# Microbial Evaluation of Traumatized Teeth Treated with Triple Antibiotic Paste or Calcium Hydroxide with 2% Chlorhexidine Gel in Pulp Revascularization

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## Abstract

**Introduction:** Revascularization outcome depends on microbial elimination because apical repair will not happen in the presence of infected tissues. This study evaluated the microbial composition of traumatized immature teeth and assessed their reduction during different stages of the revascularization procedures performed with 2 intracanal medicaments. **Methods:** Fifteen patients (7–17 years old) with immature teeth were submitted to the revascularization procedures; they were divided into 2 groups according to the intracanal medicament used: TAP group ($n$ = 7), medicated with a triple antibiotic paste, and CHP group ($n$ = 8), dressed with calcium hydroxide + 2% chlorhexidine gel. Samples were taken before any treatment (S1), after irrigation with 6% NaOCl (S2), after irrigation with 2% chlorhexidine (S3), after intracanal dressing (S4), and after 17% EDTA irrigation (S5). Cultivable bacteria recovered from the 5 stages were counted and identified by means of polymerase chain reaction assay (16S rRNA). **Results:** Both groups had colony-forming unit counts significantly reduced after S2 ($P < .05$); however, no significant difference was found between the irrigants (S2 and S3, $P = .99$). No difference in bacteria counts was found between the intracanal medicaments used ($P = .95$). The most prevalent bacteria detected were *Actinomyces naeslundii* (66.67%), followed by *Porphyromonas endodontalis, Parvimonas micra,* and *Fusobacterium nucleatum,* which were detected in 33.34% of the root canals. An average of 2.13 species per canal was found, and no statistical correlation was observed between bacterial species and clinical/radiographic features. **Conclusions:** The microbial profile of infected immature teeth is similar to that of primarily infected permanent teeth. The greatest bacterial reduction was promoted by the irrigation solutions. The revascularization protocols that used the tested intracanal medicaments were efficient in reducing viable bacteria in necrotic immature teeth. *(J Endod 2014;40:778–783)*

## Key Words

Antibiotic paste, calcium hydroxide, chlorhexidine, endodontics, microorganisms, polymerase chain reaction, pulp revascularization

Immature traumatized teeth may become infected through crown fractures or cracks (1). Bacteria invasion gradually promotes inflammation of pulp tissues, and if these factors are not treated, pulp necrosis will happen. This, in turn, will promote the destruction of odontoblasts, resulting in interruption of the root development. The treatment for immature teeth with pulp necrosis may be performed through apexification or revascularization (2).

Revascularization therapy brings more advantages than apexification, inducing root-end development and reinforcement (3–5). However, apical repair will not happen in the presence of inflamed and infected tissues (6). A variety of decontamination protocols for pulp revascularization have been reported, and most of them used sodium hypochlorite as irrigant solution and triple antibiotic paste (TAP) (metronidazole, ciprofloxacin, and minocycline) as intracanal medicament (7–9). Both substances present antimicrobial action against endodontic pathogens (10, 11). Recently, chlorhexidine (CHX) has also been tested as an irrigant solution and calcium hydroxide as an intracanal medicament, with promising results in revascularization therapy (12–15). Considering the relevance of infection removal for apical repair of immature teeth, it is important to know the bacteria most commonly found in this sort of infection. To date, there are very few studies on microbial colonization of immature necrotic teeth that suffered dental trauma; there are even fewer studies that used molecular methods to identify microorganisms (16). In addition, there are no conclusive studies comparing the efficacy of intracanal medicaments in revascularization therapy. Therefore, the aim of this study was to evaluate the microbial composition of immature teeth that suffered pulp necrosis as a result of dental trauma and to assess their reduction during different stages of endodontic revascularization therapy performed with TAP and calcium hydroxide combined with 2% CHX gel as intracanal medicaments.

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## Materials and Methods

### Patient Selection

Patients who attended the Dental Trauma Service of the Piracicaba Dental School, State University of Campinas (UNICAMP), Piracicaba, SP, Brazil for dental trauma treatment were included in this research. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the previous 3 months or who had any general disease were excluded. The Human Research Ethics Committee of the Piracicaba Dental School approved the protocol describing the sample collection for this investigation, and all volunteer caregivers signed an informed consent form. The age of the patients ranged from 7–17 years old. Inclusion criteria were composed of immature teeth diagnosed with pulp necrosis caused by hard tissue dental trauma and/or some severe luxation (extrusive luxation, lateral luxation, intrusive luxation, and avulsion). The time between the trauma and the first visit varied from 2 days–8 years. With regard to the immature root development, only teeth with apical opening larger than 1.1 mm as seen on the initial radiograph were included. Pulp necrosis was verified by cold pulp sensibility test (Endo Ice; Roeko, Langenau, Germany), electric test (Pulp Tester; Analytic Technology, Redmond, WA), and/or radiographic evidence of periapical lesion and/or presence of sinus tract. In addition, teeth with root fracture and/or periodontal disease were excluded.

Microbial samples were collected from 15 upper incisor teeth with pulp necrosis, all showing radiographic evidence of immature root. The following clinical/radiographic features were collected for further analyses: spontaneous pain, tenderness to percussion, pain on palpation, presence of radiolucent area, sinus tract, mobility, and swelling. Teeth were randomly allocated into one of the treatment groups according to the intracanal medicament that was used, the group medicated with TAP ($n = 7$) and the group medicated with combination of calcium hydroxide and 2% CHX gel (CHP) ($n = 8$).

### Sampling Procedures for TAP and CHP Groups

The method used for disinfection of the operative field has been previously described (17). Briefly, the teeth were anesthetized with 2% lidocaine with 1:100,000 epinephrine (Alphacaine; DFL, Rio de Janeiro, RJ, Brazil) and isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% $H_2O_2$ (V/V) for 30 seconds, followed by 2.5% sodium hypochlorite (NaOCl) for the same period of time, and then inactivated with 5% sodium thiosulfate. The sterility of the samples taken from both external and internal surfaces of the crown and its surrounding structures was checked by taking swab samples from the crown surface and streaking one on blood agar plates, which were incubated aerobically and anaerobically. The other one was used for DNA detection. Polymerase chain reaction (PCR) assay was performed with universal primers for 16S rRNA. The access cavity preparation was made without the use of water spray but under manual irrigation with sterile saline solution and by using a sterile high-speed diamond bur. Before entering the pulp chamber, the access cavity was disinfected according to the protocol previously described. The sterility of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically.

Five samples were collected from each of the 15 single-rooted incisors. The microbial sample was taken by introducing 3 sterile paper points (size #30; Dentsply Maillefer, Ballaigues, Switzerland) into the full length of the canal (determined radiographically) and retaining them in position for 60 seconds. Next, the paper points were pooled in a sterile tube containing 1 mL VMGA III transport medium for microbial cultivation and for DNA extraction to detect target bacteria by using the molecular method (16S rRNA).

### Clinical Procedures (Revascularization)

The first microbial sampling (S1) was performed soon after the cavity access before any treatment. Next, root canal systems were irrigated with 20 mL 6% NaOCl, which was inactivated with 5 mL sterile 5% sodium thiosulfate for a 1-minute period. The canals were then irrigated with 5 mL sterile saline solution, and a second sampling was performed (S2). Root canals were then irrigated with 10 mL 2% CHX before being neutralized with 5 mL 5% Tween 80 and 0.07% soy lecithin (to reduce the carryover effect of CHX). Next, the canals were irrigated with 10 mL sterile saline solution, and a third sampling was performed (S3). The resulting samples were dried with paper points. In the TAP group, the teeth were dressed with a mixture of 250 mg ciprofloxacin, 400 mg metronidazole, and 50 mg minocycline as described by Hoshino et al (18), which was left in the canals for 21 days. For the CHP group, the canals were dressed with a paste composed of calcium hydroxide (Biodinâmica, Ibiporã, PR, Brazil) and 2% CHX gel (Endogel, Itapetininga, SP, Brazil) in the proportion of 1:1 in a creamy consistency, which was also left in the canals for 21 days. The access cavities of all teeth were sealed with Coltosol (Coltene-Whaledent, Langenau, Germany) and composite resin (Z250 Filtek; 3M ESPE, Sumaré, SP, Brazil). After a period of 21 days, the teeth were anesthetized with mepivacaine anesthetic 3% without vasoconstrictor (MEPISV; DFL), isolated, and reassessed. Before accessing, the operative field was disinfected with the same protocol previously described, with the sterility of the samples taken from both external and internal surfaces of the crown and its surrounding structures. Intracanal medicament was removed with 10 mL saline solution irrigation, and the fourth sampling was performed (S4). Next, a final irrigation with 3 mL 17% EDTA solution (Fórmula e Ação, São Paulo, SP, Brazil) was applied for 3 minutes, followed by 5 mL irrigation with saline solution before the final sampling (S5). A manual K-file (Dentsply Maillefer) was introduced into the root canal and placed at 2 mm beyond the working length to induce bleeding into the canal. The bleeding was allowed to reach a 3-mm level below the cementoenamel junction, and teeth were left at rest for 5 minutes so that a blood clot could be formed. CollaCote (Zimmer Dental, Carlsbad, CA) fibers were placed on the blood clot, and then a 3-mm white mineral trioxide aggregate (Angelus, Londrina, Brazil) barrier was placed. The access opening was sealed with Coltosol and restored with composite resin (Z250 Filtek).

### Microbiological Cultivation

Inside an anaerobic chamber, the samples were vortexed for 60 seconds and diluted in Fastidious Anaerobe Broth (Lab M, Bury, UK) by using a 10-fold serial dilution to $10^{-4}$. A volume of 50 $\mu$L of each dilution was spread onto 5% defibrinated sheep blood (Fastidious Anaerobe Agar; Lab M) containing 5 mg/mL hemin (final concentration of 5 mg/mL) and 1 mg/mL vitamin K1 (final concentration of 1 mg/mL). The plates were incubated at 37°C in an anaerobic atmosphere for up to 7 days. After this period, the colony-forming units (CFUs) were counted and then transformed into actual counts based on known dilution factors.

### DNA Extraction

Microbial DNA from all stages of revascularization samples (S1, S2, S3, S4, and S5) and control sample, as well as from ATCC bacteria, were extracted and purified by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration (absorbance at 260 nm) was determined with a spectrophotometer (Nanodrop 2000; Thermo Scientific, Wilmington, DE).

### PCR Assay

The PCR reaction was performed in a thermocycler (MyCycler; Bio-Rad, Hercules, CA) with a total volume of 25 $\mu$L containing

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