EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
Vol. 69, 2427:2436, July, 2023
Print ISSN 0070-9484 • Online ISSN 2090-2360
Conservative Dentistry and Endodontics
Submit Date : 11-05-2023 • Accept Date : 14-06-2023 • Available online: 10-07-2023 • DOI : 10.21608/EDJ.2023.210352.2544

COMPARATIVE STUDY OF THE CYTOTOXIC EFFECT OF EPOXY RESIN AND CALCIUM SILICATE BASED SEALERS (AN IN-VITRO STUDY)

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ABSTRACT

Objective: The purpose of this study is to compare the cytotoxicity of bioceramic endodontic sealers (Well-Root ST "Vericom in Gangwon-Do, Korea") with epoxy resin-based endodontic sealers (AH + sealer "Dentsply Sirona, Germany").

Methods: In sterile test tubes, the tested sealers were serially diluted twice. In order to evaluate the proper concentration where fibroblast cells would survive, extraction media were diluted many times utilizing MEM-E Medium (Eagle's minimal essential medium). The cytotoxic effect of concentrations used in the present study (0%, 6.25%, 12.5%, 25%, 50%, and 100%) was assessed by MTT essay after 24 hours using a human fibroblast cell line. Statistical analysis was performed by using One Way ANOVA test, followed by Tukey`s Post Hoc test for multiple comparisons.

Results: All concentrations, with the exception of 12.5% and 25%, did not significantly differ between the two groups. Because group I (AH Plus sealer) had a lower proportion of viable cells than group II (Well-Root ST sealer), it had a larger cytotoxic impact. The concentrations that caused a significant difference between the two groups with a P value of 0.001 were 12.5% and 25%.

Conclusion: Evaluation of the calcium silicate-based sealer (Well-Root ST) cytotoxicity showed superior biological behavior and higher cytocompatibility compared to epoxy resin-based endodontic sealer (AH plus).

KEYWORDS: Cytotoxicity, AH plus, Well-Root ST, biocompatibility, MTT essay.

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INTRODUCTION

The key to an effective root canal treatment is hermetic seal of the root canal space after mechanical preparation. Endodontic sealer is in close proximity to the periapical tissues, and its accidental extrusion could result in periapical inflammation and cellular damage, which would impact the treatment's outcome. Therefore, a sealer's biocompatibility is crucial for effective endodontic treatment. However, rapid insertion of the gutta percha may result in the extension of the sealer and gutta-percha beyond the working length and through the apical foramen, the extrusion of filling material may cause postoperative pain. [1]

Bioceramic sealers presents a better result regarding physicochemical[2,3] and antibacterial properties compared with other sealers present in the market. Moreover, the main advantages of bioceramic sealers are related to its biocompatibility[3-5] and bioactivity, which is the capability to bond chemically to the dentin of root canal, due to the formation of an apatite-like structure that enhance the sealing ability of the obturation materials [6].

Bioceramic sealers have been used in the dental field especially endodontics for many years. An injectable bioceramic cement paste called Well-Root ST (Vericom, in Gangwon-Do, Korea) was created for the purpose of permanently obturating the root canal. Calcium silicate, zirconium oxide, filler, and thickening agents are all parts of Well-Root ST, according to the manufacturer. [7]

Recently, epoxy resin-based sealers have been recognized as the gold standard for root canal obturation; however, several drawbacks for resin sealers have been revealed, including the inflammatory response, hydrophobic nature, and cytotoxicity; there are also concerns about the adverse effects of resin sealers in contact with tissues and the suspension in the periapical healing of teeth with apical periodontitis. Bioceramic sealers are new sealers that have been reported to have good hydrophilicity and biocompatibility. Because the root canal is hydrophilic, the water absorption and solubility of sealers are critical features associated with sealing ability. [8, 9]

Because one of the primary aims of root canal therapy is to restore periapical tissue, it is critical that the sealer used for obturation promotes tissue healing or does not cause extra injury to the periapical tissues. Endodontic sealers are tested for their influence on periapical tissue repair using cytotoxicity. As a result, cytotoxicity testing should be performed prior to clinical use of these compounds. [10]

The purpose of this study is to compare the cytotoxicity of bioceramic endodontic sealers (Well-Root ST "Vericom in Gangwon-Do, Korea") with epoxy resin-based endodontic sealers (AH + sealer "Dentsply Sirona, Germany").

MATERIALS AND METHODS:

This study conducted adhered to the principles outlined in declaration of Helsinki and approved by the ethics committee (Faculty of Dentistry, October 6 University, Giza, Egypt) RECO6U/17-2021.

Sample size calculation:

The sample size was estimated using Nashaat Y. et al. [11] According to this study, the responses within each subject group were distributed normally, with a standard deviation of 0.07. If the anticipated variance between the control and tested means is 0.08 and the investigation required at least 13 samples in each group for null hypothesis rejection, the sample mean of the control and tested groups is equal with a probability (power) of 0.8. The Type I error probability resulting from this null hypothesis test is 0.05. To account for the 20% dropout rate, the number of samples was raised to 16 specimens per group.

Classification of samples:

32 specimens were assigned into two groups: **Group I:** AH Plus sealer was applied into 16 specimens.

Group II: Well-Root ST sealer was applied into 16 specimens.

Preparation of samples:

5000 cells per well of 96-well plates were used for cell seeding. 5% CO2 and a humidification incubator set at 37°C. Following incubation, different concentrations of (0, 6.25, 12.5, 25, 50, and 100%) are applied to the cells. They were incubated under the same circumstances for 24 hours.

The MTT dye was applied to the wells then, incubated once more for two to three hours at 37°C. The extracted media was combined with solubilizing buffer (PBS).

ELISA reader was used to analyze the plates at 570 nm, and the findings were computed.

Cytotoxicity evaluation procedures:

(a) Cell cultures

VACSERA Company, Giza, Egypt, supplied the HSF cell line ($2x10^5$). Eagle's minimal essential medium (MEM-E) was used for growth of cells in culture flasks, nourished with 10% 1% nonessential amino acid solution, fetal bovine serum (FBS), and 100 units streptomycin and 100 IU penicillin in 0.9% saline at temperature 37°C in a atmosphere with humidity level of 5% CO2, 95% air. (All items are from Sigma-Aldrich, St. Louis, MO, USA.)

After incubation, trypsinization for Fibroblast cells was done for 2–3 minutes using 0.25% trypsin solution on the decanted growing medium. The cells were treated with trypsin until they were completely dissociated.

A hemocytometer was used for counting the suspended fibroblast cells then, the fibroblasts were dispersed in 96 well plates with 100µl of culture media with final density of $3x10^4$ cells/cm2. Incubation of the plates was performed at temperature 37°C in a 5% CO2 environment for 24 hours to allow adherence of cells.[10]

(b) Extraction procedure

Collection of the tested materials was done in safety cabinet under aseptic condition and subsequently materials were soaked in MEM-E culture medium for 7 days with concentration 10 mg/ml of each sample. Using sterile filters of 0.45 µm pore size were used for collection of extract media.

(c) Determination of cytotoxic medicament concentrations

In sterile test tubes, the tested sealers were serially diluted twice. In order to evaluate the proper concentration where fibroblast cells would survive, extraction media were diluted many times utilizing MEM-E Medium. The cytotoxic effect of concentrations used in the present study (0%, 6.25%, 12.5%, 25%, 50%, and 100%) was assessed by MTT essay after 24 hours using a human fibroblast cell line.

Methods of evaluation:

MTT ASSAY:

MTT essay is a colorimetric analysis that quantifies the color change resulted from reduction of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in active cells. Yellow MTT penetrates the cells and passes to the mitochondria, where it is converted by mitochondrial succinate dehydrogenase to an insoluble, colored formazan product (dark purple). Organic solvent (e.g., isopropanol) was used for solubilization of fibroblast cells, and the spectrophotometric measurement for produced formazan reagent was performed. The number of viable cells can be estimated from the level of activity since the reduction of MTT can only occur in cells with active metabolism, Samples were imaged using inverted microscopy *(Olympus, Japan)*

All measurements were collected in triplicate. Using Enzyme-linked immunosorbent assay (ELISA) plate reader, the optical density of dissolved crystals at 570 nm. The percentage of viability and the medicine concentration were shown opposite each other. The optical density, which represents the percentage of remaining alive cells, was calculated using the following equation:

$$\text{Viability \%} = \frac{\text{(OD TEST 1 X 100)}}{\text{OD Control}}$$

Number of residual living cells = (OD of treated cells/ OD of untreated cells) x Number of negative control cells ($1X10^4$ cells/$0.1cm^3$).

Statistical analysis:

All results were Statistically analyzed using One Way ANOVA test, and Tukey`s Post Hoc test for multiple comparisons.

RESULTS:

Group I (AH Plus sealer):

Table (1) and figure (1) show the mean and standard deviation values for the cytotoxicity of group I (AH Plus sealer) at various doses. The **One Way ANOVA test** was used to compare different concentrations, followed by the **Tukey's Post Hoc test** for multiple comparisons, which found that:

The results showed significant difference among different concentrations as P<0.0001* where the concentration of 100% (Figure 5) revealed higher level of cytotoxicity than 50% concentration (Figure 7) as the viable cells percentage was the least in 100% concentration. Also, cytotoxicity decreased as concentration of AH Plus decreased to reach zero cytotoxicity at zero concentration (negative control group) Figure (4).

TABLE (1) Mean, standard deviation values and cell viability of the cytotoxicity in (group I) at different concentrations and comparison between them:

Concentration	Mean	Standard Deviation	Cell viability %	P value
0	0.72 a	0.01	100	<0.0001*
6.25	0.69 b	0.01	93.5	
12.5	0.61 c	0.04	84.6	
25	0.56 d	0.00	77.13	
50	0.53 e	0.01	73.30	
100	0.48 f	0.01	66.39	

Significant difference as P<0.05.
Mean with the same superscript letters were insignificantly different as P>0.05.
Mean with different superscript letters were significantly different as P<0.05.

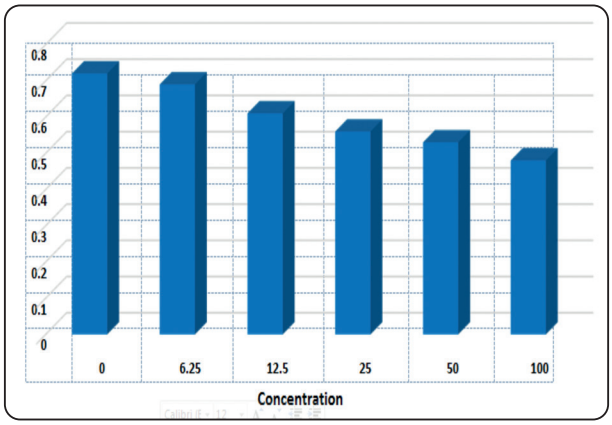

Fig. (1): Bar chart showing cytotoxicity in group I at different concentrations.

Group II (Well-Root ST sealer):

Mean and standard deviation values of the cytotoxicity of group II (Well-Root ST sealer) at different concentrations were presented in table (2) and figure (2).

The comparison of different concentrations was carried out using the One Way ANOVA test, which found a significant difference between different concentrations as P<0.0001*, followed by the Tukey's Post Hoc test for multiple comparisons, which revealed:

The results showed that 100% concentration of Well-Root ST was significantly the highest cytotoxicity among all other concentrations (Figure 6) as P<0.0001 where the percentage of viable cells was the lowest, followed by 50% (Figure 8) concentration which show significant difference but lower in cytotoxicity than concentration 100%.

There was no significant difference between 6.25% concentration and 12.5% concentration where the cytotoxic effect on comparing these concentrations results insignificance in viable cells percentages. The same result was also found between 12.5% concentration and 25% concentration.

Zero cytotoxicity was found at 0% concentration (negative control group) where the percentage of cell viability was 100% (Figure 4).

Comparison between both groups:

Table (3) and figure (3) show the mean and standard deviation of cytotoxicity for both groups at different concentrations.

The Independent t test was used to compare various groups, and the findings revealed that:

There was no significant difference between both groups in all different concentrations except 12.5% and 25%.

The concentrations resulting a significant difference among both groups with P=0.001* was 12.5% and 25% where group I (AH Plus sealer) showed higher cytotoxic effect than group II (Well-Root ST sealer) as the percentage of viable cells was lower in group I than in group II.

TABLE (2) Mean, standard deviation values and cell viability of the cytotoxicity in group II at different concentrations and comparison between them:

Concentration	Mean	Standard Deviation	Cell viability %	P value
0	0.72 a	0.01	100	<0.0001*
6.25	0.68 ab	0.04	95.11	
12.5	0.66 bc	0.04	91.05	
25	0.62 c	0.07	85.2	
50	0.55 d	0.06	76.0	
100	0.48 e	0.03	66.89	

Significant difference as P<0.05.
Mean values with the same superscript letters were insignificantly different as P>0.05. Mean values with different superscript letters were significantly different as P<0.05.

TABLE (3) Mean and standard deviation values of the cytotoxicity in both groups at different intervals and comparison between them:

Concentration	Group I (AH Plus sealer)		Group II (Well-Root ST sealer)		P value
	M	SD	M	SD	
0	0.72	0.01	0.72	0.01	1.00 ns
6.25	0.69	0.01	0.68	0.04	0.33 ns
12.5	0.61	0.04	0.66	0.04	0.001*
25	0.56	0.00	0.62	0.07	0.001*
50	0.53	0.01	0.55	0.06	0.19 ns
100	0.48	0.01	0.48	0.03	0.99 ns

M: mean SD: standard deviation
Ns: Non-significant difference as P>0.05 * Significant difference as P<0.05

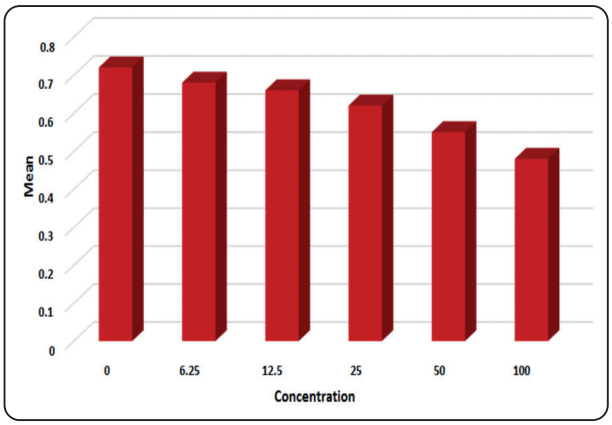

Fig. (2) Bar chart showing mean of cytotoxicity in group II at different concentrations.

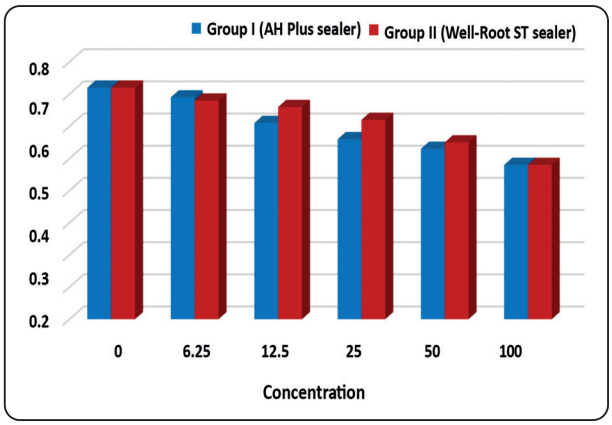

Fig. (3) Bar chart showing Mean of cytotoxicity in both groups at different intervals.

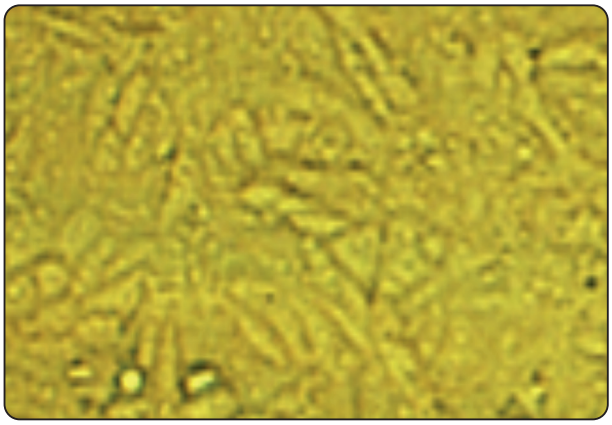

Fig. (4) Inverted microscopic image for HFB4 Control

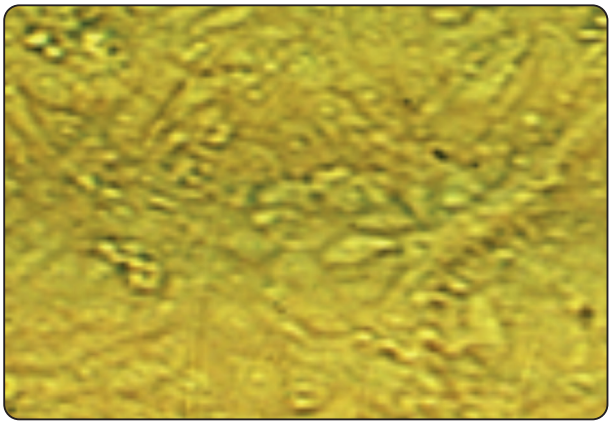

Fig. (5) Inverted microscopic image for AH Plus sealer in 100%c

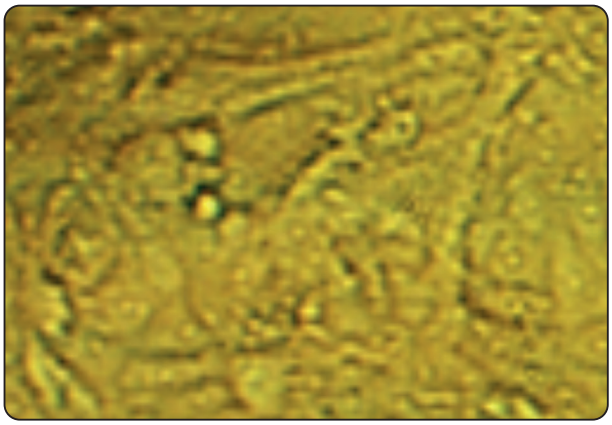

Fig. (6) Inverted microscopic image for Well- Root ST sealer in 100% c

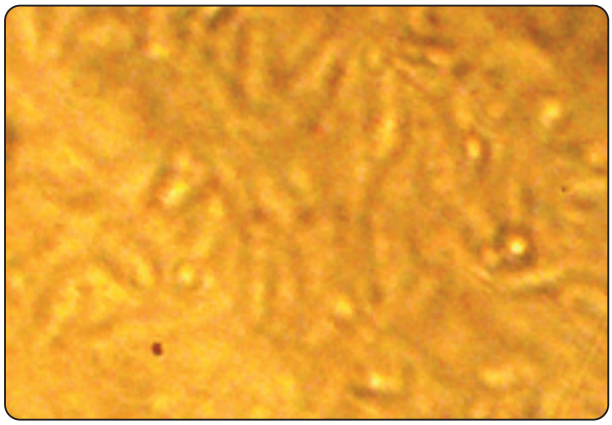

Fig. (7) Inverted microscopic image for AH Plus sealer in 50% c

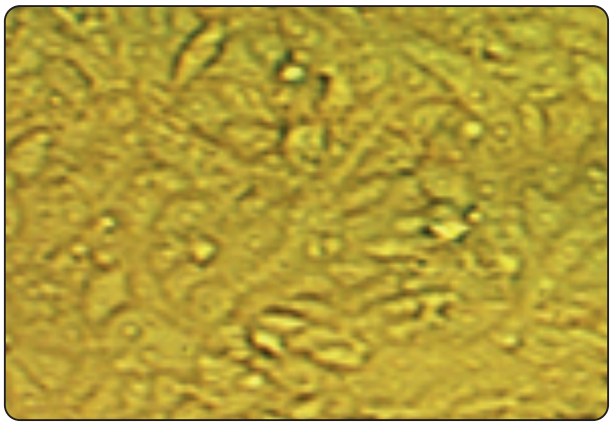

Fig. (8) Inverted microscopic image for Well-Root ST sealer in 50% c

DISCUSSION

The goal of endodontic obturation is to create an effective barrier that protects the periapical tissue from oral microbiota. Previous attempts to construct a tight barrier involved filling the root canal space with gutta percha, which has superior properties and biocompatibility than the sealer. [12]

Multiple varieties of endodontic sealers are available; the most common are resin endodontic sealers, followed by bioceramic endodontic sealers, which were released recently.

Because of their better physicochemical qualities, resin-based sealers were frequently employed in endodontics. AH+ is an epoxy resin-based sealer that was supposed to be the gold standard of endodontic obturation. This sealer's flowability and long-term polymerization time allow it to penetrate deeper into the dentinal tubules and produce strong mechanical interlocking between dentin and sealer. [13]

AH Plus presents higher solubility, pH and calcium ion release and less flow and radiopacity. Since one of the main purposes of root canal treatments is the repair of the periapical tissues, it is necessary that the materials used inside the root canal favor this repair or at least does not promote any additional harm to these tissues. [14]

Oure study purpose is to compare the cytotoxicity of bioceramic endodontic sealers (Well-Root ST "Vericom in Gangwon-Do, Korea") with epoxy resin-based endodontic sealers (AH + sealer "Dentsply Sirona, Germany").

Epoxy resin is a component of AH Plus sealer that exhibits cytotoxic properties, even with slightly diluted doses. The epoxy resin component present in AH+ is mutagenic and has the potential to disrupt the continuity of cellular DNA. Formaldehyde is not one of the components of AH Plus as claimed by the manufacturer. However, a prior study found that a negligible quantity of formaldehyde emission (3.9 ppm) occurred. The formaldehyde release in conjunction with the amine and epoxy resin ingredients release may explain the cytotoxicity of freshly mixed AH+ sealer. [15]

However, important disadvantages of the current epoxy resin endodontic sealers include leakage, cytotoxicity on both short- and long-term, in addition to their prolonged curing times. [16] Contact with unset paste with resin-based sealers can cause an acute inflammation of the oral mucosa, which can cause irritation and discomfort. Local and systemic allergies have occasionally also been noted. [17]

Due to these drawbacks, new sealing agents must be created that employ nontoxic, fast, and antimicrobial lasting antimicrobial qualities to reduce secondary inflammation and infection rates. [16]

Bioceramic endodontic sealers were recently developed to solve the disadvantages of epoxy resin-based sealing compounds. Calcium silicates, zirconia particles, hydroxyapatite, alumina, bioactive glass, and resorbable calcium phosphates are typically found in bioceramic sealers. These components provide the sealers their ability to be biocompatible, have an antibacterial impact, and even promote dynamic intratubular mineralization. Additionally, bioceramic sealers improve conventional root canal treatment results by assisting odontoblast development and the release of bioactive compounds. [18]

Comparing modern bioceramic sealers, which set quickly, to older bioceramic sealers, which took hours

or days to set, increases clinical efficiency. Clinical uses of previous generations of bioceramic sealers beyond obturation were constrained because of the possibility of material washout during operations including pulp capping, perforation repair, and root-end surgery. Additional consultations would be necessary for these treatments to validate the whole set of the material. [19]

Additionally, compared to standard methods, there is a larger likelihood that calcium silicate-based sealers may be apically extruded when clinicians administer them directly using a syringe and needle in the root canal. Excess sealers can cause periapical tissues irritation and postoperative discomfort if they are extruded outside the apical constriction. This might impact periapical tissue repair and bone metabolism. Consequently, it is essential to assess the biocompatibility of sealers. [12]

Since a single cytotoxicity assay is not satisfactory for the evaluation and prediction of the material's cytotoxicity, and as the colorimetric assay for compound analysis alone is not indicative for cytotoxicity examination, cytotoxicity was better to be evaluated by flowcytometry and MTT assay. According to its ability to exclude dye from live cells, flowcytometry provides a steady and trustworthy indication of the true cell viability. [20]

Since fibroblasts behave more like in-vivo cells, they were employed to assess the cytotoxic effect of endodontic sealers. Fibroblasts play a crucial role in local inflammatory and immunological responses as immune-regulatory cells. They create cytokines, chemokines, growth factors, and other physiologically active chemicals; the microenvironment could influence how these molecules operate. [21]

The MTT test was used to examine how AH Plus and Well-Root ST affected cytotoxicity. Cell viability is a crucial factor to consider while analyzing the biological impact of sealants. The measurement of the number of alive cells is known as cell viability, and it is often given as a percentage of the control. When first seeding the cells onto a plate and when determining the cytotoxicity of the sealers, viability tests are essential. [22]

We chose the MTT test because its measurements rely on living cells and it has been frequently used to evaluate cell growth. Mitochondrial succinate dehydrogenase act as a catalyst to reduce 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into MTT-formazan.

The MTT assay depends on mitochondrial activity and analyses a cell's capacity to create energy inferentially. The MTT test may easily be performed on cell monolayers that have been plated on multiple well plates using a colorimetric reaction, that quantify the reduction of yellow MTT by mitochondrial succinate dehydrogenase. MTT passes to the mitochondria, where it is converted to an insoluble, dark purple formazan product. Since the transformation can only take place in these cells, the amount of formazan is related to the quantity of living cells. The amount of formazan generated is calculated spectrophotometrically at 570 nm. Results were calculated using the viability percentage in compared to untreated cells. [13]

According to ISO's recommendation (10993-5, 2009) if the cell viability less than 70%, the tested material should be regarded as cytotoxic to that kind of cell. Sealer concentrations of 100 g/ml was considered harmful by the ISO since test findings for these concentrations showed that cell viability was less than 70%. [22]

Our findings revealed that the cytotoxicity of the two sealers, with the has no significant difference in all tested concentrations except 12.5% and 25%. There are less viable cells in group I (AH plus sealer) compared in group II (Well-Root ST sealer).

Even bioceramic endodontic sealer (Well-Root ST) in the current investigation displayed reduced number of viable cells over time in culture media, which may be related to high alkalinity in the fresh condition. When they come into communication with soft tissues, they turn into calcium hydroxide. Although root canal sealers' high pH may have

this detrimental impact on cell viability, it may also have certain biological benefits. The high alkalinity of these sealers may alter the environment to encourage the growth of hard tissues while inhibiting osteoclastic activity in the surrounding tissues, which is beneficial for healing. [23]

This was consistent with the findings of ***Jung S. et al***. [15], who found that immediately introduced AH+ was highly cytotoxic at a high concentration of the extract (1:2). After setup, AH+ was ceased to be cytotoxic. In contrast, other investigations found that AH+ was minimally cytotoxic after one week and harmless after two weeks in fresh settings. The least cytotoxic of the resin-containing sealers tested, AH+ nevertheless resulted in a 26% reduction in cell viability. However, periapical tissues surrounding root canals filled with epoxy-resin in animal research with induced apical periodontitis displayed less inflammation than those around those filled with alternative sealants (zinc oxide eugenol and silicone).

According to ***Wuersching SN. et al.*** [24], fibroblasts and osteoblasts identified in the periodontal ligament were highly susceptible to the cytotoxicity of AH+. The 7-day eluates were often less hazardous than the 24-h eluates, especially when diluted, according to our research on the variations in cytotoxicity between the two types of root canal sealers eluates. The toxic effects of the RCS may have diminished after only a few days due to the comparatively poor molecular stability of the poisons released within the first 24 hours of elution, according to this result.

Our results were disagreed with ***Dhopavkar.V.V. et al.*** [25], who assessed the cytotoxicity and the genotoxicity of the AH plus at both intervals 24 hrs and 48 hrs, showed the resin-based sealer has the lowest variables than the bioceramic sealer examined. Also, ***Benetti F. et al***. [26], who agreed in his article that the resin sealer has lower cytotoxicity than the bioceramic sealer but he also reported that bioceramic sealer is more biocompatible, after he compared between them.

These were in opposition to the findings of ***da Silva EJNL et al.*** [27], who reported that MTA Fillapex were more cytotoxic in comparison to AH Plus, EndoSequence BC sealer, and EndoSeal. The findings could be related to ingredients in MTA Fillapex's formulation like silica, salicylate resin, and diluting resin.

However, ***Lim.E.S. et al.*** [28] revealed that the calcium silicate-based sealer shown higher biocompatibility when compared to AH plus, a well-known resin-based sealer. Additionally, this injection-type, self-setting root canal sealer is easier to manipulate and use in a clinical environment, both of which are clinical advantages.

CONCLUSION

Evaluation of the calcium silicate-based sealer (Well-Root ST) cytotoxicity showed superior biological behavior and higher cytocompatibility compared to epoxy resin-based sealer (AH plus).

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