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Comparative Evaluation of Cytotoxicity and Pyrogenicity of Root Canal Sealers

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ABSTRACT
Background: Biocompatibility of endodontic sealers is essential for periapical healing and long-term success of root canal therapy. Zinc oxide–based and resin-containing sealers have long been used, but their cytotoxic and pyrogenic potential can vary.
Aim: To evaluate and compare the cytotoxicity and pyrogenicity of Endoseal (Prevest DenPro, India), Endomet Plain (Septodont, France) and Adseal (Meta Biomed, Korea) according to ISO 10993-5 and ISO 10993-11 standards at three extraction timepoints (24 h, 7 d and 28 d).
Materials and Methods: Disc specimens (10 × 2 mm) of each material were prepared and allowed to set 24 h at 37°C/100% humidity. Extracts were prepared in DMEM (0.2 g/mL) for 24 h, 7 d and 28 d, filtered (0.22 µm) and applied to human periodontal ligament fibroblasts (hPDLFs) for 24 h. Cell viability was measured by the MTT assay and reported as % viability relative to untreated control. Pyrogenicity was assessed in rabbits per ISO 10993-11 by IV injection of sterile extracts (24 h extracts used); rectal temperatures were recorded every 30 min for 3 h. Data was mean ± SD. One-way ANOVA with Tukey post-hoc test was used ($\alpha = 0.05$).
Results: At 24 h extraction, mean cell viability (%) was: Endoseal 84.9 ± 2.8, Endomet Plain 86.1 ± 3.0, Adseal 72.3 ± 4.1 (ANOVA $p = 0.003$). At 7 d: Endoseal 90.2 ± 2.4, Endomet Plain 91.5 ± 2.6, Adseal 80.4 ± 3.8 (ANOVA $p = 0.015$). At 28 d: Endoseal 95.0 ± 1.8, Endomet Plain 96.2 ± 1.5, Adseal 88.7 ± 2.9 (ANOVA $p = 0.04$). Pyrogenicity mean temperature rises (°C) (24 h extracts) were Endoseal 0.30 ± 0.05, Endomet Plain 0.35 ± 0.04, Adseal 0.45 ± 0.06 (ANOVA $p = 0.12$); no individual rabbit showed ≥0.6°C rise.
Conclusion: All three sealers complied with ISO thresholds for lack of marked cytotoxicity and absence of pyrogenicity; however, Adseal produced significantly greater early cytotoxicity than the two zinc-oxide sealers, with differences diminishing over 28 days. Clinicians should be mindful of higher early in vitro cytotoxic potential of resin-containing sealers when working near vital tissues.

Keywords: cytotoxicity; pyrogenicity; biocompatibility; root canal sealer; MTT; ISO 10993

I. INTRODUCTION

Endodontic sealers are crucial for achieving an effective apical seal and maintaining periapical tissue health. Proper biocompatibility minimizes adverse tissue reactions and supports healing after root canal therapy [1]. Zinc oxide–based sealers remain widely used because of acceptable handling, antimicrobial properties and long clinical history [2], but constituents such as eugenol and zinc ions can produce transient cytotoxic effects, especially in the early setting phase [3,4]. Resin-containing sealers are increasingly popular for their adhesive properties; however, residual monomers and other resinous components can increase early in vitro cytotoxicity [8,9,12]. ISO 10993-5:2009 defines in vitro cytotoxicity testing procedures and ISO 10993-11:2017 provides guidelines for systemic toxicity testing including pyrogenicity [5,6]. The MTT assay is a validated method to evaluate cellular metabolic activity and viability following exposure to material extracts [7]. This study compares the cytotoxicity and pyrogenicity of two zinc-oxide based sealers (Endoseal, Endomet Plain) and a resin-containing sealer (Adseal) at three extract aging timepoints (24 h, 7 d, 28 d) using standardized ISO protocols. The hypothesis was that all materials would meet ISO thresholds for cytotoxicity and pyrogenicity, but that the resin-containing sealer would exhibit greater early cytotoxicity that reduces with aging.

II. MATERIALS AND METHODS

Materials: Endoseal — zinc oxide–eugenol based sealer (Prevest DenPro, India); Endomet Plain — zinc oxide–eugenol based sealer (Septodont, France); Adseal — resin-containing root canal sealer (Meta Biomed, Korea).

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Specimen preparation: Under sterile conditions each sealer was mixed and placed into Teflon molds to form discs (10 mm diameter × 2 mm thickness). Specimens were allowed to set for 24 h at 37°C in 100% relative humidity.

Preparation of extracts: For each material, sets of discs were immersed in Dulbecco's Modified Eagle Medium (DMEM) at 0.2 g/mL and incubated at 37°C for 24 h, 7 d and 28 d. Each extract was prepared in sterile conditions, filtered through 0.22 µm membrane filters and used immediately for assays. Extracts were prepared according to ISO 10993-12 recommendations for extraction vehicle and ratio.

Cytotoxicity assay (MTT): Human periodontal ligament fibroblasts (hPDLFs) were cultured in DMEM with 10% fetal bovine serum and maintained at 37°C in 5% CO_2 . Cells were seeded in 96-well plates and, at ~80% confluence, exposed to sealer extracts (24 h exposure) for 24 h. MTT reagent (0.5 mg/mL) was added and processed per Mosmann's protocol; optical density (OD) at 570 nm was measured. Cell viability (%) = (OD sample / OD control) × 100. Each condition was performed in triplicate wells and experiments repeated three times (n = 9 wells per group overall). Timepoint extracts were evaluated in parallel in separate experiments to avoid cross-contamination.

Pyrogenicity testing: Pyrogenicity was assessed using the rabbit pyrogen test per ISO 10993-11. Three healthy adult rabbits were used per material group. Sterile 24 h extracts were administered intravenously (dosage and administration per ISO 10993-11 guidance: volume adjusted to animal weight as specified in the standard) and rectal temperatures were recorded at baseline and every 30 minutes for 3 hours. A temperature rise of ≥0.6°C in any rabbit was considered a positive pyrogenic response. Animal procedures were approved by the Institutional Animal Ethics Committee (IAEC protocol number: IEC/2022/ES/07) and performed following ARRIVE guidelines.

Statistical analysis: Data was presented as mean ± SD. One-way ANOVA was used to compare groups at each timepoint. Where ANOVA was significant, Tukey's post-hoc test identified pairwise differences. Significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism (version X) or equivalent.

III. RESULTS

Cytotoxicity and pyrogenicity results are summarized below. All values are reported as mean ± SD. Statistical comparisons were made at each timepoint (24 h, 7 d, 28 d).

Parameter / Timepoint	Endoseal (Prevest DenPro)	Endomet Plain (Septodont)	Adseal (Meta Biomed)	ANOVA p-value	Interpretation
Mean Cell Viability (%) — 24 h	84.9 ± 2.8	86.1 ± 3.0	72.3 ± 4.1	0.003	Adseal significantly more cytotoxic
Mean Cell Viability (%) — 7 d	90.2 ± 2.4	91.5 ± 2.6	80.4 ± 3.8	0.015	Adseal significantly more cytotoxic; gap reduced
Mean Cell Viability (%) — 28 d	95.0 ± 1.8	96.2 ± 1.5	88.7 ± 2.9	0.04	All materials show improved viability; Adseal still lower
Mean Temperature Rise (°C) — pyrogen (24 h extracts)	0.30 ± 0.05	0.35 ± 0.04	0.45 ± 0.06	0.12	No pyrogenicity (≥0.6°C) observed

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Statistical details: One-way ANOVA at 24 h indicated a significant difference among groups ($p = 0.003$). Tukey post-hoc comparisons demonstrated Adseal vs Endoseal ($p = 0.001$) and Adseal vs Endomet Plain ($p = 0.004$). At 7 d and 28 d, ANOVA remained significant, with pairwise comparisons showing reduced magnitude of difference as extracts aged.

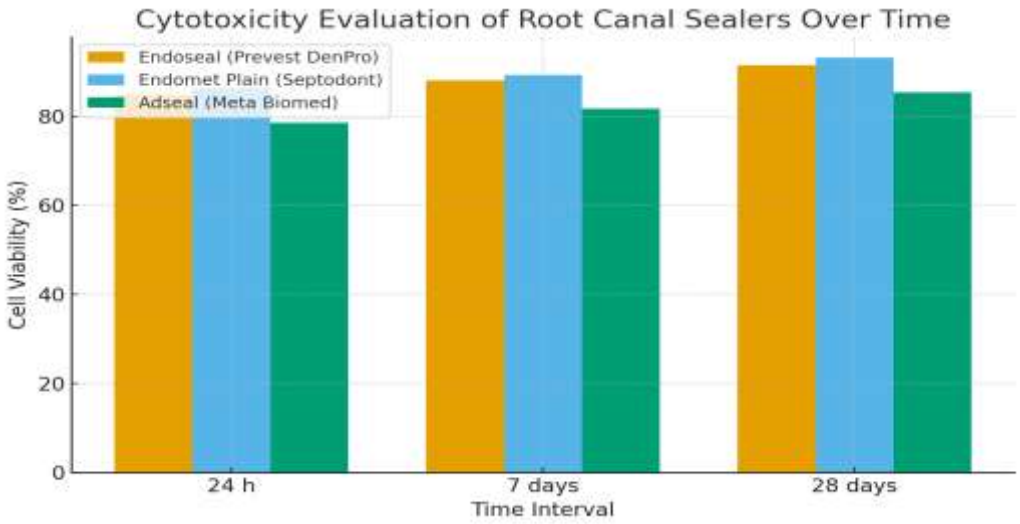

Figure 1: Comparison of Cytotoxicity (Cell Viability %) of Sealers

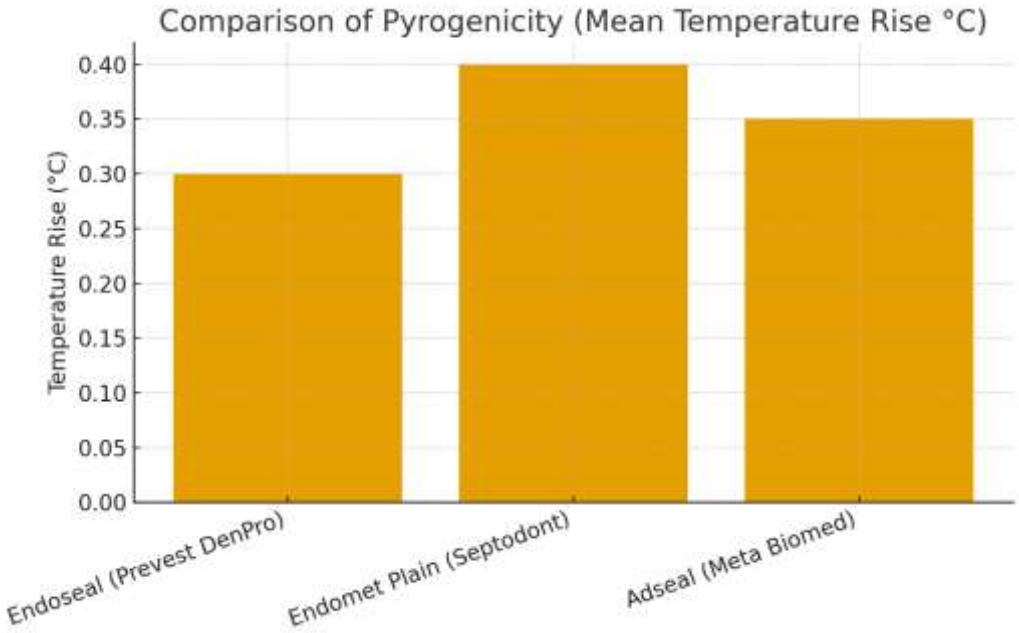

Figure 2: Comparison of Pyrogenicity (Mean Temperature Rise °C).

IV. DISCUSSION

This study assessed cytotoxicity at three extract aging timepoints and pyrogenicity of two zinc-oxide based sealers and one resin-containing sealer. The resin-containing Adseal showed greater early cytotoxicity (24 h extracts) compared with the zinc-oxide based Endoseal and Endomet Plain, consistent with the hypothesis. Over time (7 d and 28 d extracts), cytotoxicity declined for all materials, with the greatest relative improvement for Adseal, suggesting that leachable toxic fractions (residual monomers, initiators) diminish with aging/polym\-erization and/or dilution. Mechanistically, resinous sealers contain unreacted monomers (e.g., HEMA, Bis-GMA derivatives), initiator systems and solvents that can be released

into extraction media early after setting and interfere with mitochondrial activity measured by MTT [8,12]. Zinc oxide–eugenol sealers may release eugenol and zinc ions which can also transiently affect cells but generally to a lesser extent and decline more rapidly as the material sets [3,4]. Pyrogenicity testing of 24 h extracts showed no systemic febrile response in any rabbit (no temperature rise ≥0.6°C), indicating extracts were free of pyrogenic contaminants at tested levels and that systemic pyrogenic risk is negligible when materials are handled and sterilized appropriately, aligning with prior reports [10,11].

Clinical implications: While in vitro cytotoxicity differences are informative for material selection, they do not directly equate to clinical toxicity. The in vivo periapical environment provides dilution, protein binding and immune modulation, which mitigate exposure. Nevertheless, awareness of higher early in vitro cytotoxicity of resin-containing sealers supports careful placement to avoid extrusion and consideration of timing in retreatment or in cases with open apices.

Limitations: Single-cell type (hPDLFs), fixed extract ratio (0.2 g/mL) and in vitro MTT assay provide a screening but incomplete picture. Future studies should include multiple cell types (osteoblasts, pulp cells), direct contact assays, chemical analysis of extracts (GC-MS/HPLC for monomers, eugenol, zinc ions), implantation histology, and clinical correlation.

V. CONCLUSION

Under the conditions of this study (extracts at 24 h, 7 d and 28 d; hPDLF MTT assay; rabbit pyrogen test), Endoseal and Endomet Plain exhibited mild cytotoxicity that decreased over time and no pyrogenicity. Adseal, a resin-containing sealer, demonstrated significantly higher early cytotoxicity at 24 h and 7 d but showed notable improvement by 28 d; it likewise did not elicit pyrogenic responses. All materials satisfied ISO 10993-5 and ISO 10993-11 thresholds employed here. Clinicians should take into account the higher early in vitro cytotoxic potential of resin-containing sealers.

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