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Original Research

In-Vitro Comparison of Cytotoxic Effects of Two Zinc Oxide Eugenol Containing Root Canal Sealers on Human Periodontal Ligament Fibroblasts

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ABSTRACT

Objective: To evaluate and compare the cytotoxicity of two zinc oxide eugenol (ZOE) based root canal sealers—Endoflux (Ammdent, India) and Endoseal (Prevest DenPro, India)—on cultured human periodontal ligament fibroblasts (hPDLFs).

Materials and Methods: Primary human periodontal fibroblasts were cultured and exposed to freshly mixed and set specimens of the two ZOE sealers. Cell viability was assessed at 24 h, 48 h, and 72 h using the MTT assay. Data were statistically analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Results: Both ZOE sealers demonstrated time-dependent reduction in cytotoxicity. Endoseal exhibited significantly higher cell viability at all time intervals compared to Endoflux ($p < 0.05$). Cytotoxicity decreased after setting in both groups.

Conclusion: Endoseal (Prevest DenPro) demonstrated comparatively lower cytotoxic effects on human periodontal fibroblasts than Endoflux (Ammdent), suggesting a more favorable biocompatibility profile for clinical use.

Introduction

Root canal sealers play an essential role in achieving a hermetic seal within the root canal system by filling voids between gutta-percha and canal walls. However, due to their potential extrusion beyond the apex, their biocompatibility with periapical tissues is of critical importance [1]. Zinc oxide eugenol (ZOE) based sealers have been widely used because of their antimicrobial properties, satisfactory sealing ability, and radiopacity [2,3]. Nonetheless, the release of eugenol, known for its cytotoxic and inflammatory potential, may adversely affect periapical healing by damaging fibroblasts and osteoblasts [4,5]. Advancements in sealer formulations have attempted to reduce the cytotoxic effects of traditional ZOE sealers by optimizing the ratio of eugenol, incorporating plasticizers, or adding resinous modifiers [6]. Endoflux (Ammdent,India) and Endoseal (Prevest DenPro, India) are commercially available ZOE-based sealers used in endodontic practice. While both rely on eugenol's chelation with zinc oxide for setting, their composition and additives differ, potentially influencing their biocompatibility. Cytotoxicity assays using cultured human periodontal ligament fibroblasts (hPDLFs) provide a relevant in-vitro model for evaluating biocompatibility because these cells are in direct contact with materials extruded beyond the apical foramen [7,8]. Therefore, the present study aimed to comparatively evaluate the cytotoxicity of Endoflux and Endoseal on cultured human periodontal fibroblasts using the MTT assay at different time intervals.

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Materials and Methods

Two ZOE-based root canal sealers were evaluated:

- Group I: Endoflux (Ammdent, India)
- Group II: Endoseal (Prevest DenPro Ltd., India)

Component	Endoflux (Ammdent)	Endoseal (Prevest DenPro, India)
Base	Zinc oxide–eugenol with calcium hydroxide	Zinc oxide–eugenol based formulation
Powder Components	Zinc oxide, calcium hydroxide, iodoform, barium sulphate	Zinc oxide, barium sulphate, thymol iodide, magnesium stearate
Liquid Component	Eugenol	Eugenol
Radiopacifier	Barium sulphate	Barium sulphate
Manufacturer	Ammdent, India	Prevest DenPro Ltd., India

Table 1: Composition of Zinc Oxide Eugenol Sealers Evaluated

Cell Culture

Primary human periodontal ligament fibroblasts (hPDLFs) were obtained from healthy premolars extracted for orthodontic reasons after informed consent and institutional ethics approval. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Preparation of Sealer Extracts

Each sealer was prepared following the manufacturer's instructions and placed into sterile cylindrical Teflon molds (5 mm diameter × 2 mm thickness). After setting (24 h, 37 °C, 100% humidity), specimens were sterilized under UV light for 30 min. Extracts were prepared by immersing the discs in 5 mL of DMEM (1 cm^2/mL surface area to volume ratio) and incubating for 24 h [9].

Cytotoxicity (MTT) Assay

Cells were seeded into 96-well plates (1 × 10^4 cells/well) and incubated overnight for attachment. Then, the culture medium was replaced with 100 µL of sealer extracts. Cell viability was assessed at 24 h, 48 h, and 72 h by adding 10 µL of MTT solution (5 mg/mL) and incubating for 4 h. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post-hoc test with significance set at $p < 0.05$ (SPSS v26.0, IBM Corp., USA).

Results

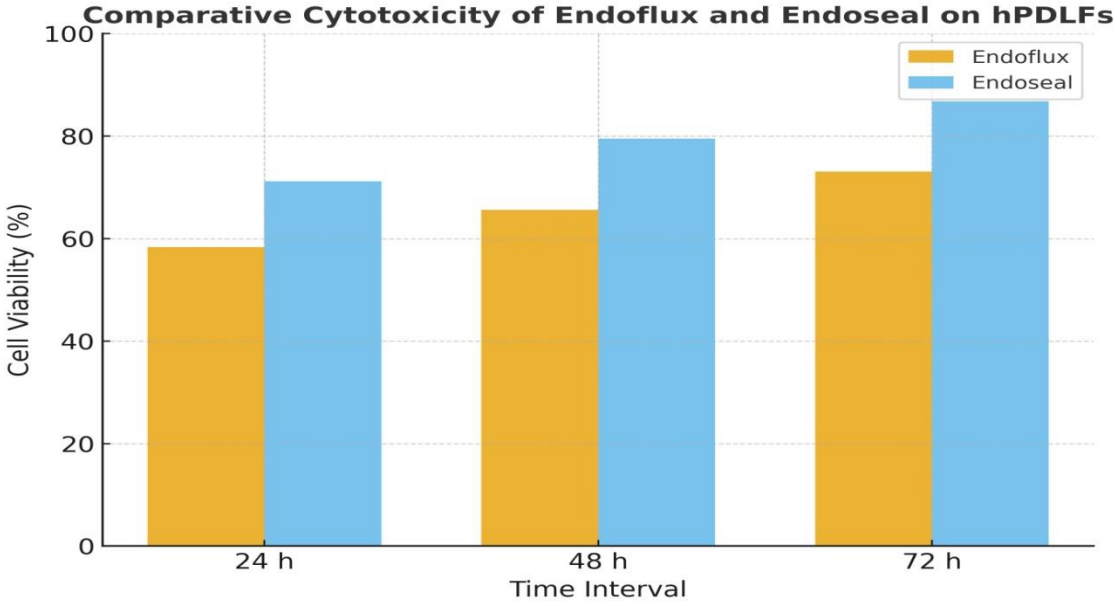
The results demonstrated that both ZOE sealers exhibited initial cytotoxicity that decreased with time.

At 24 hours, Endoflux showed significantly lower cell viability (mean ± SD = 58.3 ± 3.4%) compared to Endoseal (71.2 ± 2.8%) ($p < 0.05$).

At 48 hours, cell viability increased for both materials, with Endoseal showing higher values (79.5 ± 3.1%) than Endoflux (65.6 ± 2.9%).

At 72 hours, the cytotoxicity of both materials further decreased, and Endoseal maintained significantly higher cell viability (86.8 ± 2.4%) compared to Endoflux (73.1 ± 3.3%).

The differences between time intervals within each group were statistically significant ($p < 0.05$), indicating time-dependent reduction in cytotoxicity.



Discussion

The present study evaluated the cytotoxic response of two commercially available zinc oxide eugenol sealers—Endoflux and Endoseal—on cultured human periodontal fibroblasts. Both materials demonstrated moderate cytotoxicity after mixing, which gradually reduced with time, in agreement with earlier reports [10,11]. The initial cytotoxicity can be attributed to the release of free eugenol, which is known to disrupt cell membrane integrity and inhibit mitochondrial activity [12]. However, as setting progresses, eugenol becomes more tightly bound to zinc oxide, reducing its availability and thereby decreasing cytotoxicity [13]. Endoseal showed significantly higher cell viability compared to Endoflux across all time intervals, suggesting possible formulation modifications leading to reduced eugenol release or improved polymeric matrix stability. Previous studies have shown that minor compositional changes—such as inclusion of resinous components or lower eugenol-to-zinc ratio—can markedly influence cytocompatibility [14,15]. Human periodontal fibroblasts were chosen for this study as they represent the cell type most likely to be affected by apical extrusion of sealers [16]. The MTT assay was employed as it measures mitochondrial dehydrogenase activity, providing a sensitive indicator of cell metabolic function [17]. Our findings are in line with those of Gambarini et al. [18], who observed time-dependent decrease in cytotoxicity of ZOE sealers, and Mutoh et al. [19],

who reported that lower eugenol release correlates with improved biocompatibility. Within the limitations of this in-vitro study, it can be inferred that Endoseal demonstrates comparatively better biocompatibility than Endoflux. However, long-term in-vivo studies are necessary to confirm these observations and assess the inflammatory and healing responses in periapical tissues.

Conclusion

Both Endoflux and Endoseal exhibited time-dependent reduction in cytotoxicity to human periodontal fibroblasts. Endoseal demonstrated significantly higher cell viability, suggesting that it may be the more biocompatible choice among ZOE-based sealers for clinical use.

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