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A Comparative Analysis of Cytotoxicity of Three Different Root Canal Sealers

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Abstract

Aim: This study aims to evaluate Well-Root ST in comparison with CeraSeal and AH Plus regarding cytotoxicity using (MTT) assay on human epithelial type 2 (HEp-2) cells. **Materials and Methods:** Teflon mold was used to fabricate disc samples of each root canal sealer. Samples were divided into 3 groups: group (A) (Well-Root ST), group (B) (Ceraseal) and group (C) (AH Plus) in two evaluation periods (24h and 72h). All samples of root canal sealers were mixed according to the manufacturer's instructions and the discs were allowed to set in a at 37°C for 24 hours before extraction. Extract collected at each time point was diluted to various concentrations of 12.5%, 25%, 50% and 100% with DMEM to create a total of four different concentrations of each extract. DMEM incubated for 24 hours served as the control. (HEp-2) cell line was seeded in 96 well micro-titer plates and cultures were then subjected to 100 µL of the (12.5%, 25%, 50% and 100%) extracts medium while cell cultures with supplemented DMEM were used as controls. The plates were left in the incubator for 24 or 72 h before the cytotoxicity evaluation was carried out by (MTT) assay. Viability percentage was calculated and cytotoxicity was evaluated by rating according to cell viability relative to control group, non-cytotoxic (more than 90%), slightly cytotoxic (from 60 to 90%), moderately cytotoxic (from 30 to 59%) and severely cytotoxic (less than 30%) cell viability. **Statistics:** Data were collected, tabulated and statistically analyzed using one way ANOVA test and Bonferroni's post-hoc test. **Results:** According to evaluation time, Well-Root ST showed the highest viability values in all concentration percentages at both intervals (24 and 72 hours) while AH plus showed the least viability values. According to concentration percentage, The viability increased with decreasing the concentration in all tested groups. **Conclusion:** The evaluated root canal sealers showed varying degrees of cytotoxicity. However, Well-Root ST was associated with significantly highest cell viability percentages. 1- AH plus significantly showed less cell viability in comparison to calcium silicate-based root canal sealers.

Keywords: Root canal sealer, Bioceramic, Cytotoxicity, MTT assay.

INTRODUCTION

The obturation of root canals is a critical step in root canal therapy. This procedure consists of filling the root canals three-dimensionally to inhibit leakage and bacterial proliferation. Root canal sealers have always been a necessary component in the obturation phase of root canal treatment. Sealers are chemically classified into zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, and bioceramic-based sealers.

Bioceramic-based root canal sealers have been used in the dental field especially endodontics for many years. Well-Root ST (Vericom, Gangwon-Do, Korea) is a premixed, ready-to-use, injectable bioceramic cement paste developed for permanent obturation of the root canal. The composition of Well-Root ST as described by the manufacturer includes zirconium oxide, calcium silicate, filler, and thickening agents [1]. Ceraseal (Meta Biomed, Korea) is a recently introduced premixed calcium silicate-based [2] root canal sealer. To our knowledge, relatively limited information has been published in the scientific literature regarding the cytotoxic effects of these root canal sealers.

AH Plus (Dentsply) was launched into the market in 1997 as two pastes, packaged in tubes (of 4 ml each), and composed of epoxy resin and amines. Over the past years, this root canal sealer has been considered a benchmark in comparative studies of endodontic sealers.

Although root canal sealers are manufactured to be used within the root canals, sometimes they might pass through the apical foramen to the periapical region. Based on this possibility, root canal sealers should offer biocompatibility and non-cytotoxic properties as a crucial factor for safe usage of the material and long-term success of root canal treatment [3]. Therefore, this study aims to compare the cytotoxicity of Well-Root ST, Ceraseal and AH plus sealers.

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MATERIALS AND METHODS

Cell culture

The method used in this study was approved by the regional ethical committee and the study was conducted. The cytotoxic effect of the root canal sealers used in this study was evaluated on human laryngeal carcinoma. The human laryngeal carcinoma which is known as human epithelial type 2 (HEp-2) cell line was kindly supplied from Department of Cell Culture at Vacsera-Egypt. Standard protocols were followed in establishing and maintaining the cultures. The cell line was cultured in RPMI-1640 medium supplemented with 10% fetal biovin serum (FBS), 25mM sodium bicarbonate, 20 mM HEPES, 100 U/ml penicillin and 100µg/ml streptomycin. Cells were incubated at 37°C in 5% CO2 atmosphere humidified incubator (Jouan-France**)**.

Cytotoxicity Assay (MTT assay)

This study evaluated the cytotoxicity of three endodontic sealers which are Well-Root ST (Ivoclar Vivadent, Schaan, Liechtenstein), Ceraseal (Septodont, Cedex, France), AH Plus (Dentsply/De Trey, Konstanz, Germany) **Figure (1)**. Teflon mold was used to fabricate disc samples of each root canal sealer. Samples were divided into 3 groups: group (A) (Well-Root ST), group (B) (Ceraseal) and group (C) (AH Plus) in two evaluation periods (24h and 72h). All samples of root canal sealers were mixed according to the manufacturer's instructions and the discs were allowed to set at 37°C for 24 hours before extraction. After complete setting, sealers were removed from Teflon blocks and samples were exposed to UV light for 24 hours to prevent contamination and ensure sterility. The extract of root canal sealers was made in cell Dulbecco's Modified Eagle's Medium (DMEM) using the ratio of approximately 250 mm2/mL between the surface of the samples and the volume of medium according to ISO standard 10993-5 [4].

Figure 1: Showing tested root canal sealers. A: Well-Root ST B-Ceraseal C-AH Plus

After 24 hours, the medium was removed and labeled as the first solution to be tested. Another fresh DMEM was added with the same root canal sealer disc and kept for another 3 days for extraction. After 72 hours, the medium was removed which was labeled as the second solution to be tested. The extract collected at each time point was diluted to various concentrations of 12.5%, 25%, 50% and 100% with DMEM to provide a total of 4 concentrations of each extract. DMEM without the sealers incubated for 24 hours served as the control.

(HEp-2) cell line was seeded in 96 well micro-titer plates (5×10^3 cells/well). Cultures were then subjected to 100 µL of the extracts medium (12.5%, 25%, 50% and 100%) while cell cultures with supplemented DMEM were used as controls. The plates were left in the incubator for 24 or 72 h before the cytotoxicity evaluation was carried out.

For assessment of cell viability, Methyl Thiazol Tetrazolium (MTT) assay which is a quantitative colorimetric method to determine cell proliferation was used. It utilizes yellow tetrazolium salt (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) which is a water-soluble salt reduced to an insoluble purple formazan complex by cleavage of the tetrazolium ring by lactate dehydrogenase (LDH) within the mitochondria of viable cells [5].

After the incubation, the detached cells were washed out using phosphate-buffered saline (PBS). MTT as 0.05 ml / well (0.5mg/ml) in PBS were added to all wells. Plates were incubated at 37ºC for 4 hours, and DMSO (50 µL) was added to dissolve the developed formazan crystals. Treated plates were read at 570 nm using ELISA plate reader (ELX-800, Biotek-USA) and the absorbance values were determined. The viability percentage was calculated using the following equation:

$$\text{Viability percentage} = \frac{\text{Mean OD of Test Dilution X100}}{\text{Mean OD of Neg. Control}}$$

Cytotoxicity was evaluated by rating according to cell viability relative to the control group, non-cytotoxic (more than 90%), slightly cytotoxic (from 60 to 90%), moderately cytotoxic (from 30 to 59%) and severely cytotoxic (less than 30%) cell viability [6].

Data were collected, tabulated and statistically analyzed using one way ANOVA test and Bonferroni's post-hoc test.

RESULTS

Cytotoxicity Assay results:

A) According to evaluation time: Figure (2)

1. Viability at 24 hours

a) Between the three groups at 100% concentration

In group A, the mean and standard deviation values of viability were (14.17% ± 1.09%) (severely cytotoxic), while in group B they were (11.94% ± 1.65%) (severely cytotoxic) and in group C they were (12.71% ± 1.54%) (severely cytotoxic). There was no statistically significant difference between the three groups. (p = 0.143).

b) Between the three groups at 50% concentration

In group A, the mean and standard deviation values of viability were (56.39% ± 2.12%) (moderately cytotoxic), while in group B they were (23.47% ± 1.79%) (severely cytotoxic) and in group C they were (19.31% ± 1.76%) (severely cytotoxic). There was a statistically significant difference between the three groups. (p <0.001).

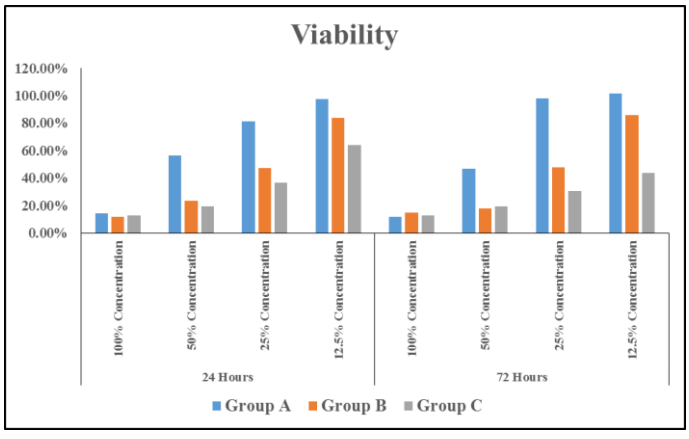

Figure 2: Bar chart showing viability percentage at different concentration percentages

Pairwise comparison showed that there was a statistically significant difference between all group pairs. Table (1)

Table 1: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	<0.001*
Group A - Group C	<0.001*
Group B - Group C	0.04*

**Significant at p<0.05*

c) Between the three groups at 25% concentration:

In group A, the mean and standard deviation values of viability were (81.04% ± 1.54%) (slightly cytotoxic), while in group B they were (47.22% ± 2.49%) (moderately cytotoxic) and in group C they were (36.46% ± 1.7%) (moderately cytotoxic). There was a statistically significant difference between the three groups. (*p* <0.001).

The post hoc comparison test showed that there was a statistically significant difference between all group pairs. Table (2)

Table 2: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	<0.001*
Group A - Group C	<0.001*
Group B - Group C	<0.001*

**Significant at p<0.05*

d) Between the three groups at 12.5% concentration:

In group A, the mean and standard deviation values of viability were (97.43% ± 1.14%) (non-cytotoxic), while in group B they were (84.03% ± 1.9%) (slightly cytotoxic) and in group C they were (64.1% ± 6.56%) (slightly cytotoxic). There was a statistically significant difference between the three groups. (*p* <0.001).

The post hoc comparison test showed that there was a statistically significant difference between all group pairs. Table (3)

Table 3: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	0.003*
Group A - Group C	<0.001*
Group B - Group C	<0.001*

**Significant at p<0.05*

2. Viability at 72 hours

a. Between the three groups at 100% concentration:

In group A, the mean and standard deviation values of viability were (11.67% ± 1.18%) (severely cytotoxic), while in group B they were (14.79% ± 2.52%) (severely cytotoxic) and in group C they were (12.71% ± 1.54%) (severely cytotoxic). There was no statistically significant difference between the three groups. (*p* = 0.1).

b. Between the three groups at 50% concentration:

In group A, the mean and standard deviation values of viability were (46.6% ± 2.17%) (moderately cytotoxic), while in group B they were (17.99% ± 1.63%) (severely cytotoxic) and in group C they were (19.31% ± 1.76%) (severely cytotoxic). There was a statistically significant difference between the three groups. (*p* <0.001).

Using the post hoc comparison test, there was a statistically significant difference between group A and group B (*p* <0.001) and between group A and group C (*p* <0.001), while there was no statistically significant difference between group B and group C (*p*=1). Table (4)

Table 4: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	<0.001*
Group A - Group C	<0.001*
Group B - Group C	1.00

**Significant at p<0.05*

c. Between the three groups at 25% concentration:

In group A, the mean and standard deviation values of viability were (98.19% ± 1.96%) (slightly cytotoxic), while in group B they were (47.85% ± 1.58%) (moderately cytotoxic) and in group C they were (30.63% ± 2.7%) (moderately to severely cytotoxic). There was a statistically significant difference between the three groups. (*p* <0.001).

Using post hoc comparison test, there was a statistically significant difference between all group pairs. Table (5)

Table 5: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	<0.001*
Group A - Group C	<0.001*
Group B - Group C	<0.001*

**Significant at p<0.05*

d. Between the three groups at 12.5% concentration:

In group A, the mean and standard deviation values of viability were (101.39% ± 2.15%) (Non-cytotoxic), while in group B they were (85.83% ± 15.84%) (slightly cytotoxic) and in group C they were (43.68% ± 1.92%) (moderately cytotoxic). There was a statistically significant difference between the three groups. (*p* <0.001).

Using post hoc comparison test, there was no statistically significant difference between group A and group B (*p* = 0.13), while there was no statistically significant difference between group A and group C (*p* <0.001) and between group B and group C (*p* <0.001). Table (6)

Table 6: Results of Bonferroni post hoc test for pairwise comparison of viability between the three groups:

	P – Value
Group A - Group B	0.13
Group A - Group C	<0.001*
Group B - Group C	<0.001*

**Significant at p<0.05*

B) According to Concentration percentage:

1. Within group A: Figure (3) Table (7)

a. At 24 hours:

The viability increased with decreasing the concentration. There was a statistically significant difference between all concentrations.

b. At 72 hours:

The viability increased with decreasing the concentration. There was no statistically significant difference between the 25% and the 12.5% concentration, while there was a statistically significant difference between all other concentrations.

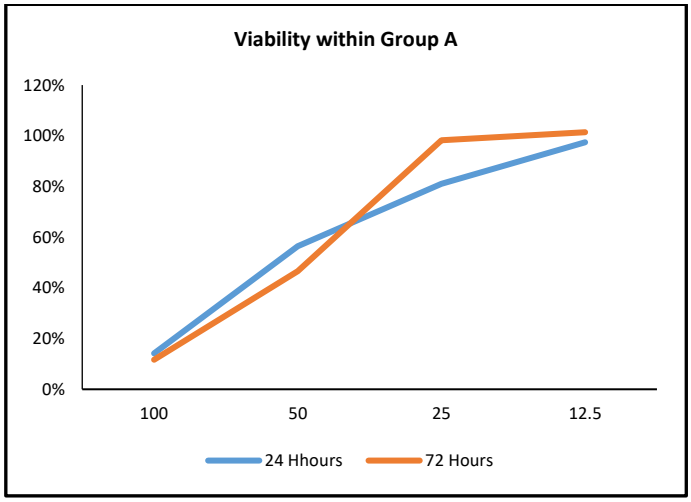

Figure 3: Line chart representing the viability changes within group A

Table 7: Mea, standard deviation (SD) and the results of ANOVA and Bonferroni post hoc test for comparison of viability within group A:

		Group A	
		24	**72**
100	**Mean**	14.17% [d]	11.67% [c]
	SD	1.09%	1.18%
50	**Mean**	56.39% [c]	46.60% [b]
	SD	2.12%	2.17%
25	**Mean**	81.04% [b]	98.19% [a]
	SD	1.54%	1.96%
12.5	**Mean**	97.43% [a]	101.39% [a]
	SD	1.14%	2.15%
	P-Value	**<0.001**	**<0.001**

**Significant at p<0.05*

2. Within group B: Figure (4) Table (8)

a. At 24 hours:

The viability increased with decreasing the concentration. There was a statistically significant difference between all concentrations.

b. At 72 hours:

The viability increased with decreasing the concentration. There was a statistically significant difference between all concentrations.

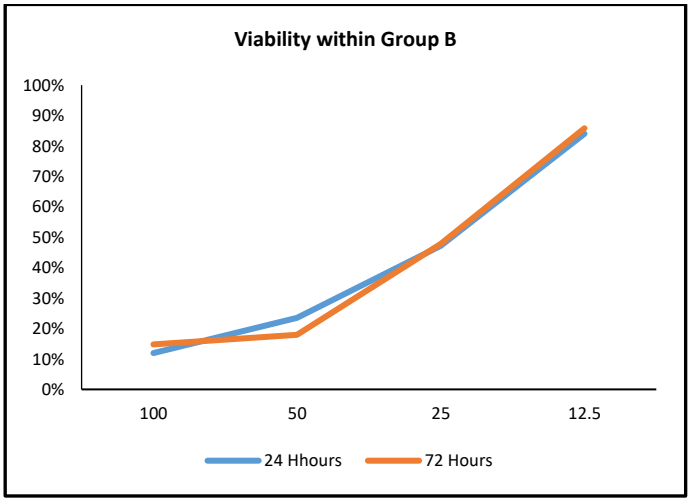

Figure 4: Line chart representing the viability changes within group B

Table 8: Mean, standard deviation (SD) and the results of ANOVA and Bonferroni post hoc test for comparison of viability within group B:

		Group B	
		24	**72**
100	**Mean**	**11.94%** [d]	**14.79%** [c]
	SD	**1.65%**	**2.52%**
50	**Mean**	**23.47%** [c]	**17.99%** [c]
	SD	**1.79%**	**1.63%**
25	**Mean**	**47.22%** [b]	**47.85%** [b]
	SD	**2.49%**	**1.58%**
12.5	**Mean**	**84.03%** [a]	**85.83%** [a]
	SD	**1.90%**	**15.84%**
	P-Value	**<0.001**	**<0.001**

**Significant at p<0.05*

3. Within group C: Figure (5) Table (9)

a. At 24 hours:

The viability increased with decreasing the concentration. There was a statistically significant difference between all concentrations.

b. At 72 hours:

The viability increased with decreasing the concentration. There was no statistically significant difference between the 100% and the 50% concentration, while there was a statistically significant difference between all other concentrations.

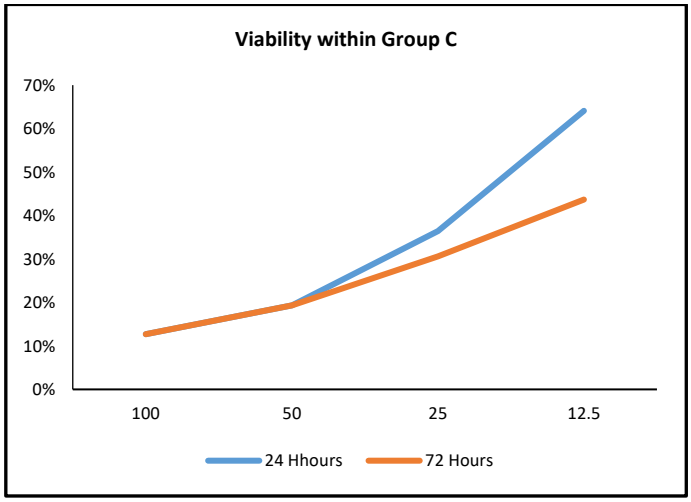

Figure 5: Line chart representing the viability changes within group C

Table 9: Mean, standard deviation (SD) and the results of ANOVA and Bonferroni post hoc test for comparison of viability within group C:

		Group C	
		24	**72**
100	**Mean**	12.71% [d]	12.71% [d]
	SD	1.54%	1.54%
50	**Mean**	19.31% [c]	19.31% [c]
	SD	1.76%	1.76%
25	**Mean**	36.46% [b]	30.63% [b]
	SD	1.70%	2.70%
12.5	**Mean**	64.10% [a]	43.68% [a]
	SD	6.56%	1.92%
	P-value	**<0.001**	**<0.001**

**Significant at p<0.05*

DISCUSSION

Different categories of root canal sealers are widely used in the dental market and clinicians select their sealers according to their properties. Cytotoxicity of root canal sealers is of prime importance and must be considered before its clinical application because these materials are in direct contact with the periapical tissues and might have negative effects if the cytotoxic effect is questionable. As sealers are mixtures that set and harden through chemical reactions, the release of toxic material during these reactions affect the biocompatibility of these sealers [7].

The cytotoxicity of sealers has been evaluated inconsistently due to differences and variations in assessment methods. In vitro cytotoxicity assays have the advantages of being simple, reproducible and suitable as a basic biological evaluation of biocompatibility [8].

In this study, the cytotoxic effect of two bioceramic root canal sealers (Well-Root ST and Cera-seal) and a gold standard epoxy resin sealer (AH Plus) has been evaluated and compared on the human laryngeal carcinoma cell line. Although the cell line used in this study is phenotypically different from periodontal cells in the periapical region, according to Geuresten *et al*. [9] there are no significant differences in the responses of different types of cells to harmful substances released from root canal sealers during the short term toxicity evaluation.

Many methods have been used for the evaluation of cell viability based on different cellular functions such as enzyme activity, cell adherence and cell membrane permeability. Among them, tetrazolium (MTT) is one of the most common methods which uses a colorimeter to determine cell viability and measures the rate of cell proliferation [10]. For each cell type, there is a linear relationship between cell number and signal produced, thus allowing accurate measurement of cell proliferation rate [11].

Results of the current study showed that there was no statistically significant difference between tested root canal sealers at full (100%) solution concentrations. However, Well-Root ST showed the highest viability values in the rest of the concentration percentages (50%, 25% and 12.5%) at both evaluation time intervals (24h and 72h). Also, it was noticed that the viability percentages values increase with decrease concentration percentages.

Furthermore, an interesting finding was noticed after 72h at 12.5% concentration of diluted ratio of extracts. The mean percentage of viable cells exceeded 100% (101.39% ± 2.15%) which could be explained by the possibility of the variable response of tested cells to mitochondrial activity compared to control cells. This is based on the fact that MTT assays and related assays (i.e. MTS) rely on a mitochondrial reductase to convert the tetrazole to formazan [12].

In the present study, AH Plus showed the least mean percentage of viable cells in both evaluation periods (24h and 72h). This is in acceptance with Silva Enjl *et al* [13, 14] who also found higher cytotoxicity levels of AH Plus. This is also in agreement with Deniz *et al* [15] and in agreement with Prati and Gandofli [16] who concluded that calcium silicate-based sealers have been shown to produce appropriate biological responses. However, this is contrary to Silva Enjl *et al* [17] who found that MTA-based sealers had higher cytotoxicity characteristics than AH Plus.

CONCLUSION

The evaluated root canal sealers showed varying degrees of cytotoxicity. However, Well-Root ST was associated with significantly highest cell viability percentages. AH plus significantly showed less cell viability in comparison to calcium silicate-based root canal sealers.

Conflict of interest

The auther reports no conflicts of interest.

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