Antibacterial Efficacy and Discoloration Potential of Endodontic Topical Antibiotics

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Abstract

Introduction: The optimal concentration for the use of endodontic topical antibiotics is not known. The aims of this study were to determine the minimum bactericidal concentrations (MBCs) and minimum inhibitory concentrations (MICs) of metronidazole, ciprofloxacin, minocycline, Augmentin (GlaxoSmithKline, Research Triangle Park, NC), and tigecycline against common endodontic pathogens and to evaluate *ex vivo* the antibacterial efficacy and discoloration effect of triple antibiotic paste (TAP), Augmentin, and tigecycline at different concentrations using a slow-release hydrogel scaffold. **Methods:** Using the Epsilometer test method (Etest; bioMérieux USA, St Louis, MO), MICs and MBCs of selected antibiotics were determined against *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus intermedius*, and *Enterococcus faecalis*. Biofilms of these bacterial species were then grown in extracted single-rooted teeth anaerobically for 3 weeks. Root canals were filled with TAP, Augmentin, and tigecycline at concentrations of 1 or 0.1 mg/mL in a degradable hydrogel scaffold or pure TAP at 1 g/mL for 7 days. Coronal discoloration was evaluated spectrophotometrically at 1, 2, and 3 weeks after dressing. **Results:** MIC/MBC data showed significant efficacy of tigecycline, Augmentin, and minocycline compared with the other antibiotics ($P < .05$). Significant differences were found when comparing the log10 colony-forming units of all experimental groups ($P < .05$). TAP at 1 g/mL had no bacterial growth but caused the greatest discoloration. Hydrogel mixtures with TAP, Augmentin, or tigecycline at 1 mg/mL significantly reduced bacterial growth and the number of positive samples compared with those at 0.1 mg/mL ($P < .05$) with minimal discoloration. **Conclusions:** TAP, Augmentin, and tigecycline in a hydrogel at 1 mg/mL reduced bacterial growth significantly with minimal color change. *(J Endod 2018;■:1–5)*

Key Words

Augmentin, bacteria, crown discoloration, regenerative endodontics, tigecycline, triple antibiotic paste

Significance

This *ex vivo* study investigated the antimicrobial efficacy and color differences for several concentrations of antibiotic/hydrogel mixtures with potential use as medicaments in regenerative endodontic therapy. The findings would guide the provider in selecting the most efficacious and least discoloring agent.

Disinfection of the pulp space has been shown to play a critical role in the success of regenerative endodontic procedures (1–3). One commonly accepted disinfection protocol for regenerative endodontic procedures is irrigation of the root canal space with sodium hypochlorite in the first visit and then dressing the root canal with triple antibiotic paste (TAP) (4–6), which includes equal proportions of ciprofloxacin, metronidazole, and minocycline (7).

Crown discoloration was reported in 40% of the studies on regenerative endodontic treatments (8). Such a complication in anterior teeth can affect patients' quality of life (9). Minocycline in TAP causes tooth discoloration (10). High concentrations of antibiotics were shown to be toxic to the stem cells of apical papilla (11, 12). Although generic, broad-spectrum, hypoallergenic antibiotics are recommended for these procedures, the efficacy of reduced concentrations of these antibiotics and their discoloration potential have not been adequately investigated. Moreover, the possibility of incorporating these antibacterial agents within scaffolds used in tissue development, which also need to be biocompatible and color neutral, has not been sufficiently explored. These scaffolds can also serve as a carrier for low-dose antibiotics to maintain them in the root canal system and allow the disinfection process to take place.

Therefore, the aims of this study were:

1. To determine *in vitro* the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of Augmentin, tigecycline, metronidazole, ciprofloxacin, and minocycline against selected common endodontic pathogens
2. To evaluate the antibacterial efficacy of TAP, Augmentin, and tigecycline at different concentrations in a slow-release hydrogel scaffold
3. To evaluate the discoloration potential of TAP, Augmentin, and tigecycline in a hydrogel scaffold *ex vivo*

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Materials and Methods

Bacterial Strain and Media

Four common endodontic pathogens were selected for the study: *Enterococcus faecalis* (ATCC 4083; American Type Culture Collection, Manassas, VA), *Streptococcus intermedius* (ATCC 31412, American Type Culture Collection), *Fusobacterium nucleatum* (ATCC 49256, American Type Culture Collection), and *Porphyromonas gingivalis* (ATCC 33277, American Type Culture Collection). All 4 bacterial strains were initially grown on anaerobic blood agar plates in an anaerobic chamber (Mini-MACS; Microbiology International, Frederick, MD) in 10% CO_2, 5% H_2, and 85% N_2 at 37°C. Then, each microorganism was cultured in its recommended broth as follows: brain-heart infusion broth for *E. feacalis*, Todd Hewitt broth for *S. intermedius*, chopped meat for *F. nucleatum*, and tryptic soy broth supplemented with hemin and vitamin K for *P. gingivalis*. All bacteria were grown to a turbidity of 1.0 McFarland density (3×10^6 colony-forming unit [CFU]/mL), which was determined using a spectrophotometer.

MBC and MIC

The MIC and MBC of metronidazole, ciprofloxacin, minocycline, Augmentin, and tigecycline were determined against the 4 study bacteria using the Epsilometer test (Etest; bioMérieux USA, St Louis, MO) as previously described (13).

Bacterial Inoculation in Root Canals

One hundred twenty intact single-rooted permanent human teeth were collected for the study. All teeth were autoclaved, and then access cavities were prepared and the canal instrumented to size 40/0.04 using Vortex nickel-titanium instruments (Dentsply Sirona, York, PA) with saline irrigation. Teeth were autoclaved again to ensure the absence of bacteria before bacterial inoculation. The root canals were then inoculated with the bacterial mixture (each at 1 McFarland concentration) using syringe delivery with a 30-G needle close to the working length. The access cavities were then sealed with a sterile sponge and white Cavit (3M ESPE, St Paul, MN). All teeth were then incubated in an anaerobic chamber. The suspensions were replenished weekly for 3 weeks to allow for biofilm formation and maturation (14).

Preparation of Hydrogel as a Scaffold for Antibiotics

We sought to incorporate the antibiotics tested into a hydrogel scaffold, which may be used as a medicament or to aid in tissue regeneration after disinfection. The tested antibiotics at concentrations of 0.1 mg/mL and 1.0 mg/mL were prepared and mixed with oxidized alginate solution to prepare the hydrogel microbeads as previously described (15). Briefly, sodium alginate was dissolved in distilled water to make a 1% solution. Then, 1.51 mL 0.25 mol/L sodium periodate was added in 100-mL alginate solution with continuous stirring to react in dark at room temperature for 24 hours. The oxidizing reaction was stopped by adding 1 g ethylene glycol, and then 2.5 g sodium chloride was added in the solution. An excess amount of ethanol (200 mL) was added to precipitate the product, which was then collected by centrifuging. The precipitates were redissolved in 100 mL distilled water and precipitated again with 200 mL ethanol. The second precipitates were collected and dissolved in 30 mL distilled water. The final product was obtained through freeze-drying under reduced pressure for 24 hours.

Initially, a 12-mg/mL solution of 7.5% oxidized alginate in 0.9% sodium chloride was prepared. Fibrinogen from bovine plasma (Sigma-Aldrich, St Louis, MO) was then added in a concentration of 1 mg/mL and incubated at 37°C from 1 to 2 hours to make a mixed solution of oxidized alginate-fibrinogen. A 100-mmol/L calcium chloride solution in distilled water was prepared, and thrombin (Sigma-Aldrich) was then added at a concentration of 1 U/mL to make a mixed, cross-linked solution. The 2 kinds of solutions were sterilized separately by filtering through a 0.2-μm hydrophilic polytetrafluoroethylene membrane (Millipore-Sigma, Burlington, MA). Hydrogel bead formation was accomplished by extruding oxidized alginate-fibrinogen solution droplets into the mixed cross-linking solution of calcium chloride and thrombin.

Antibiotic solutions were prepared and suspended in the oxidized alginate-fibrinogen solution. The suspension was then loaded into a syringe, which was placed into a syringe pump and connected to a bead-generating device (Var J1; Nisco, Zurich, Switzerland). Nitrogen gas was fed to the gas inlet, and a pressure of 8 psi was established to form a coaxial airflow to break up the alginate-fibrinogen-cell droplets. The $CaCl_2$-thrombin solution was filled in a wide plate to collect the droplets and simultaneously stirred by a magnetic bar. This produced small, oxidized alginate-fibrin-antibiotic beads.

Root Canal Dressing with Hydrogel/Antibiotic Mixtures

Preliminary experiments were conducted in which inhibition zones were measured around the various hydrogel/antibiotic mixtures or hydrogel alone with each of the 4 tested microorganisms. This was done to assure that the resultant mixtures still maintained antimicrobial properties against the tested bacterial species. These experiments showed that all hydrogel/antibiotic mixtures, except metronidazole and the hydrogel by itself, produced inhibition zones that ranged from 3–36 mm in diameter.

Root canals (n = 15 per group) were then filled randomly with antibiotics in hydrogel scaffolds as follows:

Group 1: Empty canals (negative control)
Group 2: Hydrogel + Augmentin (0.1 mg/mL)
Group 3: Hydrogel + tigecycline (0.1 mg/mL)
Group 4: Hydrogel + TAP (0.1 mg/mL)
Group 5: Hydrogel + Augmentin (1 mg/mL)
Group 6: Hydrogel + tigecycline (1 mg/mL)
Group 7: Hydrogel + TAP (1 mg/mL)
Group 8: TAP (1 g/mL) paste mixed with normal saline not the hydrogel (positive control)

Next, access cavities were sealed with a sterile sponge and Cavit (3M ESPE) and incubated anaerobically for 1 week.

Posttreatment Microbial Sampling

One week after antibiotic dressing, root canals were irrigated with 20 mL sterile saline to the working length for 10 minutes to remove the canal contents. Root canal walls were then instrumented with a size 40 K-file to disrupt any residual biofilms and were transferred to a vial containing normal saline for sampling. Size 2 Gates Glidden burs were used in the root canal dentin followed by 3 consecutive sterile paper points, which were transferred to the same vial for sampling. The samples were then serially diluted and plated on anaerobic blood agar plates, and CFUs were counted. Log10 CFUs were used for statistical analysis. As previously described (16), a spiking method was used to rule out antibiotic contamination in samples with a negative culture.

Color Change Measurement

A VITA Easyshade Compact (Vident, Yorba Linda, CA) was used to measure crown color as described previously (17). All color measurements were performed in a darkened room inside an anaerobic chamber. The instrument was calibrated before measurement in each group.

The mean baseline color measurements were taken before filling the root canal with the antibiotics and then at 1 week, 2 weeks, and