

Comparative Evaluation of Cytotoxicity of Zno-eugenol, Calcium Hydroxide, Resin and Bioceramic Endodontic Sealers After the Incorporation of Magnetic Nanoparticle- an in Vitro Study

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Abstract

Aim

To evaluate and compare the cytotoxicity of four endodontic sealers epoxy resin-based (AH Plus), calcium hydroxide-based, zinc oxide-eugenol (ZOE)-based, and bioceramic, following the incorporation of magnetic nanoparticles (MNPs), using dental pulp stem cells (DPSCs) as the biological model.

Methodology:

36 specimens were prepared and divided into four groups (n = 9 per group), each representing one sealer formulation modified with MNPs. Specimens were set in standardised cylindrical moulds (5 mm × 5 mm) at 37°C under 95% relative humidity for 48 hours, followed by UV sterilisation. DPSCs were seeded in 96-well plates at 10,000 cells/well and exposed to the respective sealer groups for 48 hours. Cytotoxicity was quantified using the MTT assay, with absorbance measured at 570 nm. Cellular morphology and adherence were assessed by phase-contrast microscopy. Data were analysed using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

Results

The bioceramic sealer demonstrated the highest mean percentage cell viability among all experimental groups. A statistically significant difference in cell viability was observed between the AH Plus and bioceramic groups (mean difference: -428.1; 95% CI: -833.5 to -22.65; $p = 0.034$). No statistically significant differences were identified among the remaining pairwise comparisons.

Conclusion

Within the limitations of this in vitro study, the bioceramic sealer incorporated with MNPs demonstrated superior biocompatibility compared to the epoxy resin-based sealer. All tested formulations exhibited acceptable cytotoxicity profiles relative to the control. Further in vivo investigations are warranted to validate these findings.

INTRODUCTION

The biological and physicochemical characteristics of the obturation materials used are just as important to the long-term effectiveness of endodontic therapy as the careful implementation of chemo-mechanical preparation and irrigation procedures.¹ Root canal sealers represent an important constituent of the obturation system, which creates a hermetic seal at the apical part of the tooth preventing further colonization by bacteria.²

There are numerous kinds of endodontic sealers that vary widely in terms of their chemical nature and setting. The various types include ZOE-containing sealers, sealers based on calcium hydroxide, epoxy resin sealers, silicone-based sealers, and more recently, bioceramics or calcium silicate sealers.³ Each type of sealer demonstrates its own characteristics. However, despite extensive studies conducted over many years, none have fulfilled the criteria for a perfect endodontic sealer, and further comparative studies on commercially available sealers still need to be carried out.⁴

In terms of biological properties for endodontic sealers, biocompatibility is crucial. Cytotoxic components released by sealers may trigger inflammation, hinder the process of healing, and negatively impact the tissues of the periodontium.⁵ In vitro cytotoxicity assays provide a standardised and reproducible means of screening endodontic materials for biological safety prior to their clinical application. A popular technique for evaluating the cytotoxicity of root canal filling materials, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay measures mitochondrial metabolic activity as a surrogate of cell viability and has been widely used in comparative studies of sealer biocompatibility.⁶

Zinc oxide-eugenol-based sealers, amongst the oldest formulations in clinical use, release eugenol upon setting, which, despite its well-documented antimicrobial properties, has been associated with cytotoxic and in some cases an inflammatory effects on the tissues of peripheral origin. The Calcium hydroxide-based sealers are recognised for their alkaline pH, which confers antimicrobial activity and may promote periapical healing; however, their cytotoxic behaviour is contingent upon the concentration of liberated hydroxyl ions and the specific formulation used.⁷ The most extensively researched epoxy resin-based sealer is AH Plus (Dentsply DeTrey, Konstanz, Germany), which exhibits outstanding dimensional stability, adherence to dentinal walls, and long-term sealing capability. Because bisphenol-A diglycidyl ether and monomers are released during the first stage of polymerisation, they show significant cytotoxicity in the freshly combined form.⁸ These cytotoxic effects to attenuate with progressive setting. Their clinical significance in cases of apical extrusion requires attention.⁹

An important development in endodontic biomaterials is the advent of bioceramic-based sealers made of calcium phosphate, zirconium oxide, dicalcium silicate, and tricalcium silicate. The hydration of the materials during the setting process produces calcium hydroxide and silicate hydrate, which causes hydroxyapatite to accumulate between the material and tissue interfaces. The bioactive setting process is characterized by alkalinity and mineralization, two processes that promote periapical tissue regeneration.³ Numerous in vitro and in vivo investigations have demonstrated that bioceramic sealers are far more biocompatible than those derived from epoxy resins, resulting in minimal cytotoxicity in a variety of cell types, including fibroblasts, osteoblasts, periodontal ligament cells, and even stem cells.¹⁰

The selection of cell model in cytotoxicity investigations is a determinant of validity of experimental research. Dental pulp stem cells (DPSCs) are an appropriate model for the evaluation of endodontic materials, due to their mesenchymal origin, multipotent differentiation capacity, and anatomical

proximity to the root canal environment. DPSCs are characterized by expression of mesenchymal stromal cell markers as well as their ability to differentiate into osteocytes, dentocytes, and adipocytes.¹¹ The published studies which used DPSC cells as a source for assessing cytotoxicity have shown that such cell model is sensitive enough to detect biological distinctions in the behavior of sealers.¹²

Alongside improvements in sealer compositions, nanotechnology is gaining attention as an area where further improvement of biological and functional characteristics of endodontic materials is possible. Nanoparticles possess antimicrobial effects via multiple mechanisms, such as damaging bacterial cell walls, producing reactive oxygen species, and inhibiting DNA replication of microorganisms, thus allowing them to combat biofilms in the intricate structures of dentinal tubules and accessory canals.¹³ The use of nanoparticles within the root canal sealer has been studied to determine the ability of enhancing their antibacterial properties while not having to sacrifice their biocompatibility with the base material. Several nanoparticles such as silver, chitosan, zinc oxide, and chlorhexidine have been tested with success, showing their effectiveness in increasing antibacterial properties without compromising cytotoxicity.¹⁴

Magnetic nanoparticles (MNPs), usually made up of ferrimagnetic iron oxide (Fe_3O_4), form a class of materials that possess special characteristics applicable in endodontics. When subjected to an external magnetic field, MNPs become capable of directional migration, which allows them to migrate deep into the dentinal tubules and lateral canals further than what is possible through mere diffusion. The ability to migrate under the influence of a magnetic field has been recommended for improving the adhesion of the sealant to the microbe-infested areas of the root canal system.¹⁵

Although there is considerable scientific evidence indicating the antimicrobial and penetration-promoting properties of MNPs in the endodontic field, the biological safety of the commercial endodontic sealers after the addition of MNPs has yet to be systematically examined among different types of endodontic sealers. The comparative cytotoxic behaviour of ZOE, calcium hydroxide, epoxy resin, and bioceramic sealers following uniform MNP modification has not been assessed in a standardised in vitro model using dental pulp stem cells, and the majority of the literature currently in publication focuses on single sealer types or experimental formulations. Furthermore, the long-term biocompatibility of residual iron oxide nanoparticles within the periapical environment, including the potential for cytotoxicity arising from sustained ion release, remains incompletely understood.⁸ This gap in the literature represents a critical barrier to the translation of MNP-enhanced sealers into clinical practice, as cytotoxicity data across sealer categories are necessary to guide material selection and inform risk-benefit considerations prior to any in vivo investigation.

In order to assess and compare the cytotoxicity of four endodontic sealers—an epoxy resin-based sealer (AH Plus), a calcium hydroxide-based sealer, a zinc oxide-eugenol-based sealer, and a bioceramic sealer—after magnetic nanoparticles were consistently added to each formulation, the current in vitro study was conducted. Cytotoxicity was assessed on cultured dental pulp stem cells using the MTT assay after 48 hours of exposure, with cell viability serving as the primary outcome measure. Secondary observation

involved the examination of cell morphology and cell adhesion using phase contrast microscopy. Results from this study will be used as initial biological safety information about MNP incorporated endodontic sealers which have varied chemical compositions, in order to develop the scientific information needed for the creation of new generation obturation materials.

MATERIALS AND METHODS

Study design

In order to assess and compare the cytotoxicity of four endodontic sealers following the addition of magnetic nanoparticles (MNPs), an in vitro experimental investigation was designed. Epoxy resin-based, calcium hydroxide-based, zinc oxide-eugenol-based, and bioceramic sealers were the sealers evaluated. The MTT assay was used to assess cytotoxicity in cultivated dental pulp stem cells following exposure to the test materials.

Study groups and sample size

36 specimens were prepared and divided into four groups (n = 9 per group):

Group 1

AH Plus sealer with incorporation of magnetic nanoparticles

Group 2

Calcium hydroxide-based sealer with incorporation of magnetic nanoparticles

Group 3

Zinc oxide-eugenol sealer with incorporation of magnetic nanoparticles

Group 4

Bioceramic sealer with incorporation of magnetic nanoparticles

Test materials

Four commercially available endodontic sealers representing different chemical formulations were used in the study: epoxy resin-based, calcium hydroxide-based, zinc oxide-eugenol-based, and bioceramic sealers. Magnetic nanoparticles were manually incorporated into each sealer formulation prior to specimen preparation. The proportion of nanoparticles added to each sealer should be standardized according to the study protocol.

Preparation of the sealer specimens

The sealers were manipulated according to the manufacturers' instructions, following incorporation of magnetic nanoparticles into each material. The modified sealers were then placed into cylindrical molds measuring approximately 5 mm in diameter and 5 mm in height. In total, nine samples were created per sample group.

All samples were incubated for 48 hours in 37°C at 95% relative humidity to allow the sample to set completely. Once all samples were allowed to fully set, they were then taken out of the mold and sterilized via UV light for 12 hours.

Cell culture

The cytotoxicity testing was carried out using dental pulp stem cells. The cells were seeded in 96-well plates in a concentration of 10,000 cells per well and incubated in culture conditions. Subsequently, after providing sufficient time for the attachment of the cells, the cells were subjected to each sealer group for 48 hours.

Cytotoxicity assessment by MTT assay

After 48 hours of treatment with the test chemicals, the MTT assay was used to determine the cytotoxic effect. Each well received 10 µL of MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; concentration 5 mg/mL] after the test material had acted for the specified amount of time. The mixture was then incubated for four hours at 37°C. The blue formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO) after the MTT solution was aspirated. At a wavelength of 570 nm, each well's absorbance value was calculated.

Microscopic evaluation

In addition to the MTT assay, adherence and morphology of the cells were also noted after exposure to the test samples using a phase contrast microscope.

Outcome measure

The primary outcome measure for this study was the percentage of cell viability after exposure to endodontic sealers impregnated with magnetic nanoparticles on dental pulp stem cells. Secondary measurement was the qualitative examination of cellular adherence and morphology by phase contrast microscope.

Statistical analysis

The mean $\pm$ standard deviation was used to express all data. One-way analysis of variance (ANOVA) was used in the statistical study to compare the groups' mean cell viability. Tukey's multiple comparisons test was used to perform post hoc pairwise comparisons. GraphPad Prism software was used for statistical analysis, and a p-value of less than 0.05 was deemed statistically significant.

RESULTS

Using the MTT cell viability assay, this study evaluated the cytotoxic effects of four root canal sealers on human mesenchymal stem cells: AH Plus, Calcium Hydroxide (Cal), Zinc Oxide Eugenol (ZoE), and Bioceramic (Bio). Using GraphPad Prism, a one-way ANOVA with Tukey's multiple comparisons post-hoc test was used to analyse all data, which were reported as mean $\pm$ standard deviation. Statistical significance was defined as a p-value of less than 0.05. AH Plus and the Bioceramic sealer showed one statistically significant difference out of ten pairwise comparisons, with a mean difference of -428.1 (95% CI: -833.5 to -22.65 ; $p = 0.0340$).

Cell Viability By MTT Assay

As seen in Fig. 1, the MTT test was performed to calculate each group's cell viability percentage. For the percentage of cell viability, the Control was used as the benchmark. With a mean difference of 33.05 when compared to the Control (95% CI: -372.4 to 438.5 ; $p\text{-value} = 0.9993$), AH Plus demonstrated the lowest percentage viability of cells among all sealers tested, suggesting that there is no meaningful difference between the two groups. The other two sealers tested, calcium hydroxide and zinc oxide eugenol sealers, showed intermediate cell viability, with mean differences of -110.7 (95% CI: -516.1 to 294.8 ; $p\text{-value} = 0.9351$) and -84.17 (95% CI: -489.6 to 321.3 ; $p\text{-value} = 0.9754$), respectively, both of which were not statistically significant when compared to the control group. The bioceramic sealer showed the greatest percentage viability of cells among all tested groups, with a mean difference of -395.1 as compared to the control (95% CI: -800.5 to 10.40 ; $p\text{-value} = 0.0593$).

Intergroup Comparisons

Tukey's multiple comparisons test revealed that the sole statistically significant difference across all pairwise comparisons was observed between the AH Plus and Bioceramic groups, with a mean difference of -428.1 (95% CI: -833.5 to -22.65 ; $p = 0.0340$). All remaining comparisons – including AH Plus versus Calcium Hydroxide (mean difference: -143.7 ; 95% CI: -549.2 to 261.7 ; $p = 0.8482$), AH Plus versus Zinc Oxide Eugenol (mean difference: -117.2 ; 95% CI: -522.7 to 288.2 ; $p = 0.9211$), Calcium Hydroxide versus Zinc Oxide Eugenol (mean difference: 26.48 ; 95% CI: -379.0 to 431.9 ; $p = 0.9997$), Calcium Hydroxide versus Bioceramic (mean difference: -284.4 ; 95% CI: -689.8 to 121.1 ; $p = 0.2830$), and Zinc Oxide Eugenol versus Bioceramic (mean difference: -310.9 ; 95% CI: -716.3 to 94.57 ; $p = 0.2044$) - did not reach statistical significance as depicted in Table 1.

Table 1
Tukey's multiple comparisons test across groups

Tukey's Multiple Comparisons Test	Mean Diff.	95.00% CI of Diff.	Adjusted P Value
Control vs. AH Plus	33.05	-372.4 to 438.5	0.9993
Control vs. Cal	-110.7	-516.1 to 294.8	0.9351
Control vs. ZoE	-84.17	-489.6 to 321.3	0.9754
Control vs. Bio	-395.1	-800.5 to 10.40	0.0593
AH plus vs. Cal	-143.7	-549.2 to 261.7	0.8482
AH plus vs. ZoE	-117.2	-522.7 to 288.2	0.9211
AH plus vs. Bio	-428.1	-833.5 to -22.65	0.0340
Cal vs. ZoE	26.48	-379.0 to 431.9	0.9997
Cal vs. Bio	-284.4	-689.8 to 121.1	0.2830
ZoE vs. Bio	-310.9	-716.3 to 94.57	0.2044

DISCUSSION

Using the MTT cell viability assay, the current study assessed the cytotoxic potential of four root canal sealers on human mesenchymal stem cells: AH Plus, Calcium Hydroxide, Zinc Oxide Eugenol, and Bioceramic. One-way ANOVA with Tukey's post-hoc multiple comparisons test was used for statistical analysis; significance was determined at $p < 0.05$. The principal finding of this investigation was that the Bioceramic sealer demonstrated significantly higher cellular viability compared to AH Plus (mean difference: -428.1 ; $p = 0.0340$), suggesting a superior biocompatibility profile for the bioceramic formulation. All remaining pairwise comparisons did not reach statistical significance, indicating comparable cytotoxic profiles among the other tested materials under the conditions of this study.

The reduced cellular viability observed with AH Plus in the present study is consistent with findings reported in the existing literature. Leyhausen et al. (1999)⁸ evaluated the cytotoxic effects of AH Plus on human periodontal ligament cells and reported that the sealer induced significant cytotoxicity in the freshly mixed state, attributing this effect to the release of bisphenol-A diglycidyl ether and formaldehyde during the initial polymerisation phase. This was further validated by Theiszová et al. (2005)¹⁶, who found that the cytotoxic response from AH Plus was dose-dependent through use of the MTT test on murine fibroblasts. The epoxy resin formulation of AH Plus has been identified as the cause for its cytotoxic nature due to the leaking of uncured oligomers in the setting process into the surrounding environment.⁹ These findings collectively observe that AH Plus maintained lower cellular viability relative to bioceramic-based formulations.

On the other hand, the highest mean percentage cellular viability was found in the Bioceramic sealer group in comparison to all experimental groups, a result that is also consistent with previous studies.¹⁷ Guerra et al. (2025)¹⁸ on the evaluation of biocompatibility of bioceramic sealers with human osteoblasts, they reported that bioceramics had low cytotoxic properties and their biocompatibility was due to the formation of calcium silicate hydrate and hydroxyapatite upon setting, which promote cell activity. Similarly, Piscopo et al. (2026)⁵ bioceramic sealers were compared to epoxy resin sealers for their cytotoxicity against human periodontal ligament fibroblasts through MTT assay method, and it was discovered that bioceramic sealers showed significantly lower cytotoxicity compared to the control group. The bioactivity of bioceramics, which is reflected in their high alkalinity and ability to undergo mineralization, was believed to be responsible for their higher cellular compatibility, a phenomenon supported by statistical significance obtained in this study comparing the Bioceramic and AH Plus groups.

The Calcium Hydroxide and Zinc Oxide Eugenol groups in this study demonstrated intermediate cellular viability values that did not show significant deviation from the control or from one another. Fonseca (2010)¹⁹ reviewed the biocompatibility of Calcium Hydroxide-containing materials and observed that although these sealants are normally well-tolerated by periapical tissues, the cytotoxicity of these sealants depends on the number of free hydroxyl ions and their formulation. This could explain why the tested sealants were not statistically significant in this study due to variations in concentration, time of incubation, and sensitivity of the human mesenchymal stem cells used.

Several limitations of the present study should be acknowledged while interpreting its results. As an in vitro investigation, the findings may not directly be extrapolated to the complex biological environment encountered in vivo, where factors such as tissue buffering capacity, immune responses, and material degradation over time can substantially influence biocompatibility outcomes.²⁰ The experimental conditions, including concentration of the sealer and duration of cell exposure, were standardised for comparison and may not fully duplicate clinical scenarios. The variability in the sample size found across comparable published literature also introduces challenges while comparing results across studies.

The findings of this study indicate that the Bioceramic sealer demonstrated significantly superior biocompatibility compared to AH Plus when evaluated using the MTT cell viability assay on human mesenchymal stem cells. While all materials exhibited acceptable overall viability profiles relative to the control, the statistically significant difference between AH Plus and Bioceramic ($p = 0.0340$) supports the growing body of evidence favouring bioceramic-based sealers as biocompatible alternatives in endodontic practice. Further in vivo and clinical investigations are warranted to validate these findings and to better understand the long-term biological behaviour of these materials in the periapical environment. The findings suggest that nanotechnology can mitigate the risk of persistent apical periodontitis by improving the hermetic seal and enhancing antimicrobial efficacy within the root canal system.

CONCLUSION

Within the limitations of this in vitro study, the incorporation of magnetic nanoparticles into endodontic sealers represents a promising modification to improve the hermetic seal of the root canal system. The expected increase in tubular penetration depth across various chemical groups suggests that MNP-doped sealers may offer superior resistance to bacterial re-infection compared to conventional formulations. These findings highlight the potential of nanotechnology to enhance the long-term success of endodontic therapy.

Abbreviations

MNPs (Magnetic Nanoparticles), zinc oxide-eugenol (ZOE)-based, dental pulp stem cells (DPSCs)

Declarations

Human Ethics and Consent to Participate: The study protocol was reviewed and approved by the Institutional Ethics Committee Dr. D.Y. Patil Dental College and Hospital (Approval No. DYPDCH/IEC/37/2025). All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki and its later amendments.

Ethical approval was waived by the Institutional Ethics Committee of Dr. D.Y. Patil Dental College and Hospital (Approval No. DYPDCH/IEC/37/2025) for use of dental pulp cells.

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Figures

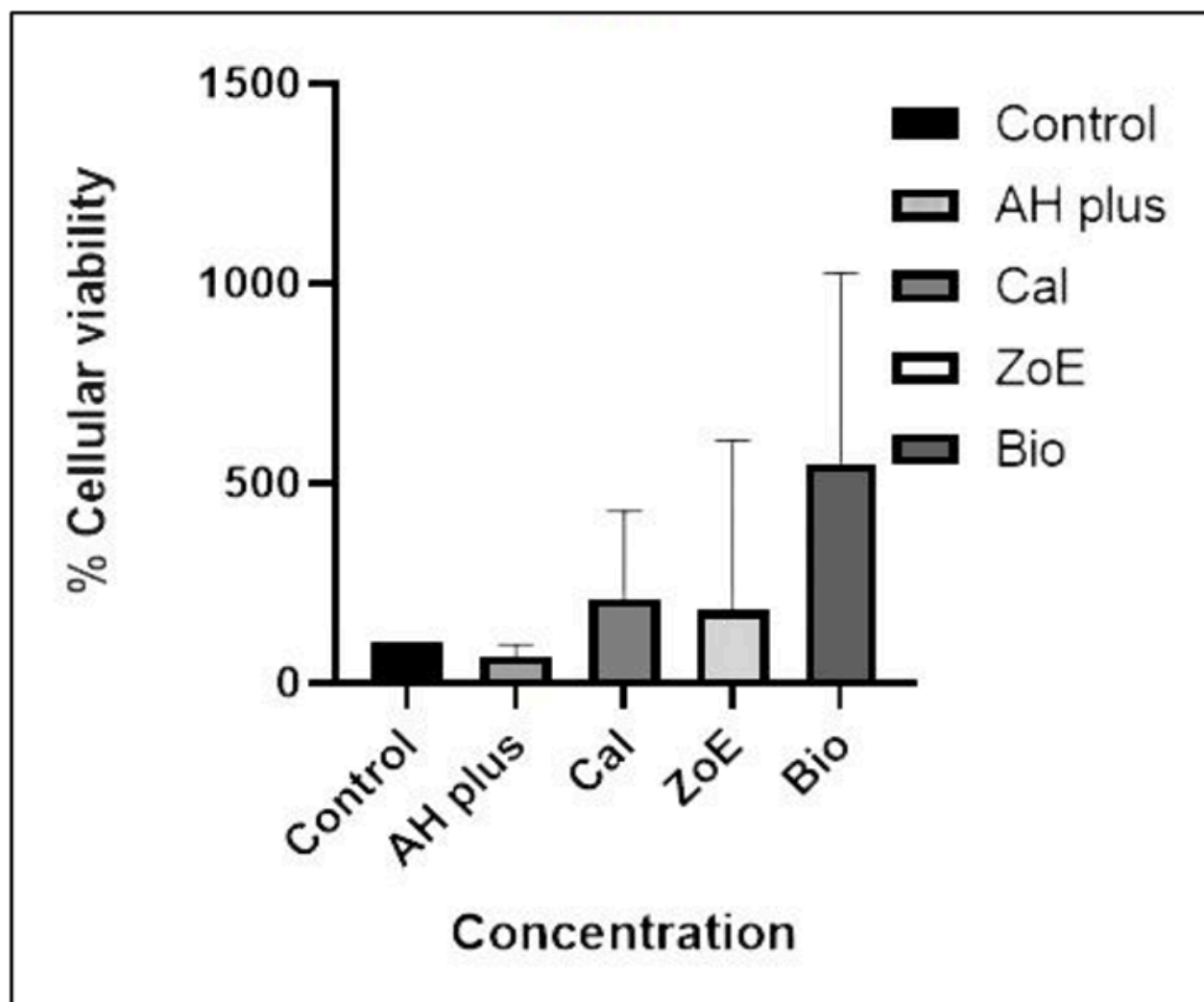


Figure 1

Concentration dependent % cellular viability by MTT assay