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CYTOTOXICITY OF DIFFERENT BIOCERAMIC SEALERS

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ABSTRACT

Aim: This study seeks to assess the cytotoxic impact of various bioceramic sealers, namely Well-Root ST, CeraSeal, and NeoSEALER Flo, in comparison to AH Plus sealer. The evaluation encompasses both the fresh and set states of these sealers, employing the MTT assay on human periodontal ligament fibroblasts.

Materials and methods: Following the manufacturers' instructions, the four tested sealers were blended in a sterile environment and then placed into standardized plastic rings. Extracts derived from the tested sealers were applied to human periodontal ligament fibroblasts. The freshly mixed sealers were examined immediately after mixing, with their extracts prepared at that moment. On the other hand, the remaining specimens, designated as set specimens, were incubated in a humidified environment with 5% CO2 and 95% air at 37º C for 24 hours before extraction to create extracts of the tested sealers. The extracted material was then diluted with DMEM to achieve twelve distinct concentrations of each extract 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.4%, 0.2%, 0.1%, 0.05%, and 0.025%. The cytotoxicity of all root canal sealers was evaluated using the MTT assay, followed by the calculation of cell viability percentages.

Results: AH Plus showed the highest toxicity followed by the NeoSEALER Flo then Well-Root ST and the least toxicity was CeraSeal.

Conclusion: The assessed root canal sealers exhibited differing levels of cytotoxicity, and the rise in cell viability percentages was contingent on the concentration.

KEYWORDS: Cytotoxicity, AH Plus, biocompatibility, CeraSeal, Well-Root St, NeoSEALER Flo.

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INTRODUCTION

Following root canal preparation, it is imperative to occupy the space originally filled by the dental pulp.[1] Inadequate filling of the space may lead to the development of resilient microorganisms, the persistence of infection, or the infiltration of the root canal and periapical tissues, resulting in a secondary infection.[1,2] Prolonged contact between periradicular tissues and sealers or their components can lead to irritation and potentially contribute to delayed wound healing. Moreover, excess sealer that extends beyond the intended area can directly interact with surrounding tissues.

The extrusion of sealer into periradicular tissue can cause significant irritation. Therefore, ensuring the effective sealing of the root canal is crucial for the long-term success of endodontic treatment.[3] To achieve this goal, endodontic sealers are employed in root canal filling and must possess characteristics that enable effective sealing, including dimensional stability, low solubility, and appropriate flow. [2,4] Additionally, the sealer should exhibit biocompatibility and be well-tolerated by periradicular tissues. When freshly mixed sealers are applied in the root canal, they should promptly engage in local elution processes because of their interaction with extracellular fluids. The impact of eluents on periradicular tissue depends on both concentration and time, influencing bone metabolism and regeneration. However, current literature asserts that, to date, all types of root canal sealers show toxicity when freshly mixed. Nevertheless, upon setting, their toxicity significantly diminishes, and the majority of sealers become comparatively inactive. Epoxy resin-based sealers are considered as the gold standard sealers in endodontics.[5] Nevertheless, they still exhibit a certain degree of cytotoxicity.[1] Calcium silicate-based sealers emerge as a viable alternative, demonstrating physicochemical properties similar to epoxy resin sealers [6,7] with the potential for improved biological properties.

Various formulations of calcium silicate-based sealers have recently been brought to the market, with ongoing assessments of their properties to establish their clinical safety. So, in this study we compared the cytotoxicity of some bioceramic sealers with AH Plus sealer.

MATERIALS AND METHODS

Human periodontal ligament fibroblasts

Human periodontal ligament fibroblasts (HPDLF) (Cat. No. ABC-TC3750, Accegen Biotechnology, New Jersey, USA) were employed in this investigation. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Thermo Fisher Scientific, USA) enhanced with 10% fetal bovine serum (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA) in a humidified environment with 5% CO2 at 37°C (Jouan SA, Saint-Herblain, Pays de la Loire, France). Cells were kept in accordance with the manufacturer's protocol, involving the removal of the growth medium and subsequent washing of the cells with phosphate-buffered saline. (Adwia Pharmaceuticals, El Sharkeya, Egypt). Cells underwent treatment with 0.25% trypsin enzyme and 0.05% (v/v) EDTA (GIBCO) for 5 minutes at 37°C. Detached cells were dispersed as required.

Sealers

The experiment was performed using 4 endodontic sealers: NeoSEALER Flo (Avalon Biomed, Houston, TX USA), Well Root ST (VERICOM, *South Korea*), CeraSeal (META BIOMED, Korea), and AH plus sealer (Dentsply DeTrey, Konstanz, Germany).

NeoSEALER® Flo is a bioactive bioceramic root canal sealer known for its excellent handling characteristics. It facilitates the formation of hydroxyapatite, promoting the healing process. In contrast to traditional sealers, NeoSEALER Flo is biocompatible, possesses antimicrobial properties, is dimensionally stable, and is entirely resin-free.

Well Root ST is a pre-mixed, ready-to-use injectable bioactive calcium silicate paste designed for long-term sealing of the root canal. Formulated with a calcium silicate composition, it relies on the existence of water for setting and hardening. Importantly, it does not undergo shrinkage during the setting process and exhibits outstanding physical properties.

CeraSeal is a bioceramic root canal sealer based on calcium silicate. Offering an ideal biocompatible environment for tissues within the root canal, CeraSeal stands out as the next generation bioceramic sealer, renowned for its exceptional sealing ability and biocompatibility.

AH Plus is a traditional root canal sealer based on epoxy resin, extensively utilized and thoroughly researched.

The sealers were prepared following the guidelines provided by the manufacturers, maintaining aseptic conditions. Subsequently, they were placed into standardized plastic rings with a diameter and height both measuring 5 mm for creating uniform sealer specimens with comparable volumes.

One set of specimens, comprised of freshly mixed sealers, underwent testing immediately after mixing, with extracts prepared at that moment. Meanwhile, the remaining specimens, classified as set specimens, were placed in a humidified environment with 5% CO2 (pH regulator) and 95% air atmosphere for 24 hours at 37°C. (Szczurko G, 2018)

Preparation of extracts from the tested sealers involved immersing them in Dulbecco's Modified Eagle's Medium (DMEM) cell culture within 24-well plates, maintaining a surface area-to-volume ratio of around 150mm^2/ml between specimen surfaces and the medium volume. Subsequently, the plates were incubated in darkness at 37°C for one week.

Cell viability evaluation (MTT Assay)

Cells were initially placed in 96-well cell culture plates at a concentration of 2 × 10^5 cells/ml and incubated at 37°C for 24 hours to attain confluence. The growth medium was removed and fresh medium containing two-fold serially diluted sealer extracts added o the pre-cultured plate of human periodontal ligament fibroblasts (HPDLF) After 24 hours, the removal of dead cells was carried out by washing with phosphate-buffered saline (PBS, pH = 7.2±0.2), followed by the addition of 50 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) stock solution at a concentration of 0.5 mg/ml per well..

After incubating for four hours at 37°C, the supernatant was removed, and the formazan precipitate was dissolved by adding 50 µl/well of dimethyl sulfoxide(DMSO).

The plates were subjected to a 30-minute incubation in the dark at 37°C, and the absorbance was measured at a wavelength of 570 nm using a microplate reader (ELx-800, Bio-Tek Instruments, Inc, Winooski, VT, USA). The cell viability percentage was computed using the formula:

Viability percentage (%) = Mean OD of test dilution × 100/Mean OD of control wells.

The IC50 value was calculated using GraphPad Prism software (v.6, GraphPad Software, La Jolla, CA, USA).

RESULTS

From the presented data, it clearly indicated that the viability percentage was influenced by both concentration and composite, as viability increased when the concentration decreased. Additionally, the IC50 values was composite dependent where AH Plus showed the highest toxicity; lowest IC50 (2.28 μgm/ml) followed by NeoSealer Flo. (5.43 μgm/ml), well-Root ST(9.56 μgm/ml) and CeraSeal (21.19 μgm/ml). Similarly, Well-Root ST showed a significant ($P<0.05$) decreased IC50 value (9.56 μgm/ml) compared with CeraSeal (21.19 μgm/ml). The IC50 of both NeoSealer Flo and AH Plus was significantly ($P<0.05$) decreased than that of Well-Root ST and CeraSeal.

TABLE (1) Evaluation of the minimum inhibitory concentration of 50% viable cells

Cone mg/ ml	Ah plus	Neosealer	wellroot	Ceraseal
50	18.33	10.74	16.25	39.59
25	21.46	24.26	49.79	58.99
12.5	34.58	45.74	65.35	69.32
6.25	49.31	57.50	80.56	95.81
3.12	68.33	71.42	101.39	98.29
1.56	87.36	78.38	101.18	97.36
0.78	101.88	101.08	101.88	97.43
0.4	101.94	100.47	101.94	99.39
0.2	101.32	100.41	101.60	100.41
0.1	101.11	100.81	101.74	100.00
0.05	101.39	101.76	101.46	101.55
0.025	101.53	101.01	100.33	100.27

	Ah plus	Neosealer	wellroot	Ceraseal
IC50	2.28	5.43	9.56	21.19

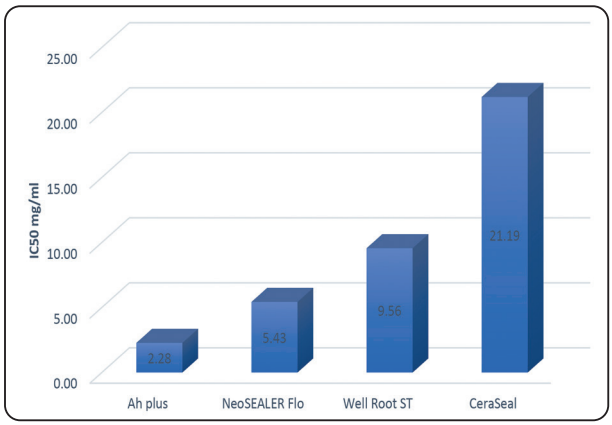

Fig. (1) Evaluation of the minimum inhibitory concentration of 50% viable cells. The IC50 values were root canal sealer dependent.

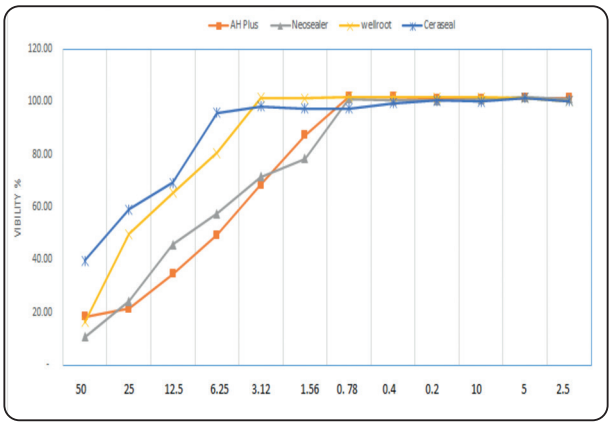

Fig. (2) Evaluation of cytotoxic effect / Viability % of different dental sealers extract against T concentration. Viability % was concentration and Sealer dependent.

DISCUSSION

Achieving a fluid-tight seal in the root canal through 3-dimensional obturation of the pulp space is crucial for the success of root canal treatment. Root canal sealers are intended to remain within the root canal; however, there is a risk of inadvertent extrusion into periradicular tissue, potentially leading to tissue irritation and delayed healing.[8] Therefore, root canal sealers must exhibit biocompatibility, Since they may closely interact with periapical tissues over an extended period, root canal sealers can have direct contact and undergo gradual degradation. This process may lead to cytotoxic damage to cells and tissues, potentially affecting the overall success of root canal treatment.[9,10]

Several sealers are currently available for use in conjunction with gutta-percha. The preferred choices among these are sealers with adhesive properties and modern bioceramic sealers. Bioceramic sealers offer numerous advantages compared to other root

canal sealers, such as improved biocompatibility, enhanced root strength after obturation, potent antibacterial properties, non-toxicity, bioinert, bioactive, or biodegradable characteristics, ease of application, and excellent sealing properties.[11]

Different cell lines have been utilized for cytotoxic assessments of endodontic sealers. Nevertheless, this study specifically opted for fibroblast cells, as they are regarded as the predominant cell type in the periodontal ligament.[12] Fibroblasts have a significant role in the function and regeneration of periodontal connective tissues. Furthermore, human periodontal ligament fibroblast cell line serves as a precise emulation of the clinical context. This cell line replicates the actual clinical scenario in which sealers may come into contact with these cells.

The MTT assay is a standardized technique for evaluating its impact on cell viability by measuring the conversion of yellow water-soluble methylthiazol tetrazolium salt (MTT) to insoluble dark blue formazan crystals within the mitochondria of living cells. Necrotic and dead cells cannot release the colored formazan so, this MTT assay is used to accurately differentiate between dead and viable cells. Subsequently, the formazan can be quantified spectrophotometrically by measuring at a specific wavelength (500–600 nm). The MTT cell proliferation assay gauges the rate of cell proliferation, and conversely, when metabolic events result in apoptosis or necrosis, cell viability decreases. An increase in cell number correlates with a higher quantity of MTT formazan produced, resulting in an elevation in absorbance.[13]

AH Plus exhibited elevated cytotoxicity which is attributed to the existence of formaldehyde and Bisphenol A in AH Plus, along with epoxy resins, all of which have cytotoxic profiles.[14] The cytotoxicity associated with resin-based materials is often linked to the release of un-polymerized monomers due to incomplete polymerization.[15] The epoxy resin found in AH Plus exhibits mutagenic properties and can potentially induce breaks in the chain of cellular DNA, leading to cell death.[16] This observation aligns with earlier studies that have reported the cytotoxic effects associated with AH Plus.[16,17] While freshly prepared AH Plus displayed strong cytotoxicity that gradually diminished over extended time intervals, it sustained a significant impact on cell viability even after the sealer had fully set. This finding aligns with Silva Enjl et al.'s research [18,19] which also reported heightened cytotoxicity levels associated with AH Plus. Additionally, this corresponds with the observations of Deniz et al.[20] and is consistent with the conclusions of Prati and Gandofli[21], who noted that calcium silicate-based sealers elicit appropriate biological responses.

NeoSEALER Flo showed lower cytotoxicity when compared with AH Plus and this was in accordance other study.[22]

Also, in this study Well-Root ST show low cytotoxicity and this in accordance with other study which showed that Well-Root ST is less cytotoxic than AH Plus. [23]

The findings of this study align with research conducted by López-García et al.[24], indicating that CeraSeal exhibited superior outcomes in terms of cell viability, cell attachment, cell migration rates, and ion release rates. Additionally, other studies by Oh et al.[25] assessing the levels of TGF-β, an anti-inflammatory cytokine, revealed that CeraSeal consistently demonstrated higher TGF-β levels compared to AH plus, supporting the notion of CeraSeal excellent cell viability and biocompatibility.

The minimal cytotoxicity exhibited by bioceramic sealers can be ascribed to the release of calcium ions from the bioceramic materials, potentially contributing to cell viability. Conversely, the cytotoxic effects observed with AH Plus in this study align with prior findings[26,27]. This cytotoxicity could be linked to the emission of formaldehyde resulting from the amines added to expedite epoxy resin polymerization and the presence of bisphenol A, a substance known for its toxicity.[28]

In comparison to the reference standard AH Plus, calcium silicate sealers generally exhibit minimal cytotoxic effects. The calcium silicate materials undergo a hydration reaction, as described by Camilleri in 2007:

$2(3CaO{\times}SiO_2) + 6H_2O$ ® 3CaO×2SiO2×3H2O + 3Ca (OH)2

$2(2CaO{\times}SiO_2) + 4H_2O$ ® 3CaO×2SiO2×3H2O + Ca (OH)2

This reaction produces Ca (OH)2, which significantly raises the pH to 12.5.[29] In acidic conditions, osteoblasts have demonstrated pH-related cell death and increased expression of pro-inflammatory cytokines .[30]This phenomenon may elucidate the reduced cytotoxic effects observed with calcium silicate sealers. Studies have indicated that the pH of AH Plus is more acidic than that of calcium silicate sealers.[31,32,33]

CONCLUSIONS

Based on the findings of this study

1- In terms of cytotoxicity, CeraSeal demonstrated the lowest cytotoxicity, whereas AH Plus exhibited the highest cytotoxicity among the root canal sealers.

2- The rise in percentages of cell viability presented by all tested sealers was concentration dependent.

Limitations in this study

The current study has limitations as it did not investigate the influence of sealers on the success rate of endodontic treatment when interacting with periapical tissues. Consequently, future research is essential to ascertain whether the reduced cytotoxicity of calcium silicate-based sealers might yield more favorable clinical outcomes compared to epoxy resin-based sealers.

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