



Evaluation of the Cytotoxicity of Orthodontic Composite Incorporating Copper and Magnesium Hydroxyapatite Nanoparticles

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ABSTRACT:

Introduction: Nanoparticle-enhanced orthodontic adhesives have gained increasing attention for their potential to reduce enamel demineralization and provide antibacterial benefits without compromising material performance. Copper (Cu) and magnesium hydroxyapatite [Mg(OH)₂] nanoparticles, in particular, exhibit promising antimicrobial and biocompatible properties suitable for dental applications. However, integrating these nanoparticles into orthodontic bonding systems necessitates careful evaluation of their biological safety.

Objectives: This study aims to assess the cytotoxicity of Transbond XT adhesive incorporated with Copper (Cu) and Magnesium Hydroxyapatite Mg(OH)₂ nanoparticles.

Methods: An orthodontic composite comprising equal proportions of Cu and Mg(OH)₂ nanoparticles was synthesized using a hydrothermal method. Cu and Mg(OH)₂ nanoparticles were mixed in ethyl alcohol, and the pH was adjusted to 10 - 10.5 with liquor ammonia. The mixture was then added to light-cured orthodontic adhesive (Transbond XT). After ultrasonic treatment and hydrothermal processing, the composite was characterized through SEM, FTIR, and EDAX analyses. The cytotoxicity test was performed on human fibroblast cells using various concentrations of the composite.

Results: SEM analysis revealed distinct surface characteristics. Orthodontic adhesive with Cu and Mg(OH)₂ nanoparticles exhibited a smooth surface with rod-like structures. Cu and Mg(OH)₂ mixture displayed a coarse, granular surface topography. FTIR confirmed specific functional groups. Peaks indicated the presence of Cu and Mg(OH)₂ functional groups within the composite. EDAX analysis yielded quantitative elemental composition. Cytotoxicity assessment demonstrated an increasing cytotoxicity trend with rising particle concentration for all samples. Cell viability consistently above 60% for all concentrations. Notably, fibroblast cell viability at 50% concentration was 87%, indicating cytocompatibility.

Conclusions: The incorporation of Cu and Mg(OH)₂ nanoparticles into Transbond XT adhesive yielded a composite with altered surface characteristics. FTIR and EDAX analysis confirmed the presence of specific functional groups and elemental composition. Cytotoxicity assessment indicated that the composite exhibited favorable cell viability, particularly at a 50% concentration, suggesting its potential for cytocompatibility.



1. Introduction

The emergence of white-spot lesions (WSL) is a significant concern during fixed appliance treatment, affecting a wide range of individuals, with reported incidence rates spanning from 2% to 96%. These lesions represent one of the most undesirable outcomes of orthodontic intervention[1–8]. The heightened susceptibility to WSL development among patients undergoing orthodontic treatment with brackets, bands, and archwires arises from compromised oral hygiene due to hindrance in effective cleaning and increased retention of plaque [1,2,9,10]. This plaque accumulation fosters an environment conducive to an elevated count of microorganisms known to contribute to enamel lesions [11–14]. Furthermore, the lowered pH resulting from retained plaque adjacent to orthodontic brackets disrupts the enamel remineralization process, potentially leading to decalcification [11]. Notably, early enamel decalcifications have been observed within just 4 weeks of commencing orthodontic treatment. [15,16]

Early attempts to counter enamel demineralization involved incorporating adhesives or cements with soluble antimicrobial compounds like chlorhexidine. However, subsequent research revealed that this approach yielded only transient anti-carries effects, often at the expense of mechanical qualities such as bond strength [17,18].

In recent times, nanoparticles have garnered attention as potential additions to orthodontic bonding systems to mitigate enamel demineralization around appliances. Nanoparticles not only exhibit promising synergistic effects on enamel remineralization but also demonstrate the potential to enhance bond strength, flexural strength, and tensile strength[18–22] Among these nanoparticles Mg(OH)₂, nanoparticles have emerged as compelling candidates due to their potent antibacterial properties and compatibility with dental materials[22,23].

Research has highlighted Copper (Cu) ability to maintain antibacterial effectiveness even after composite synthesis[24,25]. Copper nanoparticles have also shown promise in enhancing shear bond strength and antibacterial properties of dental adhesives without compromising their physical attributes[26,27]. However, the integration of nanoparticles into orthodontic bonding systems raises concerns about their unique structural and chemical characteristics, which could potentially introduce additional health risks.

Thus, a thorough evaluation of the biological properties of these materials is imperative before their adoption in oral applications. In light of this, the primary objective of this study is to investigate the cytotoxicity of Transbond XT adhesive containing Cu and Mg(OH)₂ nanoparticles. Through this investigation, we aim to contribute valuable insights into the safety and viability of utilizing these nanoparticles in orthodontic adhesive formulations.

2. Methods

This was an in-vitro study conducted in the Saveetha Dental College and Hospitals during the period of October 2022 to February 2023. Equal amounts of Cu and Mg(OH)₂, nanoparticles were dispensed in a glass jar and was dissolved in a ethyl alcohol solvent. In the present study, Zinc oxide and Tin oxide nanoparticle infused light cured orthodontic adhesive was synthesized using a hydrothermal method. 0.1 g of Cu and Mg(OH)₂ nanoparticles were synthesized adopting a hydrothermal method. The nanoparticle mixture was subjected to constant stirring using a magnetic stirrer (MS 500, REMI, India) in ethyl alcohol which was the solvent. The pH of the resultant solution was maintained at a pH range of 10 - 10.5 with the help of liquor ammonia. To this mixture, 1g of light cured orthodontic adhesive (Transbond XT 3M Unitek, Monrovia, CA, USA); i.e. by a weight ratio of 1% to the nanoparticles. Before complete mixing, the suspension was subjected to ultrasonic treatment in an ultrasonic bath for 10 min. Then the treated suspension was transferred to an autoclave and hydrothermal treatment was given at 180°C for 48 h. The resultant precipitate was filtered and washed repeatedly with deionized water to get rid of unwanted inorganic residues. The washing step was continued until the removal of organic residues limiting agglomeration. Then the precipitate was washed with ethanol several times and air-dried at room temperature. Further, the samples devoted to phase analysis were dried in a hot air oven at 100°C for 24 h. The final precipitate was segregated, light cured according to the manufacturer instructions grated into fine particles with the help of mortar and pestle (Figure 2).

The obtained sample was then subjected to the following tests

- SEM ANALYSIS (Nanoparticle and also for the composite+nanoparticle mix)
- FTIR
- EDAX



- Cytotoxicity test.

SEM Analysis and Functional Group Analysis:

Initially, a small quantity of the nanoparticles was meticulously dissolved in deionized water to create a homogeneous suspension. This suspension was subjected to ultrasonic treatment for a minimum duration of one hour to disperse the nanoparticles uniformly and eliminate any potential agglomerations. Following sonication, the prepared suspension was meticulously applied onto a glass substrate, which served as the foundation for subsequent analysis. To enhance the sample's characteristics for further characterization, a thin layer of gold was deposited onto the substrate through gold-plating. This gold-plated substrate ensured improved conductivity and facilitated more precise characterization of the sample. Subsequently, the prepared sample underwent Fourier Transform Infrared Spectroscopy (FTIR) analysis. The FTIR analysis aimed to identify the presence of specific functional groups within the nanoparticles and provide insights into their molecular composition.

EDAX Analysis:

EDAX analysis was also performed to do a quantitative analysis to estimate each component by weight percentage. After the synthesis of the composite, a small portion of the adhesive containing the nanoparticles was subjected to EDAX analysis. The sample was then carefully mounted and positioned for EDAX analysis. The setup involved bombarding the sample with X-rays, resulting in the emission of characteristic X-ray spectra from the elements present in the material. These emitted X-rays were captured and analyzed, allowing for the identification and quantification of various elements within the adhesive, particularly focusing on zinc and tin along with their corresponding oxides.

Cytotoxicity Test:

Cytotoxicity test was performed on the human fibroblast cells. 96-well plates were used, incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO₂, 95% air. The experimental solution was dispensed into the wells at various concentrations and the human fibroblast cells were allowed to grow in the wells. A control well was also maintained with the Transbond XT adhesive serving as the control. After 48 hrs, the cell viability was calculated from the number of viable fibroblast cells in each well. Cell viability was calculated in percentage of control groups according to the following formula cell

viability (%) = (OD of the test group / OD of the control group) × 100 Cell viability was then scored according to the following classification: -

- a. more than 90 percent cell viability: non-cytotoxic
- b. 60–90 percent cell viability: slightly cytotoxic
- c. 30–59 percent cell viability: moderately cytotoxic
- d. less than 30 percent cell viability: severely cytotoxic



Figure 1: Transbond XT adhesive



Figure 2: The light cured nanoparticle incorporated composite block and its powdered form (from left to right)

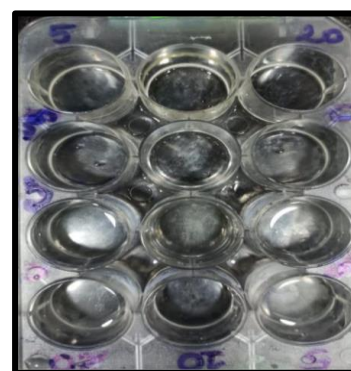


Figure 3: 12 well well plate used for cytotoxicity testing

3. Results

Surface Analysis:

Scanning Electron Microscopy (SEM) analysis revealed distinctive surface characteristics between the samples. The composite containing Cu and Mg(OH)₂ nanoparticles exhibited a smooth surface with loosely



packed rod-like structures. The Cu+ Mg(OH)₂ mixture showed a coarse and granular surface topography. (Figure 4, 5)

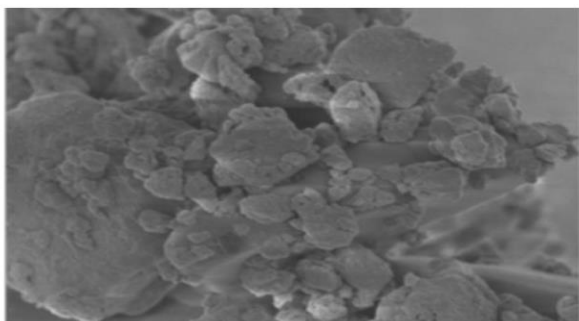


Figure 4: Picture showing SEM image of the zinc oxide and tin oxide nanoparticle mixture

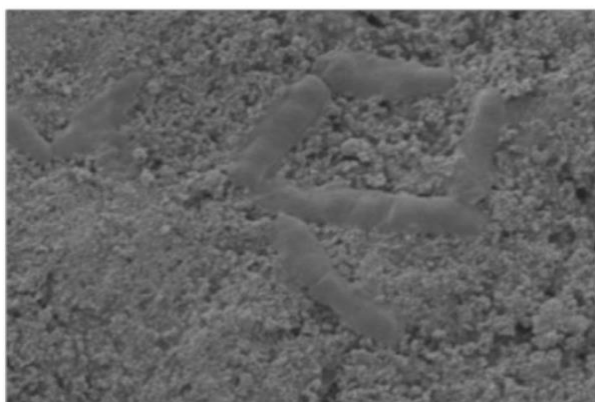


Figure 5: Picture showing SEM image of the zinc oxide and tin oxide nanoparticle mixture incorporated in Transbond XT orthodontic adhesive.

4. Elemental Analysis: Furthermore, Fourier Transform Infrared Spectroscopy (FTIR) indicated the presence of specific functional groups. Peaks in the spectrum confirmed the existence of Cu and Mg(OH)₂ functional groups within the composite. Energy-dispersive X-ray Analysis (EDAX) provided quantitative elemental composition data. The analysis demonstrated the presence of Cu at 57.8 weight percent (wt%), Mg(OH)₂ at 11.4 wt%, and oxides at 30.9 wt% in the sample.

Cytotoxicity assessment: The assessment of cytotoxicity yielded the following findings, as presented in Figure 6. A clear trend of increasing cytotoxicity was observed as the particle concentration increased across all samples.

- Despite the increasing concentration, the overall cell viability remained consistently above 60% for all concentrations.
- Particularly noteworthy was the observation that at a concentration of 50%, the cell viability of fibroblasts was 87%.
- This high cell viability percentage at the 50% concentration point strongly indicates that the composite material was cytocompatible.

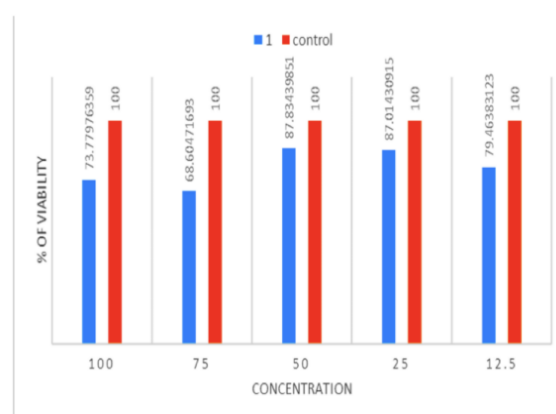


Figure 6: Cytotoxicity assessment of the zinc oxide and tin oxide nanoparticles incorporated Transbond XT orthodontic adhesive

5. Discussion

The current research presents an interesting exploration into the cytotoxicity and properties of a novel photo-initiated orthodontic adhesive containing Cu and Mg(OH)₂ nanoparticles at a concentration of 1 wt%. This study's approach is grounded in the prior work of Aydin Sevinç and Hanley (2010) [28], who demonstrated that the addition of 1 weight percent of TiO₂ nanoparticles to Transbond XT adhesive resulted in significant antibacterial effects without compromising shear bond strength. Building upon this idea, we opted for the concentration of 1 wt% for Cu and Mg(OH)₂ nanoparticles in the adhesive formulation. This decision likely stemmed from the intention to maintain a delicate balance between incorporating antibacterial attributes while retaining adhesive strength. The choice of Human gingival fibroblast cells as the model for cytotoxicity assessment is commendable. These cells are well-established as a reliable and sensitive tool to evaluate the potential toxic effects of different substances, making



them suitable for analyzing the safety of the nanoparticles used in the orthodontic adhesive.

The utilization of Cu and Mg(OH)₂ nanoparticles in dental materials is a novel aspect of this research. Studies have pointed out that Cu and Mg(OH)₂ nanoparticles possess properties that make them suitable for incorporation into dental materials. Copper (Cu) distinct chemical and physical properties, including high chemical stability, electrochemical coupling coefficient, radiation absorption range, and photostability, render it valuable for various applications[29]. Moreover, Cu well-established antibacterial and anticancer properties are noteworthy, as highlighted by various authors [30,31]. Similarly, Mg(OH)₂ nanoparticles offer an array of appealing attributes, including non-toxicity, high transmittance in the visible region, chemical stability, and antibacterial, antioxidant, and biocompatible traits[32]. The fact that Mg(OH)₂ NPs have shown efficacy against *Escherichia coli* and *Staphylococcus aureus* under both dark and UV light conditions further underscores their potential in healthcare applications [32,33]. The incorporation of a mixture of Cu and Mg(OH)₂ nanoparticles in the orthodontic adhesive is an interesting strategy to harness their synergistic properties. This approach not only capitalizes on the individual merits of each nanoparticle but also creates the potential for enhanced performance at a reasonable cost. The use of Transbond XT adhesive as the baseline for comparison is a wise choice due to its superior physical properties compared to other dental adhesives. The surface analysis of the Cu and Mg(OH)₂ nanoparticle composite, as revealed by SEM (Scanning Electron Microscopy) and FTIR (Fourier Transform Infrared Spectroscopy), provides valuable insights into the structural characteristics and functional groups present in the composite material. SEM analysis uncovered distinct variations in surface topography between the different samples. The composite material containing Cu and Mg(OH)₂ nanoparticles exhibited a relatively smooth surface with loosely packed rod-like structures, in contrast to the coarse and granular surface topography of the material without nanoparticles. This alteration in surface morphology suggests that the incorporation of nanoparticles contributes to the modification of the composite's overall structure, potentially influencing its mechanical and adhesive properties. The presence of rod-like structures might be indicative of the arrangement of nanoparticles within the composite matrix.

FTIR analysis further supported these findings by identifying specific functional groups within the composite. The presence of Cu and Mg(OH)₂ functional groups indicates successful integration of the nanoparticles into the composite material. This data suggests that the nanoparticles form chemical bonds within the composite matrix, which could contribute to the observed changes in surface topography. The emergence of these functional groups could also indicate potential interactions between the nanoparticles and the surrounding matrix, potentially influencing the material's properties.

EDAX analysis provided quantitative data on the elemental composition of the composite material. The presence of Cu, Mg(OH)₂, as well as the occurrence of oxides, confirms the successful incorporation of these nanoparticles. The relatively high weight percentages of Cu, Mg(OH)₂, along with the presence of oxides, highlight the substantial influence of these nanoparticles on the overall composition of the material. These findings are consistent with the SEM and FTIR results, collectively showcasing the successful integration of Cu and Mg(OH)₂ nanoparticles into the composite. Moving on to the cytotoxicity assessment, the results depicted in Table 1 shed light on the impact of particle concentration on cell viability. The trend of increasing cytotoxicity with rising particle concentration across all samples suggests that higher concentrations of the composite could potentially have adverse effects on cell viability. However, it's noteworthy that the overall cell viability remained above 60% for all concentrations, indicating a degree of compatibility of the composite material with the fibroblast cells. Of particular interest is the observation at the 50% concentration, where the cell viability of fibroblasts was as high as 87%. This finding underscores the cytocompatibility of the composite material even at relatively higher concentrations, suggesting that the composite has the potential for use in applications where it comes into contact with living cells or tissues.

In summary, the surface analysis techniques of SEM, FTIR, and EDAX have provided valuable insights into the structural characteristics and chemical composition of the Cu+Mg(OH)₂ nanoparticle composite. Additionally, the cytotoxicity assessment results indicate that the composite exhibits a favorable degree of cytocompatibility even at higher concentrations, paving the way for potential biomedical and healthcare applications.



Future scope: Further research could focus on investigating the long-term effects of the composite material on cell viability and exploring its applicability in specific clinical contexts. Subsequent research endeavors should be directed towards unraveling the impact of zinc oxide and tin oxide nanoparticles on critical mechanical parameters, notably compressive and tensile strength, hardness, elasticity, flow, its antimicrobial effects as well as other pertinent physical attributes. Through a systematic evaluation of these properties, a holistic understanding of how the nanoparticles influence the adhesive's behavior under diverse loading conditions can be attained.

Conclusion:

Through meticulous synthesis using a hydrothermal method, the study successfully incorporated Cu and Mg(OH)₂ nanoparticles into an orthodontic adhesive. The study demonstrated that the composite material exhibited a high degree of cytocompatibility, even at a concentration of 1 wt% for Cu and Mg(OH)₂ nanoparticles. This finding suggests the potential for safe use in biomedical and healthcare applications where contact with living cells or tissues is essential. By capitalizing on the synergistic properties of Cu and Mg(OH)₂ nanoparticles, this research introduces a novel strategy for enhancing orthodontic adhesive materials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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