Rapid Method for the Detection of Root Canal Bacteria in Endodontic Therapy

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Abstract

Introduction: Complete eradication of microorganisms is essential for successful root canal therapy. However, current methods to evaluate persistent bacteria after therapy are not widely practiced. Adenosine triphosphate (ATP) is an indicator of viable cells. The bioluminescence-based ATP assay is easy to perform, and results can be obtained in a clinically relevant time frame of 5 minutes. The aims of this study were to evaluate the sensitivity of the ATP detection method and the specificity of this assay for viable cells and to compare the ATP and culture methods from root canal samples of patients undergoing endodontic treatment. **Methods:** The sensitivity of the ATP assay was determined using bacterial species commonly isolated from root canals. Bacteria were treated with sodium hypochlorite; after which, culture plating and the ATP assay were performed. Forty-three root canal samples before (S1) and after (S2) instrumentation and 36 samples after the removal of calcium hydroxide dressing (S3) were collected from patients undergoing root canal treatment and subjected to ATP assay and anaerobic culture. **Results:** The sensitivity of the ATP assay was determined to be between 10 and 100 bacterial cells. This method of detection also correlated well with the presence of viable bacteria. The ATP readings obtained allowed clear segregation of anaerobic culture-positive and -negative samples obtained from infected root canals of patients. **Conclusions:** The ATP detection method can be used as a rapid tool to determine the presence of viable bacteria during root canal therapy. This method may be potentially useful as an adjunct to root canal treatment. *(J Endod 2015;41:447–450)*

Key Words

Adenosine triphosphate, bacterial culture, rapid detection, root canal infection

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The goal of root canal therapy (RCT) is to eliminate the pathogenic effects of bacteria in the root canal system of teeth. Treatment approaches include chemomechanical cleaning and filling to prevent microorganisms from infecting or reinfecting root canals and the periradicular tissues after treatment. The complete removal of bacteria in root canals is associated with high success rates, whereas substantial amounts of bacteria remaining after treatment are the major cause of failure (1, 2).

Single-visit RCT has recently become increasingly popular in endodontics because it is time and cost saving for both the patient and the operator. Single-visit RCT has also been associated with reduced postoperative pain, flare-up rates, and risk of interappointment infections (3, 4). Yet, the complete eradication of bacteria may not be possible in a single-visit treatment because of difficulties in reaching all sites in the canal (5, 6). Therefore, microorganisms lodged in uninstrumented areas may provide opportunity for failure (7). In contrast, multiple-visit RCT involves the placement of an antibacterial medicament such as calcium hydroxide with the aim of further reducing the bacterial load before permanent root canal filling (8). Although multiple-visit treatment should logically lead to better healing results, recent clinical studies comparing these 2 treatment regimens have yielded contradictory findings (9).

The culturing technique, based on samples from treated root canals, was a common method in the past to analyze the extent bacteria persisted in the root canal system after treatment. Although commonly taught and practiced in dental schools, the method never gained wide acceptance in the general practice of dentistry (10). An apparent reason was that culture methods are laborious to conduct, and it takes several days to weeks to identify anaerobic bacterial species. Furthermore, many species are not cultivable under laboratory conditions. On the other hand, culture-independent methods may identify these organisms. Yet, DNA-based identification methods such as polymerase chain reaction (PCR) suffer from high false-positive readings by the detection of DNA from dead bacterial cells (11, 12). Recently, the detection of RNA by reverse-transcriptase PCR has been assumed to be a better alternative to DNA to measure viable bacteria (13). It is argued that RNAs are more labile and possess a shorter half-life than DNA, thus providing a better indicator of viable cells (14). However, the detection of viable bacteria by the reverse-transcriptase PCR method is also cumbersome and requires multiple processing steps as well as substantial laboratory instrumentation and setup.

Adenosine triphosphate (ATP) is the main energy source for cellular functions in living organisms and serves as an indicator of metabolic activity in viable cells. ATP has been used to estimate the amount of biomass present in groundwater, drinking water, and biofilms (15, 16). The ATP method has further been developed to determine the contamination of enteropathogenic bacteria in food samples and for a sterility check for decontamination of medical devices (17, 18). More recently, the ATP assay was used to quantify oral bacteria in plaque samples (19). The potential of the ATP method in clinical endodontics to check persisting bacterial organisms in conjunction with treatment has not been explored. The procedure is simple to perform, and its results can be obtained in a clinically relevant time frame of less than 5 minutes. The assay is also sensitive and capable of detecting as few as 10 bacterial cells (20). Hence, if clinically feasible, the ATP assay might serve as a valuable adjunct to RCT to control the bacterial status of treated root canals.

The specific aims of this study were as follows:

1. To determine the sensitivity of the ATP assay to bacterial species commonly found in endodontic infections as a proxy for the clinical presence of bacteria
2. To determine specificity of the ATP assay for viable bacteria
3. To compare ATP and culture methods for the determination of viable bacteria from root canal samples of patients undergoing endodontic treatment

Materials and Methods

Sensitivity of the ATP Assay

Streptococcus mutans UA159, *Streptococcus sanguinis* ATCC 10556, *Enterococcus faecalis* ATCC 29212, *Porphyromonas gingivalis* W50, *Fusobacterium nucleatum* ATCC 25586, and *Prevotella intermedia* ATCC 25611 obtained from the American Type Culture Collection (Manassas, VA) were used as an estimate of the sensitivity of the ATP assay when used for bacterial detection of infected root canal samples. *P. gingivalis*, *F. nucleatum*, and *P. intermedia* were cultured anaerobically at 37°C on sheep blood agar plates in an anaerobic chamber supplemented with 80% N_2, 10% H_2, and 10% CO_2. *S. oralis*, *S. mutans*, *S. sanguinis*, and *E. faecalis* were cultured on brain-heart infusion agar and incubated at 37°C in an incubator supplemented with 5% CO_2. The amount of bacteria was determined by serial dilution and plating on agar plates. Serially diluted microorganisms were subjected to the ATP assay using BacTiter Glow reagent (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, 100 μL bacterial suspension was added to 100 μL BacTiter Glow reagent in a 96-well white plate (Greiner, Monroe, NC) followed by incubation at room temperature at 5 minutes. The luminescence produced was measured with a luminometer (GloMax, Promega).

Specificity of the ATP Assay for Viable Bacteria

E. faecalis was treated with freshly prepared 1% NaOCl for 2.5, 5, or 10 minutes; after which, NaOCl was neutralized with sodium thiosulfate to a final concentration of 5%. Bacterial cell viability was determined by culture as described previously. The ATP assay was performed on an aliquot of the treated sample as described earlier.

Patient Recruitment and Selection Criteria

Ethics approval was obtained from the Domain Specific Review Board, National Healthcare Group. Patients referred for endodontic treatment at the Faculty of Dentistry graduate clinic, National University Hospital were invited to participate in the study after obtaining written and informed consent. Inclusion criteria included single-rooted or multirooted teeth with pulp necrosis and apical periodontitis based on clinical and radiographic findings. Teeth with previously initiated therapy were accepted if root canal instrumentation was not performed or was incomplete. Teeth with severely broken down coronal tooth structures that could jeopardize leakage-free sampling conditions, teeth with prior root canal fillings, teeth with root canals filled with calcium hydroxide, and teeth presenting with vital or inflamed pulp tissues upon access were excluded. Patients who had taken antibiotics 4 weeks before sample collection were excluded from the study as well. A total of 50 S1 samples were collected; 43 samples fulfilled sterility control requirements.

Clinical Sampling Procedures

Root canal samples included a sterility control before endodontic treatment, bacterial sampling before (S1) and after (S2) root canal instrumentation, and bacterial sampling after removal of the calcium hydroxide dressing and before root canal obturation (S3). Plaque, calculus, caries, and defective restorations were removed, and the tooth was isolated using a rubber dam appropriately secured to ensure leakage-free sampling conditions. After isolation, the tooth was scrubbed with 30% hydrogen peroxide followed by 5% iodine tincture according to the method described by Möller (21). Disinfectants were deactivated with 5% sodium thiosulfate, and a sterility control sample was obtained by scrubbing a sterile cotton pellet on the occlusal surface and transferring it to a vial with thioglycollate broth. To collect S1, canals were widened with K-files (Flexofiles; Dentsply, York, PA) in the presence of reduced transport fluid (RTF) to release biofilms on dentinal walls and to accommodate sampling using paper points (Henry Schein, Melville, NY). Five successive paper points were placed in the canal and transferred to an RTF vial. Samples were placed on ice until collection for laboratory processing. Complete root canal instrumentation was performed using nickel-titanium rotary instruments (ProTaper [Dentsply] or RaCe [FKG Dentaire SA, La Chaux-de-Fonds, Switzerland]) to a minimum apical size 30 and 1.25% sodium hypochlorite (NaOCl) (Clorox; The Clorox Company, Oakland, CA) with 2 mL for irrigation (Monoject Endodontic Irrigation Needles; Kendall Healthcare, Mansfield, MA) per change of instrument. At the end of instrumentation, 1 mL 5% sodium thiosulfate deactivated NaOCl before S2 collection using the same protocol described for S1 collection. In addition, canal walls were scraped with files to release dentinal shavings by the use of a sterile K-type file of an ISO size 1 step larger than that of the master apical file and inserted to the working length. The instrument was rotated according to the balanced force technique. The apical 5 mm was cut off with a sterile cutter and transferred to a second RTF vial according to the technique designed by Örstavik el al (22). The prepared and dried canal was filled with nonsetting calcium hydroxide paste (UltraCal XS; Ultradent Products Inc, South Jordan, UT) and the tooth sealed (IRM Intermediate Restorative Material; Dentsply Caulk, Milford, DE). After an interappointment period of at least 7 days, the tooth was isolated as previously described, a sterility control sample was collected, and the root canal was reaccessed. Calcium hydroxide was removed using ultrasonically activated sterile saline. Complete removal of calcium hydroxide was confirmed visually with the aid of a dental operating microscope (OPMI pico; Carl Zeiss Meditec AG, Oberkochen, Germany). The S3 sample comprising the apical 5 mm of the last K-file and 5 paper points was collected in a manner similar to that for S2.

Laboratory Procedures

All samples were transferred to the laboratory on ice for immediate processing within 1 hour. Sterility control samples were incubated anaerobically at 37°C for 7 days. Tubes containing S1, S2, and S3 were vortexed at medium speed for 5 minutes to dislodge microorganisms adhering to the paper points and files. One hundred microliters of the sample were serially diluted using prereduced brain-heart infusion broth and plated on prereduced 5% sheep blood agar and incubated anaerobically in an atmosphere of 80% N_2, 10%H_2, and 10% CO_2 for 7 days. The ATP assay was performed on 100 μL of each sample in triplicate as described earlier.

Analysis of Data

Results obtained were presented as the mean $\pm$ standard deviation. R^2 values were calculated using GraphPad Prism software, version 6.0 (GraphPad Software, San Diego, CA).

Results

Sensitivity of ATP Assay

The sensitivity of the ATP assay was dependent on several factors such as efficiency of extraction of ATP from the target organism and