

Cytotoxicity of Two Calcium Silicate-Based Sealers and AH Plus Resin-Based Sealer for Human Gingival Fibroblasts

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Abstract

Background and Aim: The main goal of root canal treatment is to three-dimensionally seal the root canal system. Since sealer may contact the periapical tissue, it should be biocompatible and safe for the body. This study aimed to assess the cytotoxicity of MTA Fillapex and Endoseal MTA calcium silicate-based sealers and AH Plus resin-based sealer for human gingival fibroblasts (HGFs).

Materials and Methods: In this in vitro study, extracts of AH Plus, Endoseal MTA, and MTA Fillapex were obtained, serially diluted 1:2, 1:4, and 1:8, and were exposed to HGFs. Cytotoxicity was assessed by the methyl thiazolyl tetrazolium (MTT) assay. Data were analyzed by ANOVA and Tukey's test ($\alpha=0.05$).

Results: No significant difference existed between AH Plus 1:2 and MTA Fillapex 1:2 concentrations regarding the cell viability percentage ($P>0.05$). However, the difference between these two sealers in other concentrations was significant ($P<0.05$). No significant difference existed in AH Plus 1:4, Endoseal MTA 1:2, and MTA Fillapex 1:2 in cell viability ($P>0.05$); however, the difference among other concentrations of the three sealers was significant ($P<0.05$). The difference among AH Plus 1:8, Endoseal MTA 1:4, and MTA Fillapex 1:4 and 1:8 concentrations was not significant ($P>0.05$) but the difference among other concentrations was significant ($P<0.05$). Endoseal MTA 1:8 showed the highest and AH Plus 1:2 showed the lowest cell viability.

Conclusion: Endoseal MTA in all concentrations had lower cytotoxicity than MTA Fillapex and AH Plus and resulted in higher viability of HGFs.

Key Words: Root Canal Obturation; Calcium Compounds; Epoxy Resins; Dental Cements; Fibroblast

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Introduction

The main goal of root canal therapy is three-dimensional obturation of the root canal system. A hermetic seal minimizes coronal, apical, and lateral leakage of fluids and bacteria, prevents the development of apical

periodontitis, and eliminates the residual irritants from the root canal system [1].

Different endodontic materials are available for obturation and sealing of the root canal system. Endodontic sealers are imperative to seal the interface of root dentin and root filling

materials, and the gaps between them. Sealers should be able to apically and laterally seal the root canal system and fill the gaps and irregularities [2]. Since sealers may contact the periapical tissue, they must be biocompatible and safe for the human body [3].

Biocompatibility of endodontic sealers is highly important for a successful endodontic treatment. A toxic and necrotizing sealer can adversely affect tissue healing and provides a suitable environment for bacterial invasion and leads to treatment failure in the long-term [4].

Different sealer types with various advantages and disadvantages, and different physical and biological properties are available in the market; among which, resin-based sealers, zinc oxide eugenol, calcium hydroxide, glass ionomer, and silicone sealers may be named [5]. Recently, calcium silicate-based sealers were introduced to the market [6]. Resin-based sealers such as AH Plus are optimal sealers, since they are radiopaque, have optimal dimensional stability, good resistance, high flow, and low solubility [7]. On the other hand, MTA Fillapex is a calcium silicate-based sealer that enhances hard tissue mineralization by releasing calcium and creating an alkaline environment [8].

Endoseal MTA is a pozzolan-based sealer in combination with a calcium silicate sealer, which has optimal biocompatibility and results in good obturation quality [9].

The methyl thiazolyl tetrazolium (MTT) assay is a popular tool for estimation of the metabolic activity of viable cells. It is based on enzymatic reduction of tetrazolium salt into purple formazan crystals, which is measured spectrophotometrically [10].

Due to the novelty of calcium-silicate based sealers and the significance of an optimal sealer to maximize the success of endodontic treatment, this study aimed to assess the cytotoxicity of Endoseal MTA and MTA Fillapex by the MTT assay, in comparison with AH Plus resin-based sealer as the gold standard for human gingival fibroblasts (HGFs).

Materials and Methods

This in vitro, experimental study was conducted at Mazandaran University of Medical Sciences and was approved by the

ethics committee of the university (IR.MAZUMS.REC.1399.8114). HGFs with normal proliferation and no fungal or bacterial contamination in logarithmic phase of growth were used for this study. Cells with fungal or bacterial contamination were excluded.

Preparation of sealers:

Three commercially available sealers were evaluated in this study including Endoseal MTA (Maruchi; Wonju, Korea), MTA Fillapex (Angelus Soluções Odontológicas, Londrina, PR, Brazil), and AH Plus (Dentsply, De-Trey Konstanz, Germany). AH Plus and MTA Fillapex were prepared as instructed by the manufacturers [11,12]. Endoseal MTA was premixed and injected [13].

Preparation of culture medium:

To prepare the culture medium, 13.48 g of Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) and 7.3 g of sodium bicarbonate were dissolved in 1 L of distilled water. The pH of the culture medium was adjusted at 7.4 by using hydrochloric acid and NaOH. The culture medium was then filtered through a filter with 0.2 μ m pores under a laminar hood and kept refrigerated in sterile plates. Prior to use, 10% fetal bovine serum (FBS; 1 mL per each 9 mL of the culture medium) and antibiotic (100 U/mL penicillin and 100 μ g/mL streptomycin) were added to the culture medium [14].

Cell preparation:

The cells were initially obtained from the Pasteur Institute of Tehran. HGFs were removed from the nitrogen tank and cultured in 75 cm² flasks (Nunc, Denmark) containing DMEM supplemented with FBS and antibiotics. The cells were incubated at 37°C in presence of 5% CO₂. The culture medium was refreshed every 3 days. After reaching confluence, the cells were passaged and transferred to several flasks [14].

Cell passage:

The cell culture flask was rinsed twice with phosphate buffered saline. Next, 2 mm of trypsin (Merck, Germany) was added to the cells in a 75 cm² flask, and they were incubated at 37°C for 5 minutes. Next, 2 mL of the culture medium containing 10% FBS was added to the flask to stop the activity of trypsin. The cells detached from the bottom of the flask were transferred into a 15 mL sterile tube and

centrifuged at 2000 rpm for 5 minutes (Hettich Universal, Germany). The supernatant containing trypsin was discarded, and the cell sediment was transferred to a culture medium containing antibiotic and 10% FBS. The cells were then distributed among three flasks, and the flasks containing cells were incubated at 37°C and 5% CO₂ [14].

Cell counting by using trypan blue:

After detachment of cells from the bottom of the flask by using trypsin, the percentage of viable cells was determined by trypan blue. For this purpose, 20 µL of the suspension was transferred into a Neubauer chamber (Tiefe Depth Profondeur, Marienfeld, Germany 0.01 mm). The number of cells was counted in a large square composed of 16 smaller squares, and the following formula was used to calculate the percentage of viable cells:

Number of cells per each 1 mL of the suspension = number of cells in the larger square $\times 10^4$ [14]

Preparation of sealer extracts:

Endoseal MTA, MTA Fillapex, and AH Plus were prepared as instructed by the manufacturers, and were placed in wells of a 24-well plate (16.2 mm diameter and 2 mm height) prior to setting. Next, 2.5 mL of DMEM containing antibiotic and without FBS was added to the wells. The plate was then incubated at 37°C and 5% CO₂ for 48 hours. Next, all three sealers were transferred into test tubes, and 10% FBS was added to them (0.05 mL per each 5 mL of the sealer extract). These test tubes served as sealer extracts with 1:1 concentration. To prepare culture media with lower concentrations of sealer extract, the 1:1 samples were serially diluted using DMEM containing antibiotic and 10% FBS to obtain 1:2, 1:4, and 1:8 concentrations of each sealer [14].

Cytotoxicity:

HGFs were removed from the nitrogen tank and transferred to a 75 cm² flask containing DMEM enriched with FBS and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) and incubated at 37°C and 5% CO₂. After cell passage and ensuring normal proliferation of cells, they were detached from the flask using trypsin, and after ensuring their viability by

using trypan blue, they were transferred to a 96-well culture plate (8000 cells/0.5 mL/well). The plates were then incubated under standard conditions for 24 hours until cell seeding. They were then incubated along with sealer extracts at the aforementioned concentrations for 48 hours. The percentage of cell viability was then calculated using the MTT assay [14].

MTT assay:

After exposure of cells to the sealer extracts, 20 mL of 5 mg/mL MTT solution was added to each well of the plate. The plates were then incubated for 4 hours. Next, the culture medium was removed, and 100 µL of dimethyl sulfoxide (Merck, Germany) was added to each well to dissolve the formed formazan crystals. The color intensity was then quantified by measuring the optical density of each well at 545 nm wavelength (with 630 nm reference wavelength) using an ELISA Reader. The percentage of cell viability was calculated as follows:

Percentage of viable cells = mean optical density of control wells / optimal density of each well $\times 100$ [15-17].

All statistical analyses were conducted using PRISM version 3 by two-way ANOVA considering the presence of two factors to be analyzed, followed by post-hoc Tukey test for pairwise comparisons (due to normal data distribution). $P < 0.05$ was considered statistically significant.

Results

The cytotoxicity of Endoseal MTA, MTA Fillapex, and AH Plus in 1:2, 1:4, and 1:8 concentrations was assessed for HGFs in the present study. The negative control group included the culture medium alone which is not cytotoxic.

Table 1 shows the mean cell viability in presence of different concentrations of the three sealer types. Table 2 presents the results of pairwise comparisons of different concentrations of the three sealer types regarding cell viability. Figure 1 shows the histogram of cell viability following exposure to different concentrations of sealers and the control group.

Table 1. Mean cell viability in presence of different concentrations of the three sealer types

Concentration of sealers	Control Mean (SD)	AH Plus Mean (SD)	Endoseal MTA Mean (SD)	MTA Fillapex Mean (SD)
1/2	100.3±1.52	21±3.6	46±3.21	32.00 8.88
1/4	100.3±1.52	34.67±8.73	67.67±10.26	52.33±4.933
1/8	100.3±1.52	66.67±4.72	90.33±3.78	82.67±3.215

SD: Standard deviation

Table 2. Pairwise comparisons of different concentrations of the three sealer types regarding cell viability

Tukey's multiple comparisons test	P value
control - vs. AH Plus 1/2	P<0.0001
control - vs. AH Plus 1/4	P<0.0001
control - vs. AH Plus 1/8	P<0.0001
control - vs. Endoseal MTA 1/2	P<0.0001
control - vs. Endoseal MTA 1/4	P<0.0001
control - vs. Endoseal MTA 1/8	No
control - vs. MTA Fillapex 1/2	P<0.0001
control - vs. MTA Fillapex 1/4	P<0.0001
control - vs. MTA Fillapex 1/8	P<0.05
AH Plus 1/2 vs. AH Plus 1/4	No
AH Plus 1/2 vs. AH Plus 1/8	P<0.0001
AH Plus 1/2 vs. Endoseal MTA 1/2	P<0.001
AH Plus 1/2 vs. Endoseal MTA 1/4	P<0.0001
AH Plus 1/2 vs. Endoseal MTA 1/8	P<0.0001
AH Plus 1/2 vs. MTA Fillapex 1/2	No
AH Plus 1/2 vs. MTA Fillapex 1/4	P<0.0001
AH Plus 1/2 vs. MTA Fillapex 1/8	P<0.0001
AH Plus 1/4 vs. AH Plus 1/8	P<0.0001
AH Plus 1/4 vs. Endoseal MTA 1/2	No
AH Plus 1/4 vs. Endoseal MTA 1/4	P<0.0001
AH Plus 1/4 vs. Endoseal MTA 1/8	P<0.0001
AH Plus 1/4 vs. MTA Fillapex 1/2	No
AH Plus 1/4 vs. MTA Fillapex 1/4	P<0.05
AH Plus 1/4 vs. MTA Fillapex 1/8	P<0.0001
AH Plus 1/8 vs. Endoseal MTA 1/2	P<0.05
AH Plus 1/8 vs. Endoseal MTA 1/4	No
AH Plus 1/8 vs. Endoseal MTA 1/8	P<0.01
AH Plus 1/8 vs. MTA Fillapex 1/2	P<0.0001
AH Plus 1/8 vs. MTA Fillapex 1/4	No
AH Plus 1/8 vs. MTA Fillapex 1/8	No
Endoseal MTA 1/2 vs. Endoseal MTA 1/4	P<0.05
Endoseal MTA 1/2 vs. Endoseal MTA 1/8	P<0.0001
Endoseal MTA 1/2 vs. MTA Fillapex 1/2	No
Endoseal MTA 1/2 vs. MTA Fillapex 1/4	No
Endoseal MTA 1/2 vs. MTA Fillapex 1/8	P<0.0001
Endoseal MTA 1/4 vs. Endoseal MTA 1/8	P<0.01
Endoseal MTA 1/4 vs. MTA Fillapex 1/2	P<0.0001
Endoseal MTA 1/4 vs. MTA Fillapex 1/4	No
Endoseal MTA 1/4 vs. MTA Fillapex 1/8	No
Endoseal MTA 1/8 vs. MTA Fillapex 1/2	P<0.0001
Endoseal MTA 1/8 vs. MTA Fillapex 1/4	P<0.0001
Endoseal MTA 1/8 vs. MTA Fillapex 1/8	No
MTA Fillapex 1/2 vs. MTA Fillapex 1/4	P<0.05
MTA Fillapex 1/2 vs. MTA Fillapex 1/8	P<0.0001
MTA Fillapex 1/4 vs. MTA Fillapex 1/8	P<0.001

*P<0.05 was considered statistically significant. All data had a normal distribution.

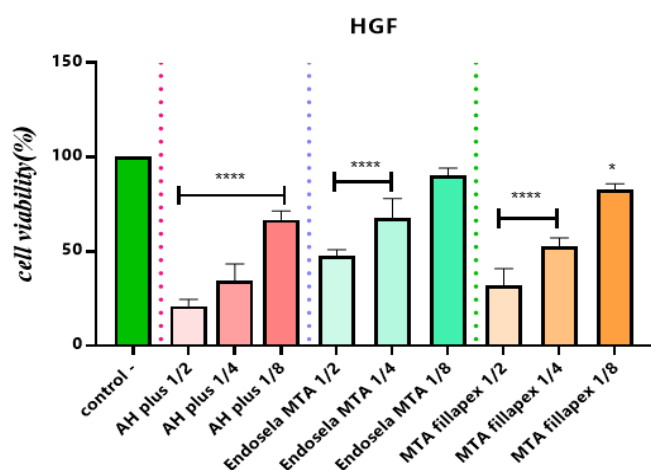


Figure 1. Histogram of cell viability following exposure to different concentrations of sealers and the control group

Discussion

Sealers should have some important characteristics, such as biocompatibility, bacteriostatic properties, and insolubility in oral fluids. Optimal setting time, minimal polymerization shrinkage, not causing tooth discoloration, and opacity are among the criteria for a suitable sealer [18,19]. Extrusion of sealer and gutta-percha into the periapical tissue during root canal obturation can lead to development of a periapical lesion. Apical periodontitis can cause moderate to severe pain.

Immunologically, extrusion of sealer into the periapical tissue elicits a periodontal ligament response characterized by activation of different inflammatory cells, and release of inflammatory mediators such as histamine, cytokines, and prostaglandins [20,21].

Different cell types are used for assessment of cytotoxicity of dental materials and endodontic sealers, such as human periodontal ligament stem cells [3,22], human gingival fibroblasts [23], V79 Hamster fibroblasts [7], and L929 murine fibroblasts.

Also, several methods are available for assessment of cell metabolism, such as assessment of enzymatic activity,

membrane permeability, cell adhesion, ATP production, production of coenzymes, and nucleotide excision repair, which can be classified as follows:

(I) Dye removal techniques, such as Trypan blue test, (II) Metabolic activity tests, (III) ATP test, (IV) sulforhodamine B test, (V) protease viability assay, (VI) clonogenic cell survival assay, (VII) DNA synthesis cell proliferation assay, (VIII) and micro-Raman spectroscopy [24].

The MTT assay is fast, quantitative, and accurate, since the results are reported spectrophotometrically. This method is based on enzymatic reduction of tetrazolium salt with a light color to purple-blue formazan crystals [10]. The MTT assay was used in the present study for assessment of cell viability.

In the present study, the percentage of viable cells was lower in AH Plus group than the other two bioceramic sealers. It means that the calcium content of bioceramic sealers increase their biocompatibility while the resin component decreases the biocompatibility. Between the two bioceramic sealers tested in the present study, Endoseal MTA showed higher biocompatibility. MTA Fillapex is a MTA paste-catalyst that includes resin. Thus, it is a resin-based bioceramic sealer. It contains natural resin, salicylate resin, diluted resin, bismuth trioxide, silica nanoparticles, and MTA [25]. On the other hand, Endoseal MTA is a pozzolan-based bioceramic sealer [26]. Pozzolan is a silicon that chemically reacts with calcium hydroxide and forms a cement. The chemical reactions of pozzolan occur between calcium hydroxide and silica, leading to formation of C-S-H hydration reaction as a cement. The pozzolan reaction takes longer than cement formation, and in the setting process, calcium hydroxide is released. Thus, its effects remain longer before setting [27]. Thus, the resin content of MTA Fillapex may decrease biocompati-

bility, while the pozzolan reaction increases the biocompatibility of Endoseal MTA sealer. Erdogan et al. [28] compared the biocompatibility of AH Plus, MTA Fillapex, and iRoot SP using XTT cytotoxicity measurement test. They reported that AH Plus and MTA Fillapex showed the lowest percentage of viable cells in higher concentrations (1:1, 1:2, 1:4), and iRoot SP resulted in significantly higher number of viable cells in all concentrations compared with the other two sealers. Although their cytotoxicity assessment test was different from that used in the present study, they showed equally high cytotoxicity of AH Plus and MTA Fillapex in 1:2 concentration, which was almost in line with the present findings. Lee et al. [3] compared three calcium silicate-based sealers (Endoseal MTA, nano-ceramic sealer, and WellRoot ST), and two epoxy-resin based sealers (AH Plus and AD Seal) from different aspects such as cytotoxicity for human periodontal ligament stem cells (by the MTT assay), inflammatory response, and osteogenic potential. They showed that calcium silicate-based sealers were more biocompatible and had lower cytotoxicity than epoxy resin-based sealers, which was in agreement with the present findings. Seo et al. [21] compared the cytotoxicity and mineralization activity of three calcium silicate-based sealers (Endoseal MTA, BioRoot RCS, EndoSequence BC) and AH Plus epoxy-resin based sealer using human dental pulp stem cells in monolayer. Cytotoxicity was evaluated by the MTT assay. They found no significant difference among the four sealers in cell viability. However, in the present study, the percentage of cell viability in Endoseal MTA group in all concentrations was higher than that in AH Plus group, which may be due to using a different cell line, or methodological differences (such as incubation times).

Conclusion

According to the present results, Endoseal MTA in all concentrations showed lower cytotoxicity and higher cell viability than MTA Fillapex and AH Plus.

Conflict of Interests

None to declare.

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