In vitro comparison of the cytotoxicity of different endodontic sealers; AH Plus, AdSeal, Endoseal MTA, and GuttaFlow Bioseal

Mehdi Dastorani (dastorani88@gmail.com)
Aja University of Medical Sciences
Muhammad javad Aliee
Aja University of Medical Sciences
Raheleh Halabian
Baqiyatallah University of Medical Sciences
Mostafa Solati
Aja University of Medical Sciences
Mohammadsadegh Alemrajabi
Aja University of Medical Sciences

Research Article

Keywords: Calcium Silicate, Epoxy Resin-Based Root Canal Sealer, Fibroblasts, Materials Testing

Posted Date: November 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1017752/v1

In vitro comparison of the cytotoxicity of different endodontic sealers; AH Plus, AdSeal, Endoseal MTA, and GuttaFlow Bioseal

Mehdi Dastorani[1*], Muhammad javad Aliee[2], Raheleh Halabian[3], Mostafa Solati[4], Mohammadsadegh Alemrajabi[5]

[1] Department of Endodontics, School of Dentistry, AJA University of Medical Sciences, Tehran, Iran.
[2] Research Center, School of Dentistry, AJA University of Medical Sciences, Tehran, Iran.
[3] Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[4] Department of Periodontics, School of Dentistry, AJA University of Medical Sciences, Tehran, Iran.
[5] Department of Oral Medicine, School of Dentistry, AJA University of Medical Sciences, Tehran, Iran.

*Correspondence: dastorani88@gmail.com
[1] Department of Endodontics, School of Dentistry, AJA University of Medical Sciences, Tehran, Iran.

Abstract

Background: This study aimed to assess the cytotoxicity of four commonly used endodontic sealers namely AH Plus, AdSeal, Endoseal MTA, and GuttaFlow Bioseal against human gingival fibroblasts (HGFs).

Methods: After culturing the HGFs, they were exposed to the respective sealers in set form and in five different weights, after sterilization. The cytotoxicity of the sealers was evaluated after 1, 3 and 7 days using the methyl thiazolyl tetrazolium (MTT) assay. Data were analyzed by repeated measures ANOVA.

Results: After 24 h, all sealers showed low cytotoxicity. However, all sealers in 250 mg and 500 mg weights showed significantly higher cytotoxicity than the negative control group at 72 h, and 7 days ($P<0.05$) except for AdSeal in 80 mg weight ($P>0.05$). AH Plus was significantly more

cytotoxic than other sealers at 3 and 7 days ($P<0.05$) while AdSeal had the closest results to the negative control group, and showed significantly higher biocompatibility than other sealers in 250 mg concentration.

Conclusion: AdSeal showed the highest biocompatibility while AH Plus had the highest cytotoxicity among the tested sealers. Thus, its application may delay the healing of periapical lesions.

Keywords: Calcium Silicate; Epoxy Resin-Based Root Canal Sealer; Fibroblasts; Materials Testing

Background

The success of endodontic treatment has reached 92% to 98% due to the recent advances in dental science and technology [1-3]. Endodontic treatment is performed to hermetically seal the canal three-dimensionally, and prevent coronal and apical leakage [4,5]. Such a hermetic seal is often achieved by the use of gutta-percha and sealer. Gutta-percha fills the root canal space and serves as a carrier for the sealer. Sealer provides a hermetic seal against microleakage [6,7]. Root canal filling materials should be preferably insoluble [8]. Aside from the apical foramen, numerous microscopic and macroscopic communications exist between the root canal system and the periodontal ligament and the supporting bone, which include dentinal tubules, accessory foramina, and accessory canals [9]. Thus, the tissue fluid can easily penetrate into the root canal system and degrade and wash out the sealer. The washed-out products may penetrate into the periodontal tissue and alveolar bone, and trigger local inflammatory reactions and subsequent complications [10,11]. Long-term exposure of the periodontal tissue to sealer or its constituents can cause irritation, and delay the healing of periapical lesions. Sealers can also directly interact with the adjacent tissue when the canal is over-filled (sealer puff) [6], and cause considerable irritation of the periradicular tissue [12]. Therefore, biocompatibility is an important prerequisite for endodontic sealers [13]. Following debridement of the canal content, and a correct non-surgical endodontic treatment, a granular fibrovascular tissue is formed and the periapical lesion is healed [14]. Biocompatible endodontic sealers can enhance the healing of apical periodontitis. According to Grossman [12], an ideal sealer should show optimal adhesion to root canal walls after mixing, provide a strong seal, not undergo shrinkage after setting, and not cause tooth discoloration. It should be radiopaque and visible on radiographs, easily mixable with liquid,

bacteriostatic, insoluble in tissue fluids, and biocompatible. It must have a slow setting, and no potential for irritation of periradicular tissue [12].

Considering all the above, biocompatibility is an important property for endodontic sealers [15]. Several methods are available for assessment of the possible cytotoxicity of dental materials such as cell culture, animal studies, and clinical investigations. The novel cell culture techniques aim to replace investigations on animal models [16]. The methyl thiazolyl tetrazolium (MTT) assay is the most commonly used test for assessment of the biocompatibility of dental materials following exposure to gingival fibroblasts, dental pulp stem cells, stem cells of the apical papilla, and periodontal ligament stem cells [17-20]. This test has been confirmed by the ISO standard for assessment of cytotoxicity [17].

The currently available sealers can be divided into zinc oxide eugenol, resin, glass ionomer, silicone, and calcium hydroxide-based sealers as well as bioactive sealers, depending on their chemical composition and structure [21]. AH Plus epoxy resin-based sealer has been commonly used as a gold-standard reference sealer in endodontic studies. Considering the lack of studies comparing the cytotoxicity of different sealer types, this study aimed to compare the cytotoxicity of AH Plus and AdSeal epoxy resin-based sealers, Endoseal MTA calcium silicate-based sealer, and GuttaFlow Bioseal silicone-based sealer against human gingival fibroblasts (HGFs). The null hypothesis was that no significant difference would be found in the cytotoxicity of the four sealer types.

Methods

This in vitro, experimental study evaluated the cytotoxicity of four commonly used sealers against HGFs. The study protocol was approved by the ethics committee of AJA University of Medical Sciences (IR.AJAUMS.REC.1399.190). Table 1 presents the sealers and their composition.

Table 1. Composition of sealers evaluated in this study

Sealer	Composition	Manufacturer

AH Plus	Paste A: Epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide Paste B: Amines, calcium tungstate, zirconium oxide, silica, silicon oil	Dentsply Sirona, Tulsa, OK, USA
AdSeal	Base: Epoxy resin, calcium phosphate Catalyst: amines, bismuth subcarbonate	Meta Biomed, Cheongju, Korea
Endoseal MTA	Calcium silicate, calcium aluminate, calcium aluminoferrite, calcium sulfate, radiopacifier, thickening agent	Maruchi, Wonju, Korea
GuttaFlow Bioseal	Gutta-percha, zinc oxide, barium sulfate, poly dimethyl siloxane, bioactive glass ceramic, zirconia, platinum catalyst, pigments, silver microparticles	Coltène/Whaledent AG, Altstätten, Switzerland

Cell culture:

C10459 HGF cell line was obtained from the cell bank of the Iranian National Center for Genetic and Biologic Resources and incubated in 5 mL of low-glucose Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc., Grand Island, NY, USA) with 10% fetal bovine serum (FBS) (Gibco, USA), and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C and 5% CO2. The cell flask was assessed daily, and the spindle-shaped morphology of the cells and risk of contamination were precisely evaluated. The culture medium was refreshed every 2 days. The cells were passaged 3 days following the primary culture after reaching 90% confluence. For this purpose, the overlying medium was removed, the cells were rinsed with 1 mL of phosphate buffered saline twice, and 1 mL of 0.25% trypsin/EDTA (Gibco, USA) was added to the cells. After 1.5 min, the flask was agitated twice and in the remaining 1.5 min, the cells were pipetted to obtain a single-cell suspension. To neutralize the enzyme, 2 mL of FBS was added to the cell suspension. Approximately 5000 cells were added to each flask, and the flasks were incubated at 37°C and 5% CO2. The cells were then frozen at -20°C and then -70°C.

For defrosting, the cryotubes were removed from the nitrogen tank and placed at 37°C; 1 mL of FBS (90% FBS and 10% dimethyl sulfoxide) was added to the stock and pipetted. The suspension was centrifuged (Hettich, USA) at 1600 rpm for 6 min. Next, 4 mL of the suspension, containing 1 mL of the cells, 45 mL of DMEM, 45 ʎ pen-strep + 5 mL of FBS, was added to a new flask. The Falcon tube containing the stock was centrifuged, the supernatant was removed, and 0.5 mL of FBS was added to the sediment and pipetted into the flask with complete culture medium. It was then incubated at 37°C and 5% CO2, and the medium was refreshed every 2 days. After the fourth passage, the first treatment was performed after cell counting.

To assess the cell viability, 0.4% trypan blue was used (0.2 g added to 50 mL of saline). The treated cells were detached with trypsin, collected in a Falcon tube, and centrifuged at 1500 rpm for 6 min to obtain a homogenous cell suspension; 20 µL of the cell suspension was collected and poured into a small microtube; 20 µL of trypan blue was added and after 3-5 min, 20 µL of the suspension was transferred to a Neubauer chamber. The number of cells was counted under a light microscope (zeiss, Germany).

For cell counting, the fourth passage cells were exposed to trypsin for 4 min and detached; 1 mL of complete culture medium was used to neutralize the enzymatic activity. The collected cells were poured in a Falcon tube and centrifuged at 1500 rpm for 5 min in order for the cell sediment to form. The supernatant was then discarded, and 1 mL of DMEM was added to the cell sediment to create a cell suspension; 10 µL of the cell suspension was then mixed with 10 µL of trypan blue; 10 µL of the mixture was injected between the slide and the cover slip and the number of cells was counted under a light microscope as follows:

Number of cells per 1 mL = mean number of counted cells in four squares x 10^4

The cells were counted in 16 wells on a Neubauer chamber. The mean value was then calculated and multiplied by the dilution coefficient (cell count x distance between the slide and the cover slip x concentration of culture medium x dilution coefficient of trypan blue).

The calculated number of cells and the culture medium were added to each well and the well-plates were incubated for 24 h in order for the cells to adhere to the wells.

Determining and optimizing the adequate amount of sealer for addition to HGFs:

The sealers were mixed on sterile pads according to the manufacturers' instructions, and after setting, they were weighed under a class II biosafety cabinet by a scale (Scaltec) with 0.001 accuracy to prepare sealer discs with 0.08, 0.1, 0.125, 0.25, and 0.5 g weight. Prior to use, the

sealer discs were UV-sterilized for 30 min. Next, 5 x 10^4 cells were cultured in each well of a 24-well plate, and after 12 h of culture, the cells were exposed to the set sealers and incubated at 37°C and 5% CO2 for 24 h. Cell viability was then assessed at the respective time points using trypan blue. After optimizing the experiment, the final test was performed. For this purpose, 5 x 10^4 cells were added to three 24-well plates (a total of 66 wells) and low-glucose DMEM containing 10% FBS was also added to the wells. The plates were incubated at 37°C and 5% CO2 for 24 h. To assess the cytotoxicity of five different weights of different sealers, the set sealers were added to 60 wells containing the cells. Three wells served as the positive control and were only exposed to dimethyl sulfoxide. The plates were incubated at 37°C and 5% CO2. Cell viability was assessed after 3 and 7 days of exposure of the cells to the sealers using the MTT assay. For this purpose, 50 µL of the MTT solution (5 mg/mL in the basic medium) was added to each well at 1, 3 and 7 days, and the plate was incubated at 37°C and 5% CO2 for 4 h. Next, 300 µL of the culture medium was removed by a pipette and replaced with 250 µL of dimethyl sulfoxide and placed at room temperature for 5 min. Finally, 100 µL of the solution was transferred into a 96-well plate and the formed formazan crystals were quantified by measuring the optical density of the solution at 570 nm by an ELISA reader (Anthos 2020.Austria). After measuring the optical density, cell viability was calculated (in comparison with the negative control) and reported in percentage such that viability of 0-30% of the cells indicated high cytotoxicity of the sealer, viability of 30-60% of the cells indicated moderate cytotoxicity of the sealer, and viability of 60-90% of the cells indicated low cytotoxicity of the sealer. Viability of >90% of the cells indicated that the sealer was not cytotoxic.

Statistical analysis:

Data were analyzed by repeated measures ANOVA via SPSS version 11 at $P<0.05$ level of significance.

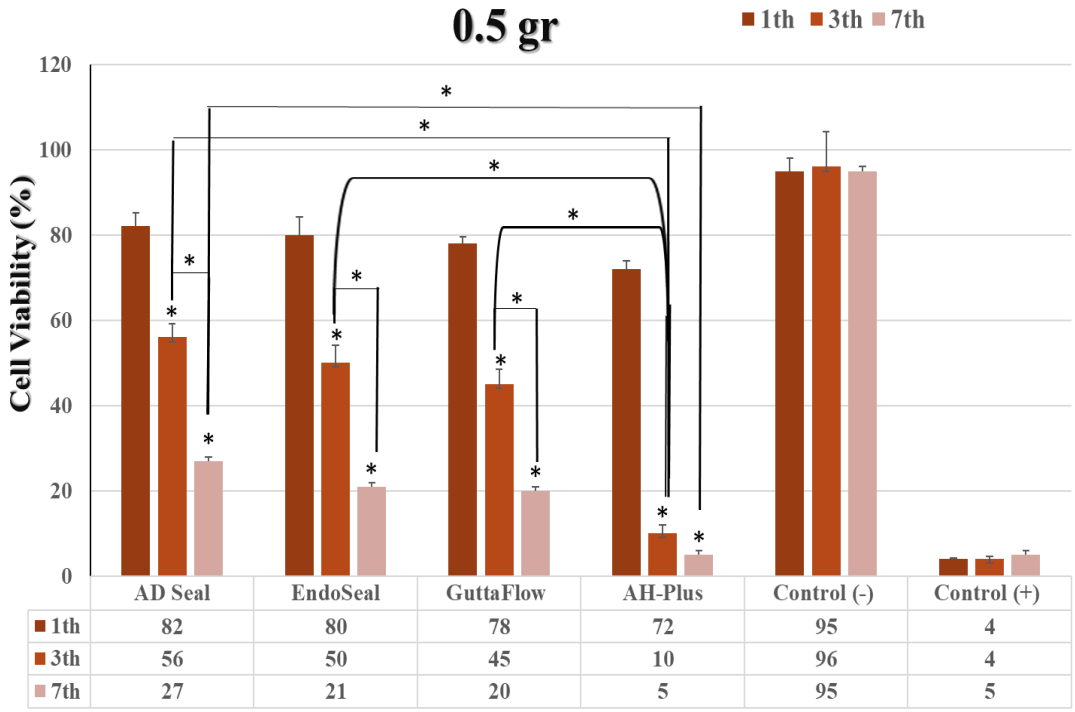
Results

Cytotoxicity of 0.5 g sealers (Figure 1):

Comparison of the cytotoxicity of each sealer at different time points revealed that all sealers were more cytotoxic at 7 days than 3 days, and caused approximately 30% reduction in cell viability at 7 days ($P<0.05$) except for AH Plus ($P>0.05$). None of the sealers had a significant difference with the negative control group at 1 day ($P>0.05$). However, all were significantly

more toxic than the negative control group at 3 days (P<0.05) and 7 days (P<0.05). At 7 days, all sealers showed cell viability < 30% (high cytotoxicity).

Comparison of sealers with each other revealed that at 3 days, AH Plus was significantly more cytotoxic than all others (P<0.05); however, at 7 days, AH Plus was only significantly more cytotoxic than AdSeal (P<0.05). As shown in Figure 1, AdSeal, Endoseal MTA and GuttaFlow Bioseal were not significantly different regarding the percentage of cell viability at all three time points (P>0.05).



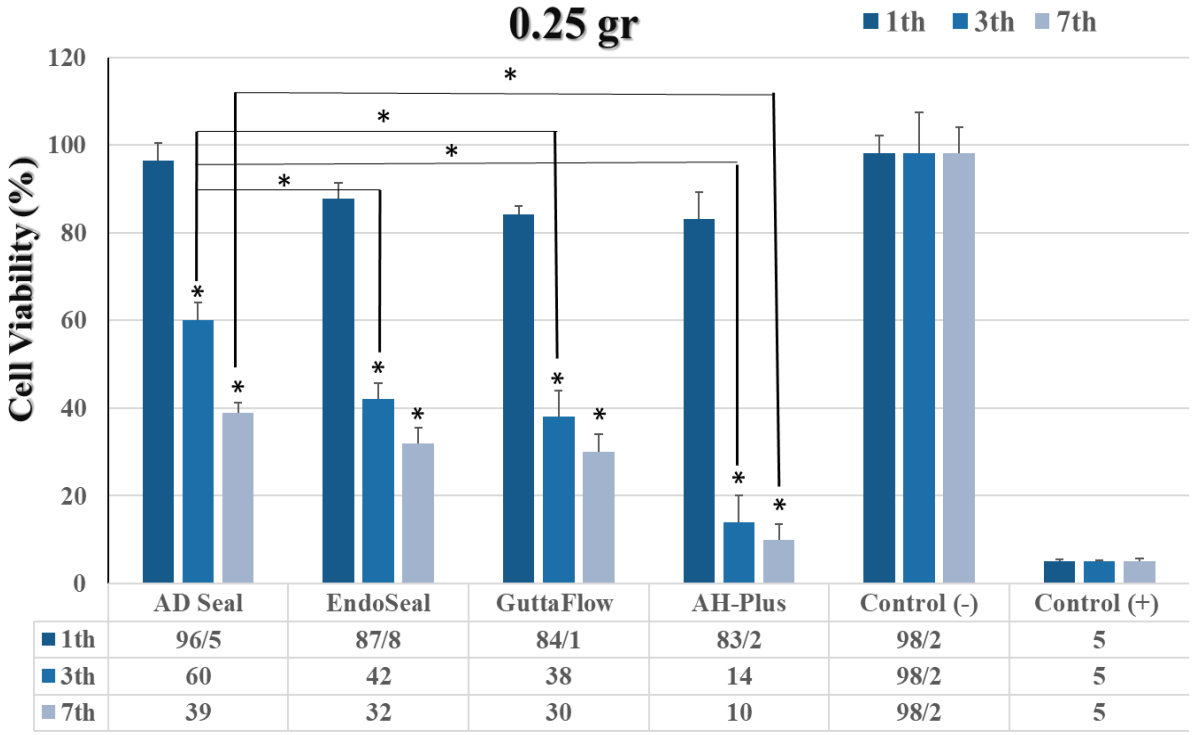
	AD Seal	EndoSeal	GuttaFlow	AH-Plus	Control (-)	Control (+)
1th	82	80	78	72	95	4
3th	56	50	45	10	96	4
7th	27	21	20	5	95	5

Figure 1. Viability of HGFs following exposure to 0.5 g of different sealers for 1, 3 and 7 days. * indicates P<0.05, and starred columns have a significant difference with the negative control group at the respective time point.

Cytotoxicity of 0.25 g sealers (Figure 2):

Comparison of the cytotoxicity of each sealer at different time points revealed no significant difference in cytotoxicity at 7 days compared with 3 days in any sealer (P>0.05). All sealers had significantly higher cytotoxicity at 3 and 7 days, compared with the negative control group (P<0.05). At 3 days, AdSeal was significantly more biocompatible than other sealers (P<0.05).

However, at 7 days, AH Plus showed significantly higher cytotoxicity than AdSeal (P<0.05). No other significant differences were noted. Endoseal MTA and GuttaFlow Bioseal showed similar cell viability with no significant difference (P>0.05). As shown in Figure 2, except for day 1 at which, the percentage of cell viability of sealer groups was close to the negative control group, AH Plus showed high cytotoxicity (< 30% cell viability) at 3 and 7 days, and other sealers showed moderate cytotoxicity (30-60%).



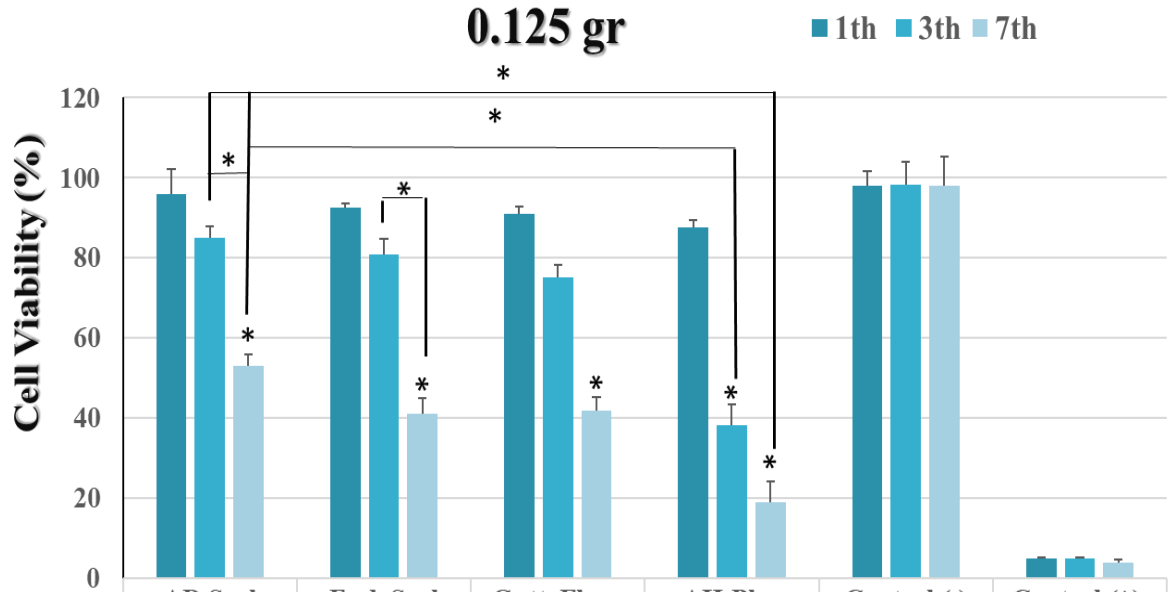
	AD Seal	EndoSeal	GuttaFlow	AH-Plus	Control (-)	Control (+)
1th	96/5	87/8	84/1	83/2	98/2	5
3th	60	42	38	14	98/2	5
7th	39	32	30	10	98/2	5

Figure 2. Viability of HGFs following exposure to 0.25 g of different sealers for 1, 3 and 7 days. * indicates P<0.05, and starred columns have a significant difference with the negative control group at the respective time point.

Cytotoxicity of 0.125 g sealers (Figure 3):

Comparison of the cytotoxicity of each sealer at different time points revealed that AdSeal and Endoseal MTA were more cytotoxic at 7 days compared with 3 days (P<0.05). At 3 days, only AH Plus showed a significant difference with the negative control group with 85% lower cell viability (P<0.05). However, at 7 days, all sealers were significantly more cytotoxic than the negative control group (P<0.05). At both 3 and 7 days, AdSeal showed higher cell viability than

AH Plus (P<0.05). The other three sealers were not significantly different (P>0.05). As shown in Figure 3, except for AH Plus, all other sealers showed low cytotoxicity (> 60% cell viability) at 3 days. However, at 7 days, they showed moderate cytotoxicity (30-60% cell viability).

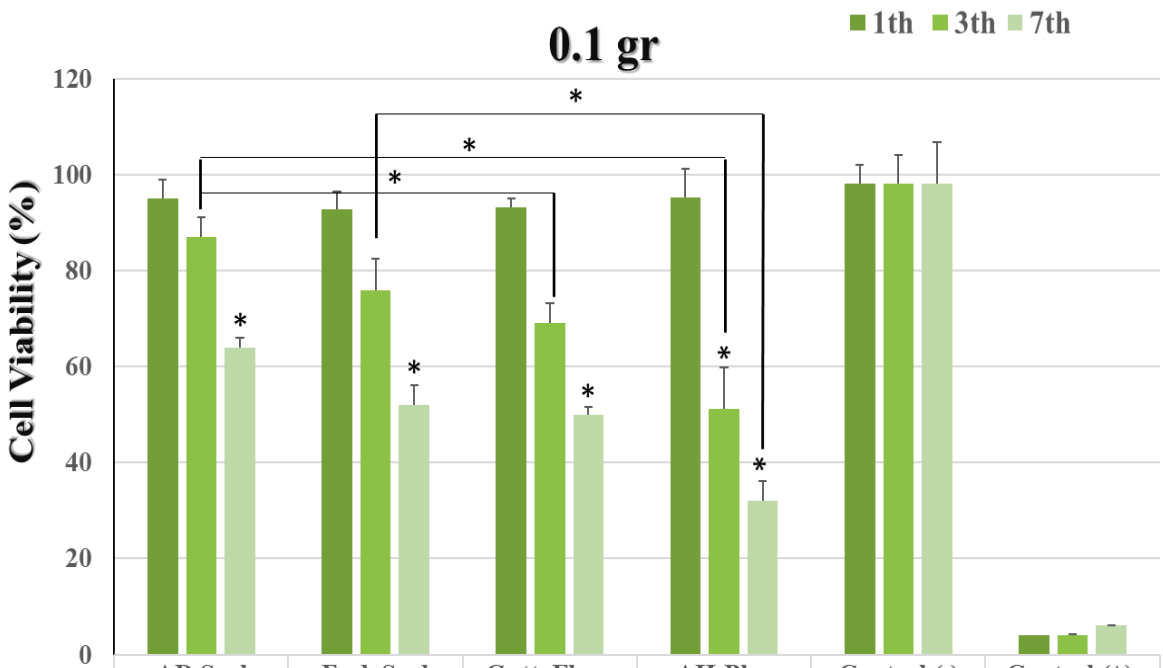


	AD Seal	EndoSeal	GuttaFlow	AH-Plus	Control (-)	Control (+)
1th	96/01	92/6	91/1	87/5	98/1	5
3th	85/01	80/8	75/1	38/2	98/2	5
7th	53	41	42	19	98/1	4

Figure 3. Viability of HGFs following exposure to 0.125 g of different sealers for 1, 3 and 7 days. * indicates P<0.05, and starred columns have a significant difference with the negative control group at the respective time point.

Cytotoxicity of 0.1 g sealers (Figure 4):

Comparison of the cytotoxicity of each sealer at different time points revealed no significant difference in cytotoxicity at 7 days compared with 3 days in any sealer group (P>0.05). On day 3, only AH Plus was significantly more cytotoxic than the negative control group (P<0.05). However, at 7 days, all sealers showed significantly higher cytotoxicity than the negative control group (P<0.05). On day 3, AdSeal showed lower cytotoxicity than GuttaFlow Bioseal and AH Plus (P<0.05). At 7 days, Endoseal MTA showed lower cytotoxicity than AH Plus (P<0.05) and cells showed higher viability by 25% in presence of Endoseal MTA. On day 3, except for AH Plus, all other sealers showed cell viability > 60% and at 7 days, all sealers showed moderate cytotoxicity (30-60%).

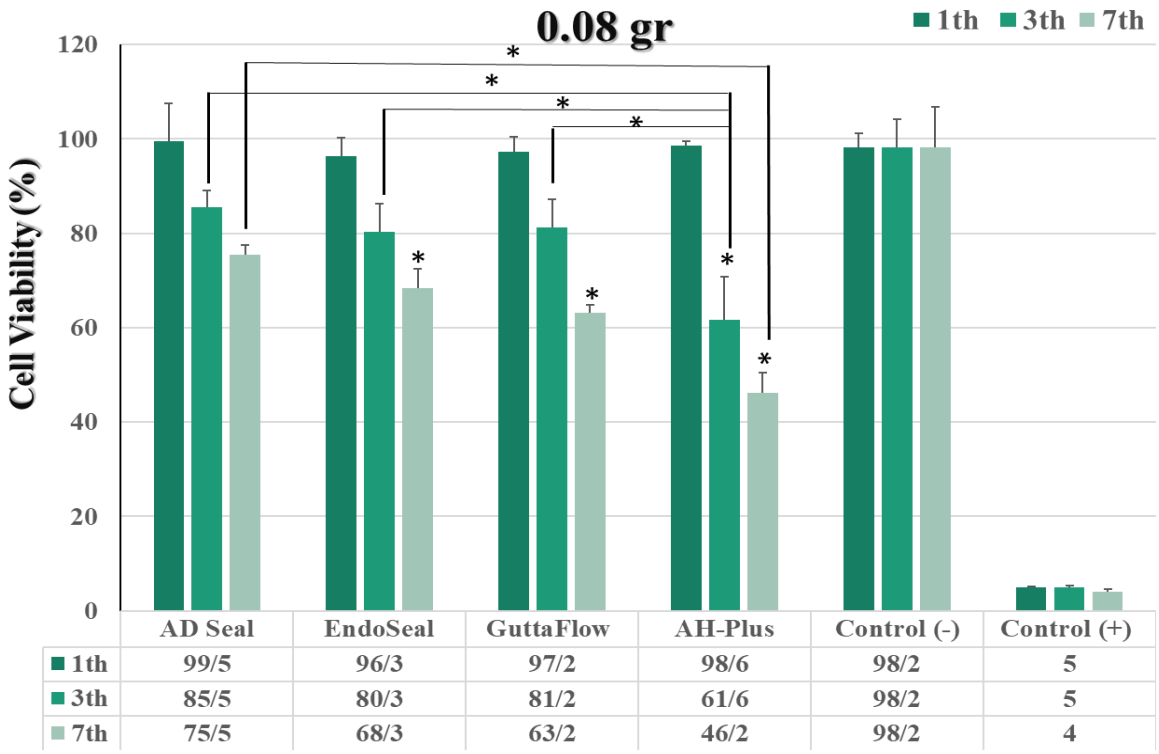


	AD Seal	EndoSeal	GuttaFlow	AH-Plus	Control (-)	Control (+)
1th	95	92/8	93/1	95/2	98/1	4
3th	87	75/8	69/1	51/2	98/1	4
7th	64	52	50	32	98/1	6

Figure 4. Viability of HGFs following exposure to 0.1 g of different sealers for 1, 3 and 7 days. * indicates P<0.05, and starred columns have a significant difference with the negative control group at the respective time point.

Cytotoxicity of 0.08 g sealers (Figure 5):

Comparison of the cytotoxicity of each sealer at different time points revealed no significant difference in cytotoxicity at 7 days compared with 3 days in any sealer group (P>0.05). On day 3, only AH Plus was significantly more cytotoxic than the negative control group (P<0.05). However, at 7 days, all sealers, except for AdSeal, showed significantly higher cytotoxicity than the negative control group (P<0.05). On day 3, AH Plus showed low cytotoxicity (>60% cell viability) and other sealers became significantly more biocompatible (P<0.05). On day 7, only AH Plus showed a significant difference with AdSeal (P<0.05), and the cells showed around 30% higher viability in presence of AdSeal. No other significant differences were noted (P>0.05). As shown in Figure 5, except for AH Plus that showed moderate cytotoxicity at 7 days, other sealers showed > 60% viability (low cytotoxicity) at all time points.



	AD Seal	EndoSeal	GuttaFlow	AH-Plus	Control (-)	Control (+)
1th	99/5	96/3	97/2	98/6	98/2	5
3th	85/5	80/3	81/2	61/6	98/2	5
7th	75/5	68/3	63/2	46/2	98/2	4

Figure 5. Viability of HGFs following exposure to 0.08 g of different sealers for 1, 3 and 7 days. * indicates P<0.05, and starred columns have a significant difference with the negative control group at the respective time point.

Discussion

This study compared the cytotoxicity of AH Plus and AdSeal epoxy resin-based sealers, Endoseal MTA calcium silicate-based sealer, and GuttaFlow Bioseal silicone-based sealer. The null hypothesis was that no significant difference would be found in cytotoxicity of the four sealer types. The results showed that AdSeal had the highest biocompatibility while AH Plus had the highest cytotoxicity among the tested sealers (P<0.05). Thus, the null hypothesis of the study was rejected. HGFs were used in this study because they are directly exposed to sealers in the oral environment and play a fundamental role in healing of wounds and periapical lesions. Injury of HGFs can significantly delay the healing course [22]. In the present study, HGFs were directly exposed to sealers according to Seo et al, [23] and the MTT assay was performed for the assessment of cell viability due to its high accuracy, simplicity, reliability, and cost-effectiveness [24]. AH Plus was used as the gold-standard sealer for the purpose of comparison with other sealers, similar to previous studies [25,26]. AH Plus has optimal physical and sealing properties

[27]. However, the present study showed its high cytotoxicity such that its cytotoxicity was higher than that of all other sealers. It also showed significantly lower biocompatibility at 3 and 7 days, compared with the negative control group. The same results were reported by Collado-González et al [28]. Similar to the present study, they found no significant difference in cytotoxicity between AH Plus and negative control group in the first 24 h but AH Plus showed significantly higher cytotoxicity at 48 h. At 1 week, AH Plus was more cytotoxic than GuttaFlow Bioseal. Oh et al. [29] evaluated the cytotoxicity of Endoseal MTA and AH Plus in freshly mixed and set forms using the CCK-8 test. They found no significant difference between the two sealers in set form at 1, 3 and 7 days, which was in agreement with our results. However, in freshly set form, AH Plus was significantly more cytotoxic at all three time points. Some other studies have also reported high cytotoxicity of AH Plus [26,30-33].

AH Plus contains epoxy resin, which is cytotoxic even in low concentrations [34]. It is also mutagenic and can break the DNA chain [35]. According to the manufacturer, it releases formaldehyde [36], which has high penetration depth and is toxic. Thus, its release along with amine and epoxy resin can explain high cytotoxicity of this sealer [33].

AdSeal showed the closest results to the negative control group in terms of cell viability. In 0.125 and 0.1 g weights, it showed insignificant cytotoxicity. In 0.08 g weight, it had no significant difference with the negative control group regarding cytotoxicity. Kim et al. [37] evaluated the biocompatibility of AdSeal compared with AH Plus and AH 26 using the agar diffusion test. Similar to the present findings, they showed that AdSeal was more biocompatible than the other two sealers; however, in contrast to the present results, they reported no cytotoxicity for AdSeal. Some other studies reported higher biocompatibility of AdSeal than AH Plus [37,38]. Although both of these sealers are epoxy resin sealers, higher biocompatibility of AdSeal compared with AH Plus may be due to the presence of calcium phosphate in its composition since calcium phosphate has high biocompatibility and induces bone formation [39].

In comparison of AdSeal with Endoseal MTA, AdSeal only showed lower cytotoxicity in 0.25 g weight on day 3. No other significant differences were noted between these two sealers at other time points, which was in agreement with the results of Lee et al [40]. They evaluated the cytotoxicity of a calcium-silicate based sealer namely Endoseal MTA, and two epoxy-resin based sealers namely AdSeal and AH Plus. They evaluated the cytotoxicity of sealers only at 1:5 concentration using the MTT assay. In line with our findings, they showed that AH Plus had the

highest cytotoxicity. They found no significant difference between AdSeal and Endoseal MTA at any time point.

In the present study, Bioseal GuttaFlow was more cytotoxic than AdSeal in 0.125 and 0.1 g weights on day 3; no other significant differences were noted between them. Search of the literature yielded no study comparing these two sealers to compare our results with.

Endoseal MTA is a calcium-silicate-based sealer, which is supplied as a paste in a syringe, and is injected into the root canal system [41]. It is recommended for root perforation repair; thus, the periodontal ligament cells would be exposed to it [42]. Also, since it is directly injected into the root canal system, it has high risk of apical extrusion and formation of sealer puff and may directly contact the periapical tissue and cause acute inflammation in the periapical region [43]. Kim et al. [44] compared the cytotoxicity of Endoseal MTA with AH Plus and Sealapex using WST-1 kit and two different cell types. They used sealer extracts in 1:10 concentration. They found no significant difference between AH Plus and the two sealers regarding cytotoxicity against MG-63 cell line at 1, 3 and 7 days. However, in exposure to HGFs, Endoseal MTA showed significantly lower cytotoxicity at all three time points. The present results confirmed their findings. In the present study, AH Plus was more cytotoxic than Endoseal MTA in 0.5, 0.1 and 0.08 g weights on day 3. In the study by Seo et al, [23] dental pulp stem cells were directly exposed to sealers for 24, 48, 72 and 120 h. The MTT assay showed higher biocompatibility of Endoseal MTA than AH Plus at 3 and 5 days.

Search of the literature by the authors yielded no study comparing Endoseal MTA and GuttaFlow Bioseal to compare our results with. In the present study, these two sealers were highly similar, and had no significant difference with each other, in comparison with the negative control group regarding cytotoxicity.

GuttaFlow Bioseal has two components, which are automatically mixed. It is easy to use and is based on silicone. Rodríguez-Lozano et al. [45] showed that this sealer can release apatite-forming ions and induce the formation of hydroxyapatite cores; this explains its higher biocompatibility even in comparison with GuttaFlow 2, which is from the same family of sealers. Saygili et al. [46] evaluated the cytotoxicity of sealers in 1:1 concentration using the MTT assay and the Tunnel test. They showed higher cytotoxicity of AH Plus compared with GuttaFlow Bioseal at 3, 24, 72, and 168 h, which was in line with the present results obtained for 0.5 and 0.08 g sealers at 3 days. In their study, GuttaFlow Bioseal showed no significant difference with

the negative control group while in the present study, such a result was only obtained on day 1. In contrast to our findings, Willershausen et al. [47] assessed the cytotoxicity of AH Plus at 24 h using the Alamar Blue colorimetric assay. However, in agreement with our results, GuttaFlow Bioseal and EndoSequence BC (calcium silicate-based) showed closer results to the negative control group at 72 and 96 h, and were significantly more biocompatible than AH Plus.
Future animal studies are required to assess the biocompatibility of sealers in vivo. Also, the cytotoxicity of sealers should be investigated on other cell types present in the periapical tissue such as the osteoblasts. Use of other tests such as WST-1 for assessment of cytotoxicity of sealers is also recommended. Furthermore, cell migration and behavior (by assessment of cell morphology) following exposure to different sealers should be investigated in future studies.

Conclusion

Within the limitations of this in vitro study, and considering the fact that the biocompatibility of AdSeal was closer to the negative control group, AdSeal appears to be the most biocompatible among all tested sealers; while, AH Plus showed the highest cytotoxicity at all time points. Thus, its application may delay the healing of periapical lesions.

Abbreviation

HGFs: Human gingival fibroblasts
MTT: The methyl thiazolyl tetrazolium
DMEM: Modified Eagle's medium
FBS: Fetal bovine serum

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of AJA University of Medical Sciences (ethical approval code: IR.AJAUMS.REC.1399.190). All methods were performed in accordance with the relevant guidelines and regulations of AJA University of Medical Sciences.

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.

Funding

This study was derived from a thesis, submitted to AJA University of Medical Sciences, School of Dentistry and was financially supported by the AJA University of Medical Sciences, Tehran, Iran. The funding body had no role in the design of the study or collection, analysis, or interpretation of data or in writing the manuscript.

Authors' contributions

MD devised the study concept, designed the study, analysis and interpretation of data, administrative, technical, and material support. MJA devised the acquisition of data, administrative, technical, and statistical analysis. RH Performed laboratory steps. MS devised the drafting of the manuscript. MSA devised the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the AJA University of Medical Sciences, Tehran, Iran.

References

1. Friedman S, Mor C. The success of endodontic therapy-healing and functionality. CDA J. 2004 Jun;32(6):493-503.
2. Friedman S, Abitbol S, Lawrence HP. Treatment outcome in endodontics: the Toronto Study. Phase 1: initial treatment. J Endod. 2003 Dec;29(12):787-93.

3. Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. Journal of endodontics. 2004 Dec 1;30(12):846-50.
4. Bodrumlu E, Tunga U. Apical leakage of Resilon obturation material. J Contemp Dent Pract. 2006 Sep 1;7(4):45-52.
5. Ghanaati S, Willershausen I, Barbeck M, Unger RE, Joergens M, Sader RA, Kirkpatrick CJ, Willershausen B. Tissue reaction to sealing materials: different view at biocompatibility. European journal of medical research. 2010 Dec;15(11):483-92.
6. Dastorani M, Malekpour B, AminSobhani M, Alemrajabi M, Mahdian A. Comparison of bacterial microleakage of three bioactive endodontic sealers in simulated underwater diving and aviation conditions. BMC Oral Health. 2021 Jul 15;21(1):345.
7. Walton RE, Torabinejad M. Principles and practice of endodontics. 3rd ed. Saunders, (2002), pp 34-7.
8. Granchi D, Stea S, Ciapetti G, Cavedagna D, Stea S, Pizzoferrato A. Endodontic cements induce alterations in the cell cycle of in vitro cultured osteoblasts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1995 Mar 1;79(3):359-66.
9. Dammaschke T, Witt M, Ott K, Schäfer E. Scanning electron microscopic investigation of incidence, location, and size of accessory foramina in primary and permanent molars. Quintessence international. 2004 Oct 1;35(9):699-705.
10. Braga JM, Oliveira RR, de Castro Martins R, Vieira LQ, Sobrinho AP. Assessment of the cytotoxicity of a mineral trioxide aggregate-based sealer with respect to macrophage activity. Dental Traumatology. 2015 Oct;31(5):390-5.
11. Geurtsen W. Biocompatibility of root canal filling materials. Australian endodontic journal: the journal of the Australian Society of Endodontology Inc. 2001 Apr 1;27(1):12-21.
12. Chandra BS, Gopikrishna V of the radicular space (chapter 15). B. Suresh Chandra, V. Gopikrishna (Eds.), Grossman's Endodontic Practice (13th ed.), Wolters Kluwer Health, New Dehli (2014), pp 343-73.
13. Spångberg L. Biological effects of root canal filling materials. 7. Reaction of bony tissue to implanted root canal filling material in guineapigs. Odontologisk tidskrift. 1969 Apr 15;77(2):133-59.

14. Heino TJ, Hentunen TA. Differentiation of osteoblasts and osteocytes from mesenchymal stem cells. Current stem cell research & therapy. 2008 May 1;3(2):131-45.
15. Johnson W, Kulild J.C, Tay F. Obturation of the Cleaned and Shaped Root Canal System. In: Hargreaves K.M, Berman L.H, editors. *Cohen's Pathways of the Pulp.* Elsevier; St. Louis, MO, USA: 2016. p. 280–322.
16. Schmalz G, Widbiller M, Galler KM. Material tissue interaction—From toxicity to tissue regeneration. Operative dentistry. 2016 Mar;41(2):117-31.
17. Zhou HM, Shen Y, Wang ZJ, Li L, Zheng YF, Häkkinen L, Haapasalo M. In vitro cytotoxicity evaluation of a novel root repair material. Journal of endodontics. 2013 Apr 1;39(4):478-83.
18. Jaberiansari Z, Naderi S, Tabatabaei FS. Cytotoxic effects of various mineral trioxide aggregate formulations, calcium-enriched mixture and a new cement on human pulp stem cells. Iranian endodontic journal. 2014;9(4):271-6.
19. Ali SA, Al-Jundi SH, Ditto DJ. In vitro toxicity of grey MTA in comparison to white MTA on human periodontal ligament fibroblasts. European Archives of Paediatric Dentistry. 2014 Dec 1;15(6):429-33.
20. Parirokh M, Forghani FR, Paseban H, Asgary S, Askarifard S, Mahani SE. Cytotoxicity of two resin-based sealers and a fluoride varnish on human gingival fibroblasts. Iranian endodontic journal. 2015;10(2):89-92.
21. Fonseca DA, Paula AB, Marto CM, Coelho A, Paulo S, Martinho JP, Carrilho E, Ferreira MM. Biocompatibility of Root Canal Sealers: A systematic review of in vitro and in vivo studies. Materials. 2019 Jan;12(24):4113.
22. Zhu Q, Haglund R, Safavi KE, Spangberg LS. Adhesion of human osteoblasts on root-end filling materials. Journal of Endodontics. 2000 Jul 1;26(7):404-6.
23. Seo DG, Lee D, Kim YM, Song D, Kim SY. Biocompatibility and mineralization activity of three calcium silicate-based root canal sealers compared to conventional resin-based sealer in human dental pulp stem cells. Materials. 2019 Jan;12(15):2482.
24. Rodríguez-Lozano FJ, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Forner L, Moraleda JM. Evaluation of cytocompatibility of calcium silicate-based endodontic sealers and their effects on the biological responses of mesenchymal dental stem cells. International endodontic journal. 2017 Jan;50(1):67-76.

25. Zhou HM, Du TF, Shen Y, Wang ZJ, Zheng YF, Haapasalo M. In vitro cytotoxicity of calcium silicate–containing endodontic sealers. Journal of endodontics. 2015 Jan 1;41(1):56-61.
26. Miletić I, Devčić N, Anić I, Borčić J, Karlović Z, Osmak M. The cytotoxicity of RoekoSeal and AH plus compared during different setting periods. Journal of Endodontics. 2005 Apr 1;31(4):307-9.
27. Schäfer E, Bering N, Bürklein S. Selected physicochemical properties of AH Plus, EndoREZ and RealSeal SE root canal sealers. Odontology. 2015 Jan;103(1):61-5.
28. Collado-González M, Tomás-Catalá CJ, Oñate-Sánchez RE, Moraleda JM, Rodríguez-Lozano FJ. Cytotoxicity of GuttaFlow Bioseal, GuttaFlow2, MTA Fillapex, and AH Plus on human periodontal ligament stem cells. Journal of endodontics. 2017 May 1;43(5):816-22.
29. Oh H, Kim E, Lee S, Park S, Chen D, Shin SJ, Kim E, Kim S. Comparison of Biocompatibility of Calcium Silicate-Based Sealers and Epoxy Resin-Based Sealer on Human Periodontal Ligament Stem Cells. Materials. 2020 Jan;13(22):5242.
30. Al-Hiyasat AS, Tayyar M, Darmani H. Cytotoxicity evaluation of various resin based root canal sealers. International Endodontic Journal. 2010 Feb;43(2):148-53.
31. Correa GT, Veranio GA, Silva LE, Hirata Junior R, Coil JM, Scelza MF. Cytotoxicity evaluation of two root canal sealers and a commercial calcium hydroxide paste on THP1 cell line by Trypan Blue assay. Journal of Applied Oral Science. 2009 Oct;17(5):457-61.
32. Huang Y, Fenech M, Shi Q. Micronucleus formation detected by live-cell imaging. Mutagenesis. 2011 Jan;26(1):133-8.
33. Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide-and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. International endodontic journal. 2007 May;40(5):329-37.
34. Schweikl H, Schmalz G, Spruss T. The induction of micronuclei in vitro by unpolymerized resin monomers. Journal of dental research. 2001 Jul;80(7):1615-20.
35. Schweikl H, Schmalz G, Federlin M. Mutagenicity of the root canal sealer AHPlus in the Ames test. Clinical oral investigations. 1998 Nov;2(3):125-9.
36. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. Formaldehyde evaluation from endodontic materials. Oral health. 1998 Dec 1;88(12):37-9.

37. Kim YB, Baek SH, Bae KS. In vivo study on the biocompatibility of new resin-based root canal sealers. Journal of Korean Academy of Conservative Dentistry. 2002 Mar 1;27(2):122-34.
38. Park SY, Lee WC, Lim SS. Cytotoxicity and antibacterial property of new resin-based sealer. Journal of Korean Academy of Conservative Dentistry. 2003 Mar 1;28(2):162-8.
39. Lim ES, Park YB, Kwon YS, Shon WJ, Lee KW, Min KS. Physical properties and biocompatibility of an injectable calcium-silicate-based root canal sealer: in vitro and in vivo study. BMC oral health. 2015 Dec;15(1):1-7.
40. Lee JK, Kim S, Lee S, Kim HC, Kim E. In vitro comparison of biocompatibility of calcium silicate-based root canal sealers. Materials. 2019 Jan;12(15):2411.
41. Tomson RM, Polycarpou N, Tomson PL. Contemporary obturation of the root canal system. British dental journal. 2014 Mar;216(6):315-22.
42. Dastorani M, Shourvarzi B, Nojoumi F, Ajami M. Comparison of Bacterial Microleakage of Endoseal MTA Sealer and Pro-Root MTA in Root Perforation. Journal of Dentistry. 2021 Jun 1;22(2):96-101.
43. Johnson W. Obturation of the cleaned and shaped root canal. Pathways of the Pulp. 2011:358-62.
44. Kim RJ, Shin JH. Cytotoxicity of a novel mineral trioxide aggregated based root canal sealer. Dental materials journal. 2014 Mar 28:2013-171.
45. Rodríguez-Lozano FJ, García-Bernal D, Aznar-Cervantes S, Ros-Roca MA, Algueró MC, Atucha NM, Lozano-García AA, Moraleda JM, Cenis JL. Effects of composite films of silk fibroin and graphene oxide on the proliferation, cell viability and mesenchymal phenotype of periodontal ligament stem cells. Journal of Materials Science: Materials in Medicine. 2014 Dec 1;25(12):2731-41.
46. Saygili G, Saygili S, Tuglu I, Capar ID. In vitro cytotoxicity of Guttaflow bioseal, Guttaflow 2, AH-Plus and MTA fillapex. Iranian endodontic journal. 2017;12(3):354-9.
47. Willershausen I, Callaway A, Briseño B, Willershausen B. In vitro analysis of the cytotoxicity and the antimicrobial effect of four endodontic sealers. Head & face medicine. 2011 Dec;7(1):1-9.