Archives of Oral Biology 84 (2017) 89–93

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research paper

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

Gisselle M. Chávez-Andrade[a], Mário Tanomaru-Filho[a,*], Elisandra M. Rodrigues[a], Ana Lívia Gomes-Cornélio[a], Gisele Faria[a], Maria Inês B. Bernardi[b], Juliane M. Guerreiro-Tanomaru[a]

[a] Department of Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araraquara, SP, Brazil
[b] Institute of Physics, University of São Paulo (USP), São Carlos, SP, Brazil

ARTICLE INFO

Keywords:
Cytotoxicity
Endodontics
Enterococcus faecalis
Genotoxicity
Farnesol
Silver nanoparticles

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

* Corresponding author at: Rua Humaitá, 1680, Caixa Postal 331, Centro, Araraquara, SP 14801-903, Brazil.
E-mail addresses: tanomaru@uol.com.br, tanomaru@foar.unesp.br (M. Tanomaru-Filho).

http://dx.doi.org/10.1016/j.archoralbio.2017.09.028
Received 8 May 2017; Received in revised form 20 September 2017; Accepted 24 September 2017
0003-9969/ © 2017 Elsevier Ltd. All rights reserved.

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

Download English Version:

https://daneshyari.com/en/article/5637937

Download Persian Version:

https://daneshyari.com/article/5637937

Daneshyari.com