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Influence of the addition of microsphere load amoxicillin in the physical, chemical and biological properties of an experimental endodontic sealer

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ABSTRACT

Aim: To develop an endodontic sealer with amoxicillin-loaded microsphere and to evaluate its properties.
Methods: Experimental sealer was obtained by mixing 70 wt% UDMA, 15 wt% GDMA and 15 wt% BISEMA. In addition, CQ, DHEPT and PB were incorporated at 1 mol% and 0.01 wt% BHT and 10 wt% YbF3. Microspheres were produced by drying in spray dryer. Two experimental groups (10 and 15 wt% of microspheres) and one control group (without microspheres) were formulated. The sealers were evaluated for the degree of conversion (n = 3), degradation in solvent (n = 3), drug release profile (n = 3), antimicrobial activity (n = 3), flow (n = 3), film thickness (n = 3) and cytotoxicity (n = 3). Data were analyzed by ANOVA and Tukey with significance level of 5%.
Results: Microespheres presented a mean size of 2.664 μm. Immediate degree of conversion ranged from 51.73% to 55.13% and the 24 h degree of conversion ranged from 60.79% to 73.80%. Upon solvent degradation 54.44% and 56.21% reduction in hardness were observed for 10% and 15% concentrations respectivelly. The drug release profile showed an average release of 73.76% of the drug in 96 h. Significant reduction in antimicrobial activity was observed for 10% concentration after 24 h, 48 h and 96 h compared to control. The flow and film thickness showed values in accordance to the ISO 6876. Cytotoxicity showed high cellular viability.
Conclusion: The addition of up to 10% of microspheres containing amoxicillin presented antimicrobial activity and did not alter the properties of the experimental endodontic cement.
Clinical significance: Amoxicillin microspheres with its antimicrobial activity in root canal sealers could reduce reintervations in endodontics when persistent bacteria or reinfection takes place in root canal system.

1. Introduction

Endodontic treatment success relies on the complete disinfection and sealing of root canal system, however, remaining bacteria could be viable even after mechanical preparation [1]. Anaerobic gram-positive and facultative microorganism are most commonly found in treated root canal and in the periapical area being the major cause of endodontic failure [2–5]. Among these microorganisms, *Enterococcus faecalis* have the ability to penetrate dentinal tubules on different environmental and nutritional conditions [6] being associated to acute infections after root canal treatment [7].

The control of the infection depends not only on bacteria removal, but also on preventing reinfection with a completely sealing of the canal system [8,9]. The use of root canal filling material with antimicrobial activity is considered beneficial to reduce the number of remaining microorganisms [10] however, most of the studies attempt to incorporate free antibacterial drug in sealers showing varied antimicrobial effect with few information on the effect of these drugs in materials properties and in antibacterial activity over the time [5,11–13]. Commercial amoxicillin has already been studied in sealers and showed a significant difference in antimicrobial response against *E. faecalis* [3,13]. Nevertheless, no encapsulated amoxicillin was added to

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endodontic cements.

New approaches involve the use of local drug-carrier system based on micro or nano particles to allow the incorporation of antimicrobials with prolonged release. This system improves the stability of the drug [14], its biocompatibility and bioavailability [15] and the control of drug delivery [16] which is related to reduction in drug dosage and thus, reduction of potential controversial effects [17]. Despite its beneficial effects, the use of drug carrier systems is Dentistry is little explored [18].

The aim of this study was to develop a microsphere containing amoxicillin (Amx-ME), incorporate into an experimental sealer and evaluate the properties.

2. Materials and methods

2.1. Ethics

This study was an in vitro study approved by the local ethics committee (n° 1.739.340).

2.2. Preparation of experimental endodontic sealer

The experimental endodontic sealer was obtained by mixing 70 wt% urethane dimethacrylate (UDMA), 15 wt% ethoxylated bisphenol A glycol dimethacrylate (BISEMA30) and 15 wt% glycerol 1,3-dimethacrylate (GDMA). Camphorquinone (CQ), *N,N*-dihydroxyethyl-para-toluidine (DHEPT) and benzoyl peroxide (BP) were added as photoinitiator/activator system to produce a dual-cure resin-based experimental sealer. Ytterbium trifluoride (YbF3; 10 wt%) was also added as radiopacifier, and Butylated hydroxytoluene (BHT; 0.01 wt%) was added as polymerization inhibitor.

2.3. Preparation of microsphere

Amoxicillin-loaded microspheres (Amx-ME) were prepared with Eudragit® S100, poly(MMA-co-MAA) (3.0 g) and trihydrate amoxicillin (0.3 g). The polymer was first dissolved in acetone (200 mL) and subsequently the amoxicillin was added under magnetic stirring at 25 °C. The suspension was spray dried (B-290, Buchi, Flawi, Switzerland) in the closed cycle dryer, using nitrogen as an inert gas. The inlet temperature in the drying chamber was maintained at approximately 60 ± 4 °C, and the outlet temperature was 40 ± 4 °C. Microspheres were measured through laser diffraction (Mastersizer 2000, Malvern, Worcestershire, UK). The distribution of the particle size (span) values was calculated by (d0.9 – d0.1)/d0.5, where d0.9, d0.5, and d0.1 were the particle diameters at the 90th, 50th, and 10th percentile of particles. Amoxicillin loaded microspheres presented mean size of 2.664 μm, d_{10} of 1.369 μm, d_{50} of 2.430 nm and d_{90} of 4.315 μm and were added to resin-based sealers in 10 wt% and 15 wt% concentrations.

2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to evaluate the morphology of the amoxicillin loaded microspheres using JSM 5800 microscope (JEOL, Tokyo, Japan).

2.5. Drug release

Three discs measuring 2 mm diameter x 1 mm height of experimental sealer containing 15% of Amx-ME were immersed in volumetric flasks with 10 mL of simulated body fluid (SBF) under magnetic stirring at 37 °C. After 24, 48 e 96 h, 1 mL of released medium was collected and fresh SBF was replaced. The aliquots were filtered using a 0.45-μm (Millipore) filter. Free amoxicillin was measured using high-performance liquid chromatography (HPLC, Shimadzu LC 10-A Shimadzu, Kyoto, Japan) with injector S-200, a UV/visible detector (λ = 280 nm), a guard column, and Nova-Pak® C18 3.9 × 150 mm (4 μm) Waters column. The mobile phase (60/40 v/v acetonitrile/water solution, pH 4.5, adjusted with acetic acid) was filtered and pumped at a constant flow rate of 1 mL min-1. After injection of 20 μL, amoxicillin was detected with a retention time of 1.9 s.

2.6. Antimicrobial test

Antimicrobial test was based previous study [19]. For antibacterial activity evaluation of experimental sealer, three specimens (disks of 3 mm diameter x 2 mm thickness) of each group were fixed on teflon matrixes on the lid of a 48-well plate and sterilized by ethylene oxide. In the sterile 48-well plate, 900 μL of brain heart infusion (BHI) broth (Sigma-Aldrich, St Louis, MO, USA) with 90 μL of a suspension of an overnight broth culture of *Enterococcus faecallis* (ATCC 29212), adjusted to optical density of 0.3 (550 nm) were added to each one of the wells. The plate was closed and incubated at 37 °C for 24, 48, and 96 h. Daily medium changes were performed for during all experimental times. The samples from each group were then removed from the lid's teflon matrixes and placed inside a micro-tube containing 900 μL of saline and vortexed [20]. Dilutions were made up to 10–6. Two 25 μL-drops of each dilution were platted in BHI agar Petri dishes and incubated for 48 h at 37 °C. The number of colony forming units (CFU) was visually counted by optical microscopy and transformed to log CFU/mL.

2.7. Cytotoxicity sulforhodamine B (SRB) colorimetric assay

Primary pulp fibroblasts were derived from two intact human third molars with incomplete root formation of different patients without systemic health problems that agreed to donate tooth for this study and signed the Informed Consent. After the extractions, teeth were immersed in 1 mL of Dulbecco's Modified Eagle Medium (DMEM), supplemented with Hepes, fetal bovine serum 10% and 100 IU/mL penicillin, 100 μg/mL streptomycin (Thermo Fischer Scientific, Waltham, Massachusets, EUA), at room temperature. The pulp was removed using endodontic instruments and dentin excavator. The collected tissue was sectioned into small fragments with scalpel blade. Tissue fragments were placed in cell culture plates in DMEM with Hepes, fetal bovine serum 10% and 100 IU/mL penicillin, 100 μg/mL streptomycin (Thermo Fischer Scientific, Waltham, Massachusets, EUA), and then incubated at 37° C humidified atmosphere with 5% CO_2. Culture medium was changed 24 h after the initial plating and from this moment on, every 2 days. After expansion to reach sufficient numbers of cells, experiments were performed with cells in fifth passage.

For the cytotoxicity assay, eluate was prepared immersing the specimen (3 mm diameter x 1 mm thickness) for 10% and 15% Amx-ME sealers and for the control group in 1 mL of medium during 24 h and 72 h. Cells were seeded in triplicate at a concentration of 5×10^3 in 96 well plates, and then treated with 100 μL of eluate. After 72 h cells were fixed with a concentration of 10% trichloroacetic acid (TCA), they were incubated at 4° C for 1 h and subsequently were washed 6 times under running water and dried at room temperature. Sulforhodamide B (SRB, Sigma-Aldrich, St. Louis, USA) at 4% was added to stain the cells and the plate was incubated for 30 min at room temperature. The plate was washed 4 times with 1% acetic acid to remove unbound excess dye and allowed to dry completely at room temperature. Trizma solution was added and the plate incubated for 1 h to allow complete solubilization of the dye. At the end of this process the microplates were read at 560 nm. Cell viability was normalized against viability of cells in wells without treatment. Three samples were used to test cell viability and tests were performed in triplicate.

2.8. Flow test

The flow test was conducted according to ISO 6876 [21]. A total of 0.05 mL of each experimental sealer was placed on a glass plate