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Influence of biosynthesized silver nanoparticles on vital pulp therapy of New-Zealand rabbits: In Vivo study

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Abstract

Background: Direct pulp capping success depends on materials that promote healing while preventing infection. This study evaluated silver nanoparticle (AgNP)-enhanced resin composite for pulp capping compared to mineral trioxide aggregate (MTA) and conventional resin.

Methods: Eighteen New Zealand rabbits were divided into three groups (n=6/group): Control (resin only), MTA, and AgNPs (resin with 1% w/w 50nm AgNPs). After pulp exposure and capping, teeth were extracted at 4 weeks for histological analysis using H&E and Masson's trichrome staining. Inflammatory cell area percentage was quantified via Leica QWin image analysis.

Results: Histological and histochemical analyses were conducted after four weeks. The AgNPs group demonstrated minimal inflammatory cell infiltration, no necrosis, and prominent reparative dentin formation with thick, continuous dentin bridges. Collagen fiber organization was superior in the AgNPs group, indicating enhanced tissue regeneration. One-way ANOVA revealed statistically significant differences in inflammatory cell area percentage among the experimental groups, the AgNPs group showing the lowest inflammation ($p < 0.001$). These findings suggest that AgNPs not only reduce bacterial infection risks but also promote pulp healing and regeneration, outperforming traditional MTA in key areas.

Conclusion: AgNP-modified resin composites significantly reduced pulpal inflammation and enhanced tissue regeneration compared to both MTA and conventional resin, suggesting their potential as an advanced pulp capping material combining antimicrobial properties with biocompatibility.

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1 Introduction

The field of dentistry has always been at the forefront of innovation, constantly seeking new ways to improve patient outcomes and enhance the quality of care. One of the most critical challenges in restorative dentistry is preserving the vitality of dental pulp following exposure due to caries or trauma. Direct pulp capping, a procedure designed to protect and heal the pulp, plays a pivotal role in maintaining tooth health and function. However, the success of this procedure hinges on the materials used, which must not only promote healing and tissue regeneration but also guard against bacterial infection, which is a common cause of treatment failure. [1]

For decades, materials like calcium hydroxide and mineral trioxide aggregate (MTA) have been the go-to choices for pulp capping due to their proven biocompatibility and ability to stimulate reparative dentin

formation. [2] Yet, despite their advantages, these materials are not without limitations. MTA, for instance, while highly effective, is costly and can be challenging to handle, making it less accessible for widespread use.[3] On the other hand, resin composites, which offer superior aesthetic and mechanical properties, have raised concerns regarding their biocompatibility and ability to prevent infections when used in pulp capping.[4]

Nanotechnology is a groundbreaking advancement that has transformed countless industries, including medicine and pharmaceuticals. Among the many innovations it has brought, silver nanoparticles (AgNPs) stand out for their remarkable antimicrobial properties and versatility.[5] These tiny particles, with their unique physical and chemical characteristics, have shown immense potential in combating bacterial infections, enhancing drug delivery, and even aiding in cancer therapy.[6] In dentistry, AgNPs have emerged as a promising tool to address some of the most pressing challenges, including infection control and material performance.[7, 8]

By integrating AgNPs into resin composites, researchers and clinicians are exploring a novel approach that combines the best of both worlds: the aesthetic and mechanical benefits of resin composites with the powerful antimicrobial action of AgNPs. [9, 10] This innovative strategy has the potential to revolutionize direct pulp capping, offering a material that not only supports pulp healing but also significantly reduces the risk of post-operative infections. [1]

This study delves into the integration of silver nanoparticles into bonding material of resin composites, examining their impact on the biological outcomes of direct pulp capping. By bridging the gap between advanced nanotechnology and restorative dentistry, this research aims to pave the way for more effective, durable, and patient-friendly dental materials, ultimately improving clinical outcomes and enhancing the standard of care in dentistry.

2 Materials and Methods

2.1 Ethical Approval

The study adhered to the ethical guidelines for animal research established by the Faculty of Dentistry at MSA University (approval no. REC-D 113612-4).

All procedures were conducted in the MSA University animal house under strict sterile conditions. The rabbits were housed individually in clean cages, maintained at a controlled temperature of 20–24°C with a 12-hour light-dark cycle. They were provided with a standard diet and water ad libitum throughout the study period.[11]

2.2 Sample size calculation

The sample size was determined using PS software, with the primary outcome being histomorphometric analysis of pulp tissue, specifically inflammatory cell count. Based on an effect size of 0, 80% power, and a 5% significance level ($\alpha = 0.05$), the required sample size was calculated to be 15 rabbits. To account for potential dropouts, the sample size was increased to 18 rabbits, allowing for a 15% attrition rate.[12]

2.3 Samples selection

The study involved 18 healthy male New Zealand rabbits, aged 6–8 months and weighing 2.5–3 kg. The rabbits were randomly assigned to one of three groups (six rabbits per group):

- Control Group: consisted of six rabbits; cavities were sealed with resin composite alone, without any pulp capping material.
- MTA Group: consisted of six rabbits; pulp was capped with Mineral Trioxide Aggregate (MTA), followed by resin composite coverage. [13]
- AgNPs Group: consisted of six rabbits; pulp was capped using resin composite with a bond infused with silver nanoparticles (50 nm in size, at a concentration of 1% w/w). [14]

2.4 Preparation & characterization of silver nanoparticles

Silver nanoparticles (50nm) were obtained from Nano Gate Lab. The preparation of silver nanoparticles (AgNPs) was carried out using the chemical reduction method as described by Turkevich, Lee, and Meisel. In this process, a solution of silver nitrate ($AgNO_3$) was used as the source of Ag^+ ions. Polyvinylpyrrolidone (PVP) was employed as a stabilizer, while sodium borohydride ($NaBH_4$) acted as a mild reducing agent. The reduction of Ag^+ ions to silver nanoparticles was visually confirmed by the change in the solution's color to yellow, indicating the formation of AgNPs. [15-17]

A self-etch adhesive (Single Bond Universal, SBU; 3M ESPE) was used as the parent bonding system and control group. The silver nanoparticles were dissolved in 2-(tert-butylamino) ethyl methacrylate (TBAEMA; Sigma) at a concentration of 0.08 g of silver salt per 1 g of TBAEMA. The prepared Ag-TBAEMA solution was then mixed with the bond in a ratio of 0.1% by weight. To study the dispersion of silver nanoparticles in adhesives, the adhesives were applied to slides and cured using a light-emitting diode (LED) polymerization unit woodpecker light cure I LED Woodpecker, China. After

curing, the slide surfaces were coated with gold using a sputter coater and analyzed with a transmission electron microscopy (TEM) conducted using a JEOL JEM-2100 high-resolution transmission electron microscope operated at an accelerating voltage of 200 kV to analyze the size and shape of the nanoparticles. [18]

Fourier-transform infrared (FTIR) spectroscopy was utilized for simple, non-destructive, and reliable analysis of samples, enabling both qualitative and quantitative assessments. The analysis was performed using a Bruker FT-IR Vertex 70 spectrometer equipped with a RAM II module.

2.5 Experimental Procedures

2.5.1 Cavity Preparation

Under general anesthesia, deep cavities (approximately 4 mm in diameter and 3 mm in depth) were prepared on the first mandibular molars of each rabbit using a high-speed handpiece and sterile diamond burs. The pulp tissue was intentionally exposed to simulate conditions requiring direct pulp capping. [1]

2.5.2 Pulp Capping

The exposed pulp was gently dried using sterile cotton pellets. Each group received a specific treatment:

- Control Group: The cavity was sealed with resin composite alone.
- MTA Group: The pulp was capped with MTA, followed by resin composite coverage. [13]
- AgNPs Group: The pulp was capped using resin composite with a bond infused with silver nanoparticles. [14]

2.5.3 Animal Sacrifice and Sample Collection

Four weeks after the procedure, all rabbits were humanely euthanized using a ketamine overdose. The treated teeth were carefully extracted for histological and histochemical analyses.[3]

2.6 Histological and Histochemical Evaluations

2.6.1 Histological Evaluation

The extracted teeth were fixed in 10% neutral buffered formalin for 24 hours, dehydrated in a series of ethanol solutions, cleared in xylene, and embedded in paraffin wax. Thin sections (3–4 microns) were cut and stained with hematoxylin and eosin (H&E) to assess the histopathological changes in the pulp tissue. [19]

2.6.2. Histochemical Evaluation

Additional Sagittal Sections of 3-4 microns were cut in rotary micrometer, mounted on clean slides and then, stained with Masson trichrome stain where three dyes (hematoxylin, fuchsin and light green) were used. Masson trichrome stain was used to detect the formation of collagen fiber bundles in the dental pulp.[20]

Histological & histochemical specimens were observed under a digital microscope, Leica DM3000 LED S.N 346986, camera DFC295, S.N 0705530414, made in Germany.

2.6.3 Histomorphometric Analysis

The distribution of inflammatory cells in the area beneath the applied material in pulp capping (control, MTA, and silver groups) was analyzed using Leica QWin Image Processing and Analysis Software (Part No. 872705, Version V3.5.1; Leica Microsystems Ltd., CH-9435 Heerbrugg, Switzerland). The area percentage of inflammatory cells was calculated in accordance with the method described by Kaczmarek et al. [21].

The analytical procedure involved several key steps. First, high-resolution images of H&E-stained sections were acquired under standardized microscopy conditions. The software's thresholding function was then employed to differentiate inflammatory cells from surrounding tissue based on their distinct colorimetric and grayscale characteristics. Following optimal threshold adjustment, the region of interest (ROI) encompassing the area immediately beneath the capping material was delineated using the software's selection tools, including both automated detection and manual polygonal outlining functions for precision. [19]

To ensure measurement reliability, each sample was analyzed across multiple representative fields (minimum of five fields per section) with consistent ROI dimensions. The software automatically calculated the area percentage of inflammatory cell infiltration using the formula: (threshold-positive area/total ROI area) × 100. This approach provided quantitative data for comparative analysis between experimental groups while minimizing observer bias. [22] All measurements were performed by two independent investigators to verify inter-rater consistency, with discrepancies resolved through consensus review.

2.7 Statistical Analysis

Quantitative analysis of inflammatory cell distribution was performed by calculating the area percentage for each experimental group. All data were first assessed for normality using both the Kolmogorov-Smirnov and Shapiro-Wilk tests, which confirmed parametric distribution ($p > 0.05$ for all groups).

Intergroup comparisons were conducted using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. This analytical approach was selected as it is appropriate for normally distributed data with more than two independent groups.

The significance level was set at $P < 0.05$. Statistical

analysis was performed with IBM® SPSS® Statistics Version 25 for Windows.

3 Results

The findings of this study revealed significant differences in the biological outcomes of direct pulp capping among the three experimental groups.

3.1 Histological Results

Histopathological examination of the pulp tissue using hematoxylin and eosin (H&E) staining demonstrated distinct tissue responses across the groups:

3.1.1 Control Group

Pulp tissue in the control group exhibited severe inflammatory cell infiltration, indicating a persistent inflammatory response. Vasodilatation and congested blood can be noticed, and the pulp tissue showed signs of disorganization and necrosis in some areas; odontoblasts appeared evacuated. **(Fig. 1)**

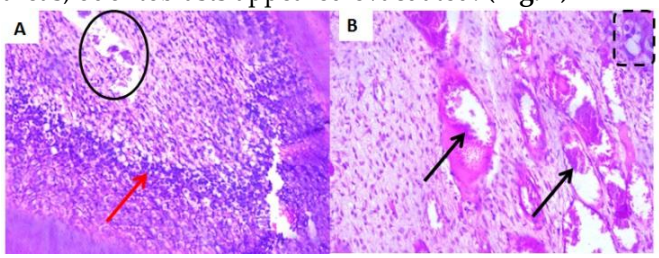

Figure 1. A photomicrograph of control group (A) showing severe infiltration of inflammatory cells (red arrow) (X200 orig. Mag.), areas of necrosis can be seen (circle), (B) the odontoblasts are evacuated (Rectangle), vasodilated blood vessels (black arrows) (X400 orig. Mag.)

3.1.2 MTA Group

The MTA group displayed mild inflammatory cell infiltration, with a noticeable reduction in inflammatory cells compared to the control group. Reparative dentin formation was evident; very thin, not continuous dentin bridges were observed in most samples. The pulp tissue appeared healthier than the control group, with minimal signs of necrosis and minimal vasodilated blood vessels; in some areas, odontoblasts appeared evacuated **(Fig. 2)**.

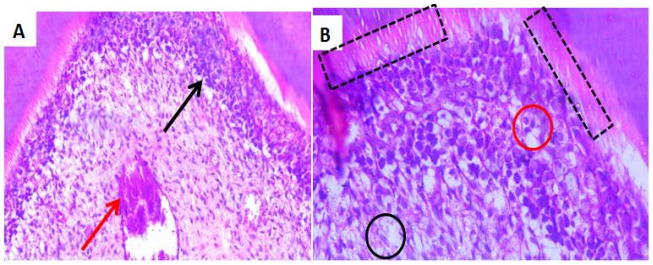

Figure 2. A photomicrograph of MTA group (A) showing moderate infiltration of inflammatory cells (black arrow), vasodilated blood vessel (red arrow) (X200 orig. Mag.) (B) Showing fewer and smaller areas of necrosis can be seen (black circle), thin layer of reparative dentin (rectangle), and evacuated odontoblasts (red circle) (X400 orig. Mag.)

3.1.3 AgNPs Group

The AgNPs group showed the most favorable outcomes, with minimal inflammatory cell infiltration and no signs of necrosis. Reparative dentin formation was prominent, with thick and continuous dentin bridges observed in all samples. The pulp tissue maintained its vitality and structural integrity, demonstrating the biocompatibility and healing potential of AgNPs-infused bonding material. **(Fig. 3)**

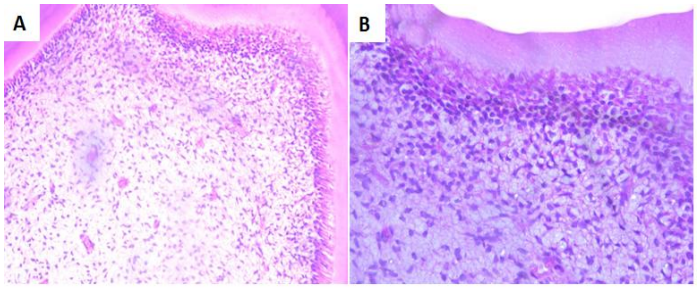

Figure 3. A photomicrograph of AgNP group (A) showing mild infiltration of inflammatory cells (black arrow), thick continuous reparative dentin (rectangle) (X200 orig. Mag.) (B) Showing fewer infiltration of inflammatory cells (black arrow), thick continuous reparative dentin (rectangle) (red circle) (X400 orig. Mag.)

3.2 Histochemical Results

Masson's trichrome staining was used to assess collagen fiber organization within the pulp tissue:

3.2.1 Control Group

Collagen fibers in the control group appeared disorganized and fragmented, indicating poor tissue healing. **(Fig. 4A)**

3.2.2 MTA Group

The MTA group showed improved collagen fiber organization compared to the control group, a discontinuous arrangement of fibers, and a very thin layer of reparative dentin **(Fig. 4B)**.

3.2.3 AgNPs Group

The AgNPs group exhibited the most organized continuous collagen fiber bundles closing the cavity and a well-structured reparative dentin. This finding suggests enhanced tissue regeneration and healing in the presence of silver nanoparticles**. (Fig. 4C)**

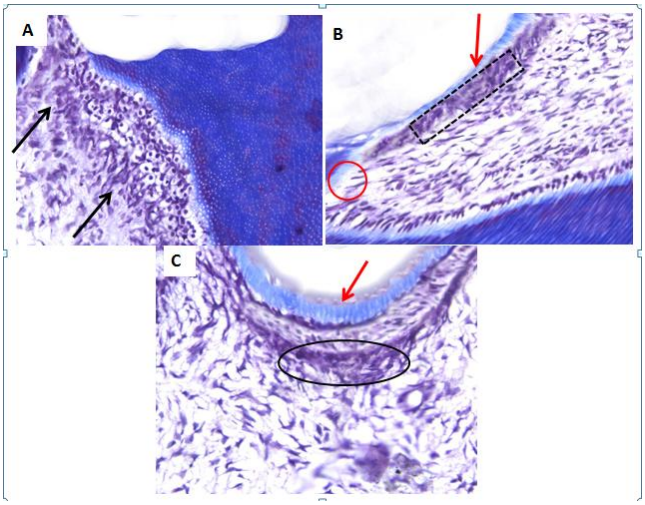

Figure 4. A photomicrograph of histochemical results (A) control group showing disorganized and fragmented collagen fibers, (B) MTA group showing improved collagen fiber organization compared to the control group(rectangle) , the band of fibers is discontinuous (red circle) and very thin layer of reparative dentin (red arrow), (C) AgNP group showing organized continuous collagen fiber bundles closing the cavity (black circle) and a well-structured reparative dentin(red arrow) (X400 orig. Mag.)

3.3 Statistical Analysis

Statistical analysis of the histomorphometric data (inflammatory cell area percentage) revealed significant differences among the groups:

3.3.1 Area percentage of inflammatory cells evaluation

There was a statistically significant difference between the control, MTA, and silver groups, where $p<0.001$. A statistically significant difference was found between the control) group and each of the MTA and silver groups, where $p<0.001$ and $p<0.001$, respectively. Also, there is a statistically significant difference between the MTA and silver groups, where $p<0.001$. The highest mean value was found in the control group, 43.93 ± 1.87, followed by the MTA group with a mean value of 20.12 ± 1.20, while the lowest mean value was found in the silver group 14.7 ± 1.07. **(Fig. 5, Table 1)**

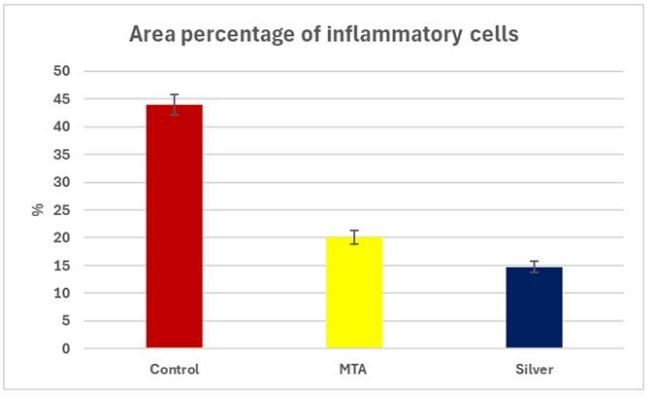

Figure 5. Bar chart representing Area percentage of inflammatory cells

Table 1. The mean, standard deviation (SD) values of Area percentage of inflammatory cells of different groups.

Variables	Area percentage of inflammatory cells	
	Mean	SD
Control	43.93 [a]	1.87
MTA	20.12 [b]	1.20
Silver	14.76 [c]	1.07
p-value	<0.001*	

Means with different small letters in the same column indicate significant difference.
*; significant ($p<0.05$)

4 Discussion

Nanotechnology represents a groundbreaking multidisciplinary field that has revolutionized dental treatments by utilizing the unique properties of nanomaterials. These highly versatile nanoparticles are being applied across various aspects of endodontic therapy, including their incorporation into irrigants, obturating materials, and intracanal medicaments. Their exceptional antimicrobial, mechanical, and regenerative properties have enabled significant advancements in infection control, material performance, and tissue healing, making nanotechnology a pivotal innovation in modern dentistry. By integrating nanoparticles into endodontic procedures, researchers and clinicians are addressing critical challenges and enhancing the efficacy and durability of dental treatments. [23-25]

A notable example is the use of silver nanoparticles, which exhibit potent antibacterial properties, making them highly effective in combating oral pathogens and preventing infections. These nanoparticles disrupt bacterial cell membranes and inhibit their growth, offering a promising solution for dental caries, periodontal diseases, and other oral health challenges. By integrating silver nanoparticles into dental materials and treatments, nanotechnology has paved the way for more effective and long-lasting oral care solutions. [26]

Histological examinations of the pulp tissue in the control group revealed severe inflammatory cell infiltration, indicative of a persistent inflammatory response. Vasodilation, vascular congestion, and necrosis were observed in certain areas, with odontoblasts appearing vacuolated. Histochemical analysis further demonstrated disorganized and fragmented collagen fibers, suggesting impaired tissue healing. These findings are consistent with the outcomes in untreated or poorly managed pulp tissue in many studies, where inflammation and tissue degradation dominate. [27, 28]

In contrast, the MTA group exhibited mild inflammatory cell infiltration, with a significant reduction in inflammatory cells compared to the control group. Reparative dentin formation was evident, although the dentin bridges observed were thin and discontinuous in

most samples. The pulp tissue appeared healthier overall, with minimal necrosis and only slight vasodilation of blood vessels. However, odontoblast vacuolation was still noted in some areas. Histochemically, the MTA group showed improved collagen fiber organization compared to the control group, though the arrangement remained discontinuous. These results align with the well-documented biocompatibility and dentinogenic potential of MTA by Hilton TJ et al [29], MTA has long been considered the gold standard for pulp capping. [30, 31]

The AgNPs group demonstrated the most favorable outcomes, with minimal inflammatory cell infiltration and no signs of necrosis. Prominent reparative dentin formation was observed, characterized by thick and continuous dentin bridges in all samples. The pulp tissue maintained its vitality and structural integrity, indicating a superior healing response compared to both the control and MTA groups. Histochemically, the AgNPs group exhibited the most organized and continuous collagen fiber bundles, further supporting its regenerative potential.

Statistical analysis of the inflammatory cell area percentage revealed significant differences among the groups. A statistically significant difference was observed between the control, MTA, and AgNPs groups ($p < 0.001$). Specifically, there was a significant difference between the control group and each of the MTA and AgNPs groups ($p < 0.001$ for both comparisons). Additionally, a significant difference was found between the MTA and AgNPs groups ($p < 0.001$). The highest mean inflammatory cell area percentage was observed in the control group, while the lowest was in the AgNPs group. These findings underscore the superior anti-inflammatory and healing properties of AgNPs compared to both the control and MTA groups.

The favorable outcomes in the AgNPs group can be attributed to the well-documented antibacterial properties of silver nanoparticles by Gupta et al. [9]. Furthermore Burdușel et al.[7] stated the causes of anti-bacterial potentiality of AgNPs, they found the silver nanopaticles disrupt bacterial cell membranes, inhibit biofilm formation, and reduce the risk of postoperative infections, making them an ideal additive for pulp capping materials. This is particularly critical in direct pulp capping, where bacterial contamination is a leading cause of treatment failure. The antimicrobial efficacy of AgNPs likely contributed to the minimal inflammatory response and absence of necrosis observed in this group. [1]

The thick and continuous dentin bridges observed in the AgNPs group suggest that silver nanoparticles not only protect the pulp from infection but also actively promote tissue regeneration. This is consistent with previous studies indicating that AgNPs stimulate the differentiation of odontoblast-like cells and enhance the secretion of extracellular matrix components, such as collagen.[10] The well-organized collagen fiber bundles observed in the AgNPs group histochemically further support this regenerative potential. These findings highlight the dual role of AgNPs in both antimicrobial protection and tissue regeneration, making them a promising candidate for pulp capping applications.

Although MTA has long been considered the gold standard for pulp capping, thanks to its excellent biocompatibility and ability to promote dentin formation as demonstrated by Huang *et al.*, [2] this study sheds light on its limitations, particularly in terms of anti-inflammatory and regenerative capabilities. These shortcomings have also been noted by Parirokh M. et al., [32] who identified several drawbacks, including MTA's prolonged setting time, challenging handling properties, relatively low compressive strength, potential for causing tooth discoloration, lack of a known solvent for easy removal, and high cost. While MTA remains a valuable material, these limitations highlight the need for further research and development of alternative solutions in pulp capping.

In current study the AgNPs group not only matched but exceeded the performance of MTA in several key areas, including inflammation control and reparative dentin thickness. This suggests that AgNPs may offer a viable alternative or adjunct to MTA in clinical practice.

Although this study provides promising results, it is not without limitations. The use of an animal model may not fully replicate the clinical conditions encountered in human patients. Additionally, the study focused on short-term outcomes (4 weeks), and longer-term evaluations are needed to assess the durability and biocompatibility of AgNP-infused materials.

5 Conclusion

The integration of silver nanoparticles into the bonding of resin composites represents a significant advancement in direct pulp capping. AgNPs not only provide potent antimicrobial protection but also enhance tissue regeneration, making them a promising alternative to traditional materials like MTA. This innovative approach has the potential to improve clinical outcomes, reduce treatment failures, and elevate the standard of care in restorative dentistry. Further research, including long-term studies and human trials, is essential to validate these findings and optimize the use of AgNPs in

clinical practice.

Authors' Contributions

Hinar Hani Al Moghazy (HH Al Moghazy) conducted the experimental procedures and contributed to manuscript drafting.
Samah Mohamed Kamel (SM Kamel) designed the study, led the manuscript writing, and interpreted the data.
Moataz Mohamed El-Kholy was responsible for data collection, analysis, and interpretation.

Conflict of interest

The authors declare that they hold no competing interests.

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