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Research Article

The Effect of Bioceramic-Based Root Canal Sealers on the Cell Viability and Cytotoxicity of Human Periodontal Ligament Fibroblast cell line (An in vitro study)

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Abstract: The current study aimed to assess the cell viability and cytotoxicity of bioceramic-based root canal sealers by comparing them to a zinc oxide-eugenol-based sealer (Endovit) in the set state of human periodontal ligament fibroblasts for one day, three days, and seven days. **Materials and Methods**: Sealers were prepared following the manufacturer's directions and inserted into cylindrical rubber molds (5 mm diameter, 2 mm height) to get uniform sealer specimens. The specimens were kept for 24 hours at 37Cº. Human periodontal ligament fibroblasts were cultured with extracts from the tested sealers. Cell viability and Cytotoxicity were assessed for extracts of one-day, three-day, and seven-day immersion. The cell viability of all root canal sealers was determined using an MTT test, and cytotoxicity was determined using an LDH test. Data were statistically evaluated with One way-analysis of variance ANOVA followed by Duncan's MRT to determine if group variance was significant. **Results**: Each tested sealer demonstrated a statistically significant variance in cell viability and cytotoxicity at various time intervals. NeoSEALER Flo had the highest cell viability and lowest cytotoxicity percentages, followed by Cerafill RCS, MTA fillapex, and Endovit, respectively, at each evaluation time interval. **Conclusions**: NeoSEALER Flo was the least cytotoxic while ENDOVIT was the most cytotoxic root canal sealer. Cell viability decreased over time.

Keywords: Cell viability; Cytotoxicity; LDH assay; MTT assay

INTRODUCTION

Root canal sealers have an essential function in biological reactions with the tissues that surround them, since they encourage attachment between gutta-percha and dentinal walls, in addition to enabling the sealing of lateral, accessory canals, and multiple foramina, which promote healing when they come in touch with periapical tissues [1, 2]. Root canal filling materials should be biocompatible as they can come into contact with the periapical tissues directly (in the case of material extrusion) or indirectly through the products released during the setting reaction [3]. Therefore, their potential cytotoxicity and genotoxicity should be examined before using the material in clinical practice[4] .

Sealers are categorized by their primary chemical composition into the following groups: Zinc oxide-eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based sealers [5]. Recently, calcium silicate-based sealers (CSBS, bioceramic) have gained popularity (6). Several varieties of CSBS have been developed as a powder-liquid and pre-mixed composition. During the setting, Bioceramic-based sealers release calcium and hydroxyl ions, which leads to an increase in pH (> 12). Thereby, they exhibit long-term antibacterial properties [8 -6]

Additionally, bioceramic sealers chemically bond to the dentinal tubules through the production of hydroxyapatite [9, 10]. During the treatment of root canals, sealers may project through the apical foramen or accessory canals, as well as the periapical tissues [11, 12]. As a result, they should exhibit an appropriate cyto- and biocompatibility, which means that they should not negatively affect the surrounding tissues when they come into contact with periodontal tissues, and they also should not decrease the viability of cells, migration, proliferation, or differentiation [13]. Additionally, root canal sealants should promote bioactivity.[15 ,14]

The biocompatible material used in dentistry, according to the International Organization for Standardization (ISO) 1942, stimulates a proper host response when it comes into contact with vital tissues without causing undesirable local or systemic reactions .[16 ,12]

From a physical-chemical perspective, a bioactive substance needs to have the ability to promote hydroxyapatite precipitation on its surface through an ionic exchange with surrounding tissue fluids. This improves the development of a mineral connection to the dentin substrate at the intra-coronal or intra-radicular level.[17]

Root canal sealer's toxicity can be attributed to their components, such as eugenol, bisphenol A, and resin monomers; chemical substances formed during the setting process (e.g., formaldehyde) or afterward due to their solubility, such as calcium hydroxide. Although sealers are intended to remain in the root canal, due to their flow

properties, they may be inadvertently driven into the periapical tissues via the apical foramen, or lateral and auxiliary canals.[4 ,18]

When root canal sealers come into contact with tissue fluids, they dissolve, allowing components to penetrate them. Sealant degradation products may remain in contact with the periapical tissues for a long time and lead to cytotoxic and genotoxic consequences.[20 ,19]

NeoSealer Flo is a bioceramic sealer that contains bioactive components including tricalcium silicate and dicalcium silicate, as well as radiopacifiers such as calcium aluminate, calcium aluminum oxide (grossite), tricalcium aluminate, and tantalite. The manufacturer also reported small amounts of calcium sulfate (<1%). It is bioactive, biocompatible, resin-free, and does not discolor teeth.

Recently, a novel bioceramic-root canal sealer called "Cerafill" (Prevest DenPro, Jammu, India) was developed. According to the manufacturer's guidelines, it is a premixed calcium silicate sealer including aluminum-free calcium phosphate, bioactive glass particles, and zirconium oxide (as a radio-opacifier). It is said to have superior bioactive and biocompatible qualities with excellent handling characteristics. There is currently insufficient research to demonstrate their bioactivity and cytotoxicity characteristics.

The purpose of this in vitro study was to assess the cytocompatibility and cytotoxicity of bioceramic-based sealers on the human periodontal ligament fibroblast cell line (HPDLFs) and compare them with the zinc oxide eugenol-based sealant. The null hypothesis stated that there would be no difference in cell viability and cytotoxicity among the tested sealers.

MATERIALS AND METHODS

Root canal sealers

In the current study, four root canal sealers were investigated: one zinc oxide-eugenol-based (ENDOVIT) and three Bioceramic-based sealers (NeoSEALER flo, Cerafill RCS, and MTA-FILLAPEX) (Table 1). All the experiments have been approved by the Committee of Ethics of the University of Mosul/College of Dentistry (UoM.Dent.24/1011; 6/2/2024).

Table (1): Materials utilized in this study

Root canal sealer	Manufacturer	Composition
ENDOVIT	Celit Dental Company, Voronezh, Russia	Powder: Zinc oxide, radiopaque filler Liquid: Eugenol and thymol
NeoSEALER flo	Avalon Biomed, Houston, Texas, USA	Tricalcium silicate, dicalcium silicate, calcium aluminate, calcium aluminum oxide (grossite), tricalcium aluminate, and tantalite as radiopacifiers.
Cerafill RCS	Prevest Denpro, Jammu, India	Calcium silicates, Calcium phosphates, Zirconium oxide, Calcium Sulphate, Fillers, Accelerators, and thickening agents
MTA-FILLAPEX	Angelus, Londrina, PR, Brazil	Base Paste: Salicylate resin, Natural resin, Calcium tungstate, Nanoparticulated Silica, Pigments. Catalyst Paste: Diluting resin, Mineral trioxide aggregate, Nanoparticulated Silica, and Pigments.

Sample and extract preparation:

Sealers were prepared according to the manufacturer's guidelines and inserted in cylindrical rubber molds (5 mm diameter, 2 mm height). Molds had been sterilized with UV light for 15 minutes. Samples were then left to set for 24 hours in an incubator (37ºC, 5% CO2, and 95% humidity) [21].

Sample extracts from tested sealers were obtained by immersing them in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Invitrogen, Waltham, MA, USA) with 10% of fetal bovine serum (FBS) for 24 h, 72 h, and 168 h in a humid atmosphere (37ºC, 5% CO_2) in a ratio of 3 cm^2 of sample surface per milliliter of volume of medium [22]. Undiluted extracts were used in this study.

Cell culture procedure

All in vitro assays were carried out on human periodontal ligament fibroblast cell lines (HPdLFs) (ATCC; Manassas, VA, USA).

Cells were grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (ATCC; Manassas, VA, USA), 100 μg/mL penicillin, and 100 μg/mL streptomycin. Cell cultures were kept at typical environments (37 °C, 5% pCO2; 95% humidity) according to the manufacturer's directions. Cells were split when the culture achieved 90–95% growth.

Cell viability and cytotoxicity analysis

The cell viability and proliferation assessment of the four sealer extracts cultured with HPDLFs (test groups) was evaluated and compared to HPDLFs cultured in

DMEM medium without any sealer extracts, which served as a negative control. The analysis utilized a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as reported in prior investigations [23, 24].

HPDLFs were seeded onto 96-well culture plates at 1×10^4 cells per well ($n = 6$ per test group) and incubated in various sealer-conditioned DMEM (conditioned for 1, 3, and 7 days) and incubated for 24 h at 37 °C, 5% CO2, and 95% humidity. To develop the MTT assay, each well received 10 μL MTT solution (0.5 mg/mL) (Sigma Aldrich, USA) and was incubated for 4 hours at 37◦C, with 5% CO_2.

After removing the medium, dimethylsulfoxide (DMSO; Sigma-Aldrich, USA) was applied to each well (100 μl/well) to dissolve the purple formazan crystals formed by metabolically active/viable cells. Absorbance was measured at 570 nm wavelength by a microplate reader (ELx800; Bio-Tek Instruments, Winooski, VT, United States). Cell viability was determined using this formula:

(Test sample absorbance / Control sample absorbance) × 100.

Cytotoxicity – Lactate Dehydrogenase (LDH) Assay

An LDH Assay kit (Promega, Madison, WI, USA) was used to determine the amount of lactate dehydrogenase (LDH) released from the mitochondria of dead cells. A total of 50 μL of the supernatant was combined with 50 μL of reagent and incubated for 30 min at room temperature. A 50 μL stop solution was subsequently added, the color transformed from blue to yellow, and the absorbance at 450 nm was determined with a spectrophotometer. The cytotoxicity rate was computed using the formula:

Cytotoxicity (%) = 100 × Experimental LDH Release absorbance / Maximum LDH Release absorbance (negative control).

The viability of HPDLFs was utilized to determine the cytotoxicity of root canal sealers. Cytotoxicity responses were classified as major (≤30%), medium (30%-60%), minor (60%-90%), or non-cytotoxic (≥90%) [25].

Statistical Analysis

A one-way analysis of variance ANOVA followed by Duncan's Multiple Range Test was used to determine whether group variance was significant; statistical significance was interpreted as $p \leq 0.05$. Data were reported as mean± standard deviation, and statistical significances were calculated using Graph Pad Prism version 9.4 (Graph Pad Software Inc., La Jolla, CA).

RESULTS

Analysis of the cell proliferation

To determine the effect of sealants on cell proliferation, DMEM was conditioned in the presence of various sealants for 1, 3, and 7 days. HPDLFs were then incubated

in sealant-conditioned DMEM for 24 hours before evaluation of cell proliferation and compared to HPDLFs grown in non-sealant-conditioned DMEM. HPDLFs exposed to DMEM conditioned with NeoSEALER Flo and Cerafill RCS showed a high cell proliferation, with no significant differences relative to the negative control groups (cells cultivated without sealer extracts) ($p > 0.05$). However, media conditioned with both NeoSEALER flo and Cerafill RCS for 7 days (168 h) significantly decreased the proliferation of HPDLFs ($p < 0.0001$), relative to the negative control.

Similar to the NeoSEALER flo and Cerafill RCS, HPDLFs grown in media conditioned with MTA-FILLAPEX and ENDOVIT root canal sealers for 168 hours, proliferated less compared to cells grown in non-conditioned DMEM ($p < 0.0001$), Figure (1).

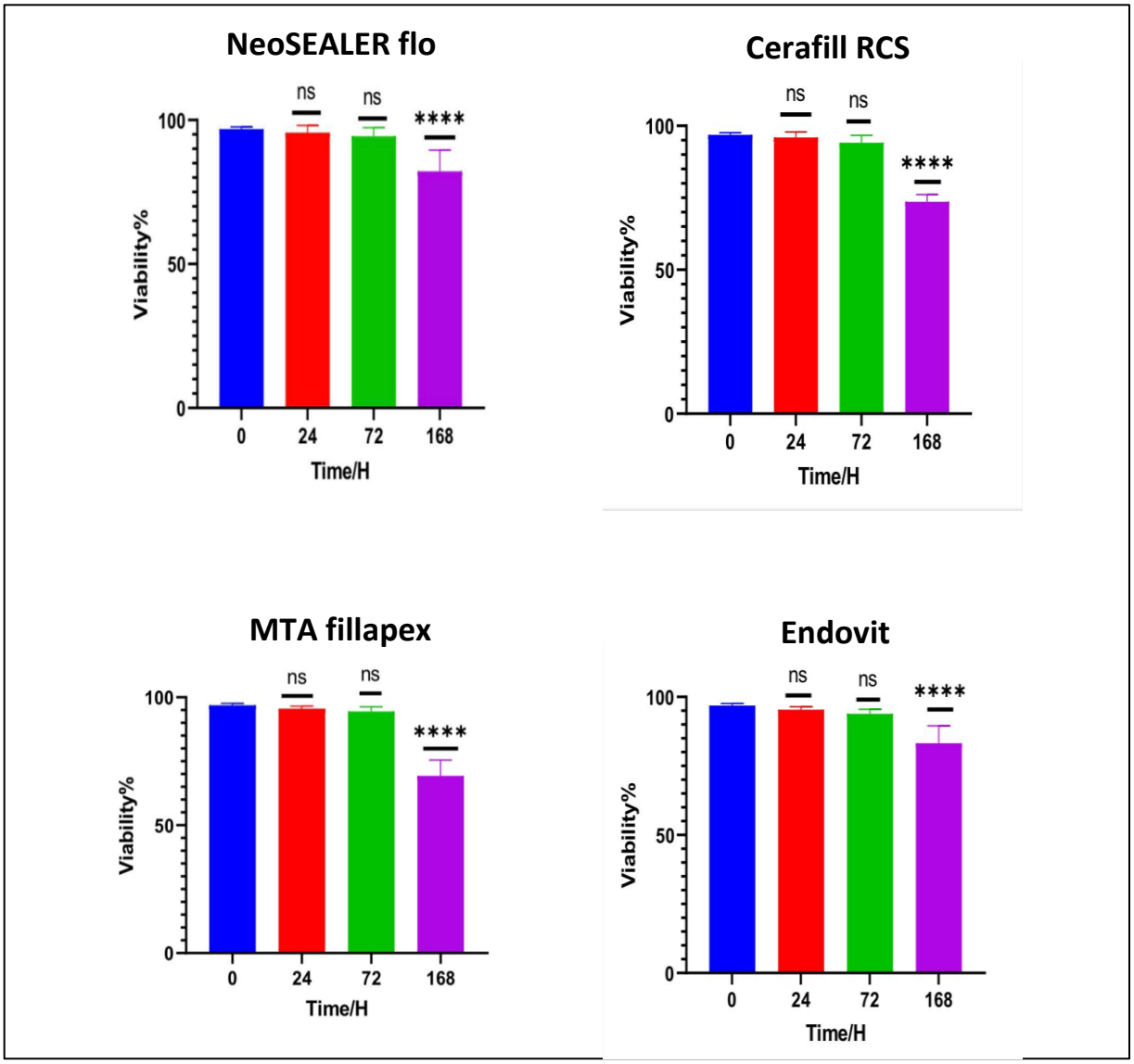

Figure (1): The cell viability assay of NeoSEALER flo, Cerafill RCS, MTA-FILLAPEX, and ENDOVIT root canal sealers (for extracts of 24 h, 72 h and 168 h) following 24 h of incubation with HPDLFs. Statistical significance on the charts: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; **** $p <$ 0.0001 ns; (Non significance, $p > 0.05$) against negative control (0).

The mean and standard deviation values of cell viability (%) (mean ± SD) for various sealers are listed in (Table. 2)

Table (2): The cell viability (%) (mean ± SD) for various sealers.

Sealer	0 Control	24 h	72 h	168 h
NeoSEALER flo	96.91± 0.71^{A}	95.62± 2.48^{A}	94.34± 3.04^{A}	82.21± 7.32^{B}
Cerafill RCS	96.91± 0.71^{A}	95.92± 1.92^{A}	94.15± 2.55^{A}	73.64± 2.45^{C}
MTA-FILLAPEX	96.91± 0.71^{A}	95.58± 0.92^{A}	94.46± 1.79^{A}	69.30± 6.16^{D}
ENDOVIT	96.91± 0.71^{A}	95.38± 1.03^{A}	93.88± 1.68^{A}	83.19± 6.34^{B}

Various superscript letters indicate statistically significant variations along the same horizontal row. NS indicates non-significant ($p > 0.05$), * indicates Significant ($p<0.05$), **** indicates highly significant ($P<0.0001$).

Analysis of the cytotoxicity of sealers

To determine the effect of sealants on cell death, DMEM was conditioned in the presence of various sealants for 1, 3, and 7 days. HPDLFs were then incubated in sealant-conditioned DMEM for 24 hours before evaluation of cell toxicity and compared to HPDLFs grown in non-sealant-conditioned DMEM. The LDH test revealed that NeoSEALER Flo was noncytotoxic in all the sealant-conditioned DMEM treatments, with no statistically significant alteration in LDH leakage values in relation to the control group ($P>0.05$). However, all treatments with Cearafill RCS-conditioned DMEM showed significant cytotoxicity compared to the control group ($P<0.01$).
Media conditioned with MTA-FILLAPEX for 24 hours was non-cytotoxic following incubation with HPDLFs for 24 h, showing no statistically significant variation in LDH leakage values compared to the control group ($P>0.05$). However, media conditioned with MTA-FILLAPEX for 72 h and 168 h were significantly cytotoxic compared to the control group ($p<0.05$) .

Media conditioned with ENDOVIT sealer was cytotoxic to HPDLFs at all periods of sealer extracts, with time-dependent cytotoxicity after conditioning DMEM with sealant for 24 h (Figure 2).

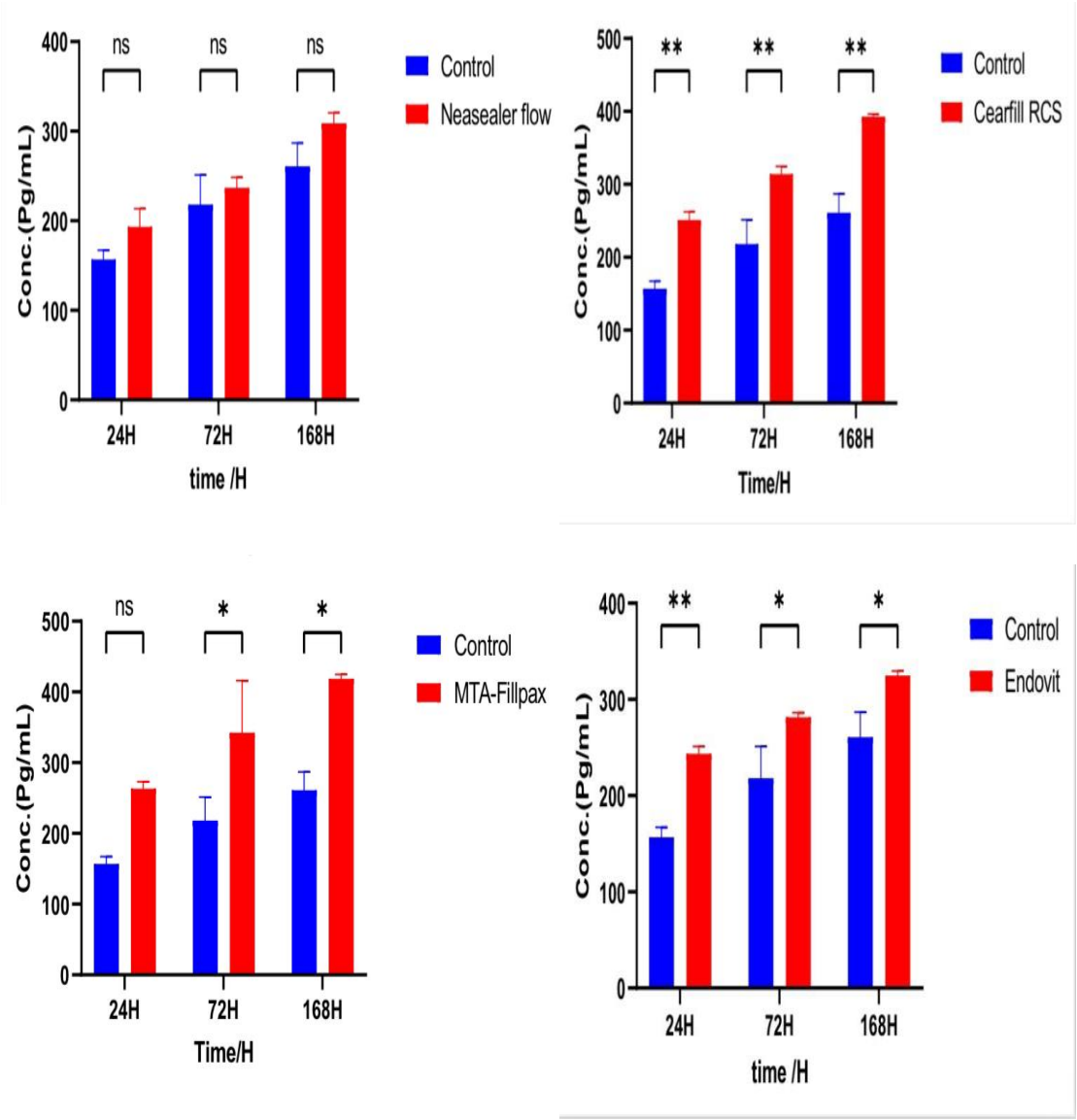

Figure (2): The cytotoxicity assay of NeoSEALER flo, Cerafill RCS, MTA-FILLAPEX, and ENDOVIT root canal sealers via LDH assay at different periods of sealer extracts following incubation with HPDLFs for 24 h. Statistical significance on the charts: ns indicates Non-significance ($p > 0.05$); * indicates $P<0.05$; ** indicates $p < 0.01$, against negative control.

The mean and standard deviation values of cytotoxicity (%) (mean ± SD) for various sealers are listed in (Table 3).

Table (3): The mean and standard deviation values of the LDH test for various tested sealers are documented as (mean ± SD).

Sealer	24 h	72 h	168 h
NeoSEALER flo	193.16±20.18	236.91±11.72	308.75±11.72
Cerafill RCS	251.19±11.07	313.82±11.07	392.57±3.90
MTA-Fillapex	263.16±9.76	342.377±73.59	418.36±6.51
Endovit	243.36±7.81	281.58±4.55	324.87±4.55
Control	156.78±10.42	218.03±33.21	260.86±26.05

DISCUSSION

In the present study, the null hypothesis was rejected because statistically significant differences in cell viability and cytotoxicity were found between the tested sealers.

A key feature of root canal sealers is appropriate biocompatibility, as sealers can come into direct contact with the periapical tissues (in case of material extruding) or indirect contact via compounds released during the setting reaction [3]. Hence, before employing the material in clinical applications, cytocompatibility and possible cytotoxicity must be studied[4]

Cerafill RCS is a novel injectable bioceramic sealer. To the best of our knowledge, no research has yet been done to assess the bioactivity and cytotoxicity of this sealer.
A variety of cell lines have been used to investigate the cytotoxicity of root canal sealers such as fibroblasts, osteoblasts, and periodontal ligament cells [26, 27, 28]. With that said, fibroblast cells are thought to be the most common type of cells in the periodontal ligament [29], and they have an essential role in the function and regeneration of periodontal connective tissues; hence, they were chosen specifically for this investigation.

In the present study, cell viability was assessed using the MTT assay. MTT assay is a colorimetric method based on the capacity of mitochondrial dehydrogenase enzymes in living cells to transform the yellow water-soluble tetrazolium salt MTT into purple formazan crystals. The MTT assay is a simple and precise approach for assessing in vitro cell viability and proliferation.[30]

Cytotoxicity was assessed via the LDH test. LDH is a soluble cytosolic enzyme found in the majority of cells that quickly leaks into the cell culture media upon cell death because of plasma membrane disruption. The rise in LDH activity in culture media is related to the number of lysed cells. Thus, higher levels of LDH reflect an elevated cytotoxicity impact.(31)

In the present study, NeoSEALER Flo overall presented the highest cell viability, exhibiting above 90% of viable hPLFCs after exposure to 24 h and 72 h sealer-conditioned media and above 80% of viable cells after incubation with 168 h sealer-conditioned media. Thus, it can be considered non-cytotoxic, because based on ISO 10993 criteria, a sealer is cytotoxic when cell viability is less than 70%.[32 ,1]
The findings in this study are in line with those of Sebastian et al. [30]

NeoSEALER Flo presented the significantly highest cell viability and lower cytotoxicity values in comparison with Cerafill RCS and MTA-FILLAPEX, which agrees with Elgendy and Badr [25]

In contrast, López-García et al. [33] showed that undiluted concentrations of NeoSEALER flo were associated with a significant reduction in mitochondrial activity compared to the control.

The discrepancies between studies may be attributed to changes in experimental conditions, including preparation of samples, exposure period, and culture of cells (2D or 3D). In 3D cell aggregates, there is a greater interaction between the cells and the matrix as compared to 2D cell culture; therefore, the ability to penetrate the sealant-conditioned media is reduced, resulting in lower cytotoxic impact.[34]

Cerafill RCS exhibited less cell viability than NeoSEALER flo, with 73% of cells viable after incubation with 168 h sealant-conditioned DMEM. Furthermore, incubation of cell media conditioned with MTA- FILLAPEX for 168 h exhibited lower than 70% viability. Thus, with the results of this study, it can be concluded that MTA Fillapex was the most cytotoxic sealer among the three tested bioceramic sealers, which is in agreement with other studies .[38 ,37 ,36 ,35]

MTA Fillapex includes resin in its composition, which can have a negative impact on biocompatibility [39], as demonstrated by the viability results.

A significant degree of cytotoxicity to HPdLFs was observed following incubation of cells with media conditioned with the zinc oxide-eugenol-based sealer (ENDOVIT) that was utilized for comparison in this study. This was proven to be particularly cytotoxic after 24 hours in cell culture studies, which is in line with other studies. [40-42]

All the sealers exhibited a decrease in cell viability over time. Due to the eugenol and zinc oxide are the primary components of sealers with recorded cytotoxicity. Araki et al confirmed the detrimental effect of eugenol, demonstrating that eugenol reduced the survival of human periodontal ligament fibroblasts significantly more than fatty acids. The irritating effect of zinc oxides is due to the release of Zn2+ ions, which promote inflammation in connective tissues and are already toxic at 10 µg/mL concentrations [43]. Over time, the cytotoxic effect of bioceramic sealers could return to their high pH values.[44]

Bioceramic-based sealers showed the highest amounts of Ca+2 release and alkalizing activity, which can be related to changes in the quantities of calcium silicates and calcium aluminates .[45]

The significant calcium hydroxide release and alkaline pH of these sealers might produce severe inflammatory reactions that impair the viability of neighboring cells .[46]

The cytotoxic impact of bioceramic sealers may also be caused by the incorporation of radiopacifiers or thickening agents [47], the concentration of oxides, particularly barium oxide [48], and the production of hydroxyapatite.[47]

These results are in agreement with the studies of Lee et al. and Jagtap et al., which found a decline in cell viability of the bioceramic sealers with time.[49 ,44]

CONCLUSIONS

Within the constraints of the current study, it is possible to conclude that:

1 .Bioceramic sealer NeoSEALER flo presented higher cell viability and lower cytotoxicity, while ENDOVIT was the most cytotoxic root canal sealer.

2 .Cell viability decreases with time.

The findings of this study may help select the proper material for endodontic therapy.

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Ethical statement: All the experiments were approved by the Committee of Ethics of the University of Mosul/College of Dentistry (UoM.Dent.24/1011; 6/2/2024).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

REFERENCES

1. de Souza, L. C., Neves, G. S. T., Kirkpatrick, T., Letra, A. & Silva, R. Physicochemical and biological properties of AH plus bioceramic. J. Endod. 49, 69–76 .(2023)

2. Zamparini, F., Spinelli, A., Cardinali, F., Ausiello, P., Gandolfi, M. G., & Prati, C. (2023). The use of premixed calcium silicate bioceramic sealer with warm carrier-based technique: A 2-year study for patients treated in a master program. Journal of Functional Biomaterials, 14(3), 164.

3. Kapralos, V. et al. On the biocompatibility of endodontic sealers. Swiss Dent. J. 132, 586–597.(2022)

4. Fonseca, D. A. et al. Biocompatibility of root canal sealers: A systematic review of in vitro and in vivo studies. Materials (Basel). 12, 1–34.(2019)

5. Paz JER, Costa PO, Souza AAC, de Oliveira IM, Falcão LF, Falcão CAM, et al. Bone repair in defects filled with AH Plus sealer and different concentrations of MTA: a study in rat tibiae. Restor Dent Endod. 2021;46(4):e48.

6. Radwanski, M. et al. A micro-CT analysis of initial and long-term pores volume and porosity of bioactive endodontic sealers. Biomedicines 10, 2403.(2022)
7. Chopra, V., Davis, G. & Baysan, A. Physico-chemical properties of calcium-silicate vs. resin based sealers—A systematic review and meta-analysis of laboratory-based studies. Materials (Basel, Switzerland) 15, 229.(2021)
8. Zavattini, A., Knight, A., Foschi, F. & Mannocci, F. Outcome of root canal treatments using a new calcium silicate root canal sealer: A non-randomized clinical trial. J. Clin. Med. 9, 782.(2020)
9. Kharouf, N. et al. Physicochemical and mechanical properties of premixed calcium silicate and resin sealers. J. Funct. Biomater.(2023)
10. Farrayeh, A. et al. Effectiveness of two endodontic instruments in calcium silicate-based sealer retreatment. Bioengineering (Basel, Switzerland) 10, 362.(2023)
11. Li J, Chen L, Zeng C et al (2022) Clinical outcome of bioceramic sealer iRoot SP extrusion in root canal treatment: a retrospective analysis. Head Face Med 18:28.
12. Kim J, Cho S-Y, Choi Y et al (2022) Clinical Efficacy of Sealer-based Obturation Using Calcium Silicate Sealers: A Randomized Clinical Trial. J Endod 48:144–151.
13. Yang X, Zheng T, Yang N et al (2023) A Review of the research methods and progress of biocompatibility evaluation of root canal sealers. Aust Endod J 49:508–514.
14. Rengo S (2023) Bioceramics, a turning point. G Ital Endod 37.
15. Estivalet MS, de Araújo LP, Immich F et al (2022) Bioactivity Potential of Bioceramic-Based Root Canal Sealers: A Scoping Review. Life 12:1853.
16. Williams, D. F. On the mechanisms of biocompatibility. Biomaterials 29, 2941–2953.(2008)
17. Vallittu PK, Boccaccini AR, Hupa L, Watts DC (2018) Bioactive dental materials—Do they exist and what does bioactivity mean? Dent Mater 34:693–694.
18. Özdemir, O. & Kopac, T. Cytotoxicity and biocompatibility of root canal sealers: A review on recent studies. J. Appl. Biomater. Funct. Mater. 20, 22808000221076324.(2022)
19. Camilleri, J., Atmeh, A., Li, X. & Meschi, N. Present status and future directions: Hydraulic materials for endodontic use. Int. Endod. J. 55, 710–777.(2022)
20. Elyassi, Y., Moinzadeh, A. & Kleverlaan, C. Characterization of leachates from 6 root canal sealers. J. Endod. 45, 623–627.(2019)
21. Sanz, J. L., Lopez-Garcia, S., Forner, L., Rodriguez-Lozano, F. J., Garcia-Bernal, D., Sanchez-Bautista, S., Puig-Herreros, C., Rosell-Clari, V., & Oñate-Sánchez, R. E. (2022). Are endodontic solvents cytotoxic? An in vitro study on human periodontal ligament stem cells. Pharmaceutics, 14, 2415.

22. Rodríguez-Lozano FJ, López-García S, García-Bernal D et al (2020) Chemical composition and bioactivity potential of the new Endosequence BC Sealer formulation HiFlow. Int Endod J 53:1216–1228.
23. Kim M-A, Rosa V, Neelakantan P et al (2021) Characterization, Antimicrobial Effects, and Cytocompatibility of a Root Canal Sealer Produced by Pozzolan Reaction between Calcium Hydroxide and Silica. Materials 14:2863.
24. Zheng J-J, Kang X-Y, Li S-M et al (2020) Effects of MTA, iRoot SP and AH Plus on proliferation and differentiation of human periodontal ligament stem cells. Shanghai journal of stomatology 29:449–455.
25. Elgendy, A., & Badr, B. (2023). Cytotoxicity evaluation of different root canal sealers at different time intervals (an in vitro study). Egyptian Dental Journal, 69(2), 1687-1693.
26. López-García, S., Myong-Hyun, B., Lozano, A., García-Bernal, D., Forner, L., Llena, C., ... & Rodríguez-Lozano, F. J. (2020). Cytocompatibility, bioactivity potential, and ion release of three premixed calcium silicate-based sealers. Clinical oral investigations, 24, 1749-1759.
27. Poggio, C., Riva, P., Chiesa, M., Colombo, M., & Pietrocola, G. (2017). Comparative cytotoxicity evaluation of eight root canal sealers. Journal of clinical and experimental dentistry, 9(4), e574.
28. Willershausen, I., Wolf, T., Kasaj, A., Weyer, V., Willershausen, B., & Marroquin, B. B. (2013). Influence of a bioceramic root end material and mineral trioxide aggregates on fibroblasts and osteoblasts. Archives of oral biology, 58(9), 1232-1237.
29. Yadav S., Jhingran R., Srivastava R., Madan R.: Role of fibroblast in periodontal health and disease: an overview. Asian J Oral Health Allied Sc., 7 : 22-31, 2017.
30. Sebastian, Sabatiny , et al. Evaluation of the antibacterial and cytotoxic properties of TotalFill and NeoSEALER flo bioceramic sealers. Journal of Conservative Dentistry and Endodontics 27(5):p 491-497, May 2024. | DOI: 10.4103/JCDE.JCDE_103_24
31. De-Deus G Canabarro A Alves G Linhares A Senne MI Granjeiro JM Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. J Endod 200935101387–1390
32. Mann, A. et al. Evaluation of the physicochemical and biological properties of EndoSequence BC Sealer HiFlow. J. Endod. 48, 123–131.(2022)
33. López-García, S and et al., (2024). Premixed calcium silicate-based ceramic sealers promote osteogenic/cementogenic differentiation of human periodontal

ligament stem cells: A microscopy study. Microscopy Research and Technique, 87(7), 1584–1597.

34. Szczurko, G., Pawińska, M., Łuczaj-Cepowicz, E., Kierklo, A., Marczuk-Kolada, G., & Hołownia, A. (2018). Effect of root canal sealers on human periodontal ligament fibroblast viability: ex vivo study. Odontology, 106, 245-256.

35. Marques, E. F., da Silva Benigno, M. B., Macedo, C. P., & Bitencourt, L. (2020). Cytotoxicity and genotoxicity analysis of two endodontic cements in human fibroblast culture in vitro. Int J Adv Eng Res Sci, 7, 103-108.

36. Mestieri, Letícia Boldrin, et al. "Cytocompatibility and cell proliferation evaluation of calcium phosphate-based root canal sealers." Restorative Dentistry & Endodontics 45.1.(2020)

37. Rodríguez-Lozano FJ,et al, Evaluation of cytocompatibility of calcium silicate-based endodontic sealers and their effects on the biological responses of mesenchymal dental stem cells. Int Endod J. 2017; 50:67–76.

38. Collado-González M, Tomás-Catalá CJ, Oñate-Sánchez RE, Moraleda JM, Rodríguez-Lozano FJ. Cytotoxicity of Guttaflow Bioseal, Guttaflow 2, MTA Fillapex, and AH Plus on human periodontal ligament stem cells. J Endod. 2017; 43:816–822.

39. Borges RP, Sousa-Neto MD, Versiani MA, Rached-Júnior FA, De-Deus G, Miranda CE, Pécora JD. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. Int Endod J. 2012; 45:419–428.

40. Pawinska M, et al, Cytotoxicity and Oxidative Stress Induction by Root Canal Sealers in Human Periodontal Ligament Fibroblasts: An in vitro Study. Iran Endod J. 2021 Summer;16(3):164-175. doi: 10.22037/iej.v16i3.29449. PMID: 36704398; PMCID: PMC9735252.

41. Andrade, Leonardo Lemos, et al. "Evaluation of the cytotoxicity of two endodontic cements based on calcium silicate and Pulp Canal Sealer cement in human fibroblasts." Cell 99349218 (2020): 0000-5374.

42. Loushine, B. A., Bryan, T. E., Looney, S. W., Gillen, B. M., Loushine, R. J., Weller, R. N., ... & Tay, F. R. (2011). Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. Journal of Endodontics, 37(5), 673-677.

43. Pascon EA, Spångberg LS. In vitro cytotoxicity of root canal filling materials: 1 Gutta-percha. J Endod. 1990;16(9):429–33.

44. Lee JK, Kim S, Lee S, Kim HC, Kim E. In vitro comparison of biocompatibility of calcium silicate-based root canal sealers. Materials (Basel) 2019; 12:2411.

45. Zamparini F, Prati C, Taddei P, Spinelli A, Di Foggia M, Gandolfi MG. Chemical-physical properties and bioactivity of new premixed calcium silicate-bioceramic root canal sealers. Int J Mol Sci 2022; 23:13914.
46. Almeida MM, Rodrigues CT, Matos AA, Carvalho KK, Silva EJ, Duarte MA, et al. Analysis of the physicochemical properties, cytotoxicity and volumetric changes of AH plus, MTA fillapex and TotalFill BC sealer. J Clin Exp Dent 2020;12:e1058–65.
47. Janini, A. C. P. et al. In vitro evaluation of cytotoxicity in human osteoblastic cells and antimicrobial activity in the biofilm of different root canal sealers. Res. Soc. Dev. 11, e430111032842.(2022)
48. Shokrzadeh, M., Motafeghi, F. S., Lotfizadeh, A., Ghorbani, M. & Haddadi Kohsar, A. Cytotoxicity and mineralization activity of calcium silicate-based root canal sealers compared to conventional resin-based sealer in human gingival fibroblast cells. Int. J. Dent. 2023, 4376579.(2023)
49. Jagtap P, Shetty R, Agarwalla A, Wani P, Bhargava K, Martande S. Comparative Evaluation of Cytotoxicity of Root Canal Sealers on Cultured Human Periodontal Fibroblasts: In vitro Study. J Contemp Dent Pract 2018; 19:847–852. PMID: 30066690.

تأثير ســـدادات قناة الجذر المعتمدة على الســـيراميك الحيوي على حيوية الخلية والســـمية الخلوية في خطوط الخلايا الليفية في أربطة اللثة البشرية

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الملخص

الأهداف: تهدف الدراسة الى تقييم حيوية الخلية والسمية الخلوية لسدادات قناة الجذر المعتمدة على السيراميك الحيوي ومقارنتها مع سدادات قناة الجذر المعتمدة على اوكسيد الزنك والأوجينول على الخلايا الليفية في أربطة اللثة البشرية على مدى يوم واحد ،وثلاثة أيام ، وسبعة أيام. **المواد وطرائق العمل**: تم تحضير سدادات قناة الجذر وفقاً لتعليمات الشركة المصنعة ، ووضعها في قوالب مطاطية إسطوانية (قطرها 5 ملم وإرتفاعها 2 ملم) للحصول على عينات متطابقة ، وتم تخزين العينات لمدة 24 ساعة عند 37 درجة مئوية ، وتم تحضين الخلايا الليفية في أربطة اللثة البشرية باستخدام مستخلصات سدادات قناة الجذر التي تم إختبارها. تم تقييم حيوية الخلية والسمية الخلوية لمستخلصات يوم واحد ،وثلاثة أيام ،وسبعة أيام وفقاً لوقت الغمر ، وتم تحديد حيوية الخلية لجميع سدادات قناة الجذر باستخدام مقايسة MTT ، وتم تحديد السمية الخلوية باستخدام مقايسة LDH . تم تحليل البيانات إحصائياً باستخدام تحليل التباين أحادي الاتجاه ANOVA متبوعا باختبار Duncan لايجاد الفروق وبمستوى إحتمالية $p \leq 0.05$. **النتائج:** كان هناك فرق ذو دلالة إحصائية في حيوية الخلية والسمية الخلوية التي أظهرتها كل مادة تم إختبارها على فترات زمنية مختلفة .أظهرت مادة NeoSEALER flo أعلى نسبة حيوية للخلية ونسب سمية أقل للخلايا تليها كلاً من Cerafill RCS , MTA fillapex , Endovit على التوالي في كل فترة زمنية للتقييم. **الاستنتاجات**: كانت مادة NeoSEALER flo هي الأقل سمية للخلايا ، بينما كانت مادة Endovit هي الأكثر سمية للخلايا ،وتقل حيوية الخلية مع مرور الوقت.