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Research paper

Cytotoxicity, genotoxicity and antibacterial activity of poly(vinyl alcohol)-coated silver nanoparticles and farnesol as irrigating solutions

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ABSTRACT

Objective: To evaluate the cytotoxicity, genotoxicity and antibacterial activity of poly(vinyl alcohol)-coated silver nanoparticles (AgNPs-PVA) and farnesol (FAR).

Design: The cytotoxicity (% of cell viability) was evaluated by MTT assay and the genotoxicity (% of DNA in the tail) was evaluated by Comet assay. Root canal disinfection with different irrigating protocols was evaluated ex vivo in human teeth contaminated with *Enterococcus faecalis* for 21 days. Three microbiological samples were collected: initial (after contamination); post-irrigation (after irrigation); and final (after 7 days). After each sample, the number of log 10 CFU mL^{-1} was determined. Statistical analyses was performed using two-way ANOVA and Bonferroni post-hoc tests for MTT assay, Kruskal-Wallis and Dunn post-hoc tests for Cometa and antibacterial assays ($\alpha = 0.05$).

Results: The MTT assay showed that AgNPs and FAR were less cytotoxic that sodium hypochlorite (NaOCl) and showed a lower% of DNA in the tail, in comparison with H_2O_2 (positive control − C+). In the post-irrigation microbiological sample, all the irrigating protocols were more effective than C+ (without irrigation). NaOCl/saline, NaOCl/saline/AgNPs-PVA and NaOCl/saline/FAR led to complete bacterial elimination ($p > 0.05$). In comparison with the initial sample, both the post-irrigation and the final samples showed microbial reduction ($p < 0.05$).

Conclusions: AgNPs-PVA and FAR showed low cytotoxicity and genotoxicity, and exhibit potential for use as a final endodontic irrigation protocols.

1. Introduction

The root canal instrumentation aims to promote root canal cleaning, shaping and disinfection of root canal system (RCS) (Blattes et al., 2016; Sakamoto, Siqueira, Rocas, & Benno, 2007). Persistence of micro-organisms in the RCS contributes to treatment failure (Sakamoto et al., 2007; Siqueira & Rocas, 2008). Sodium hypochlorite (NaOCl) is the most used irrigant solution due to its antimicrobial action and capacity for dissolving organic tissue (Goncalves, Rodrigues, Andrade Junior, Soares, & Vettore, 2016; Mohammadi, 2008). Nevertheless, it may present cytotoxicity when in contact with periapical tissues (Gondim, Setzer, Dos Carmo, & Kim, 2010; Mehdipour, Kleier, & Averbach, 2007). Furthermore, NaOCl may decreases the elastic modulus and flexural strength of human dentin by the proteolytic action on the collagen matrix (Lahor-Soler, Miranda-Rius, Brunet-Llobet, Farre, & Pumarola, 2015; Zehnder, 2006). Differences in the dentin surface affect adhesion of the endodontic sealers (Lahor-Soler et al., 2015; Vilanova, Carvalho-Junior, Alfredo, Sousa-Neto, & Silva-Sousa, 2012). Therefore, chemical substances and associations have been studied as alternative to the use of NaOCl (Alves, Neves, Silva, Rocas, & Siqueira, 2013; Moghadas, Shahmoradi, & Narimani, 2012; Wu, Fan, Kishen, Gutmann, & Fan, 2014).

Silver nanoparticles (AgNPs) present antimicrobial properties and biocompatibility, and has shown potential for biomedical applications. However, AgNPs may show toxic effects (Haase et al., 2012). Antibacterial activity has been related to the release of Ag^+ ions from the surface of AgNPs (Dankovich & Gray, 2011). The AgNPs may have a toxic effect by inactivating essential microbial enzymes (Mohanty et al., 2012), increasing the permeability of the membrane and damaging the cytoplasm (Haase et al., 2012). The Ag^+ ions may catalyze the

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production of oxygen radicals, inhibiting multiplication of the microorganisms (Zhang, Chen, Zhang, Zhang, & Liu, 2013). Moreover, the smaller particle size increases the area of contact with the microbial membrane, providing a greater bactericidal effect (Monteiro et al., 2011).

Coating agents on the surface of AgNPs and the particle size contribute to toxicity (Ahn, Eom, Yang, Meyer, & Choi, 2014). Polyvinyl-alcohol (PVA) has been used as a stabilizing agent in the synthesis of AgNPs (Mahmoud, 2015; Paino & Zucolotto, 2015) because it is biocompatible and non-mutagenic (Baker, Walsh, Schwartz, & Boyan, 2012).

Farnesol (FAR), is a sesquiterpene alcohol naturally found in propolis and in essential oils of aromatic plants and citric fruits, that presents antitumor, antimicrobial and antibiofilm activity (Alves, Silva, Rocas, & Siqueira, 2013). FAR deserves to be highlighted due to its elevated level of antimicrobial activity against bacterial and fungal species, but its mechanism of action is not yet fully elucidated (Cordeiro et al., 2013). In Endodontics, there are not many studies, however this solution has the potential for use as coadjuvant in endodontic treatment (Alves, Silva et al., 2013).

The aim of the present study was to evaluate the cytotoxicity, genotoxicity and effectiveness of AgNPs-PVA and FAR solutions for root canal disinfection. The null hypotheses tested were as follows: (1) there is no cytotoxicity and genotoxicity of these irrigants against L929 mouse fibroblasts, (2) there is no difference in antibacterial efficacy of AgNPs-PVA and FAR against *E. faecalis* when compared with NaOCl.

2. Material and methods

2.1. Synthesis and characterization of AgNPs-PVA

Synthesis and characterization of the AgNPs-PVA (50 μM) solution was performed at the Institute of Physics − USP (São Carlos, São Paulo, Brazil). Silver nitrate − $AgNO_3$ (1 mmol L^{-1}) was dissolved in ultrapure water, and then the PVA was added. The AgNPs-PVA solution was synthesized by means of reduction of the $AgNO_3$ solution in sodium borohydride − NaBH4 under constant agitation for 2 h. The solution was centrifuged at 15,000 rpm for 1 h to remove the excess of reducing agent. The mean size of AgNPs-PVA (4–11 nm) was determined by Dynamic Light Scattering (DLS, Malvern instruments, UK) (Paino & Zucolotto, 2015).

2.2. Farnesol (FAR) solution preparation

The FAR solution was obtained from the commercial product 3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol, Ref. F203 (Sigma-Aldrich, St Louis, MO, USA) with concentration of 95%. To perform the experiments, the 1% FAR solution was prepared in sterile distilled water immediately before use.

2.3. Fibroblast L929 cell culture

Fibroblast cells (L929 line) were grown in Dulbecco Modified Eagle Medium − DMEM (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum − FBS (Gibco/Invitrocell-Life Technologies Brazil Ltda., SP, Brazil), penicillin (100 IU/mL), and streptomycin (100 mg/mL), and maintained at 37 °C and modified atmosphere containing 5% CO_2 and 95% humidity. The cells were detached by a mixture of trypsin/EDTA (Gibco-BRL/Life Technologies, Gaithersburg, MD, USA) at 37 °C, the supernatant was centrifuged (1000 rpm − 10 min), the pellet was resuspended in DMEM containing 10% FBS and the cells were counted in a hemocytometer (KASVI, K5-0111, Curitiba, PR, Brazil).

Table 1
Dilutions in physiological solution of the solutions evaluated in the MTT assay.

Dilutions	AgNPs-PVA (μM)	FAR (%)	NaOCl (%)
1:1	50	1	1
1:2	25	0.5	0.5
1:4	12.5	0.25	0.25
1:8	6.25	0.125	0.125

2.4. MTT assay

To determine the viability/metabolism of cells in contact with the solutions (Table 1), the methyl thiazol tetrazolium-MTT assay was used. In DMEM culture medium supplemented with 5% FBS, 1×10^5 cells/mL were plated in 96-well plates (JET BIOFIL®, Guangzhou, China) and incubated for 24 h. The solutions were diluted in sterile physiological solution (0.9% sodium chloride solution, JP Indústria Farmacêutica S.A. − Riberão Preto, SP), with exception of the AgNPs-PVA solution in the 1:1 dilution (stock solution). After 3 min exposure to the different solutions (100 μL/each) and controls (negative: 5% DMEM and physiological solution; positive: hydrogen peroxide (H_2O_2 − 1 mM), the contents of the wells were removed and the wells filled with 200 μL of 5% DMEM culture medium, and the plates were again incubated for 4 h (Giannelli, Chellini, Margheri, Tonelli, & Tani, 2008). After incubation, the culture medium was replaced with 100 μL DMEM without FBS containing 5 mg/mL of MTT (Sigma Chemicals, MO, USA) and the plates were incubated for 3 h. Afterwards, 100 μL of acidified isopropyl alcohol (HCl: isopropyl alcohol, 0.04N) was added. The optical density (OD 570 nm) was measured by a spectrophotometer UVM 340 (ASYS, Nova Analítica Importação e Exportação Ltda., SP, Brazil). The assay was performed in triplicate and repeated at three different time intervals. Data were exported to the *GraphPad Prism 5.03* program (GraphPad Software Inc., San Diego, CA, USA) and submitted to statistical analysis by the two-way ANOVA with Bonferroni post-hoc tests ($\alpha = 0.05$).

2.5. Comet assay

Single Cell Gel Electrophoresis (SCGE) or Comet assay, under alkaline conditions, is a technique for quantifying and detecting damage to the DNA (Collins, 2004; Lorenzo, Costa, Collins, & Azqueta, 2013). To evaluate the genotoxic effect of AgNPs-PVA and FAR solutions, the Comet assay was used (Singh, McCoy, Tice, & Schneider, 1988), with some modifications, according to published studies (Paino & Zucolotto, 2015; Patlolla, Hackett, & Tchounwou, 2015).

Cells were plated in 24-well culture plates (JET BIOFIL®) in triplicate at a concentration of 2×10^6 cells/mL. After 24 h of incubation, the cells were exposed to the different dilutions of the solutions for 3 min (Table 2). After this, the solutions were removed from the wells, and the wells immediately filled with 200 μL of DMEM culture medium containing 5% FBS, and the plates were kept in an oven at 37 °C, 95% humidity and 5% CO_2 for an additional 4 h. The negative controls were the cells treated with sterile physiological solution or with DMEM culture medium, and the positive control was the cells treated with H_2O_2 (1 mM). After incubation, cells were detached and centrifuged (4000 rpm/8 min). The cells of each group were resuspended in 200 μL

Table 2
Dilutions in physiological solution of the solutions evaluated in the Comet assay.

Dilutions	AgNPs-PVA (μM)	FAR (%)
1:1	50	1
1:2	25	0.5
1:4	12.5	0.25
1:8	6.25	0.125

of agarose low melting point − LMP (Sigma-Aldrich, St Louis, MO, USA) and transferred to identified slides, prepared with agarose normal melting point (Sigma-Aldrich, St Louis, MO, USA). The slides were immersed in lysis solution (NaCl 2.5 M, EDTA 100 mM, Tris 10 mM/1% Triton X-100, 10% DMSO) and kept at 4° C overnight. The slides were placed in the horizontal cube (BIO-RAD, PowerPac™ Basic, Hercules, CA, USA), immersed for 20 min in the electrophoresis solution − pH 13 (EDTA − 0.2 M, NaOH − 10 M, H_2O milliQ) to allow DNA unwinding. The source was set to 25 V/400 mA and the run continued for another 25 min. The slides were removed and immersed in the neutralization solution − pH 7.5 (distilled H_2O, Tris − 0.4 M) for 15 min, fixed in pure ethanol and left to dry overnight at ambient temperature.

The slides were stained with 70 μL ethidium bromide at 0.02 mg/mL (Sigma-Aldrich, St. Lois, MO, USA), and were immediately observed under a fluorescence microscope (EVOS-fl, AMG-Advanced Microscopy Group, Bothell, WA, USA), at 40× magnification. The images were analyzed in the *Comet Score™ Freeware* v1.5 program, to obtain the percentage (%) values of DNA in the tail. The same group of solutions was compared with both the negative (physiological solution) and positive (H_2O_2) controls. The data were statistically analyzed by the Kruskal-Wallis and Dunn *post-hoc* tests ($\alpha = 0.05$).

2.6. Effectiveness of RCS disinfection: an ex vivo study

The roots of 60 extracted human single-root teeth, with a single straight root canal, and standardized at 15 mm of length, were used. A size 20 K-file (Dentsply-Maillefer, Ballaingues, Switerland) was used to standardize the foraminal diameter. The working length (WL) was established 1 mm short of the apical foramen and the apical stop was prepared up to a size 40/.04 rotary file of the Mtwo system (VDW, Endodontic Synergy, Germany). Manual irrigation was performed with 2.5 mL of 2.5% NaOCl solution at each change of instrument. The root canals were filled with 17% trisodium EDTA solution (Biodinâmica, Ibiporã, PR, Brazil) for 3 min, followed by irrigation with 5 mL of saline solution. Afterwards, the apical region of each root was sealed with Z100-Restaurador Universal (3 M ESPE, USA) light polymerizing resin composite and the external root surface was made impermeable with two layers of epoxy adhesive (Araldite, Brascola Ltda, Taboão da Serra, SP, Brazil), except the root canal access. The specimens were randomly distributed into 24-well cell culture plates (Corning Incorporated, Corning, NY, USA) and fixed in the wells with self-curing acrylic resin (Clássico Artigos Odontológicos, SP, Brazil). The plates containing the specimens were wrapped and sterilized by ethylene oxide (ACECIL, Campinas, SP, Brazil).

Standardized *E. faecalis* strains were cultured overnight in Tryptic Soy Broth (TSB), seeded onto Tryptic Soy agar (TSA) and incubated at 37 °C for 24 h. The sterile TSB culture medium was mixed to the bacterial suspension (1×10^8 CFU mL^{-1}) at a 1:1 ratio and root canals were contaminated with 20 μL of this mixture. The plates were kept in a microaerophilic environment at 37° C. The period of contamination was 21 days, and every two days sterile culture medium was added in the interior of the root canals (Guerreiro-Tanomaru, Chavez-Andrade, de Faria-Junior, Watanabe, & Tanomaru-Filho, 2015). After this period, the initial sample of all the root canals was collected by using a sequence of two size 40 sterile absorbent paper points (Tanari Industrial Ltda., SP, Brazil) per specimen. The paper points remained in the root canal for 1 min, and afterwards were transferred to microtubes containing 1 mL sterile saline solution. The microtubes were shaken for 1 min in a vortex (AP56, Phoenix, Araraquara, SP, Brazil). After this, ten-fold serial dilutions were made and 20 μL aliquots were seeded, in triplicate, onto Petri dishes containing Tryptic Soy Agar. The plates were incubated in a microaerophilic environment at 37° C for 48 h. Bacterial growth was determined by the CFU mL^{-1} counts of *E. faecalis*.

The irrigation protocols were performed with 5 mL syringe (Ultradent Products, South Jordan, UT, USA) and 31-G irrigation needle (NaviTip − Double Sideport Irrigator Tip, Ultradent Products, USA), placed 1 mm short of the WL. The final irrigation protocols were performed: **G1**: 5 mL of 2,5% NaOCl + 1 mL of 17% EDTA (agitated with size 40 K-file for 3 min) + 5 mL of SS; **G2**: 5 mL of NaOCl + 1 mL of EDTA (agitated with size 40 K-file for 3 min) + 3 mL of SS + 2 mL of AgNPs-PVA (3 min); **G3**: 5 mL of NaOCl + 1 mL of EDTA (agitated with size 40 K-file for 3 min) + 3 mL of SS + 2 mL of 1% FAR (3 min); **G4**: 9 mL of SS + 2 mL of AgNPs-PVA (3 min); **G5**: 9 mL of SS + 2 mL of FAR (3 min); **G6**: without irrigation (C +); and **G7:** sterile culture medium (C-).

After irrigation, the root canals were filled with 1% sodium thiosulfate solution used as neutralizing agent (Alves, Neves et al., 2013; Moghadas et al., 2012). After this, the post-irrigation sample was collected, as described for the initial sample, and the microplates containing the specimens were incubated in a microaerophilic environment at 37 °C for 7 days. After this period, the final sample was collected. Data obtained were submitted to base-10 logarithmic transformation and statistically analyzed by the Kruskal-Wallis and Dunn *post-hoc* tests ($\alpha = 0.05$).

3. Results

3.1. MTT assay

The results are represented in Fig. 1. The cells treated with physiological solution (control) showed higher cell viability values than those of the AgNPs-PVA, FAR and NaOCl solutions. NaOCl was shown to be cytotoxic in all the dilutions evaluated. At the 1:1 dilution, AgNPs-PVA (stock solution) showed cytotoxicity similar to that of NaOCl. There was no difference between AgNPs-PVA and FAR at the 1:4/1:8 dilutions.

3.2. Comet assay

The results, expressed in% of DNA in the tail (DNA fragmentation of the cellular nucleus), are represented in Fig. 2. This parameter allows evaluating the genotoxic effects of the solutions on the fibroblast cells; thus, a higher% of DNA in the tail expressed a greater genotoxic effect. The 1:1 dilution of AgNPs-PVA (50 μM) was excluded from the test, because it was not possible to obtain sufficient cells to count. This may be explained because this concentration was not diluted in physiological solution. The stock solution was used. The cells treated with AgNPs-PVA and FAR showed a lower% of DNA in the tail (at all the dilutions evaluated) in comparison with the positive control (H_2O_2). The 1:1 dilution of FAR showed the greatest genotoxic effect (16.13%) when compared with the other dilutions, the AgNPs-PVA solution and with the negative control (5.08%).

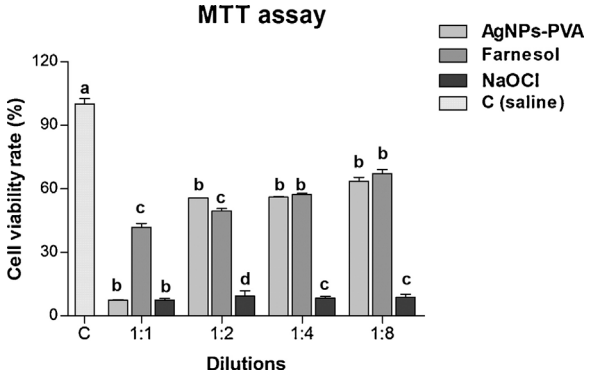

Fig. 1. Cell viability rate (%) in L929 fibroblasts exposed to AgNPs-PVA, FAR and NaOCl in different dilutions (1:1, 1:2, 1:4 and 1:8) for 3 min. Different letters represent significant differences among the solutions within each dilution and the negative control (C). *two-way* ANOVA, Bonferroni *post-hoc* ($p < 0.05$).

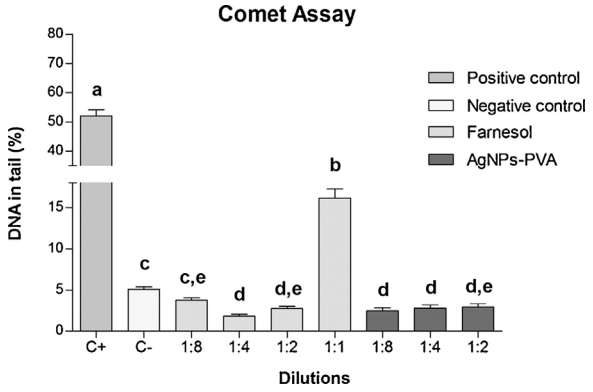

Fig. 2. Genotoxic effect represented by the percentage (%) of DNA in the tail in L929 cell line treated with different dilutions of solutions (AgNPs-PVA and FAR) for 3 min. C+ represents the positive control (1 mM hydrogen peroxide); C- represents the negative control (physiological solution). Treated *vs.* C-, Kruskal-Wallis, Dunn *post-hoc* ($p < 0.05$).

3.3. Microbiological test

Contamination of the specimens with the *E. faecalis* strain was confirmed in the initial sample, collected after 21 days of incubation, in which all the groups had a similar CFU mL^{-1} log_{10}, counts, as shown in Table 3. Bacterial viability in the C+ was confirmed throughout the entire experimental period, as was shown in the initial, post-irrigation and final samples ($P > 0.05$). The C- group showed no contamination during the experiment. In the post-irrigation sample, there was statistically significant difference between C+ and the other groups ($P < 0.05$), with exception of G5- SS/FAR ($P > 0.05$), however, G4- SS/AgNPs-PVA and G5- SS/FAR were similar ($P > 0.05$). Moreover, the groups with 2.5% NaOCl showed inhibition of bacterial growth, without statistical difference ($P > 0.05$). In the final sample, the groups with SS (G4 and G5) were similar to C+ ($P > 0.05$) which, in turn, differed from the groups with NaOCl ($P < 0.05$). Table 3 also shows the comparison between the microbiological samples within each group, in which we may observe that in the post-irrigation and final samples, all the experimental groups differed statistically from the initial sample ($P < 0.05$). There was no difference between the post-irrigation and final samples in the experimental groups ($P > 0.05$). However, there was an increase in the number of bacteria 7 days after the irrigation protocols. In the comparison with the initial sample, both the post-irrigation and the final samples showed significant microbial reduction ($P < 0.05$).

4. Discussion

The AgNPs may be synthesized with different stabilizing and coating agents. PVA was used in synthesis of the AgNPs-PVA solution in the present study, because it presented biocompatibility, high solubility in water and chemical resistance, which made it adaptable for use as a stabilizing agent and for coating the AgNPs (Mahmoud, 2015; Paino & Zucolotto, 2015). Therefore, this agent could improve the possible cytotoxic and genotoxic effects of the AgNPs (Ahn, Eom, Yang, Meyer, & Choi, 2014), corroborating the results of the present study.

The AgNPs solution has been recommended as a root canal irrigant solution (Gomes-Filho et al., 2010; Moghadas, Shahmoradi, & Narimani, 2012; Wu, Fan, Kishen, Gutmann, & Fan, 2014), not only because of its bactericidal potential, but also due to its biocompatibility, especially in low concentrations (Gomes-Filho et al., 2010). An AgNPs-based irrigant solution has been evaluated against *E. faecalis* and *S. aureus*, demonstrating efficacy similar to that of NaOCl (Moghadas, Shahmoradi, & Narimani, 2012). This result may be explained by the addition of ethanol and sodium hydroxide in the solution prepared by the authors, which differed from the solution used in our study. In another study, the structure of the *E. faecalis* biofilm was not destroyed after irrigation with the AgNPs solution (Wu, Fan, Kishen, Gutmann, & Fan, 2014).

An ideal irrigant solution must associate maximum antibacterial efficacy and solvent effect on both organic and inorganic tissue, with a minimum toxic effect on the periapical tissues (Blattes et al., 2016; Navarro-Escobar, Gonzalez-Rodriguez, & Ferrer-Luque, 2010). To make an in vitro evaluation of cell viability in solutions with bactericidal action, dilutions are made in physiological solution (Barsoumian et al., 2013), in culture medium or undiluted (medium-free) (Ryu, Matsumura, Quan, & Furuta, 2013). In the present study, dilutions were made in physiological solution.

In this study, the option taken was to use fibroblast cells L929, as was done in previous studies (Abbaszadegan et al., 2015; Lin, Lin, Dong, & Hsu, 2012; Zhang, Torabinejad, & Li, 2003) and the MTT assay that is one of the methods most used in the literature for evaluating the cytotoxicity of a material, including irrigant solutions (Navarro-Escobar, Gonzalez-Rodriguez, & Ferrer-Luque, 2010; Prado et al., 2015; Zhang, Torabinejad, & Li, 2003) and AgNPs (Abbaszadegan et al., 2015; Kar et al., 2014; Kaur & Tikoo, 2013; Lin, Lin, Dong, & Hsu, 2012). Prado et al. (2015) compared the cytotoxic effects of irrigant solutions simulating the clinical situation of irrigant use during biomechanical preparation. In the present study, the solutions were evaluated as a final irrigation protocol after root canal preparation. Our results are in agreement with Abbaszadegan et al. (2015), who showed that AgNPs were less cytotoxic, in comparison with NaOCl.

The Comet assay is a sensitive method that has become one of the standard methods for evaluating and quantifying lesions and/or detecting the effects of repair on DNA (Collins, 2004; Lorenzo, Costa, Collins, & Azqueta, 2013). The time of contact of solutions with the cells was similar to that used in the MTT assay, simulating the clinical situation of final irrigation after root canal prepration, and afterwards the treated cells were incubated in 5% DMEM medium for 4 h. This incubation time allows the cells to recover and for possible damage to the DNA to be reverted (Lorenzo et al., 2013). Thereby, this modified method allowed us to show that the AgNPs-PVA and FAR solutions presented no genotoxic effects.

Recently, was evaluated the cytotoxicity and genotoxicity of the AgNPs (Souza, Franchi, Rosa, da Veiga, & Takahashi, 2016). The results showed that the AgNPs of 10 nm were more cytotoxic and genotoxic than those of 100 nm, which suggested possible adverse biologic effects on bacterial and human cells. To our knowledge, no previous studies have evaluated the cytotoxicity and genotoxicity of FAR.

The success of endodontic treatment depends on root canal disinfection (Kamocki, Nor, & Bottino, 2015). In the present study, AgNPs and FAR were less cytotoxic than NaOCl and does not show genotoxicity. NPsAg and FAR seem to be more favorable for tissue repair in comparison with NaOCl, promoting also an effective disinfection of the SCR when used in the final irrigation after root canal preparation.

Table 3
Comparison among groups and samples: initial, post-irrigation and final (mean and standard deviation of log_{10} CFU mL^{-1}).

Groups/Samples	Initial	Post-irrigation	Final
G1– NaOCl/EDTA/SS	6.01 (± 0.59)^{A,a}	0.0 (± 0.0)^{C,b}	1.01 (± 1.75)^{B,b}
G2– NaOCl/EDTA/SS/AgNPs-PVA	6.08 (± 0.45)^{A,a}	0.0 (± 0.0)^{C,b}	1.28 (± 1.72)^{B,b}
G3– NaOCl/EDTA/SS/FAR	5.69 (± 0.37)^{A,a}	0.0 (± 0.0)^{C,b}	1.42 (± 1.33)^{B,b}
G4– SS/AgNPs-PVA	6.26 (± 0.52)^{A,a}	1.93 (± 1.61)^{BC,b}	4.14 (± 0.60)^{A,b}
G5– SS/FAR	5.69 (± 0.36)^{A,a}	3.27 (± 0.57)^{AB,b}	4.23 (± 0.75)^{A,b}
G6–without irrigation (control)	5.68 (± 0.66)^{A,a}	5.67 (± 0.65)^{A,a}	5.11 (± 0.59)^{A,a}

Different letters indicate statistically significant differences ($P < 0.05$). Capital letters in the same column for comparison between groups and lower case letters on the same line for comparison among samples. NaOCl, sodium hypochlorite; SS, saline solution; AgNPs-PVA, poly(vinyl alcohol)-coated silver nanoparticles; FAR; farnesol.

The AgNPs-PVA and FAR solutions showed low cytotoxicity, without genotoxic effects on the fibroblast cells, and had antimicrobial efficacy in reducing *E. faecalis*. This solutions exhibit potential for use as a final irrigation protocols in the root canal treatment.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Experimental protocols were approved by the Araraquara Dental School – Univ Estadual Paulista, UNESP Research Ethical Committee (# 07827012.0/0000.5416).

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