RESEARCH PAPER

Comparative Evaluation Of Cytotoxicity Of Epoxy – Resin Based Sealer And Bio Ceramic Sealer, An In-Vitro Study

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ABSTRACT
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INTRODUCTION

Endodontic therapy encompasses a series of precisely orchestrated clinical procedures designed to remove infected or necrotic dental pulp, thereby eradicating microbial contamination and preventing subsequent bacterial invasion into the root canal system [1]. The selection of root canal filling materials is a decisive factor in ensuring long-term therapeutic success. Ideally, these materials should demonstrate excellent dimensional stability, provide a hermetic seal to prevent microleakage, and possess the capacity to stimulate biological cellular responses that support tissue repair and regeneration [1].

Root canal sealers are formulated in a variety of types, including those based on bioceramics, glass ionomer, silicone, epoxy resin, calcium hydroxide, and zinc oxide–eugenol, each characterized by distinct physicochemical properties and setting mechanisms [2]. The biological behavior of these sealers is influenced by several critical parameters, such as cytotoxicity, cytocompatibility, overall biocompatibility, cellular plasticity, differentiation potential, and intrinsic bioactivity [2].

According to the classic criteria proposed by Grossman, an ideal endodontic sealer must fulfill multiple essential requirements. It should be capable of forming a robust bond with dentinal walls, thereby establishing a hermetically sealed interface upon setting, which is crucial for preventing microbial infiltration and ensuring long-term clinical success. Radiopacity is another vital property, as it enables accurate radiographic visualization and verification of the quality of the obturation. The material should possess a fine particle size to facilitate easy and homogeneous mixing with liquid components, ensuring a consistent and workable mixture. Furthermore, it must remain dimensionally and chromatically stable after setting to maintain structural integrity and aesthetic compatibility. Insolubility in tissue fluids is essential to prevent disintegration and maintain the longevity of the root canal filling. Additionally, the sealer should be bioactive, promoting hydroxyapatite formation when in contact with physiological fluids, which enhances the biological seal and supports periapical healing. Finally, it should exhibit excellent biocompatibility, characterized by non-mutagenic, non-sensitizing, and non-cytotoxic behavior, thereby minimizing the risk of adverse tissue reactions [2].

It is well established that extruded sealers can come into direct contact with periapical tissues, leading to potential cytotoxic effects and delayed wound healing [3]. Therefore, to ensure a favorable biological response, ideal sealers must be immunologically compatible, non-cytotoxic, and non-mutagenic [3]. However, certain materials may still elicit local irritation and inflammatory responses, especially when they interact with adjacent tissue fluids or enter the circulatory system, which can compromise healing and tissue regeneration [4].

Among the available endodontic sealers, AH Plus®, an epoxy resin–based formulation, remains the gold standard due to its exceptional mechanical strength, dimensional stability, and advantageous physicochemical characteristics [4]. Nonetheless, its limited tissue-specific bioactivity has spurred the development of novel biomaterials such as bioceramic sealers, which are engineered to enhance the biological response of apical and periapical tissues. One notable example is **CeraSeal** (Meta Biomed Co., Cheongju, Korea), a premixed calcium silicate–based sealer designed specifically for endodontic use [4]. Despite its promising bioactive properties, current data regarding its biological performance are limited due to its relatively

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recent introduction, underscoring the need for more comprehensive in vivo and in vitro studies [4].
A thorough evaluation of endodontic filling materials, particularly in terms of cytotoxicity, bioactivity, and their potential to support reparative and regenerative processes, is therefore essential for optimizing clinical outcomes and guiding the development of next-generation biomaterials [5].

MATERIALS AND METHODS

Materials

The materials used in this study comprised commercially available root canal sealers, including a bioceramic root canal sealer (CeraSeal), an epoxy resin–based sealer (Adseal), and AH Plus®, which served as the positive control. The L929 mouse fibroblast cell line was utilized for cytotoxicity evaluation. Additional reagents included fetal bovine serum (FBS), antibiotics and antifungal agents, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide), Dulbecco's Modified Eagle Medium (DMEM), L-glutamine, trypsin, and trypan blue. The experimental setup also required standard laboratory equipment and consumables such as 12-well and 96-well culture plates, microtips, coverslips (No. 1), distilled water, a microplate absorbance reader (FluoSTAR Omega, BMG Labtech), and sterile cell culture facilities.

Experimental Design

The study was structured into four experimental groups. Group 1 consisted of cells cultured without exposure to any sealer and served as the negative control. Group 2 comprised cells treated with Adseal, an epoxy resin–based root canal sealer. Group 3 included cells exposed to the bioceramic sealer CeraSeal, and Group 4 contained cells treated with AH Plus®, a standard epoxy resin–based sealer that functioned as the positive control. This design allowed for a comparative evaluation of cytotoxicity and cellular responses among different sealer formulations.

Preparation of Sealer Specimens

All root canal sealers were prepared strictly according to the respective manufacturers' instructions. The mixed materials were transferred into molds of uniform dimensions to ensure consistency across samples. After setting completely, the specimens were subjected to sterilization by immersion in ethanol, followed by ultraviolet (UV) irradiation for 30 minutes to eliminate potential microbial contaminants. This preparation ensured that subsequent cellular responses were attributable solely to the material's properties rather than external contamination.

Cell Culture Conditions

L929 mouse fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% antibiotic solution. The cultures were maintained under optimal physiological conditions at 37 °C in a humidified incubator with a controlled atmosphere of 5% CO_2. These conditions ensured proper cell growth, confluence, and viability before experimental exposure (Figure 1).

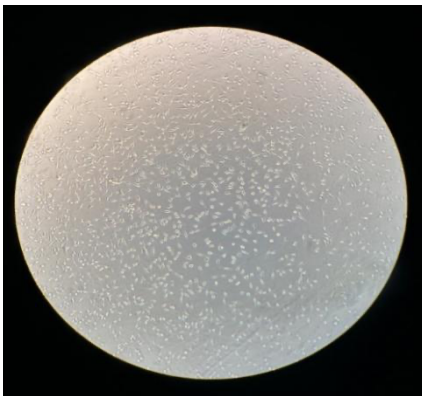

Figure 1. Morphology of L929 fibroblast cell lines under standard culture conditions

Cytotoxicity Evaluation

MTT Assay Procedure

Cytotoxicity was assessed using the MTT assay, a well-established quantitative technique that evaluates cell metabolic activity based on mitochondrial enzyme function (Mosmann, 1983). This assay measures the ability of mitochondrial succinate dehydrogenase to convert MTT into insoluble formazan crystals, which serve as an indirect indicator of viable cell count.
L929 cells were seeded into 12-well culture plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hours. Following the adhesion phase, the sterilized sealer specimens were placed into the wells, and the cultures were incubated. Cytotoxicity was evaluated at three distinct time intervals to monitor temporal changes in cellular responses. After each incubation period, MTT reagent was added, and the cells were further incubated to allow for formazan formation. The crystals were subsequently solubilized, and absorbance was measured at 570 nm using a FluoSTAR Omega microplate reader (BMG Labtech) (Figure 2).

Figure 2: Multimode microplate reader (FluoSTAR Omega, BMG Labtech)

The percentage of cell viability was calculated using the following equation:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated cells at } 570\text{nm}}{\text{Absorbance of control cells at } 570\text{nm}} \times 100$$

This calculation enabled a quantitative comparison of cellular metabolic activity between treated and control groups, thereby reflecting the cytotoxic potential of each sealer.

Statistical Analysis

To validate the use of parametric statistical tests, data normality within each experimental group at all three time points was assessed using the Shapiro–Wilk test. Based on the distribution results, appropriate parametric analyses were conducted to compare differences between groups. All statistical analyses were performed using GraphPad Prism software (Version 8.0.2, GraphPad Software Inc., San Diego, CA, USA). A significance level of $p < 0.05$ was considered indicative of statistically meaningful differences.

RESULTS

Cytotoxicity Assessment Using the MTT Assay

The cytotoxic potential of the tested endodontic sealers—Adseal, CeraSeal, and AH Plus—was evaluated using the Methyl Thiazolyl Tetrazolium (MTT) assay to assess cell viability and metabolic activity of L929 mouse fibroblast cells at 24, 48, and 72 hours of incubation.

At the 24-hour time point, a slight reduction in cell viability was observed in the groups treated with Adseal, CeraSeal, and AH Plus compared with the negative control group; however, these differences were not statistically significant (Figure 3). This indicates that none of the materials exerted acute cytotoxic effects during the initial phase of cell-material interaction.

At 48 hours, all experimental groups demonstrated similar cell viability profiles, and no significant differences were detected between any of the test sealers and the control group (Figure 4). This suggests that prolonged exposure up to 48 hours did not result in increased cytotoxicity for any of the tested materials.

By 72 hours, minor variations in cytotoxicity became apparent among the experimental groups (Figure 5). Although CeraSeal and AH Plus showed no statistically significant differences compared with the control, Adseal exhibited a statistically significant difference when compared with AH Plus, indicating slightly higher cytotoxicity. Despite this, the observed cytotoxic effect remained relatively low overall, suggesting that all tested materials maintained acceptable levels of biocompatibility over the experimental period.

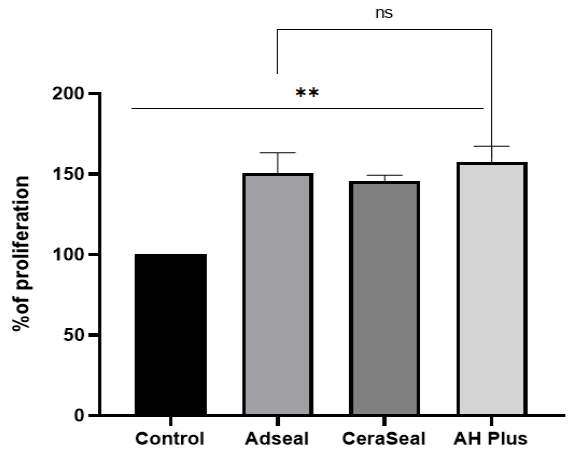

Figure 3: MTT assay results at 24 hours

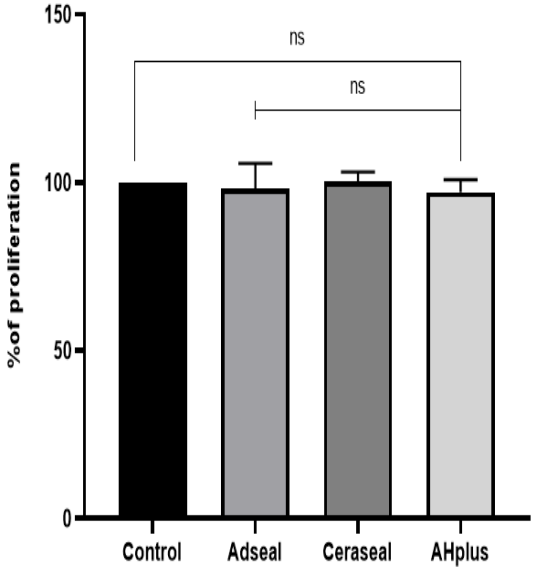

Figure 4: MTT assay results at 48 hours

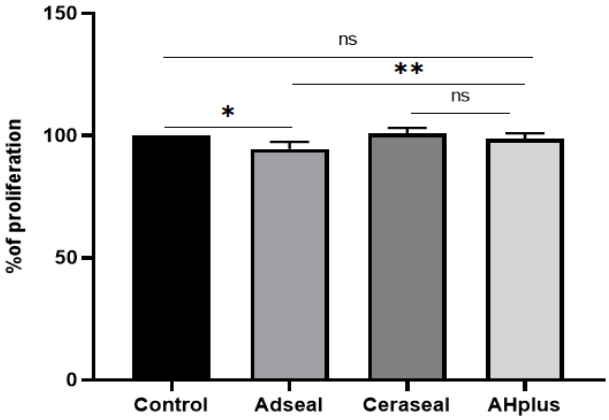

Figure 5: MTT assay results at 72 hours

Discussion

Root canal sealers are indispensable for the long-term success of endodontic therapy. While gutta-percha cones occupy the majority of the root canal space, sealers play a crucial complementary role by filling the remaining voids and ensuring a fluid-tight seal [1]. When these materials establish direct contact with periradicular tissues, or when lixiviated components diffuse into surrounding tissues via the apical foramen, accessory canals, lateral pathways, or dentinal tubules, they can induce inflammatory responses [6]. Even after the setting phase, potentially cytotoxic constituents may leach from the sealer into tissue fluids, posing risks to periapical health. In clinical scenarios, sealers are typically applied while still freshly mixed or partially polymerized, increasing the possibility of tissue interaction before complete setting. Consequently, cytotoxicity studies such as the present investigation are essential for understanding the biological behavior of diffusible components once the material has set [7]. The cytotoxicity assessment in this study was performed using an ATCC-approved and validated L929 fibroblast cell line, ensuring a reliable in vitro model for biocompatibility evaluation.

Among commercially available sealers, resin-based sealers (RBS) are widely recognized for their superior physicochemical properties, including excellent dimensional stability, low solubility, and resistance to disintegration [8]. Bioceramic sealers, on the other hand, offer distinct biological advantages in clinical applications due to their chemical and thermal stability, non-toxicity, and intrinsic biocompatibility [9].

The present study demonstrated that there were no statistically significant differences in cytotoxicity between the test sealers (Adseal, CeraSeal, and AH Plus) and the control group at 24 hours. Similarly, no significant variation was observed in cytotoxic responses among any of the groups at 48 hours. By 72 hours, however, a slight difference in cytotoxicity was detected between the Adseal and control groups. While both CeraSeal and AH Plus exhibited cytotoxicity levels comparable to the control, Adseal demonstrated a statistically significant difference when compared to AH Plus, indicating a slightly higher cytotoxic potential. These findings suggest that all tested sealers maintain acceptable levels of biocompatibility, with only minor variations in cellular responses over time.

The cytotoxic characteristics of epoxy resin–based sealers have been widely attributed to the presence of bisphenol diglycidyl ether, a component known to influence cellular responses [10]. Previous research reported negligible or minimal cytotoxicity associated with AH Plus, concluding that variations in cytotoxicity are closely linked to the chemical composition of the sealer, a conclusion that aligns with the findings of the present study [11]. Similarly, earlier investigations observed that AH Plus exhibited higher cytotoxicity after 1 hour, 24 hours, and 48 hours compared with longer setting periods of 7 days and one month. In contrast, RoekoSeal demonstrated no cytotoxic effects at any time point, further supporting the observation that material composition and setting time significantly influence cytotoxicity profiles [12].

It is important to recognize several inherent limitations associated with the current study. The cytotoxicity assessment was conducted using materials in their set form, whereas in clinical practice, sealers are applied immediately after mixing, when they are still in a freshly prepared state. Evaluating cytotoxicity under these conditions would provide a more comprehensive understanding of their biological effects during clinical application. Furthermore, CeraSeal is a relatively new premixed formulation, and limited data exist regarding its sealing capacity, physicochemical characteristics, and antimicrobial properties. This scarcity of information restricts the ability to draw definitive conclusions about its long-term clinical performance. Additionally, the sealers investigated in this study were derived from different chemical compositions, and no direct comparison was made within the calcium silicate–based sealer category. Future investigations incorporating these considerations would provide a more robust and comprehensive evaluation of sealer cytotoxicity and biological compatibility.

Conclusion

The present study demonstrated that resin-based, epoxy-derived, and bioceramic root canal sealers exhibit minimal cytotoxicity when compared under controlled in vitro conditions, with AH Plus serving as a positive control and untreated cells as a negative control. All three sealers maintained cell viability within physiologically acceptable limits, indicating their potential for safe clinical use. However, subtle differences in cytotoxic profiles highlight the importance of chemical composition in influencing biological responses. To further validate the clinical reliability, long-term safety, and functional effectiveness of these materials, more comprehensive investigations are required. Future research should incorporate both in vitro and in vivo models, with emphasis on evaluating biological behavior during different stages of material setting and assessing chemical interactions within complex periapical environments. Such studies will provide deeper insights into the regenerative potential, biocompatibility, and therapeutic efficacy of contemporary root canal sealers in endodontic practice.

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