Evaluation of Triple Antibiotic Paste Removal by Different Irrigation Procedures

Julie A. Berkhoff, DDS, Paul B. Chen, BDS, Fabricio B. Teixeira, DDS, MS, PhD, and Anibal Diogenes, DDS, MS, PhD

Abstract

Introduction: Regenerative endodontics aims to reestablish a functional pulp-dentin complex. First, the root canal system is disinfected primarily by irrigants and medicaments. Triple antibiotic paste (TAP), a commonly used intracanal medicament, has been shown to be directly toxic to stem cells at concentrations greater than 0.1 g/mL. Thus, its complete removal is a crucial step in regenerative endodontic procedures. We hypothesized that currently used irrigation techniques do not completely remove TAP from root canal system. **Methods:** TAP was radiolabeled by the incorporation of I^{125}, and calcium hydroxide (Ultracal; Ultradent, South Jordan, UT) was radiolabeled with Ca^{45}. The intracanal medicaments were placed into standardized human root segments and incubated for 28 days at 37°C. Then, canals were irrigated with EndoActivator (Dentsply, Tulsa, OK), passive ultrasonic irrigation, EndoVac (SybronEndo, Coppell, TX), or a syringe/Max-i-Probe needle (Dentsply Rinn, Elgin, IL) using a standardized irrigation protocol in a closed system. Radioactivity levels (counts per minute values) were measured for each tooth before and after the irrigation protocols. Furthermore, the canals were sequentially enlarged and dentin samples collected and evaluated for radioactivity. Data were analyzed with analysis of variance and Bonferroni post hoc testing ($P < .05$). **Results:** Approximately 88% of the TAP was retained in the root canal system regardless of the irrigation technique used (no difference among groups). Furthermore, approximately 50% of the radiolabeled TAP was present circumferentially up to 350 μm within the dentin. Conversely, up to 98% of the radiolabeled intracanal calcium hydroxide was removed, and most residual medicament was found present in the initial 50 μm of dentin. **Conclusions:** Current irrigation techniques do not effectively remove TAP from root canal systems, possibly because of its penetration and binding into dentin. However, calcium hydroxide is effectively removed with significant less residual presence. *(J Endod 2014;40:1172–1177)*

Key Words

Calcium hydroxide, $Ca(OH)_2$, endodontics, irrigation, isotope, regenerative, TAP, triple antibiotic paste

Regenerative endodontic treatment is a biologically based procedure aimed at reestablishing a functional pulp-dentin complex. The key components include disinfection of the root canal and introduction of stem cells, growth factors, and scaffolds (1) using treatment methods that impose minimal toxicity to stem cells (2). Immature teeth are at risk for pulp necrosis because of trauma, dental anomalies, or caries. Historically, root canal infections in these teeth have been treated with apexification procedures. Although these procedures treat apical periodontitis, they do not favor the continued root development often seen in teeth treated by regenerative endodontic procedures (2, 3). Both apexification and regenerative endodontic procedures rely on adequate disinfection of the root canal system for a successful outcome. It has been shown that regenerative endodontic treatment is a stem cell–based therapy (4, 5). Thus, the potential for tissue regeneration may well depend on stem cell survival and differentiation capacity. From this perspective, the first critical step in a regenerative procedure is to adequately disinfect the root canal system while creating a microenvironment conducive to stem cell survival, proliferation, and differentiation.

Many disinfection protocols include the use of intracanal medicaments. Several successful case reports used triple antibiotic (ciprofloxacin, metronidazole, and minocycline) paste (TAP) in the root canal system for several weeks before the recruitment of stem cells into the canal via induced bleeding (2). TAP is an effective antimicrobial agent (6–8) that creates conditions suitable for tissue revascularization (9). However, relatively little is known about the cellular effects of the paste at clinically used concentrations, and it is possible that different concentrations of TAP or irrigation protocols may improve clinical outcomes. This latter point is very important because the TAP constituents are administered directly into the root canal system, resulting in local concentrations many orders of magnitude greater than those found in systemic circulation after oral administration. For example, circulating levels of ciprofloxacin (2 hours after a 500-mg capsule) peak at about 2.6 μg/mL in blood (10). However, concentrations 10,000 times greater (20 mg/mL ciprofloxacin in TAP) have been applied into the root canal system in regenerative procedures (11). Thus, root canal concentrations of antibiotics are about 5000- to 10,000-fold greater than circulating levels. This offers the potential for nonselective toxicity of the antibiotics against host cells, with a particular concern regarding stem cells. A recent animal study found that TAP induced a moderate inflammatory reaction in subcutaneous tissues (12). In addition, it has been shown that the clinically used concentration of TAP is cytotoxic to the stem cells of the apical papilla (13). These results provide a strong rationale for a study to determine

From the Department of Endodontics, University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Address requests for reprints to Dr Anibal Diogenes, Department of Endodontics, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229. E-mail address: Diogenes@uthscsa.edu

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.12.027

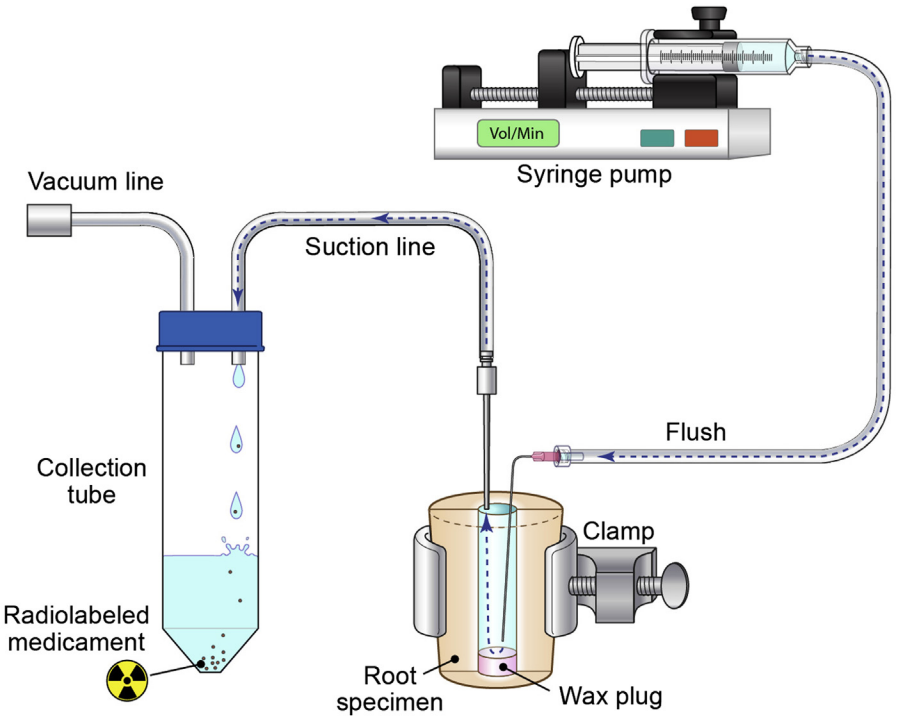

Figure 1. A schematic illustration of the fluid-closed system used for the aspiration and collection of the irrigants containing the radiolabeled medicaments. All irrigants were delivered with a standardized rate of (2 mL/min) using a syringe pump and a 28-G Max-i-Probe needle placed within 1 mm from the apex, except for the EV group, which had solutions delivered at the orifice and brought into the canal by the negative pressure created by its macrocannula placed within 1 mm from the apex (not shown).

the optimal method for removing antibiotics from the root canal system before the introduction of stem cells.

Calcium hydroxide ($Ca[OH]_2$) is another commonly used medicament in regenerative procedures, with several cases showing favorable clinical outcomes (2). The therapeutic benefits of $Ca(OH)_2$ have been appreciated for decades in endodontics long before its use in regenerative procedures. Historically, $Ca(OH)_2$ has been used as an antibacterial medicament (14) and a pulp capping agent (15) and to promote hard tissue formation in apexification procedures (16). Importantly, it has been found recently to promote stem cell survival and proliferation (13). Thus, $Ca(OH)_2$ represents another major class of intracanal medicament used in regenerative procedures.

There are many contemporary irrigation modalities that can be used to remove medicaments from the canal system. Sonic irrigation with EndoActivator (EA) has unique appeal for regenerative treatment because the tips are strong, flexible, and smooth, so they do not cut dentin. It has been reported to effectively clean debris from the canals and remove the smear layer even when used in curved canal systems (17, 18). Passive ultrasonic irrigation (PUI) is a term that was first described by Weller et al (19) in 1980. It is a technique that relies on the ultrasonic activation of irrigants for the efficient removal of debris and microorganisms (20). EndoVac (EV) is a negative-pressure irrigation system that deposits fresh irrigant into the chamber and aspirates it into the canal via an apically positioned microcannula. This system has been shown to be more effective at removing debris, particularly from the apical region, than conventional needle positive-pressure irrigation (21–23). Positive-pressure syringe irrigation with side-vented needles, such as Max-i-Probe (Dentsply Rinn, Elgin, IL), is widely accepted and used by endodontists. It is inexpensive and allows easy control of the depth of needle penetration and the volume of irrigant delivered (24). This method is proven most effective when the tip is in close proximity to the apex (25–27), a large volume of irrigant is used (28), and the apical diameter is equal or larger than ISO 40 (29, 30). All of the aforementioned irrigation techniques are feasible modalities to be used in regenerative endodontic treatment.

However, there is a gap in knowledge because the optimal irrigation method for the removal of TAP and $Ca(OH)_2$ from the root canal system remains unknown. Therefore, the aim of the study was to evaluate the effectiveness of these irrigation techniques for the removal of the 2 most commonly used intracanal medicaments, TAP and $Ca(OH)_2$, from simulated immature teeth with open apices.

Materials and Methods

Tooth Collection and Preparation

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, San Antonio, TX. Extracted teeth were collected from the clinics of the University of Texas Health Science Center at San Antonio School of Dentistry and placed in 15 mmol/L sodium azide for 24 hours at 4°C followed by copious irrigation with sterile saline and used for preparation of the simulated immature roots with open apices.

A total of 36 roots from freshly extracted human teeth were prepared for the study. The roots were sectioned from the crown at the level of the orifice. The apical-most 3 mm of the roots was resected with a #1557 bur (Komet, Rock Hill, SC) to remove ramifications and create specimens with a centered canal and standardized length of 10 mm. The canal lumen was initially cleared with a size 15 K-file and then enlarged with ortho- and retrograde instrumentation using a ProTaper Finishing File 1 (Dentsply, Tulsa, OK) to 8 mm. The walls were then paralleled and the lumen standardized to 1.0 mm in diameter with a size 100 LightSpeed LSX instrument (SybronEndo, Orange, CA).