Original Article

Evaluation of cytotoxicity and adaptability of a novel bioceramic root canal sealer: An *in vitro* and scanning electron microscope study

Soha Alaa Emam, Abeer Hashem Mahran[1], Marwa Mokbel Elshafei

Department of Endodontic, Faculty of Dentistry, Misr International University, [1]Department of Endodontic, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

Abstract

Context: Cytotoxicity and adaptability are among the highly imperative tests that should be performed on a novel endodontic material to ensure its successful implementation in endodontic treatment.

Aims: Assess a recently introduced bioceramic root canal sealer CeramoSeal with TotalFill BC and AH plus sealers regarding the cytotoxicity and adaptability.

Materials and Methods: Five sealer discs were prepared for each sealer and their extracts were cultured in 96-well plates containing human fibroblasts for 24 h. After their incubation, MTT solution was added to each well plate using an enzyme-linked immunosorbent assay plate reader was implemented to calculate the percentage of viable cells. Thirty mandibular single-rooted premolars were prepared using the Edge Endo rotary system, teeth were divided into three groups ($n = 10$) based on the sealer type: Group 1 CeramoSeal, Group 2 Totalfill, and Group 3 AH plus sealer. Teeth were sectioned longitudinally and viewed under a scanning electron microscope where the region with the gaps was identified and quantified as a percentage of the root canal's overall area.

Statistical Analysis: One-way ANOVA test was used for cytotoxicity, while Kruskal–Wallis and Friedman's tests were used for adaptability.

Results: Ceramoseal statistically significantly showed the lowest viability, at high concentrations AH plus showed the highest cell viability, while at lower concentration Totalfill BC sealer showed the highest cell viability percentage. The gap percentages were statistically significantly higher in Ceramoseal group, there was no statistically significant difference between AH Plus and Totalfill groups.

Conclusions: Ceramoseal sealer exhibited the lowest viability and highest gap percentage compared to the other sealers.

Keywords: Adaptability; bioceramics; calcium silicates; cytotoxicity; scanning electron microscope

INTRODUCTION

Root canal sealers supplement the root canal obturating material by sealing all the root canal complexities and irregularities, hence effectively depriving any residual bacteria of the nutrients required for flourishing.[1] At present, a new calcium silicate-based endodontic sealer has been introduced which according to the manufacturer can be successfully used in root canal obturation. Therefore, assessing the physical and biological properties of such bioceramic root canal sealers would be of great value.

Although endodontic sealers should be confined within the root canal space, unintentional extrusion of the

Address for correspondence:
Dr. Soha Alaa Emam,
Villa 89 District 1, Fifth Settlement, Cairo, Egypt.
E-mail: soha.emam@miuegypt.edu.eg

Date of submission : 20.01.2024
Review completed : 08.02.2024
Date of acceptance : 12.02.2024
Published : 06.03.2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcde

DOI: 10.4103/JCDE.JCDE_40_24

How to cite this article: Emam SA, Mahran AH, Elshafei MM. Evaluation of cytotoxicity and adaptability of a novel bioceramic root canal sealer: An *in vitro* and scanning electron microscope study. J Conserv Dent Endod 2024;27:326-30.

endodontic sealer beyond the apical constriction could occur.[2] The components of these sealers can behave as toxins, leading to periapical tissue damage.[3] All freshly mixed sealers exhibit some degree of cytotoxicity which on setting is markedly reduced until some sealers end up being inert.[4] This means that cytotoxicity tests are prudent when assessing new sealers.

The obturation method employed, along with the physical and chemical characteristics of the sealer, have a tremendous impact on how well the root canal sealer adapts to the dentinal walls and the gutta percha.[5,6] Therefore, proper evaluation of the adaptability of endodontic sealers is of utmost importance. Scanning electron microscopy offers advantages over other testing techniques, including the ability to view submicron defects at the required magnification and save microphotographs for final assessment.[7]

In the current study, Totalfill BC sealer was chosen as a comparator since it is also a premixed calcium silicate-based sealer with the capacity to release calcium ions and exhibits good physical and biological qualities.[8] The epoxy resin-based sealer AH plus was also selected because, for the past two decades, it has been regarded as the gold standard root canal sealer.[9] The hypothesis of the current study is that the Ceramoseal sealer has higher cytotoxicity value and gap percentages compared to the AH plus and Totalfill BC sealers.

MATERIALS AND METHODS

Materials

Three sealers have been selected for the current study; Ceramoseal Bioceramic sealer (DM Trust, Minya, Egypt) which is a bioceramic-based single-paste hydrophilic root canal sealer. It is composed of silicon dioxide, aluminum oxide calcium oxides, titanium dioxide, barium sulfate, calcium sulfate, and calcium carbonate in special particle size distribution of Nano and micro size. The other two sealers were Totalfill BC sealer (FKG, La Chaux-de-Fond, Switzerland) and AH plus epoxy resin sealer (Dentsply, New York, USA).

Methods

Cytotoxicity

Ninety-six well plates of human fibroblasts were cultured and maintained in a humid condition of 5% CO2, 95% air at 37°C for 24 h. Full concentration (100%), 50% and 25%, 12.5% and 6.25% diluted eluates from five sealer discs of each sealer were attained after immersing them in culture medium for 24 h and subsequently incubated in the cell culture well plates for 24 h. Following the removal of the media, the cells were washed with phosphate-buffered saline and the MTT solution[10] (4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue) was added to each well plate. Control samples containing medium alone were incubated similarly. Using an enzyme-linked immunosorbent assay plate reader, optical densities were determined at 570 nm. All assays were repeated three times to ensure reproducibility.

The following formula was used to get the percentage of viable cells: (A/B) × 100 = percentage of viable cells. Where A represents viable cells in the experimental wells and B represents viable cells in the control well. Less than 30% of cell viability is regarded as severely cytotoxic, 60%–90% as somewhat cytotoxic, 30%–59% as moderately cytotoxic, and more than 90% as noncytotoxic.[11]

Adaptability

Thirty mandibular single-rooted premolars were collected from the Ain Shams University teeth bank, after the acceptance of the Faculty of Dentistry Ain Shams University Research Ethical Committee with ethical number (FDASU-REC 105).[12] Each tooth was subjected to mesiodistal and buccolingual radiographs to confirm the inclusion criteria which were the presence of a single canal and a well-developed apex. The exclusion criteria were caries, previous restorations, calcifications, sharp curvatures, or resorption. The teeth were divided into three groups (n = 10) based on the sealer implemented in the obturation: Group 1 CeramoSeal, Group 2 Totalfill, and Group 3 AH plus sealer. All teeth were negotiated with a stainless steel endodontic #10 K file (Mani, Tochigi, Japan) and the entire root length was recorded followed by a subtraction of 1 mm to obtain the working length. Instrumentation was performed by Edge Endo rotary file system (Henry Schein, Albuquerque, USA) till file size 30/0.06.

Using 5 mL of 5.25% sodium hypochlorite (NaOCl), canals were irrigated during their instrumentation with a side vented needle followed by 3 mL of 17% EDTA for 1 min to eliminate the smear layer's inorganic portion, followed by 3 mL of 5.25% NaOCl for 1 min to eliminate the organic portion of the smear layer. One last rinse of 5 mL of saline for 1 min was performed. Samples were obturated using gutta-percha cone size 30/0.06 (Diadent, Gyeonggi-do, South Korea) using single-cone obturation technique then 4 mL of Coltosol F temporary filling (Coltene, Switzerland) was placed to provide a proper coronal seal. Radiographs were taken for each tooth in a mesiodistal and a buccolingual direction to confirm the absence of any voids. Samples were kept in an incubator at 37°C and 100% humidity. To ensure complete setting of the sealer for 10 days.[13] On an Isomet machine (Buehler. Illinois, USA), samples were longitudinally sectioned in a buccolingual direction using a water-cooled diamond blade. Marginal gap adaptation at the sealer and root dentin interface was evaluated using a Scanning Electron Microscope (SEM) (Quanta 250, FEI, The Netherlands) with a magnification of ×500. The samples

were mounted on an aluminum stub, gold sputter coated and viewed under SEM at the coronal, middle, and apical thirds. Each canal third was examined using Image J image analysis software (LOCI, University of Wisconsin, USA), and the gap percentage was measured at the sealer-dentin interface. Using color code thresholding, the gap region was highlighted. Before calculation, a binary image representing the required gap area was created. After calculating the gap area was fractionated as a percentage of its overall area[14] [Figure 1].

One-way ANOVA test was used for cytotoxicity, while Kruskal–Wallis and Friedman's tests were used for adaptability.

RESULTS

Cytotoxicity

As regards the concentrations 100, 50, and 25; a statistically significant distinction existed between the groups ($P < 0.001$, effect size = 0.957), ($P < 0.001$, effect size = 0.948), and ($P < 0.001$, effect size = 0.961), respectively. When the groups were compared pairwise, the Ceramoseal exhibited the statistically substantially lowest viability percentage and AH Plus showed the statistically significantly highest viability. Total Fill exhibited a significantly lower percentage compared to the AH plus [Figure 2].

For concentrations 12.5 and 6.25, there was a statistically significant difference between the groups ($P < 0.001$, effect size = 0.975) and ($P < 0.001$, effect size = 0.966), respectively. Pair-wise comparisons between the groups revealed that the Ceramoseal group showed the statistically significantly lowest viability. AH Plus showed statistically significantly lower values compared to the total fill which showed the statistically significantly highest viability percentage [Figure 2].

Adaptability

Pair-wise comparisons between the groups revealed that a statistically significant difference was seen among the groups at the coronal, middle, and apical levels, where the Ceramoseal showed the statistically significantly highest gap percentage. Between AH Plus and Total Fill, there was no statistically significant variance; both displayed statistically significantly smaller gap percentages [Table 1].

DISCUSSION

Accurate assessment of the biocompatibility as well as the physical properties of any novel endodontic sealer is of prime importance to successfully and safely implement it in clinical practice. The hypothesis of the current study was fulfilled as Ceramoseal was found to have statistically significantly higher cytotoxicity and gap percentage values compared to the other groups. In cytotoxicity investigations, extracts from endodontic sealers are diluted to varying degrees. The dilution is warranted because the leachable substances gradually lose concentration as the material comes into contact with tissue due to the extracellular fluids present in the periapical tissues.[15] The Ceramoseal

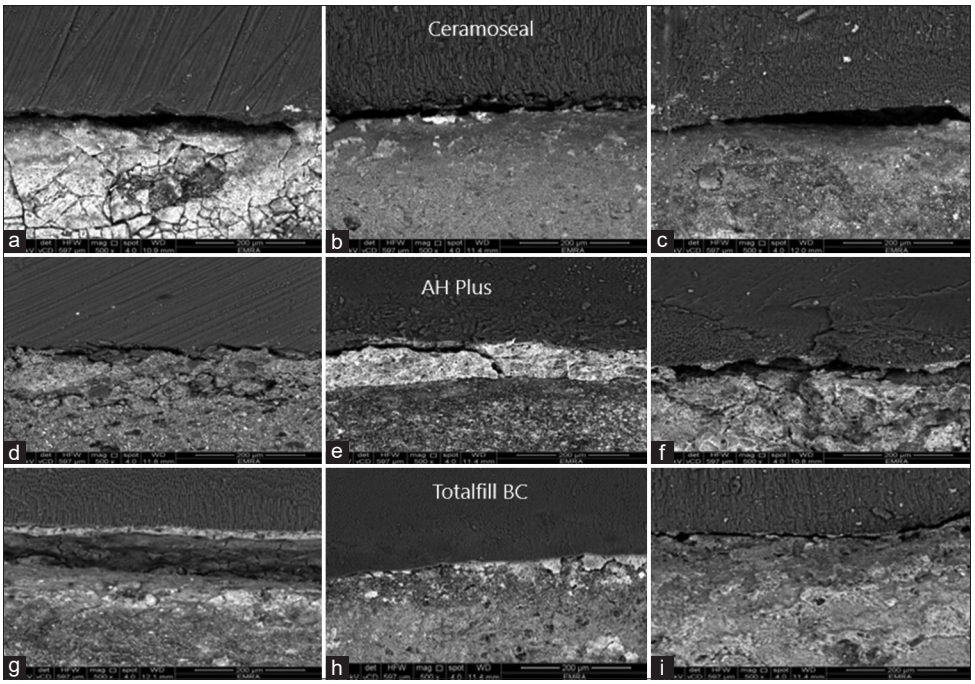

Figure 1: Scanning electron microscope images at magnification ×500 representing Ceramoseal at (a) coronal, (b) middle and (c) apical third, AH plus at (d) coronal, (e) middle and (f) apical third and Totalfill at (g) coronal, (h) middle, and (i) apical third

Table 1: Descriptive statistics Kruskal–Wallis test for comparison between gap percentages in the three groups

Root level	Ceramoseal		AH plus		Total fill		*P*	Effect size (Eta squared)
	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Median (range)	Mean (SD)		
Coronal	5.24^{A} (4.45–7.15)	5.492 (1.019)	0.001^{B} (0–0.002)	0.002 (0.003)	0.002^{B} (0.001–0.005)	0.002 (0.002)	0.007*	0.960
Middle	2.4^{A} (1.25–3.42)	2.456 (0.828)	0.338^{B} (0.267–0.524)	0.36 (0.096)	0.289^{B} (0.212–0.341)	0.291 (0.05)	0.007*	0.844
Apical	4.81^{A} (3.41–6.25)	4.814 (1.026)	0.913^{B} (0.714–1.25)	0.93 (0.197)	0.841^{B} (0.726–0.993)	0.846 (0.095)	0.007*	0.921

*Significant at $P \leq 0.05$. ^{A,B}Pair-wise comparison of the median of the coronal, middle, and apical one-thirds between the three sealer groups using different superscripts in the same row indicating statistically significant difference. SD: Standard deviation

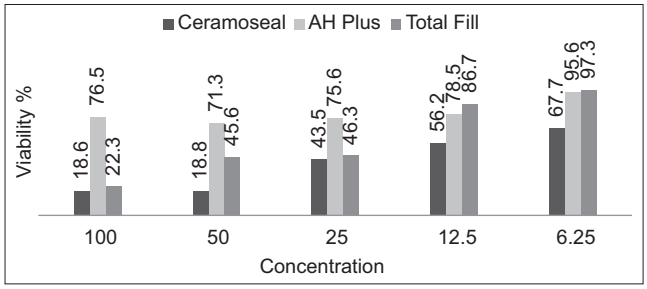

Figure 2: Bar chart representing mean values for percentage of viable cells in different groups

showed significantly the highest cytotoxic values followed by the Totallfill and the lowest was the AH Plus sealer at the 100, 50, and 25 dilution percentages. The higher cytotoxic effect of high concentrations of the calcium silicate-based sealers compared to the AH plus sealer could be attributed to their higher solubility rate. Totalfill has been found in the literature to be more soluble than the AH plus sealer.[16-18] Also, the highly alkaline pH medium created by the bioceramic sealers can also attribute to its cytotoxic effect on the fibroblasts.[19] Raising the pH level can cause damage to the cytoplasmic membrane, DNA, and proteins, which can lead to the death of bacteria as well as the host cells.[20] Results of the current study are consistent with Loushine *et al*. who found that at the 24-h period, the cytotoxicity of Endosequence BC sealer was greater compared to AH Plus and justified that by the more extended final setting time of the bioceramic sealer that could lead to the components leaching over a prolonged period and negatively influence cell viability.[21] A portion of the toxicity of the AH plus sealer can be explained by the resin's polymerization process and the presence of the epoxy resin itself as a component.[22] At lower concentrations of 12.5 and 6.25, the Totalfill BC sealer showed the least cytotoxic effect and highest viability followed by AH plus and finally, the Ceramoseal showed the least viability and highest cytotoxicity. López García *et al*.[23] and Malta *et al*.[24] accredited the compatibility of the Totalfill BC sealer to the elution of the calcium ions and the proprietary additives in the bioceramic paste that play a vital role in the improved biocompatibility of the material.

Several factors that integrate together can ultimately determine the degree of adhesion of the filling materials to dentine. These factors are the intermolecular surface energy and cleanliness of the adherent which is the dentin, the surface tension and wetting capacity of the adhesive which is the sealer.[25] These factors affect the degree of a material's dentinal tubule penetration.[26] The present study revealed that Ceramoseal showed the statistically significantly highest gap percentage. AH Plus and Total Fill did not differ statistically significantly, they both presented statistically significantly lower gap percentages. The superior adaptability of the Totalfill BC sealer is clarified by the hydration products of the calcium silicate sealer's alkaline caustic effect, which has been shown to break down the interfacial dentin's collagenous component. This could therefore make it easier for sealers to enter the dentinal tubules,[27] which is also in agreement with Patri *et al*.[13] The AH Plus sealer showed favorable adaptability results due to its chemical bonding to root dentin, the sealer forms covalent bonds between the collagen of the dentin and epoxy resin.[24] When comparing the root thirds, AH plus and Totalfill sealers demonstrated larger gaps at the apical level compared to the coronal level. The reduced diameter as well as the density of the dentinal tubules at the apical region justifies the disparity. The oval shape of the premolar root canal may be a contributing factor to the higher mean gaps at the apical region.[28]

CONCLUSIONS

1. Ceramoseal bioceramic sealer showed significantly the lowest number of viable cells with the highest cytotoxicity values at different concentrations. At higher concentrations, AH plus showed the highest cell viability, while at lower concentrations, Totalfill BC sealer showed the highest cell viability percentage
2. Ceramoseal showed statistically significantly the highest gap percentage. Between AH Plus and Total Fill sealers, there was no statistically significant variation in adaptability.

Financial support and sponsorship
Self-funded.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Baras BH, Melo MA, Thumbigere Math V, Tay FR, Fouad AF, Oates TW, *et al.* Novel bioactive and therapeutic root canal sealers with antibacterial

and remineralization properties. Materials (Basel) 2020;13:1096.
2. Kumar A, Kour S, Kaul S, Malik A, Dhani R, Kaul R. Cytotoxicity evaluation of Bio-C, CeraSeal, MTA-Fillapex, and AH Plus root canal sealers by microscopic and 3-(4, 5 dimethythiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. J Conserv Dent 2023;26:73-8.
3. Bouillaguet S, Wataha JC, Tay FR, Brackett MG, Lockwood PE. Initial *in vitro* biological response to contemporary endodontic sealers. J Endod 2006;32:989-92.
4. Jung S, Sielker S, Hanisch MR, Libricht V, Schäfer E, Dammaschke T. Cytotoxic effects of four different root canal sealers on human osteoblasts. PLoS One 2018;13:e0194467.
5. Dasari L, Anwarullah A, Mandava J, Konagala RK, Karumuri S, Chellapilla PK. Influence of obturation technique on penetration depth and adaptation of a bioceramic root canal sealer. J Conserv Dent 2020;23:505-11.
6. Bal AS, Hicks ML, Barnett F. Comparison of laterally condensed. 06 and. 02 tapered gutta-percha and sealer *in vitro*. J Endod 2001;27:786-8.
7. Eltair M, Pitchika V, Hickel R, Kühnisch J, Diegritz C. Evaluation of the interface between gutta-percha and two types of sealers using scanning electron microscopy (SEM). Clin Oral Investig 2018;22:1631-9.
8. López García S, Pecci Lloret MR, Guerrero Gironés J, Pecci Lloret MP, Lozano A, Llena C, *et al.* Comparative cytocompatibility and mineralization potential of bio-C sealer and totalfill BC sealer. Materials (Basel) 2019;12:3087.
9. Asawaworarit W, Pinyosopon T, Kijsamanmith K. Comparison of apical sealing ability of bioceramic sealer and epoxy resin-based sealer using the fluid filtration technique and scanning electron microscopy. J Dent Sci 2020;15:186-92.
10. Edmondson JM, Armstrong LS, Martinez AO. A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. J Tissue Cult Methods 1988;11:15-7.
11. ISO Standards 10993-5. Biological Evaluation of Medical Devices. Tests for Cytotoxicity: *In vitro* Methods. Geneva, Switzerland: International Organization for Standardization; 2009.
12. Caceres C, Larrain MR, Monsalve M, Peña Bengoa F. Dentinal tubule penetration and adaptation of Bio-C sealer and AH-Plus: A comparative SEM evaluation. Eur Endod J 2021;6:216-20.
13. Patri G, Agrawal P, Anushree N, Arora S, Kunjappu JJ, Shamsuddin SV. A scanning electron microscope analysis of sealing potential and marginal adaptation of different root canal sealers to dentin: An *in vitro* study. J Contemp Dent Pract 2020;21:73-7.
14. Nawrocka A, Piwonski I, Sauro S, Porcelli A, Hardan L, Lukomska Szymanska M. Traditional microscopic techniques employed in dental adhesion research-applications and protocols of specimen preparation. Biosensors (Basel) 2021;11:408.
15. Poggio C, Dagna A, Ceci M, Meravini MV, Colombo M, Pietrocola G. Solubility and pH of bioceramic root canal sealers: A comparative study. J Clin Exp Dent 2017;9:e1189-94.
16. Silva EJ, Cardoso ML, Rodrigues JP, De Deus G, Fidalgo TK. Solubility of bioceramic- and epoxy resin-based root canal sealers: A systematic review and meta-analysis. Aust Endod J 2021;47:690-702.
17. Donnermeyer D, Schemkämper P, Bürklein S, Schäfer E. Short and long-term solubility, alkalizing effect, and thermal persistence of premixed calcium silicate-based sealers: AH Plus bioceramic sealer versus total fill BC sealer. Materials (Basel) 2022;15:7320.
18. Siqueira JF Jr., Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: A critical review. Int Endod J 1999;32:361-9.
19. 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.
20. Estrela C, Cintra LT, Duarte MA, Rossi Fedele G, Gavini G, Sousa Neto MD. Mechanism of action of bioactive endodontic materials. Braz Dent J 2023;34:1-11.
21. Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, *et al.* Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. J Endod 2011;37:673-7.
22. Zhou HM, Du TF, Shen Y, Wang ZJ, Zheng YF, Haapasalo M. *In vitro* cytotoxicity of calcium silicate-containing endodontic sealers. J Endod 2015;41:56-61.
23. López García S, Myong Hyun B, Lozano A, García Bernal D, Forner L, Llena C, *et al.* Cytocompatibility, bioactivity potential, and ion release of three premixed calcium silicate-based sealers. Clin Oral Investig 2020;24:1749-59.
24. Malta CP, Silva Barcelos RC, Segat HJ, Burger ME, Souza Bier CA, Morgental RD. Toxicity of bioceramic and resinous endodontic sealers using an alternative animal model: *Artemia salina*. J Conserv Dent 2022;25:185-8.
25. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. Lasers Surg Med 2016;48:70-6.
26. Garlapati R, Gali PK, Bolla N, Guptha Anila BS, Vemuri S, Naidu BP. A confocal laser scanning microscopic evaluation of nonthermal atmospheric plasma on the dentinal tubule penetration of bioceramic and epoxy resin-based root canal sealers. J Conserv Dent 2023;26:265-70.
27. Sajini S, Atmeh AR, Banerjee A, Festy F, Cook RJ, Andiappan M, *et al.* Glass-ionomer and calcium silicate-based cements interactions with human dentine in health and disease: Two-photon fluorescence microscopy and Raman spectroscopy analysis. Dent Mater 2022;38:1710-20.
28. Arikatla SK, Chalasani U, Mandava J, Yelisela RK. Interfacial adaptation and penetration depth of bioceramic endodontic sealers. J Conserv Dent 2018;21:373-7.