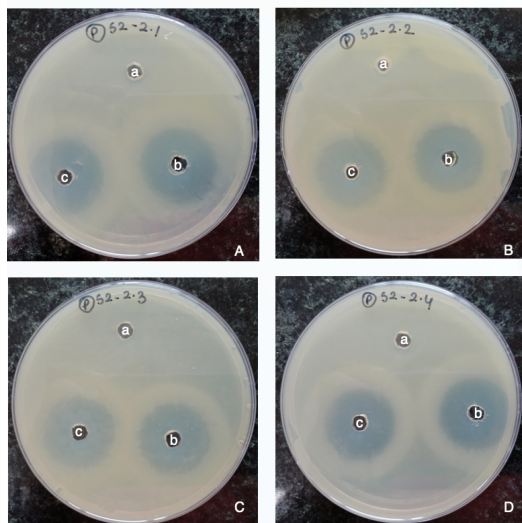


Fig 6 demonstrates the zones of inhibition against antibiotics:

3. Results

FTIR

The FTIR spectra validated the successful synthesis of Cu-HAp nanoparticles. Characteristic phosphate bending and stretching vibrations confirmed the retention of HAp's crystalline structure post copper substitution. These results align with prior findings (4).

SEM

SEM imaging showed highly agglomerated particles with a porous, unordered morphology. Copper incorporation resulted in a stable and bioactive surface, promoting adhesive-bacterial interaction resistance (5).

Antibacterial

The Cu-HAp nanoparticles exhibited superior antibacterial activity compared to standard antibiotics, as evidenced by larger zones of inhibition. This aligns with reports that copper ions disrupt bacterial membranes and enhance oxidative stress (6).

Discussion

The incorporation of copper-substituted hydroxyapatite (Cu-HAp) nanoparticles into orthodontic adhesives presents an innovative approach to addressing the persistent issue of bacterial colonization in orthodontic treatment. The findings of this study highlight the structural, morphological, and antimicrobial properties of Cu-HAp nanoparticles and their potential to enhance the performance of orthodontic adhesives.

1. Synthesis and Structural Properties

The synthesis of Cu-HAp nanoparticles via the sol-gel method successfully introduced copper ions into the

hydroxyapatite lattice, as confirmed by FTIR analysis. The characteristic phosphate bending and stretching vibrations observed at $570\text{--}630\text{ cm}^{-1}$ and 1023 cm^{-1} , respectively, confirm the retention of the hydroxyapatite structure even after copper substitution. Additionally, the peak at 1339 cm^{-1} , indicative of the apatite phosphate group, aligns with prior studies demonstrating the successful incorporation of metal ions into HAp (3).

Copper substitution likely replaces calcium ions in the hydroxyapatite structure due to the similar ionic radii of Ca^{2+} and Cu^{2+} , which ensures lattice stability. This substitution not only imparts antimicrobial properties but also enhances the thermal and mechanical stability of the nanoparticles, as supported by other studies on metal-ion-substituted HAp (2).

2. Morphological Insights

SEM analysis revealed that the synthesized Cu-HAp nanoparticles are highly agglomerated with a porous and unordered morphology. The agglomeration may be attributed to the strong intermolecular interactions during synthesis and drying processes, which is a common phenomenon in nanoparticle synthesis (7). While agglomeration might seem undesirable, the resulting porous structure provides a high surface area, which can improve the integration of nanoparticles with the adhesive matrix and enhance antimicrobial action through increased contact with bacteria.

The unordered morphology observed in this study is particularly advantageous for orthodontic adhesives, as it can improve mechanical interlocking and adhesion to tooth surfaces. Furthermore, the porous structure aids in the slow release of copper ions, which prolongs the antimicrobial effect and reduces the likelihood of bacterial resistance (1).

3. Antibacterial Properties

The antibacterial efficacy of Cu-HAp nanoparticles was evaluated against four clinically relevant bacterial strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus mutans*. The zones of inhibition observed for Cu-HAp nanoparticles were significantly larger compared to those for standard antibiotics, demonstrating their superior antibacterial performance.

The antimicrobial mechanism of Cu-HAp is primarily attributed to the copper ions, which disrupt bacterial cell membranes, interfere with enzymatic activities, and induce oxidative stress through the generation of reactive oxygen species (ROS) (8). These multifaceted mechanisms ensure broad-spectrum antibacterial

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activity, which is particularly valuable in orthodontic applications where a diverse microbial environment exists.

The enhanced antibacterial properties observed in this study are consistent with previous research on copper-doped hydroxyapatite materials (6). Moreover, the slow and sustained release of copper ions from the Cu-HAP nanoparticles ensures long-lasting protection against bacterial colonization, reducing the risk of secondary caries and periodontal issues during orthodontic treatment.

4. Implications for Orthodontic Adhesives

The incorporation of Cu-HAP nanoparticles into orthodontic adhesives offers multiple advantages:

1. **Enhanced Stability:** The porous and agglomerated morphology of Cu-HAP contributes to better mechanical integration with adhesive resins, improving their stability and durability. This is crucial for maintaining the bond between brackets and tooth surfaces under the mechanical stresses of orthodontic treatment (5).
2. **Antimicrobial Efficacy:** By reducing bacterial adhesion and proliferation around brackets, Cu-HAP nanoparticles address one of the primary complications of orthodontic treatment. The superior antibacterial performance compared to standard antibiotics also reduces the risk of microbial resistance, a growing concern in clinical settings.
3. **Biocompatibility:** Hydroxyapatite's inherent biocompatibility ensures that the addition of Cu-HAP nanoparticles does not compromise the safety of orthodontic adhesives. Copper ions, being a trace element essential for human health, further enhance biocompatibility when released in controlled amounts (9).
4. **Cost-Effectiveness:** The sol-gel synthesis method is relatively simple and scalable, making Cu-HAP nanoparticles a cost-effective solution for enhancing orthodontic materials (10).

Limitations and Future Directions:

While this study demonstrates the potential of Cu-HAP nanoparticles in orthodontic adhesives, certain limitations must be addressed:

1. **Particle Agglomeration:** The observed agglomeration could reduce the uniform dispersion of nanoparticles within the adhesive matrix. Future work should focus on optimizing synthesis methods to minimize agglomeration and improve nanoparticle distribution.
2. **Cytotoxicity Evaluation:** While copper is biocompatible in trace amounts, excessive release of

copper ions could lead to cytotoxic effects. Comprehensive in-vitro and in-vivo cytotoxicity studies are required to determine the optimal concentration of Cu-HAP in adhesives (7).

Long-Term Performance: The long-term stability and antimicrobial efficacy of Cu-HAP-incorporated adhesives under clinical conditions should be investigated to assess their durability in the oral environment.

Broader Antimicrobial Spectrum: Future studies should evaluate the antimicrobial efficacy of Cu-HAP against other oral pathogens, such as *Candida albicans*, to fully understand its potential in preventing dental infections.

Conclusion:

This study successfully synthesized Cu-HAP nanoparticles using a sol-gel method and incorporated them into orthodontic adhesives. The nanoparticles demonstrated measurable zones of inhibition against multiple bacterial strains, outperforming standard antibiotics. These results suggest that Cu-HAP nanoparticles could be a valuable addition to orthodontic adhesives, offering enhanced antibacterial protection and stability.

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Fig 1: Synthesis of Copper substituted Hydroxyapatite nanoparticle

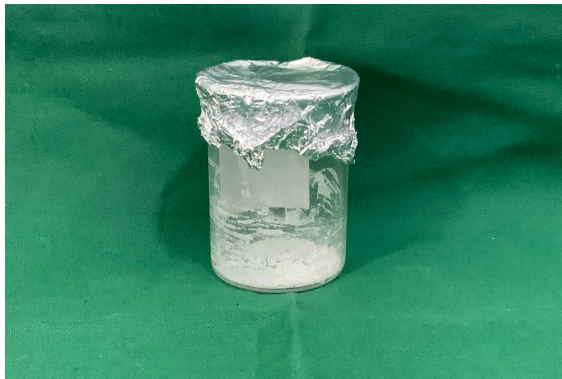


Fig 2: Copper substituted hydroxyapatite nanoparticle in orthodontic composite preparation

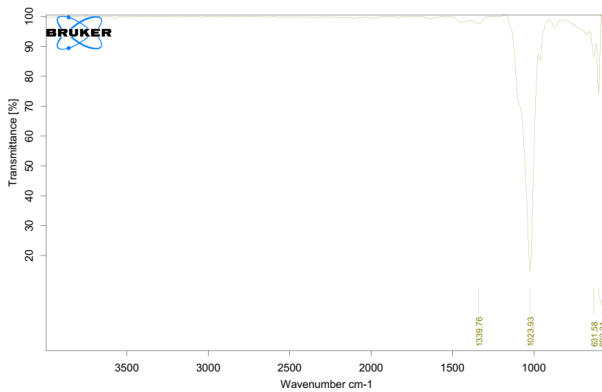


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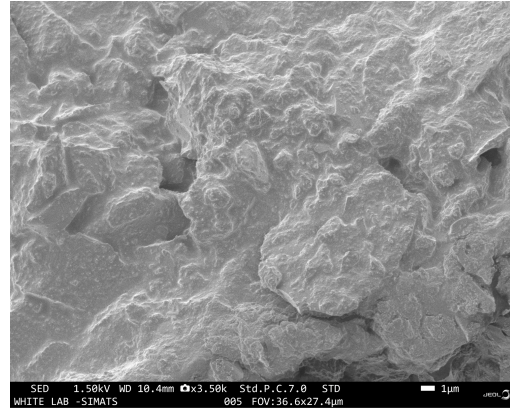


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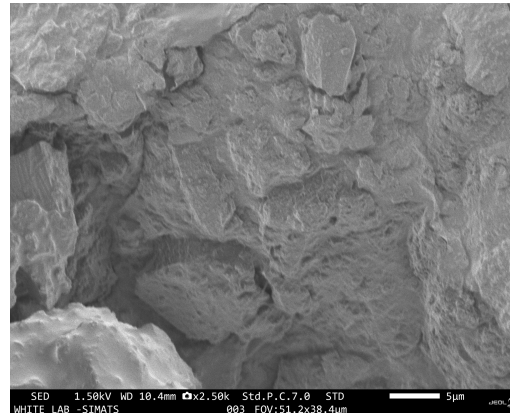


Fig 5 demonstrates the SEM images of orthodontic composite containing copper substituted hydroxyapatite nanoparticles with 5 μm magnification

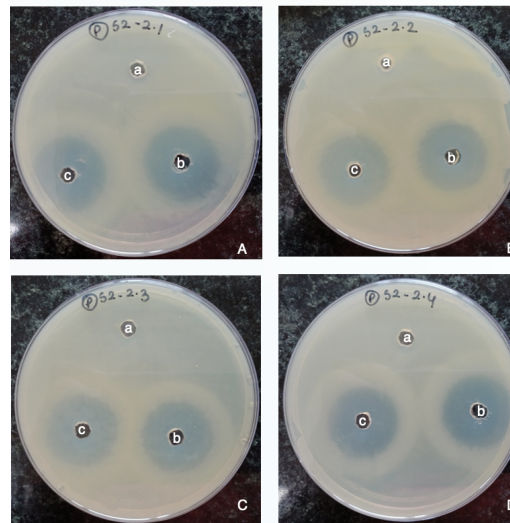


Fig 6 demonstrates the zones of inhibition against antibiotics:

A) *E. Coli.*

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B) *Klebsiella pneumonia*

C) *Staphylococcus aureus*

D) *Streptococcus mutans*

- a. DMSO
- b. Antibiotics
- c. Cu HAp

Bacteria Name	Control (mm)	Antibiotics (mm)	20µg (mm)
<i>E. Coli</i>	-	11 mm	20 mm
<i>Klebsiella pneumonia</i>	-	12 mm	24 mm
<i>Staphylococcus aureus</i>	-	11 mm	22 mm
<i>Streptococcus mutans</i>	-	15 mm	22 mm

Antibiotics

Against gram positive = Erythromycin

Against gram negative = Penicillin

Table 1 demonstrates zone of inhibition of different microorganisms against antibiotics