Cytotoxicity and Mineralization Activity of Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Gingival Fibroblast cells

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Abstract

**Background:** root canal obturation is performed by gutta-percha cones and sealer. Therefore, these materials specially sealers, must be biocompatible. This study aimed to investigate the cytotoxicity and mineralization activity of two calcium silicate-based sealers (Endoseal MTA and Ceraseal) and an epoxy resin-based sealer (AH26).

**Methods:** in this experiment, the toxicity of Endoseal MTA, Ceraseal, and AH26 on human gingival fibroblast cells was examined using Methyl-Thiazol-Tetrazolium assay at time intervals of 24, 48, 72, and 120 hours. The mineralization activity of sealers was evaluated by Alizarin Red Staining assay. Data analysis was carried out using one-way ANOVA and Tukey's post-test at a significance level of less than 0.05.

**Results:** cytotoxicity of sealers decreased gradually (P<0.0001). AH26 showed the highest level of cytotoxicity (P<0.001). in terms of cytotoxicity, no considerable differences were observed between the two calcium silicate-based sealers. AH26 showed the lowest mineralization activity (P<0.0001). among the calcium silicate-based sealers, mineralization and formation of calcium nodules were more frequently observed in the Endoseal MTA group (P<0.001).

**Conclusion:** the examined calcium silicate-based sealers had less cytotoxicity and higher mineralization activity than the resin-based sealer (AH26). There was negligible difference between the cytotoxicity of the two calcium silicate-based, but the cell mineralization caused by endoseal MTA was higher.

**Background**

Correct root canal treatment of teeth with apical periodontitis is done following complete removal of infected pulp while root canal preparation, followed by the establishment of apical and coronal seals (1). The purpose of root canal obturation as the last stage of endodontic treatment is to create a gap-free environment throughout the entire root canal system to prevent recurrent infection and any communication between the internal space of the root canal and the periapical tissues (2). Currently, in most cases, root canals are obturated by gutta-percha cones along with endodontic sealers. The main application of endodontic sealers is to fill the empty spaces between the canal walls and gutta percha cones (3). Sealers prevent the penetration of microorganisms and their reproduction inside the root canal by establishing proper sealing in the entire root canal space, especially the coronal and apical areas (4). The advantages of using sealers are not only related to filling the remaining void spaces inside the canal but also because of their antimicrobial properties and bacterial growth prevention (2). The contact between biomaterials and tissues leads to interactions; therefore, these materials should be biocompatible and not harmful to the biological environment (3). Sealer components may be cytotoxic to human cells, leading to inflammation and DNA damage, resulting in genome instability and increased carcinogenesis; these substances interfere with cellular elements including lipids, proteins, and DNA which may harm the unity of the membrane according to the chemical composition of its surface (5).
Fibroblasts have many important roles including the healing of periodontium. These cells are necessary for the regeneration of the firm fibrillar link between the tooth root, gingiva, and periodontal ligament (6).

Evaluation of cell viability after exposure to toxicants is determined by MTT (Methyl-Thiazol-Tetrazolium) which is a water-soluble tetrazolium salt that will turn to an insoluble purple formazan after the cleavage of the tetrazolium ring by succinate dehydrogenase found in the mitochondria. The formazan cannot pass through the cell membrane and thus aggregates in normal cells (7). Alizarin red staining (ARS) is a method for investigating cell mineralization activity that has been utilized for the evaluation of calcium-rich sediments in a growth medium (8).

There are various types of sealers including eugenol-based sealers (Zinc oxide eugenol), resin-based sealers (AH26 and AH plus), calcium silicate-based sealers, and bioceramic sealers (4). Currently, epoxy resin-based sealers are widely used in root canal treatment, but some limitations have been reported for this type of sealer, such as the possibility of mutation, inflammatory response, cytotoxicity, and hydrophobic nature; there are also concerns about the negative effects of AH in contact with tissues and the suspension in the periapical repair of teeth affected by apical periodontitis (1, 9). Calcium silicate-based sealers are a new group of sealers that show a high degree of hydrophilicity and biocompatibility. Since the internal environment of the root canal is hydrophilic, water absorption and solvability of sealers are essential properties related to the stability of the sealer in the root canal. Also, due to their bioactive nature, they have a positive effect on hard tissue (9). AH26 (Dentsplysirona, Tusla, Ok, USA) is an epoxy resin-based sealer that is in powder and liquid form and was initially introduced as single obturation and was widely used due to its suitable handling characteristics. Before setting, this sealer is more toxic than when it is set, and its toxicity decreases over time. Endoseal MTA sealer (Maruchi, Wonju, Gangwon-do, Korea) is a calcium silicate-based sealer that is used by injection into the root canal. It contains thickening agents, radiopacifier, calcium sulfate, calcium silicate, and calcium aluminate and has favorable characteristics such as dissolution, rapid setting time, high bond strength, resistance to dissolution, and bioactivity (2, 10, 11).

Ceraseal (Metabiomed, Cheongju, Chungcheongbuk-do, Korea) is a new bioceramic sealer that is used by injection and its ingredients include calcium silicate, zirconium oxide, and thickening agents (12). Cell migration, cell attachment, and ion release in ceraseal compared to Endoseal MTA also mineralization, gene expression, and anti-inflammatory properties have been observed to be more (13).

Considering the importance of fibroblast in the immunity system and periodontal tissue regeneration and since the application of calcium silicate sealers is increasing, the present study was conducted to examine the effect of these three sealers on the cytotoxicity and mineralization of gingival fibroblast cells.

**Methods**

**Preparation of Cells**
The human gingival fibroblast cell line was obtained from Pasteur Institute of Iran. Human gingival fibroblast cells (type C165) were removed from the nitrogen tank and cultured in a 75 cm² flask (Nunc-Denmark) containing DMEM (Dulbecco Modified Eagles Medium) enriched with FBS and antibiotics. The cells were incubated in an incubator at 37°C and 5% CO2, and their culture medium was changed every 3 days. When the bottom of the flask was filled with cells by passage, the cells were then distributed to several flasks.

**Preparation of Sealer Extract**

The sealers used in this study were AH26, Ceraseal, and EndosealMTA. EndosealMTA and Ceraseal were premixed and drawn into a syringe. The AH26 sealer was prepared in a 1:1 ratio according to the manufacturer's instructions. Discs containing sealers had a diameter of 6 mm and a height of 3 mm and were made in aseptic conditions using Teflon mold. The discs were then stationed at 37°C and standard atmosphere with 5% CO2 for 72 hours to be activated.

**Inspection of Cytotoxicity**

Human gingival fibroblast cells were removed from the nitrogen tank and incubated in a 75 cm² culture flask containing DMEM culture medium and were enriched with fetal bovine serum and antibiotics (Penicillin 100 U/ml, Streptomycin 100 µg/ml) at 37°C and 5% CO2.

After performing several cell passages and ensuring their normal proliferation, the cells were separated from the culture flask with trypsin. The viability of the culture medium containing fibroblast was assessed with trypan blue solution and moved to a 96-well culture plate (cells/0.5 ml/well 8000) without any toxic substances. Subsequently, the plate was placed in the incubator for 24 hours under the aforementioned standard conditions for the cells to be seeded on the plate. The cells were then inspected after 24, 48, 72, and 120 hours. By the end of the intended time interval, the cell viability percentage was evaluated by the MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-Diphenyl-2H-tetrazolium bromide) test (14).

**MTT (Methyl-Thiazolyl-Tetrazolium) Testing Method**

MTT is one of the standard laboratory tests used to determine the toxicity of drugs and other substances. When the MTT solution is added to the cells' environment, it is reduced to formazan (purple) by the reductase enzyme present in the mitochondria in living cells. Therefore, the intensity of the purple color indicates the number of living cells or cell proliferation.

At the end of the predetermined proximity time of cells with the sealer extract, 20 ml of MTT solution was added to each of the wells of the plate and placed in the incubator for 2–4 hours. Thereafter, the culture medium on the cells was drained and 100 microliters of dimethyl sulfoxide (DMSO) (Merk-Germany) was added to each well to dissolve the reduced formazan dye. The intensity of the resulting color was then evaluated by the light absorption of each well using the Elisa reader (Awareness Technology Inc) at a wavelength of 545 nm (with a reference of 630 nm).

The percentage of live cells was assessed from the subsequent Eq. (14, 15).
% Living cells = 100* (mean optical absorbance of wells / optical absorbance of each well)

**Alizarin Red Staining Assay**

The discs containing the sealer were moved to a conical tube containing 20 ml of fresh culture solution and placed in a standard atmosphere at 37°C with 5% CO2 for 7 days. Following these preparation steps, the solution was filtered by 0.2 um filters, and the human gingival fibroblast cells containing culture medium were incubated in 24 wells with a density of 10,000 x 2 for 24 hours to form attachments. The mineralization activity was measured after 15 days, in which the sealer extract was changed every 3 days.

The cells were stained with 2% Alizarin solution for 20 minutes and then rinsed 5 times with sterile water. In order to evaluate the results quantitively, the stained cells were soaked with 10% cetyprimidinium chloride solution for 15 minutes and measuring the absorbance at 560 nm was done utilizing an absorbance microplate reader (16).

**Statistical Data Analysis**

The data recorded throughout the research were analyzed by PRISM Ver.3 software, one-way ANOVA, and Tukey's post-test statistical tests. The significance level of less than 0.05 was considered.

**Results**

In due course, the cytotoxicity of sealers decreased and the percentage of cell vitality increased. According to the results of the experiment, AH26 showed the highest toxicity and the lowest cell viability among the experimental groups (P < 0.001). There was no considerable difference between the level of toxicity and cell viability between the two calcium silicate-based sealers (Ceraseal and Endoseal MTA) (Fig. 1).

On day zero no noteworthy difference was observed in the amount of mineralization and the calcium nodule formation between the experimental and control groups. On day 15, the amount of mineralization in all sealers was significantly different in comparison with the control group and in comparison with their equivalent group on day zero (P < 0.0001). On the 15th day, among the experimental groups, AH26 showed the lowest amount of mineralization compared to Ceraseal and Endoseal MTA sealers, and this difference was significant (P < 0.0001).

Also, a considerable difference was detected in the amount of mineralization between Ceraseal and Endoseal MTA groups on day 15 (P < 0.001). The mineralization and calcium nodule formation was higher in the Endoseal MTA group (Fig. 2).

**Discussion**

Gutta percha and sealers are used as obturation materials (17). During obturation, the endodontic sealer may be pushed out from the root apex (18). Therefore, these materials may be in close contact with the
periodontal soft tissue in the apical area for a long period and some reactions are expected in this area (19). Cytotoxicity is the ability of substances to affect cell viability, so cytotoxicity assays are in fact biocompatibility assays that evaluate cell lysis, prevention from cell growth, and other effects on cells (20).

When injecting the sealer inside the canal, some reactions occur with the periapical tissue until the setting is completed (13). Cytotoxicity emanated from sealers, by releasing toxic substances to the periapical tissue, even if the sealer is not pushed out from the apex, leads to periapical destruction, bone loss, wound healing alteration, or even tooth loss (3, 21).

Resin-based sealers like AH26 have been commonly applied in endodontics although, formaldehyde release by these sealers leads to cytotoxicity. Previous studies have shown that the maximum toxicity of this sealer is in the first 24 hours, and the toxicity of this sealer decreases as the sealer sets (22). Aluminum is one of the elements that is found in high amounts in Endoseal and since the cytotoxicity and toxicity effects of aluminum on animals have been observed, this element can be considered as the cause of cytotoxicity induced by this sealer; The effect of zirconium oxide found in Ceraseal as an opacifier has been proven to induce the proliferation of fibroblasts and accelerate the reduction of inflammatory reactions, as well as the cytotoxic effects of dental materials containing bismuth have been demonstrated. Therefore, the cytotoxic effect of sealers containing these elements can be justified (15).

Calcium silicate-based sealers like Ceraseal and Endoseal MTA have bioactive properties due to the calcium ions release and lead to the differentiation of bone marrow stem cells and osteoblast progenitor, thus the progression of osteogenesis, results in faster repair and improving of apical periodontitis (3, 14). The alkalinity of calcium silicate sealers strengthens the biocompatibility and antimicrobial properties of the sealers and prevents the dissolution of minerals by neutralizing the lactic acid produced by osteoclasts (14).

In the current study, the resin-based sealer (AH26) showed the highest cytotoxicity in comparison with both calcium silicate-based sealers during the study. in terms of cytotoxicity, there was no notable difference between Ceraseal and Endoseal MTA. In addition, the cytotoxicity of all sealers decreased over time.

Eun-Su Lim et al., noticed considerably more viable cells in the Endoseal group on day 14, by comparing the cell viability in the Endoseal and AH plus groups, which is in line with the results of the present study (23). Through inspection of cell viability using the MTT assay methods conducted by Min-Gyu Park et al., the AH plus group demonstrated lower cell viability than the Ceraseal and Endoseal TCS group throughout the study, which is similar to the findings of the current study (17).

Among the evaluated sealers by Ju Kyung Lee et al, including Endoseal MTA and AH plus, the lowest cell viability during the study was for the resin-based sealer which is the same as in the present experiment (3). Although, in contrast to the current experiment, cell viability in the AH plus and Endoseal MTA groups significantly decreased during the study. Also, in a study published by Hanseul Oh et al., ceramic sealers
had the highest cell viability compared to resin-based sealers, and similar to the previous and present studies the findings demonstrate that calcium silicate-based sealers are more biocompatible than epoxy resin-based sealers (13). However, Emmanuél João et al. concluded that both Endoseal and AH plus are biocompatible (24).

In the study conducted by Min-Gyu Park et al., Ceraseal and Endoseal TCS showed a considerably higher absorption and cell viability than the control group on the 7th day, and this cell viability increased throughout the experiment as in the current study (17).

In the current study, in terms of the calcium nodules formation and cell mineralization activity, calcium silicate-based sealers performed more desirable than resin-based sealers due to the calcium ions release, and among calcium silicate-based sealers, Endoseal MTA had higher activity than Ceraseal. In the study of S. López-García, higher cell viability and mineralization capacity of Endosequence BC and Ceraseal sealers than Endoseal were reported (15). This finding is in contrary to the findings of the present study because in this study, a higher amount of mineralization was seen in the Endoseal MTA group. Deog-Gyu Seo et al. observed that Endoseal MTA, Endosequence BC and BioRoot RCS showed the formation of more mineralized nodules than AH plus (14). Also, Hanseul Oh et al. found that AH plus resin-based sealer showed less staining after performing ARS assay than Ceraseal and Endoseal TCS (13). These findings are in line with the current study which mineralization occurred more in the calcium silicate-based sealers group than in epoxy resin-based sealers.

By reviewing the findings of the current study and other studies, it is evident that most studies emphasize the greater biocompatibility of the calcium silicate-based sealers in comparison with the epoxy resin-based sealers.

**Conclusion**

The findings show that calcium silicate-based sealers (Endoseal MTA and Ceraseal) have less cytotoxicity and more mineralization activity than AH26 and are more biocompatible. There is no considerable difference between the cytotoxicity of the two calcium silicate-based sealers, but the cell mineralization induced by Endoseal MTA was more.

**Abbreviations**

MTT
Methyl-Thiazol-Tetrazolium

ARS
Alizarin Red Staining

DMEM
Dulbeccos Modified Eagles Medium

DMSO
dimethyl sulfoxide.

**Declarations**

**Ethics approval**

The present study was approved by the Medical Ethics Committee of Mazandaran University of Medical Sciences with the ethics code IR.MAZUMS.REC.1400.581.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Competing interests**

The authors declare that they have no competing interests.

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**Author Contributions**

*Azam Haddadi Kohsar* participated in conceptualization and methodology, *Mohammad Shokrzadeh* and *Mohammad Ghorbani* performed experimental procedures and data collection, *Farzaneh Sadat Motafeghi* contributed to the interpretation of the data and data analysis, *Anahita Lotfizadeh* participated in investigation, data analysis and drafting the manuscript, *Azam Haddadi Kohsar* revised the content. All authors contributed to the final editing of the manuscript and approved the content.

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Not applicable.

**References**


Figures
Figure 1
Comparison of cell viability in each sealer group with the control group over time

![Graph showing cell viability comparison over time]

Figure 2
Comparison of mineralization activity in each sealer group with the control group

![Graph showing mineralization activity comparison]