

Review Article

Comparison of Cytotoxic Effects of Silver Nanoparticles and Calcium Hydroxide on Human Gingival Fibroblasts: An *In-vitro* Study

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Abstract

Objective: The current investigation aimed to study the cytotoxicity of silver nanoparticles (AgNPs) alone, Compared with Conventionally used calcium hydroxide [Ca(OH)₂] or in combination of both materials to Human Gingival Fibroblast cells (HGFs) at different time intervals.

Materials and Methods: Cytotoxicity of AgNPs and Ca(OH)₂ was tested, *in-vitro*, using cultured HGFs. After 24 hours and 7 days of exposure, Cytotoxicity was assessed using multiparametric assay kit including extracellular Lactate Dehydrogenase (LDHe) and 2,3- bis (2-methoxy- 4-nitro- 5-sulphophenyl)- 2H- tetrazolium- 5-carboxanilide (XTT).

Results: In cytotoxic analysis, AgNPs and Ca(OH)₂ decreased HGF. viability after 24 hr. of exposure (54.54% and 77.5%, respectively). The combination of both materials resulted in ≥ 90% viable cells after 24 hrs. of exposure. After 1 week, no significant difference was detected between all the experimental groups.

Conclusion: The exposure of HGFs to AgNPs or Ca(OH)₂ has reduced the cells viability after 24 hrs. of exposure, while viability was increased after 1 week. Additionally, the combination of both materials is nontoxic and resulted in higher cells viability in 24 hrs. and 1 week of exposure suggesting the presence of synergistic effect between these materials.

Keywords: AgNPs; Ca(OH)₂; Cytotoxicity; HGFs

Introduction

Determining the safety of dental materials through laboratory and animal testing before the widespread use of such products in humans is critical to the continued use of many materials

in dental field. Many dental materials have the potential to be in contact with oral tissues over extended periods of time. Material properties and the extended duration of material exposure are considered important factors contributing to inflammation (cytotoxic) or DNA damage and the potential for deleterious effects such as cancer (Genotoxic) [1]. Nanoparticles (NPs) are microscopic particles with one or more dimensions in the range of 1-100 nm. The advantage of using nanoparticles in biomedicine is attribute to their ability to be administered intravenously and distributed to different body organs and tissues. They can also cross biological membranes and closely interact with cells [2-4]. However, there are several types of NPs in endodontics such as biodegradable (e.g. polylactic acid), inorganic (e.g. silica) and metal (e.g. gold and silver) [5-9].

Silver nanoparticles (AgNPs) have showed strong anti-inflammatory and antibacterial potential against both Gram-positive and Gram-negative bacteria and are powerful against the multidrug resistant organisms [10-12]. Toxicity studies to determine the deleterious effects of nanoparticles on living cells, have revealed that it should be used with Caution. This is because its toxicity is concentration-dependent. Furthermore, silver can be accumulated in gingiva and gradually lead to cell death [13-14]. Some investigators have demonstrated that silver nanoparticles dose 4,10 mg/kg is safer and had no side effect as 20,40 mg/kg dose did in rats [15]. Others have observed the cellular response of human periodontal fibroblasts to different concentrations and sizes

of AgNPs, less than 10 nm, 15-20 nm and 80-100 nm. Their results showed that both groups of 10 nm and 15-20 nm have increased cytotoxicity in human fibroblasts in a dose- and time-dependent manner. On contrast, larger size of 80-100 nm did not alter the viability of primary culture cells [16]. Inkelewicz – Stepniak, et al., have examined the co-exposure of human gingival fibroblasts cells to AgNPs and fluoride. They found that AgNPs penetrated the cell membrane and localized inside the mitochondria with or without fluoride. Co exposure to both AgNPs and fluoride resulted increased oxidative stress, inflammation and apoptosis [17]. Recently, Fukuto, et al., have evaluated the toxic and antifibrotic effects of AgNPs on human corneal fibroblasts using three different sizes (10 nm, 50 nm and 100 nm). Cells were incubated for 24 hrs. with AgNPs at concentrations ranging from 0.05 to 50 µg/ml. Subsequently a collagen gel contractions assay was performed. The result revealed that cell viability was significantly decrease with 10 nm, 50 nm and 100 nm in a concentration - dependent manner. In addition, no antifibrotic effect on human fibroblasts was noticed for all sizes of AgNPs [18].

Calcium hydroxide $[\text{Ca}(\text{OH})_2]$ was introduced to dentistry by Hermann in 1920 for its pulpal - repairing ability [19]. It can be Successfully applied for other endodontic purposes or as effective intraconal medicaments against different types of microorganisms [12,20-26]. Moreover, $\text{Ca}(\text{OH})_2$ has low water solubility, high pH, can make a direct contact for long period with vital tissues before dissolved by tissue fluid, as well as generating the induction of hard tissue deposition and being antibacterial [24,26-28]. Regarding, the reaction of $\text{Ca}(\text{OH})_2$ to fibroblasts, it showed moderate cytotoxicity at concentration 0.5 mg/ml. While at concentrations of 0.25, 0.125 mg/ml it displayed slight cytotoxicity. The high pH of $\text{Ca}(\text{OH})_2$ in suspension leads to extreme cytotoxicity in screening tests [27]. Some researchers have found that $\text{Ca}(\text{OH})_2$ was significantly less toxic than betadine scrub, chlorine dioxide, iodine potassium iodide and sodium hypochlorite. It was well tolerated by cultured human gingival fibroblasts [29]. Others have evaluated the cytotoxicity of Neem oil, double antibiotic past and $\text{Ca}(\text{OH})_2$ to cultured human fibroblasts. The results showed that $\text{Ca}(\text{OH})_2$ recorded the highest significant cytotoxicity while double antibiotic past which recorded the lowest significant cytotoxicity. Cytotoxicity of the used medicaments were directly proportional to their concentration [30-32].

Review of the literature revealed lack of knowledge and only few studies were performed about the cytotoxic effect of AgNPs to HGFs. Therefore, the purpose of this work was to evaluate the cytotoxic effect of AgNPs alone, compared with conventionally used $\text{Ca}(\text{OH})_2$, or combination of both materials to HGFs at different time intervals.

Material and Methods

Cytotoxicity Effect

Cell Culture

Healthy gingival tissue was obtained during gingivectomy on tooth #36 from 29 years old patient. The patient has no medical problem and was not exposed to any medication during the past 6 months. The gingivectomy was planned for prosthetic reasons and informed consent was obtained from the patient. The procedure was performed by a periodontist. The gingival tissue was immediately placed in 15 ml Falcon tube (BD Falcon®, NJ, USA) contain phosphate buffered saline (PBS, Gibco® BRL, NY, USA) and transferred to Stem Cell Lab in the College of Medicine at King Saud University, Riyadh. A modified direct explant tissue culture technique from the study proposed by Almeida-Lopes, et al., was performed. 33 All following procedures were performed under class II laminar flow hood (Lab Gard ES 425 Biological Safety Cabinet, NuAire®, Plymouth, MN, USA). The tissue was washed with PBS including 1% Pen-strep (Penicillin- Streptomycin - 10,000 U/ML, Gibco® BRL, NY, USA). Tissue was excised through small fragments with an average size of 2X2 mm and placed in the center of each well of a 6 well tissue culture plate. Few drops of Dulbecco's Modified Eagle's Medium (DMEM:Gibco® BRL, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco® BRL, NY, USA), 1% Pen-strep and 1% 100µM MEM non-essential amino acids (Gibco® BRL, NY, USA) was applied over the tissues. The plate was incubated in humidified atmosphere of 5% CO_2 and 95% O_2 at 37°C for 24 hrs. to allow tissue attachment to well surface. Then, 1 ml of DMEM was added for each well.

Tissues were observed under inverted microscope (Observer A1, Zeiss® Gottingen, Germany) for 2 weeks with medium changed every 2 days. HGFs with spindle-shape were noticed to emerge and proliferate around the gingival tissue until it reached confluency (Fig.1). Subsequently, HGFs were subcultured according to technique proposed by Key, et al., [34]. Wells were washed with sterile PBS and HGFs cells were detached from well surface by 0.05% trypsin containing 0.1 mM Ethylene-Diamine-Tetra-Acetic Acid (EDTA) in calcium and magnesium acid free Hanks' solution (Gibco® BRL, NY, USA) which incubated at 37°C for 2 minutes. Trypsin was deactivated by adding 1 ml of new DMEM to it. The mixture of trypsin and DMEM were transferred

to 15 ml Falcon tube and centrifuged for 5 minutes at 30,000 rpm. The formed cells pellet was suspended in new culture medium and placed in T25 culture flask. Cells were subcultured until we reached the desired passage. Fibroblasts from fifth to seventh passages were used in the experiment. At this point, after cells trypsinization and centrifuging, cell pellet was suspended in 10 ml DMEM and 1 ml was used to perform viable cell count in Automated Cell Counter (Invitrogen Corporation, USA). The volume of culture medium containing fibroblast cells were adjusted to reach a concentration of 0.15×10^6 /well in 24-well plates. Two of 24-well plates were prepared with 1 ml of cell suspension/well and incubated for 24 hrs at 37°C to allow cells attachment.

Exposure to Experimental Materials

In order to receive the treatment without possible direct contact of insolubilized materials with cells, a cell insert (BD falcon®, Corning, USA) with a pore size of 1 µm was placed in each well (Fig. 2). There was clearance of 2 mm between the insert and the bottom of the well, thus allowed medicaments to freely diffuse into the media. Wells were incubated with the experimental materials for 24 hrs. and 1 week at 37°C. Viability of HGFs cells were evaluated using multi-parametric assay kit (In - Cytotox, Xenometrix, Allschwil, Switzerland) which enable us to perform different tests on the same sample. The tests that used in this study was Extracellular Lactate Dehydrogenase LDHe and Tetrazolium XTT that allows the quantification of the membrane integrity and the mitochondrial metabolism and respiratory chain activity of cells in response to pharmaceutical, chemical and environmental compounds and nutrients.

Membrane Integrity (LDHe: Extracellular Lactate Dehydrogenase)

Released LDH, a marker for cell damage, is determined kinetically in the medium (NADH consumption). Unlike some other LDHe assays, the In Cytotox LDHe assay measures the oxidation of NADH to NAD⁺ and the concurrent reduction of pyruvate to lactate. By providing an excess of pyruvate in the reaction mixture, the In Cytotox LDHe assay is therefore insensitive to pyruvate in the culture medium, which can cause product inhibition of the reverse reaction implemented in other LDHe assays. Trinton was used as negative control for LDHe analysis as proposed by the manufacture. The LDH I, II, III solutions were pre-heated at 37°C in a water bath before use as recommended from the manufacture. LDH I was used to reconstitute LDH II and LDH III. A micropipette was used to transfer 20 µl of the supernatant from each well to a new 96-well plate. A mixture of 16 ml LDH II with 3.4 ml LDH III was prepared. Two hundred forty µl/well of the LDH II / LDH III mixture was added which started the reaction. Immediately, reading was started kinetically at 340 nm for 25 minutes at 37°C.

Mitochondrial Dehydrogenase Activity

Mitochondrial dehydrogenase activity was measured by the XTT assay. This test is based on the ability of mitochondrial enzymes from metabolically active cells to reduce 2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5 carboxanilide (XTT) molecules to a soluble salt of formazan. XTT solutions was warmed at 37°C in a water bath until clear solution is obtained. Old medium was removed and wells were washed with PBS and 666 µl of fresh medium was added/well. XTT II and XTT I solutions were mixed at a 1:100 ratio by mixing 4 ml of XTT I and 40 µl of XTT II. From this mixture, 166 µl was added to each well. Plate was incubated for 2-3 hrs. at 37°C, 5% CO₂. Optical density (O.D.) was read at 480 nm with reference of 690 nm and measured by a microplate reader. The procedure was performed twice and the average was calculated. The cells viability was calculated as follow:

$$\% \text{viability} = (\text{mean OD sample} / \text{mean OD cell controls}) \times 100.$$

Statistical Analysis

Data were collected and analyzed using SPSS Pc+ version 21.0 statistical software. Descriptive statistics (mean, standard deviation and standard error) were used to describe the quantitative study variables. A one-way analysis of variance was used to compare the mean viability % values in relation to study materials levels of concentration. Tukey's test was used for multiple comparison of mean values, in relation to materials, concentration. Student's paired t-test and independent test were used to compare the mean values of CFU before and after application of drugs and between two time points (1 week and 2 weeks). A p-value of <0.05 was used to report the statistical significance of results.

Ethical Approval

This study was conducted after obtaining the approval of the Research Ethics Committee, College of Dentistry, King Saud University, Riyadh, Saudi Arabia.

Results

LDHe Assay

* Twenty-four hrs. exposure:

There were statistically significant differences in the mean inhibition values of AgNPs groups compared to other groups (Table 1 and Fig. 1-3).

* *One week exposure:* There was highly statistically significant difference in the mean inhibition values of 6 study groups at 1 week. Among the 6 study groups, the mean $\pm$ SD of inhibition value of Trintion group was significantly higher than all other 5 groups. Furthermore, the mean $\pm$ SD of inhibition values of other 5 groups were not significantly different from each other's (Table 2 and Fig. 4).

* *Comparison between 24 hrs. and 1 week exposure:* There was no statistically significant difference in the mean $\pm$ SD values of inhibition between two time period (24 hrs. and 1 week) in any of the 6 study groups (Table 3 and Fig. 5).

XTT Assay

* *Twenty-four hrs. exposure:* There was high statistically significant difference in the mean $\pm$ SD of viability % value of 6 groups at 24 hrs. Among the 6 groups, the mean $\pm$ SD of viability % value of Trintion group was significantly lower than all other 5 groups. Moreover, the mean $\pm$ SD of viability % value of AgNPs group and Ca(OH)_2 were significantly lower than cell control, Ca(OH)_2 , $\text{Ca(OH)}_2 + \text{AgNPs}$ (1:2) and $\text{Ca(OH)}_2 + \text{AgNPs}$ (1:3) and significantly higher than Trintion. Furthermore, there is no statistically significant difference in the mean $\pm$ SD of viability % value of the 3 groups [cell control, $\text{Ca(OH)}_2 + \text{AgNPs}$ (1:2) and $\text{Ca(OH)}_2 + \text{AgNPs}$ (1:3)] (Table 4 and Fig. 6).

* *On Week exposure:* There was highly statistically significant difference in the mean $\pm$ SD of viability % values of 6 study groups at 1 week. Among the 6 groups, the mean $\pm$ SD of viability %, value of Trintion group was significantly lower than all other 5 groups and the mean $\pm$ SD viability % of cell control was significantly higher than all other 5 groups, Furthermore, the mean $\pm$ SD of viability % value of AgNPs group was significantly lower than cell control, Ca(OH)_2 and $\text{AgNPs} + \text{Ca(OH)}_2$ (1:2) and significantly higher than Trintion. In addition, the mean $\pm$ SD of viability % of $\text{AgNPs} + \text{Ca(OH)}_2$ (1:3) was significantly higher than Trintion and significantly lower than cell control but not significantly different from Ca(OH)_2 and $\text{AgNPs} + \text{Ca(OH)}_2$ (1:2) (Table 5 and Fig. 7).

* *Comparison between 24 hrs. and 1 week exposure:* There was statistically significant difference in the mean $\pm$ SD values of viability % between two time points (24 hrs and 1 week) of experiment in AgNPs group. The mean viability % value was significantly higher at 1 week, when compared with the value at 24 hrs. in this group. Whereas for other 4 groups [Ca(OH)_2 , $\text{AgNPs} + \text{Ca(OH)}_2$ (1:2), $\text{AgNPs} + \text{Ca(OH)}_2$ (1:3) and Trintion] no statistically significant difference was observed in the mean value of viability % between 24 hrs. and 1 week (Table 6 and Fig. 8).

Groups	Mean $\pm$ SD of Inhibition	F-value	P-value
AgNPs	35.96 $\pm$ 1.27*	3.005	<0.0001
Ca(OH)_2	4.35 $\pm$ 0.24		
$\text{AgNPs} + \text{Ca(OH)}_2$ (1:2)	2.47 $\pm$ 0.14		
$\text{AgNPs} + \text{Ca(OH)}_2$ (1:3)	4.61 $\pm$ 1.84		
Cell control	0.00 $\pm$ 0.00		
Trintion	100 $\pm$ 0.00 #		
By Tukey's multiple comparison test			
* Statistically significant from other groups.			
# Statistically significant from other groups.			

Table 1: Comparison of mean $\pm$ SD values of inhibition among 6 study groups at 24 hours with LDHe assay.

Groups	Mean $\pm$ SD of Inhibition	F-value	P-value
AgNPs	28.11 $\pm$ 13.75	2.33	<0.001**
Ca(OH) ₂	5.91 $\pm$ 3.62		
AgNPs + Ca(OH) ₂ (1:2)	6.90 $\pm$ 3.51		
AgNPs + Ca(OH) ₂ (1:3)	5.29 $\pm$ 1.95		
Cell control	0.00 $\pm$ 0.00		
Trinton	100 $\pm$ 0.00 #		

** Statistically significant among all the 6 groups.
 By Tukey's test: # Significantly, higher than all other groups.
 No significant difference among the other 5 group.

Table 2: Comparison of mean $\pm$ SD value of inhibition among the 6 groups at 1 week with LDHe assay.

Groups	24 hour	1 Week	Difference of mean	t- value	P-value
AgNPs	35.96 $\pm$ 1.27*	28.11 $\pm$ 13.75	7.85	-1052	0.7967
Ca(OH) ₂	4.35 $\pm$ 0.24	5.91 $\pm$ 3.62	-1.56	-1.74	0.0718
AgNPs + Ca(OH) ₂ (1:2)	2.47 $\pm$ 0.14	6.90 $\pm$ 3.51	-4.44	-2.43	0.6437
AgNPs + Ca(OH) ₂ (1:3)	4.61 $\pm$ 1.84	5.29 $\pm$ 1.95	-0.68	-2.88	0.6786
Cell control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00	0.00	0.00
Trinton	100 $\pm$ 0.00 #	100 $\pm$ 0.00 #	0.00	0.00	0.00

Table 3: Comparison of mean $\pm$ SD values of inhibition between 24 hours and 1 week time with LDHe assay.

Groups	Mean $\pm$ SD of Inhibition	F-value	P-value
AgNPs	54.54 $\pm$ 4.04 #	132.03	<0.001**
Ca(OH) ₂	77.50 $\pm$ 3.27 *		
AgNPs + Ca(OH) ₂ (1:2)	93.99 $\pm$ 1.50		
AgNPs + Ca(OH) ₂ (1:3)	90.63 $\pm$ 7.36		
Cell control	100 $\pm$ 0.00		
Trinton	17.57 $\pm$ 2.37 ≠		

** Statistically significant among all the 6 groups. By Tukey's test: ≠ Significantly lower than all other group.
 # * Significantly lower than cell control, AgNPs + Ca(OH)₂ (1:2), AgNPs + Ca(OH)₂ (1:3) and significantly higher than Trinton.

Table 4: Comparison of mean $\pm$ SD values of viability among the 6 groups at 24 hours using XTT assay.

Groups	Mean $\pm$ SD of Inhibition	F-value	P-value
AgNPs	76.37 $\pm$ 3.03 #	462.45	<0.001**
Ca(OH) ₂	86.97 $\pm$ 2.52		
AgNPs + Ca(OH) ₂ (1:2)	91.18 $\pm$ 0.39		
AgNPs + Ca(OH) ₂ (1:3)	83.78 $\pm$ 2.74 *		
Cell control	100 $\pm$ 0.00		
Trinton	16.71 $\pm$ 0.33 ≠		

** Statistically significant among all the 6 groups. By Tukey's test: ≠ Significantly lower than all other groups;
 # Significantly lower than Ca(OH)₂, AgNPs + Ca(OH)₂ (1:2), cell control, and significantly higher than Trinton.
 * Significantly lower than cell control and significantly higher the Trinton, but not different from other groups.

Table 5: Comparison of mean values of viability among the 6 groups at 1 week with XTT analysis.

Groups	24 hour	1 Week	Difference of Mean	t- value	P-value	95% Confidence Interval of Difference of Mean
AgNPs	54.54 ± 4.04	76.37 ± 3.03	-21.83	-6.10	0.03	(-37.2, -6.4)
Ca(OH) ₂	77.50 ± 3.27	86.97 ± 2.52	-9.47	-9.47	0.08	(-22.0, 3.1)
AgNPs + Ca(OH) ₂ (1:2)	93.99 ± 1.50	91.18 ± 0.39	2.81	2.5	0.13	(-1.9,7.5)
AgNPs + Ca(OH) ₂ (1:3)	90.63 ± 7.36	83.78 ± 2.74	6.84	1.23	0.34	(-17.1,30.7)
Cell control	1.00 ± 0.0	1.00 ± 0.0	--	--	--	--
Trinton	17.57 ± 2.37	16.71 ± 0.33	0.86	0.51	0.66	(-6.42,8.1)

Table 6: Comparison of mean value of viability between 24 hrs. and 1 week time point in each of the 6 groups with XTT assay.

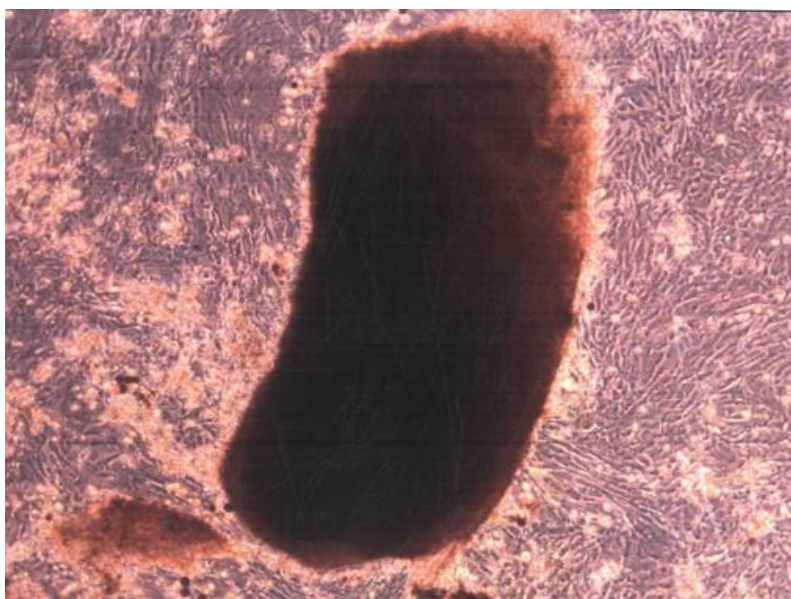


Figure 1: Showing microscopic picture of the human gingival fibroblasts cell emerge around the gingival tissue after 2 weeks of incubation.



Figure 2: Showing the 24 well plate and the cell insert that were used for application of experimental medicament over human gingival fibroblasts.

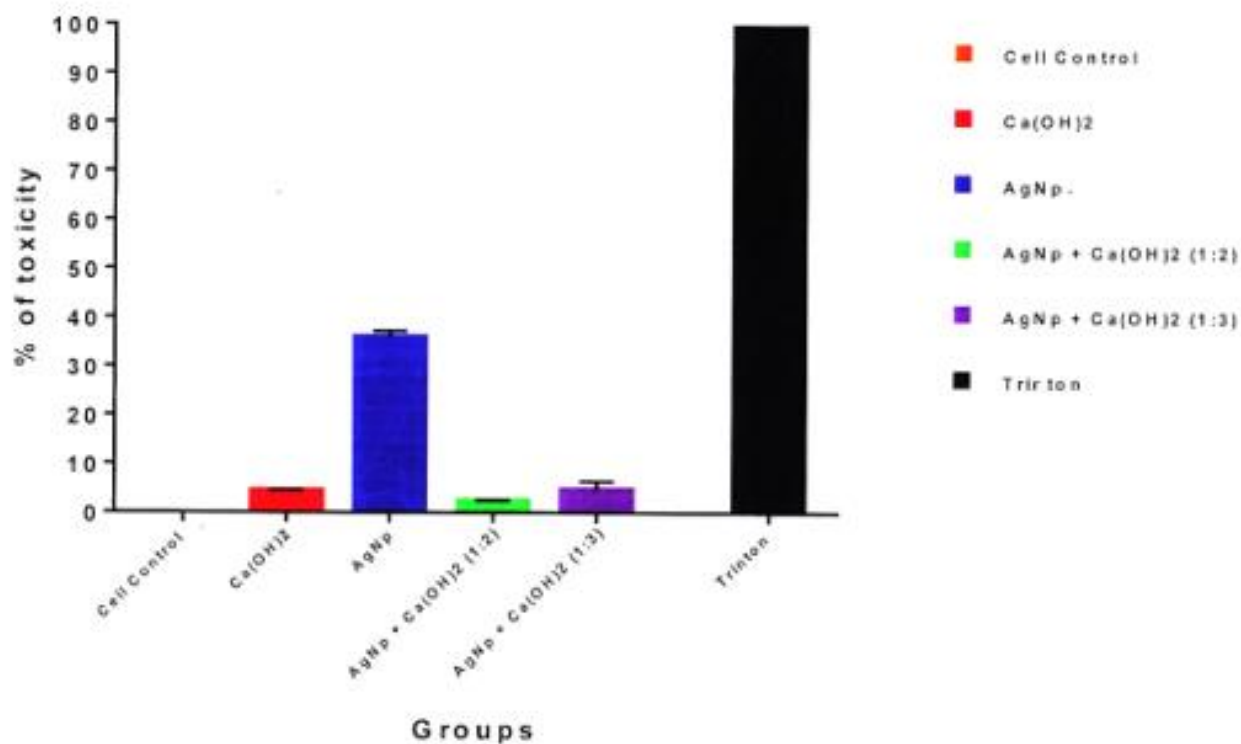


Figure 3: Showing the comparison in the percentage of inhibition among the groups after 24 hrs exposure by LDHe assay.

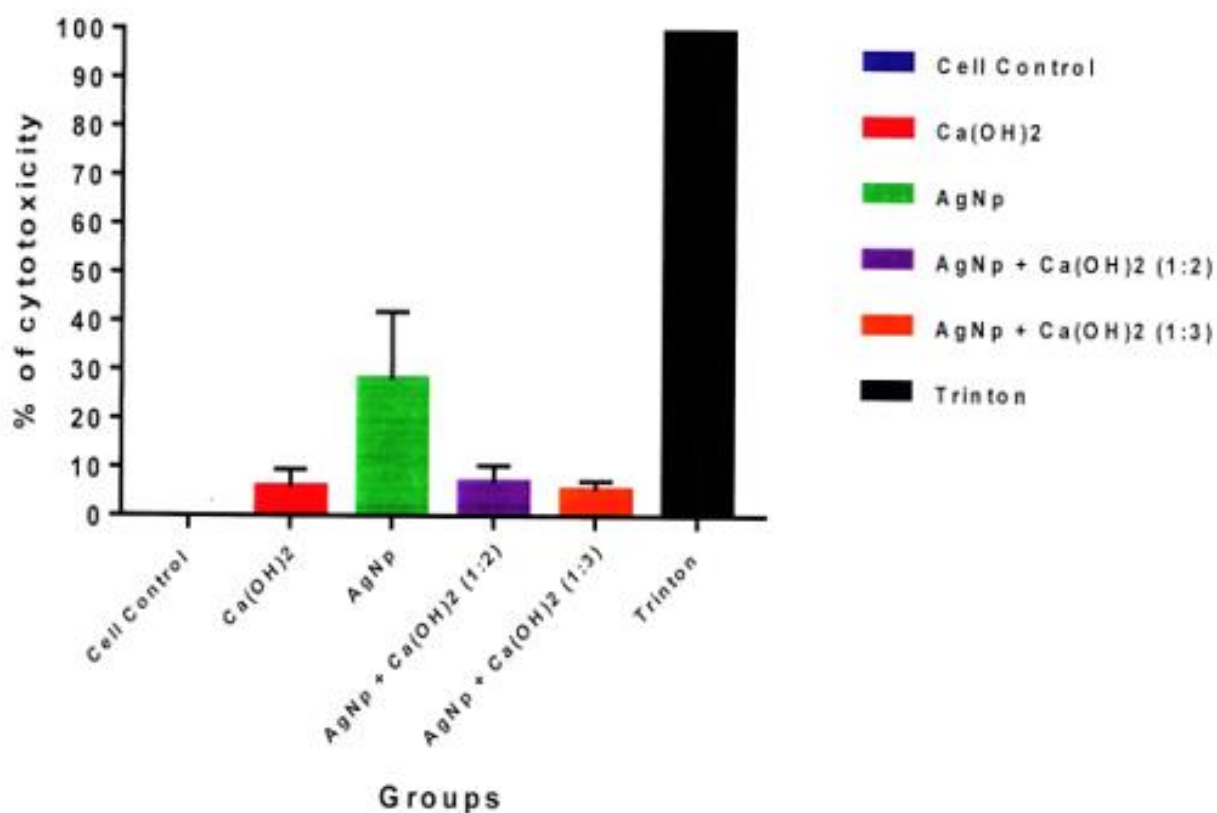


Figure 4: Showing the comparison in the percentage of inhibition among the groups after 1 week exposure by LDHe assay.

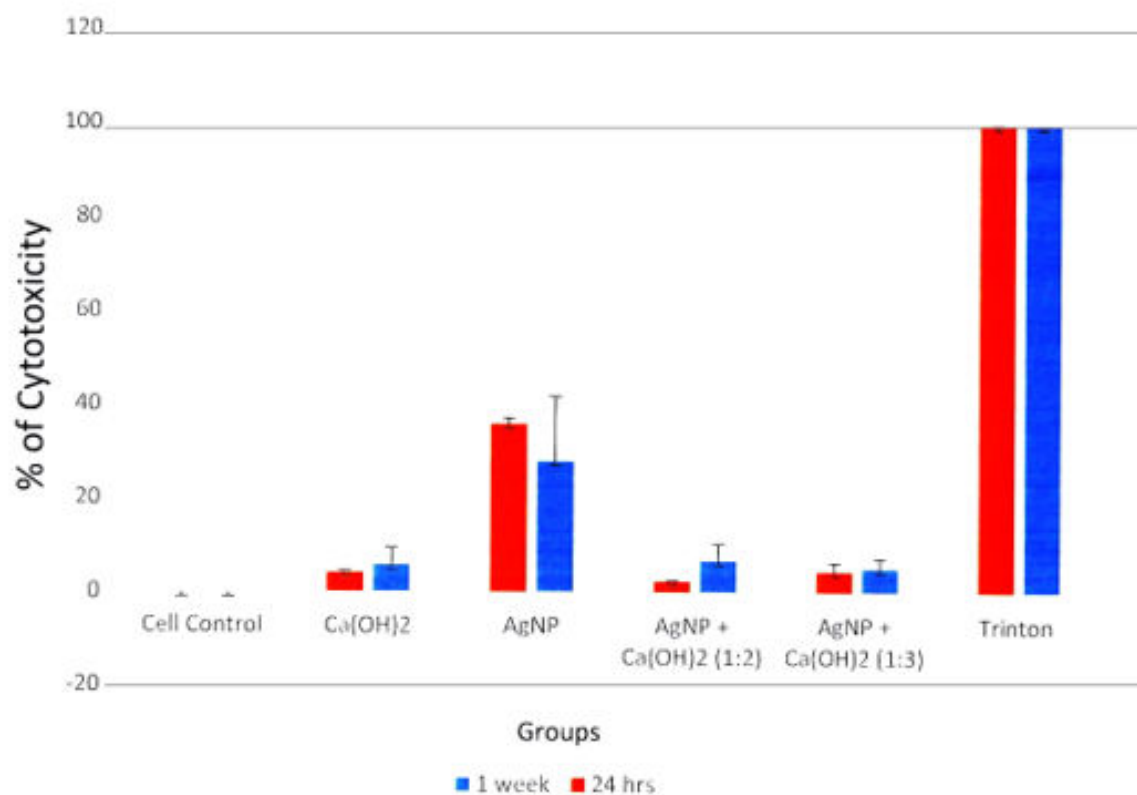


Figure 5: Showing the comparison in the percentage of inhibition among the groups in two time periods by LDHe assay.

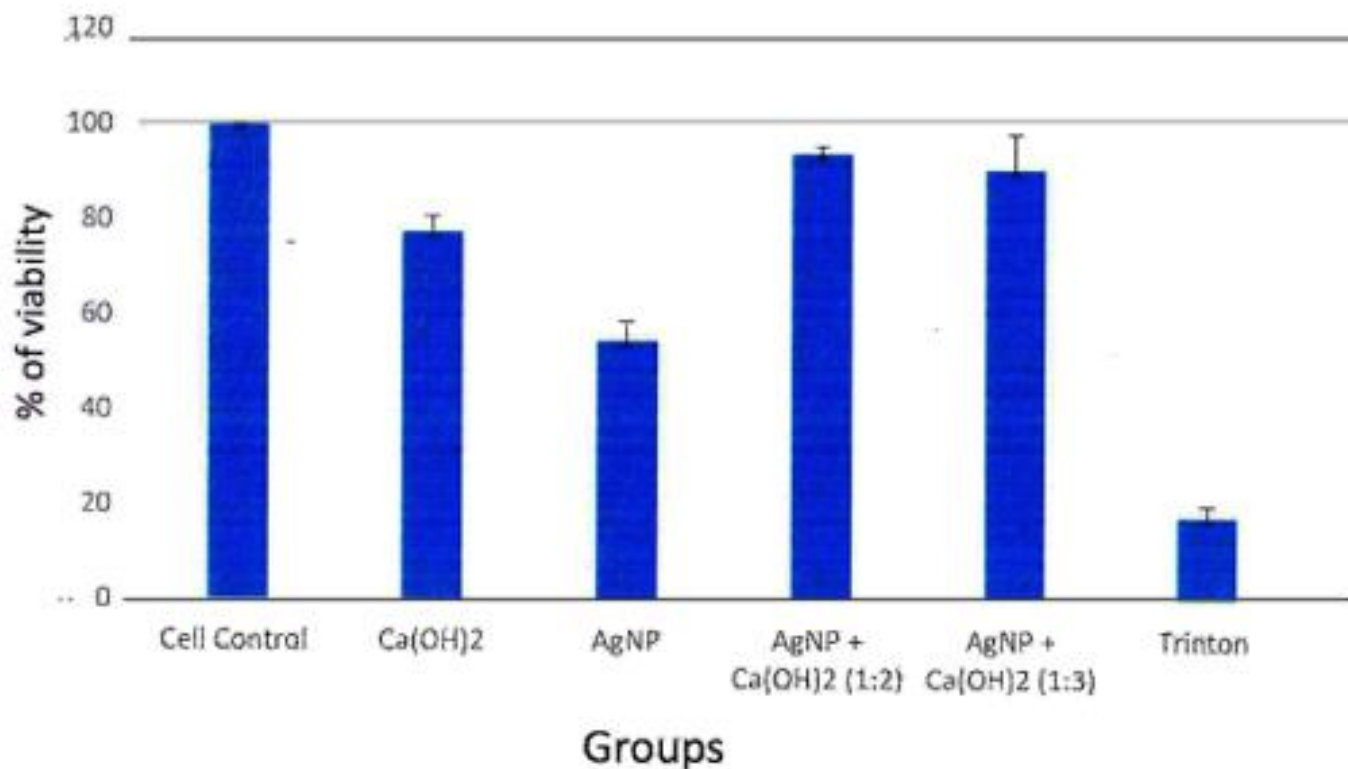


Figure 6: Showing the comparison in the percentage of inhibition among the groups after 24 hrs exposure by XTT assay.

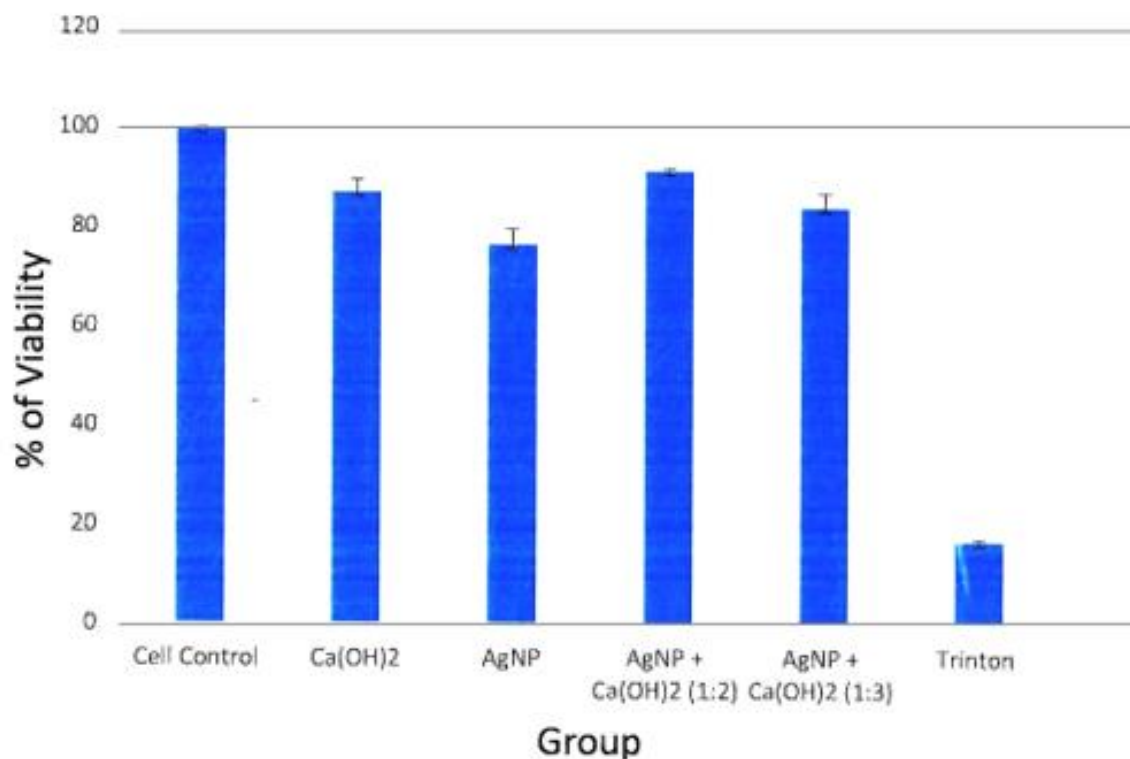


Figure 7: Showing the comparison in the percentage of inhibition among the groups after 1 week exposure by XTT assay.

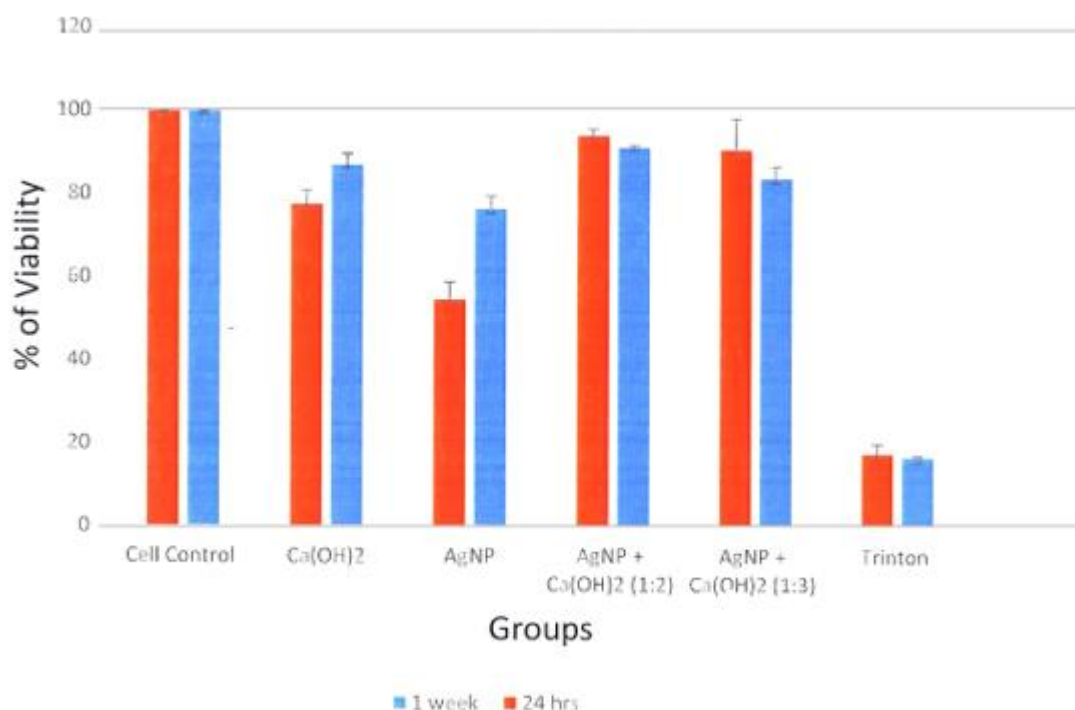


Figure 8: Showing the comparison in the percentage of inhibition among the groups two time periods by XTT assay.

Discussion

Nanotechnology is an emerging field of applied science and technology that deal with manipulation of matter at the atomic or molecular level. The growing interest in the dental application of nanotechnology is leading to the emergence of a new field which may help to maintain oral health using nanomaterial and biotechnology. Silver nanoparticles increasingly are being used

as antimicrobial agents because they are more effective than gold, zinc, copper or magnesium nanoparticles. In general, nanoparticles constitute a suitable alternative in order to deliver active compounds effectively to the target site, increasing their therapeutic efficacy [1,5,7].

In the present study, the cytotoxicity analysis demonstrated lowest fibroblast cells viability after exposure to AgNPs for 24 hrs. compared to other groups, this viability increased after 1 week of exposure and does not differ significantly from other groups. These results were similar to previous studies and indicated the biocompatibility of AgNPs [5,15,29,35]. Furthermore, others used different concentration of AgNPs to evaluate the proliferation, viability, migration, attachment and osteogenic differentiation of human mesenchymal stem cells. Favorable results were obtained supporting our finding. They attributed these data to AgNPs that enhanced the alkaline phosphatase gene expression [36]. Recently, some investigators evaluated the toxic effects of AgNPs on HGFs at different concentration and treatment duration. They revealed that AgNPs was concentration- and exposure time dependent. They added that concentration below 10 µg/ml for 2 min exposure can be considered non-cytotoxic and safe during short-term oral cavity exposure. In contrast, a 24 hrs. exposure led to a significant decrease in cell viability [18,37]. Furthermore, others demonstrated that HGFs exposed to AgNPs exhibited increased in oxidative stress, inflammation, cellular damage and cell necrosis. The differences in findings may attributed to difference in cell types (stem cells, osteogenic cells, or epithelial cells), using different form of AgNPs (solution vs. gel), difference in concentration, difference in particle size, or exposure time [13,14,16-18].

The results of the present work revealed lower cell viability after exposure to $\text{Ca}(\text{OH})_2$ for 24 hrs. which increased after 1 week of application. This was in agreement with findings reported by previous studies especially if used at low concentration [27,29,38]. In contrast, some investigators have demonstrated that even low concentration of $\text{Ca}(\text{OH})_2$ was toxic for fibroblasts of the periodontal ligament and dental pulp [39]. This discrepancy in finding may be due to the exposure time was 20 hrs. before proceeding the analysis. Moreover, the high pH of $\text{Ca}(\text{OH})_2$ (approximately 12.5-12.8) led to extreme cytotoxicity to fibroblasts cells. Additionally, its main actions are achieved through the ionic dissociation of Ca^{2+} and OH^- ions and their effect on vital tissues, the antibacterial properties and generating the induction of hard-tissue deposition [12,19-22,24,27].

To the best of our Knowledge, a single previous study had evaluated the cytotoxicity of combination between AgNPs and $\text{Ca}(\text{OH})_2$ on human mesenchymal stem cells. The results demonstrated that this combination is nontoxic, have positive effects on cell proliferation, as well as higher cell viability when compared to AgNPs or $\text{Ca}(\text{OH})_2$ [36]. These findings confirmed and supporting our current data. Furthermore, we feel that this result may be attributed to synergistic effect of both materials. Also, *in-vitro* cell culture technique has been a useful method for evaluating the biocompatibility of medical, dental materials or devices. This may be due to its simple, economic and productive advantage as reported by Hanks, et al., [40]. In addition, for the cytotoxicity experiment, we used periodontally derived cells as proposed in previous study by Al-Nazhan and Spangberg [41]. The primary HGFs were selected for many reasons. HGFs are a kind of sensitive cells and can be easily isolated and cultured in the normal culture medium. From a biological standpoint, it might be more relevant to the clinical environment by using the human cells derived directly from clinically relevant tissues. The genetic characteristics and biological trails of the cells are well preserved in the primary cultured cells [42]. Finally, HGFs are the main cells of the gingival tissue that might be approached and further damaged in endodontic treatment.

Conclusion

From the scope and limitation of this study, it appeared that;

1. The exposure of HGFs to AgNPs or $\text{Ca}(\text{OH})_2$ has reduced the cells viability after 24 hrs. of exposure, while cells viability was increased after 1 week
2. The combination of both materials resulted in higher cells viability in 24 hrs. and 1 week of exposure suggesting the presence of synergistic effect between these materials
3. Positive effect of this combination making them a potential biomaterial for various clinical applications

Conflict of Interests

The authors have no conflict of interest to declare.

Acknowledgment

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References

1. Stanford JW. Recommendations for determining biocompatibility and safety for the clinical use of metals in dentistry. *Int Dent J*. 1986;36:45-8.
2. Hall JB, Dobrovolskaia MA, Patri AK, McNeil SE. Characterization of nanoparticles for therapeutics. *Nanomedicine*. 2007;2:789-803.
3. Zhang L, Gu FX, Chan JM. Nanoparticle in medicine: therapeutic application and developments. *Clin Pharmacol Ther*. 2008;83:761-9.
4. Stander L, Theodore L, Environmental implication - An update. *Int J Environ Res Public Health*. 2011;8:470-9.
5. Roig-Sorian X, Souto EB, Elmsmary F. Nanoparticles in endodontic disinfection: State of the art. *Pharmaceutics J*. 2022;14(7).
6. García-Contreras R, Argueta-Figueroa L, Mejía-Rubalcava C, Jiménez-Martínez R, Cuevas-Guajardo S, Sanchez-Reyna PA, et al. Perspectives for use of silver- nanoparticles in dental practice. *Int Dent J*. 2011;61:297-301.
7. Priyadarsini S, Mukherjee S, Mishra M. Nanoparticles used in dentistry: A review. *J Oral Biol Craniofac Res*. 2018;8:58-67.
8. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Fard MJ. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. *J Dent*. 2015;43:1573-9.
9. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. *J Endod*. 2014;40:285-90.
10. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. *Nanomed*. 2007;3:95-101.
11. Ayala-Nunez NV, Villegas HHL, Turrent LCI, Padilla CR. Silver nanoparticles toxicity and bacterial effect against methicillin resistant *Staphylococcus aureus*: nanoscale does matter. *J Nanobiotechnol*. 2009;5:2-9.
12. Al-abdulmohsen ZA, Saad AY. Antibacterial effect of silver nanoparticles against *Enterococcus faecalis*. *Saudi Endod J*. 2017;7:29-35.
13. Sue YM, Lee JY, Wang MC. Generalized argyria in two chronic hemodialysis patients. *Am J kidney Dis*. 2001;37:1048-51.
14. Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, et al. Comparison of the abilities of the ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett*. 2006;8:1794-807.
15. Tiwari DK, Jin T, Behari J. Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats. *Toxicol Mech Methods*. 2011;21:13-24.
16. Hernández-Sierra JF, Galicia-Cruz O, Salinas-Acosta A, Ruíz F, Pierdant-Pérez M, Pozos-Guillén A. *In-vitro* cytotoxicity of silver nanoparticles on human periodontal fibroblasts. *J Clin Pediatr Dent*. 2011;36:37-42.
17. Inkielewicz-Stepniak I, Santos-Martinez MJ, Medina C, Radomski MW. Pharmacological and toxicological effects of co-exposure of human gingival fibroblasts to silver nanoparticles and sodium fluoride. *Int Nanomed*. 2014;1677-87.
18. Fukuto A, Chikama T, Kiuchi Y. The antifibrotic effect of silver nanoparticle on human corneal fibroblasts *in-vitro*. *Invest Ophthalmol Visual Sci (IOVS)*. 2023;64:23-7.
19. Hermann BW. Calcium hydroxide als Mittelzurn, Behandeln und Fullen von Wurzelkanalen. Wurzburg. 1920.
20. Saad AY. Calcium hydroxide and apexogenesis. *Oral Surg*. 1988;66:499-501.
21. Saad AY. Calcium hydroxide in the treatment of external root resorption. *JADA*. 1989;118:579-81.
22. Saad AY, Clem WH. Calcium hydroxide and endodontic therapy. *Postgrad Dentistry*. 1993;3:129-33.
23. Desai S, Chandler N. Calcium hydroxide-based root canal sealers: a review. *J Endod*. 2009;39:475-80.
24. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J*. 2011;44:697-730.
25. Hassan R, khallaf M., Effect of a silver nanoparticle intracanal-based medicament on the microhardness of human radicular dentin. *Endo (Lond Engl)*. 2018;12(2):125-31.
26. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol*. 1985;1:170-5.

27. Siqueira JF Jr, Lopes HP. Mechanism of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J*. 1999;32:361-9.
28. Elmsmari F, Delgado LM, Duran-Sindreu F, Pérez RA, García ML, Trull MT, et al. Novel strategies enhancing endodontic disinfection: Antibacterial biodegradable calcium hydroxide in an *ex-vivo* model. *Int J Pharma*. 2023;468.
29. Barnhart BD, Chuang A, Dalle Lucca JJ, Roberts S, Liewehr F, Joyce AP. An *in-vitro* evaluation of the cytotoxicity of various endodontic irrigants on human gingival Fibroblasts. *J Endod*. 2005;31:613-15.
30. Hussein SHH, Mostafa NMA, Hegazy EM, Fayyad DM, Darrag AM. Comparative evaluation of cytotoxic effect of different intracanal medicaments. *Dent Sci Upd*. 2021;2:123.
31. Heward S, Sedgley C. Effects of intracanal mineral trioxide aggregates and calcium hydroxide during four weeks on pH changes in simulated root surface resorption defects: an *in-vitro* study using matched pairs of human teeth. *J Endod*. 2011;37:40-4.
32. Yoshino P, Nishiyama CK, Modena KC, Santos CF, Sipert CR. *In-vitro* cytotoxicity of white MTA, MTA Fillapex and Portland cement on human periodontal ligament fibroblasts. *Braz Dent J*. 2013;24:111-6.
33. Almeida-Lopes L, Rigau J, Zangaro RA. Comparison of the low-level therapy effect on cultured gingival fibroblasts proliferation using different irradiance and fluence. *Lasers Surg Med*. 2001;29:179-84.
34. Key JE, Rahemtulla FG, Eleazer PD. Cytotoxicity of a new root canal filling material on human gingival fibroblasts. *J Endod* 2006;32:756-80.
35. Kim YS, Kim JS, Cho HS. Twenty-eight-day oral toxicity, genotoxicity and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol*. 2008;20:575-83.
36. Algazlan AS, Almuraikhi N, Muthurangan M, Balto H, Alsalleh E. Silver nanoparticles alone or in combination with calcium hydroxide modulate the viability, attachment, migration and osteogenic differentiation of human mesenchymal stem cells. *Int J Mol Sci*. 2023;14(1):702.
37. Puttanuparp T, Rirattanapong P, Ruangsawasdi N. Cytotoxicity of silver nanoparticles on human gingival fibroblasts: An *in-vitro* study. *M Dent J*. 2023;43(3):107-14.
38. Silva EJ, Herrera DR, Almeida JF. Evaluation of cytotoxicity and up-regulation of gelatinases in fibroblast cells by three root repair materials. *Int Endod J*. 2012;45:815-20.
39. Al-Shaher A, Wallace J, Agarwal S. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. *J Endod*. 2004;30:359-61.
40. Hanks CT, Wataha JC, Sun Z. *In-vitro* models of biocompatibility; a review. *Dent Mater*. 1996;12:186-93.
41. Al-Nazhan S, Spangberg L. Morphological cell changes due to chemical toxicity of a dental material: an electron microscopic study on human Periodontal ligament fibroblasts and L929 cells. *J Endod*. 1990;16:129-34.
42. 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. *Int Endod J*. 2007;40:329-37.

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