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Effectiveness of Sodium Hypochlorite in Preventing Inoculation of Periapical Tissues With Contaminated Patency Files

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The aim of this study was to analyze the effectiveness of 5.25% sodium hypochlorite (NaOCl) in preventing inoculation of periapical tissues with contaminated patency files. Twenty-eight extracted human permanent teeth with single canals were used in the study. Group I teeth were filled with NaOCl, and #15 stainless steel files contaminated with *Streptococcus sanguis* (ATCC #10556) were allowed to pass through the NaOCl into the culture medium. The teeth in group II were also filled with NaOCl, but the contaminated files used in group II canals were immersed in NaOCl for 10 s prior to being placed into the canals and cultured. The negative control group used sterile files (0% growth), the first positive control group used contaminated patency files in teeth with empty canals (100% growth), and the second positive control group placed contaminated files into broth next to teeth filled with NaOCl (to evaluate potential chlorine leakage; 100% growth). The experimental results showed no positive growth of *S. sanguis* for groups I and II, indicating that the NaOCl present in the canal after irrigation was sufficient to kill the test organism.

The need for endodontic therapy often arises from the presence of pulpal and periapical pathology caused by oral bacteria (1, 2). A major objective of endodontic therapy is proper debridement of the canal space, which results in a reduction of bacteria present within the canal (3). Previous studies have suggested an improved prognosis when previously infected canal spaces test negative to bacteriological sampling (4, 5). Inherent in the root canal debridement phase is the prevention of bacterial introduction into the canal through proper instrumentation and sterilization techniques.

Several instrumentation methods that are currently used to debride and shape root canals advocate the use of patency and recapitulation files (6). In 1997, 50% of 48 dental schools surveyed in the United States taught some concept of a patency file (7). Patency files can be defined as "small flexible K-file(s), which passively move through the apical constricture without widening it" (8). The small files used to obtain patency are often the same files initially used to negotiate the canal space. Because patency files are used before the canal space has been thoroughly debrided, the files are subject to contamination by bacteria initially present in the canal. Patency files can also be contaminated if they are touched by a contaminated glove or placed in an area that has inadvertently been contaminated by the patient or doctor. If these contaminated files are reused, bacteria can be introduced into the tooth after the canals have been thoroughly debrided. Because patency files are taken through the apical constricture, contaminated files can also potentially introduce bacteria into the periapical tissues.

The use of sodium hypochlorite (NaOCl) as an irrigation agent has been well documented, and 5.25% NaOCl is recommended as an irrigant due to its antimicrobial activity, the increase in effectiveness of removing pulpal debris from the root canal system, and the ability to dissolve tissue (9). NaOCl has specifically been found to be effective against a number of endodontic pathogens (10). Because of its widespread use as well as its antimicrobial properties, NaOCl may be effective in preventing contamination of the periapex with patency files. Thus, the purpose of this study was to analyze the effectiveness of NaOCl in preventing inoculation of periapical tissues with contaminated patency files.

MATERIALS AND METHODS

Tooth Preparation

Thirty-three human teeth were obtained from clinics at the University of Alabama at Birmingham School of Dentistry. All teeth were canines with a single root canal and an intact pulp chamber. No active caries or restorations were present. The teeth were extracted for periodontal and/or restorative purposes. Conventional access preparations were made with a #4 round bur. Standardized canal preparations were done with 0.06 rotary nickel-titanium files (Tulsa Dentsply, Tulsa, OK), used in a crown-down technique 1 mm short of the apical foramen to a #50 size. The apical foramina were enlarged and kept patent using a #15 K-type file (Maillefer, Tulsa, OK) approximately 1 mm beyond the apical foramen. Approximately 10 ml of 5.25% NaOCl was used as an

irrigant during instrumentation of each tooth. A final flush was completed with 17% aqueous EDTA (ethylenediaminetetracetic acid; Stone Pharmaceuticals, Philadelphia, PA) in an effort to open all dentinal tubules to facilitate bacterial ingress. The canals were then dried with paper points. The cementum was coated with two layers of nail polish, and apical patency was confirmed. Ligature wires to be used to suspend the teeth into the medium were wrapped around the cervical portion of the teeth. The teeth and culture apparatus were then autoclaved twice to kill native bacteria (11).

Bacterium and Culture Condition

An overnight culture of *Streptococcus sanguis* (ATCC #10556) in tryptic soy broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) was used to coat the patency files in this study. Initially, a stock of *S. sanguis* was streaked onto a plate of tryptic soy agar (Becton Dickinson and Company, Sparks, MD) with 5% defibrinated sheep blood (Micronet Medical, Inc., White Bear Lake, MN). The plate was cultured under anaerobic conditions for 48 h at 37°C. Single colonies were obtained and inoculated into tryptic soy broth and cultured overnight in the same conditions.

After assignment to the various experimental conditions (see below), teeth were suspended with ligature wires into individual test tubes so that the apical 3–4 mm of the tooth was immersed into the culture medium. Each test tube contained 2 ml of tryptic soy broth mixed with 1 ml 40% sodium thiosulfate (Mallinckrodt, Paris, KY). The sodium thiosulfate was added in order to neutralize any residual chlorine that may have leaked into the culture medium (13). #15 K-type files coated with *S. sanguis* were placed beside the teeth, directly into the culture medium. The test tubes were capped, and the cultures were allowed to grow for 24 h at 37°C. One hundred microliters of culture from each test tube were then spread onto plates of tryptic soy agar with 5% sheep blood and allowed to culture for 48 h at 37°C under anaerobic conditions. Visualization of positive growth on the agar plates indicated the presence of live bacteria.

Experimental Design

The teeth were randomly assigned to five different groups. The negative control group ($n = 3$) used sterile files (i.e. no *S. sanguis* contamination) passed through NaOCl in the root canal, and the tip of the file was immediately placed 2–3 mm past the apical foramen and into the culture medium. Under sterile conditions, the teeth were irrigated with 1 ml 5.25% NaOCl using a Monoject needle (Sherwood Medical, St. Louis, MO) placed about 15 mm into the canal space. No effort was made to ensure the NaOCl completely filled the canal space. The teeth were suspended into the culture medium, the test tubes were capped, and the presence of live bacteria was evaluated as described above.

The first positive control group ($n = 5$) evaluated whether contaminated files retain bacteria when passed through teeth with empty canals.

The second positive group evaluated whether the lack of growth was due to chlorine leakage from the canal system sufficient to overcome the sodium thiosulfate placed in the broth.

Group I ($n = 10$) consisted of files contaminated with *S. sanguis* and placed 2–3 mm beyond the apical foramen in teeth filled with NaOCl.

Group II ($n = 10$) consisted of files contaminated with *S. sanguis* and placed in a fresh solution of NaOCl for 10 s. This amount of time was chosen on the basis of previous research that showed that gutta percha cones could be effectively sterilized by placing them into a 2.5% solution of NaOCl for less than 10 s (12). After removal from the NaOCl solution, the files were placed 2–3 mm beyond the apical foramen of teeth filled with NaOCl.

RESULTS

None of the teeth in the negative control group exhibited growth. All of the teeth in the first positive control group (contaminated files placed in teeth filled with saline) exhibited growth. All of the teeth in the second positive control group (contaminated files placed adjacent to teeth filled with NaOCl) exhibited growth. For groups I and II, 100% of the ten teeth in each group showed no positive bacterial growth (two-sided 95% confidence interval, 69% to 100%). Fisher's exact test of a 4×2 table (14) showed that group I, group II, and the negative control group were statistically significant from the positive control groups ($p = 0.0003$).

DISCUSSION

This study demonstrated that patency files contaminated with *S. sanguis* can be disinfected by 5.25% NaOCl present in the root canal after irrigation. *S. sanguis* was chosen because it is a facultative anaerobic bacteria that is commonly found in endodontic infections (15, 16). Samples of *S. sanguis* have been isolated from root canals even after complete biomechanical instrumentation (16), and *S. sanguis* has also been isolated from blood samples taken after completion of endodontic therapy (17). Finally, *S. sanguis* is grown easily in tryptic soy broth, so the positive cultures were quickly identified.

Sodium thiosulfate was added in order to neutralize any leakage of residual chlorine into the culture medium (13). The first positive control group consisted of contaminated files placed through five teeth that did not contain NaOCl. All cultures for this group showed bacterial growth as expected. However, it was possible that leakage of NaOCl into the culture medium could prevent bacterial growth despite the addition of sodium thiosulfate. Therefore, sterilized teeth in the second positive control group were filled with NaOCl to account for possible leakage into the culture medium, and the files were placed on the outside of the teeth.

The present study focused on the ability of a single solution to prevent inoculation of periapical tissues with patency files. Other solutions may be just as effective as NaOCl, using the experimental model outlined above. Also, when the experimental design was chosen, it was assumed that the NaOCl would not be able to prevent inoculation of periapical tissues simply by passing the contaminated file through the NaOCl in the tooth. Therefore, group II was added to the design to show the results of prolonged exposure of the test organism to NaOCl. Obviously, the NaOCl exceeded the expectations of the experiment, and its effectiveness could have been shown without immersing the contaminated files for 10 s.

Patency files are generally used throughout endodontic treatment, and they can potentially contaminate the periapical tissues any time they are used. The present study is limited to examining the effect of NaOCl on patency files after the instrumentation process is complete. Further studies are needed to show the ability

of test solutions to prevent inoculation of periapical tissues throughout the instrumentation process.

As stated previously, this study demonstrated that patency files contaminated with *S. sanguis* can be disinfected by 5.25% sodium hypochlorite present in the root canal after irrigation. One of the main goals of endodontic treatment is to reduce or eliminate the bacteria present in the root canal system. The fact that all of the positive controls showed bacterial growth implies that the potential for contamination of the periapical tissues is present, and care must be taken to kill bacteria present on contaminated instruments prior to use in the root canal space.

Dr. K. Izu and Dr. Thomas completed their endodontic residency program at the School of Dentistry, University of Alabama at Birmingham, Alabama in June 2003 and are now in private practice.

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