

Disinfection/Sterilization of Extracted Teeth for Dental Student Use

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Abstract: Extracted human teeth are used in many preclinical courses. While there has been no report of disease transmission with extracted teeth, sterilization of teeth used in the teaching laboratory should be a concern. The purpose of this study was to determine the effectiveness of different sterilization/disinfection methods of extracted human teeth using *Bacillus stearothermophilus*, a bacteria resistant to heat and frequently used to test sterilizers. In this study, 110 extracted molars with no carious lesions were collected and stored in buffered saline. An endodontic occlusal access preparation was cut into the pulp chamber of each tooth. Pulp tissue in the chamber was removed with a broach. Approximately 1×10^5 *B. stearothermophilus* endospores in culture medium were injected into the pulp chamber, sealed with Cavit G, and then placed in sterile saline for twelve hours. Ten teeth were placed into each of eleven groups. Seven groups were immersed for one week in one of the following solutions: a) sterile saline (control group), b) 5.25% NaOCl, c) 2.6% NaOCl, d) 1% NaOCl, e) 10% buffered formalin, f) 2% glutaraldehyde, g) 0.28% quaternary ammonium. Four additional groups were treated by h) 10% formalin for two days, i) 10% formalin for four days, j) autoclaving at 240°F and 20 psi for twenty minutes, and k) autoclaving at 240°F and twenty psi for forty minutes. Each tooth was then aseptically split and placed in an individual test tube with growth medium. Samples were examined for evidence of growth (turbidity) at forty-eight hours. Only autoclaving for forty minutes at 240°F and 20 psi or soaking in 10 percent formalin for one week were 100 percent effective in preventing growth. A chi-square analysis of the data indicates these two methods were significantly better than all other methods ($p < 0.001$).

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Dental students must learn technical and preclinical skills before they enter the clinical environment and deliver patient care. Much of the dental student's early experience with technical procedures is learned in the preclinical laboratory. Some endodontic procedures can be taught conceptually using manufactured instructional materials such as artificial plastic blocks and teeth on mannequins and models. However, there are instances in which there is no acceptable substitute for extracted teeth for examination, preparation, or research.

Infectious disease transmission has long been a concern in the practice of dentistry. Universal precautions, as applied in the clinical setting, require that all body fluids and tissues be treated as if known to be infectious for HIV, Hepatitis B and C virus, or other bloodborne pathogens. The Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard considers human teeth used for research and teaching purposes as a potential source

of bloodborne pathogens.¹ To address this concern the Center for Disease Control recommends storing extracted teeth in 1:10 household bleach.¹ However, Tate and White² demonstrated that to be a poor disinfectant for this purpose. Ethylene oxide sterilization has been found to have 20 percent to 36 percent efficacy on *B. subtilis* spores in extracted teeth.³ Pantera and Schuster⁴ found microbial growth from the canals of extracted teeth that had been autoclaved for twenty minutes, but a forty-minute cycle eliminated all microbial growth.

Extracted teeth with amalgam restorations should not be autoclaved because of mercury vapor released in the air through autoclave exhaust and residual mercury contamination of the autoclave. It is also possible that the thermal cycling may cause teeth with amalgam restorations to fracture due to differences in their coefficients of thermal expansion. Formalin may be the most effective disinfectant, but it is a hazardous material and a potential carcinogen.⁵

The purpose of this study was to evaluate the effectiveness of various disinfection/sterilization methods for extracted human teeth.

Methods and Materials

Noncarious, unrestored molar teeth were collected and stored in saline until the start of the study (n = 110). Approval for the use of human teeth in this study was obtained from the University of Louisville Human Studies Committee. An endodontic access was prepared through the occlusal enamel and dentin into the pulp chamber using a high-speed #4 round bur. The pulp tissue from all three canals was extirpated, and gross pulpal tissue was removed with a fine broach (Union Broach, NY). The canals were then instrumented with a # 25 Hedstrom file (Kerr, Romulus, MI) to further remove additional tissue. The canals and pulp chamber were irrigated with normal saline to remove dentinal and pulpal debris. The pulp chamber and canals were dried with absorbent paper points and then inoculated with 0.05ml *Bacillus stearothermophilus* endospores (approximately 1×10^5). The occlusal access was then sealed with Cavit G (ESPE, Germany). The teeth were placed in sterile saline for twelve hours and then assigned to one of the following treatment groups (10 teeth /group): 5.25 percent, 2.6 percent, or 1.3 percent NaOCl (Clorox Bleach, Clorox Co., Oakland, CA) for one week, 10 percent formalin (Baxter Scientific Products, IL) for one week, four days, or two days, 2 percent Glutaraldehyde (JT Baker, Phillipsburg, NJ) for one week, 0.28 percent Quaternary ammonium (Envirocide, Metrex Research Co., Parker, CO) for one week, autoclaving at 240°F at 20 psi for twenty minutes or forty minutes, and buffered saline (control) for one week. Groups of ten teeth were immersed in separate containers filled with 30

ml of solution. The teeth placed in the autoclave treatment groups were placed in a glass beaker with 100 ml of saline (10 teeth/group) to prevent the teeth from dehydrating when some of the saline solution evaporated during the autoclave cycle. (See Table 1.)

Fresh solutions were prepared on the day of use. Solutions of NaOCl were changed after four days when any evidence of oxidizing effervescence had subsided.

Following the assigned treatment procedure and time period, teeth from each group were aseptically split with sterile extraction forceps, and placed into separate test tubes containing trypticase soy broth (BBL, Baltimore, MD) growth medium. The test tubes containing individual teeth were incubated for forty-eight hours at 54°C. Evidence of sporulation and growth was observed using the McFarland turbidity indicator. Data was collected, and statistical analysis was performed using a chi-squared (χ^2) analysis of contingency table.

Results

Teeth immersed in 10 percent formalin for one week or autoclaved at 240°F at 20 psi for forty minutes were the only methods that prevented the growth of *B. stearothermophilus* spores (Table 2). Autoclaving at 240°F at 20 psi for twenty minutes and immersion in 10 percent formalin for two days were effective in preventing microbial growth in nine of ten cases. Immersion in 10 percent formalin for four days was effective in 80 percent of the samples. Full strength household bleach (5.25 percent NaOCl) for one week prevented growth of spores in six of ten samples studied. Only half of the teeth immersed in 2 percent glutaraldehyde showed no growth. All other treatments were effective in less than 50 percent of the test samples. Positive controls displayed 100 per-

Table 1. Sterilization/disinfection methods

Treatment	Concentration	Time Period
NaOCl	5.25%	1 week
NaOCl	2.6%	1 week
NaOCl	1.3%	1 week
Formalin	10%	1 week
Formalin	10%	4 days
Formalin	10%	2 days
Quaternary Ammonium	0.28%	1 week
Glutaraldehyde	2%	1 week
Autoclave @240@20 psi	-	20 minutes
Autoclave @240@20 psi	-	40 minutes
Saline (control)	-	1 week

Table 2. Sterilization/disinfection methods

Treatment	Number of Teeth	Percent No Growth
5.25% NaOCl 1 wk	10	60%
2.6% NaOCl 1 wk	10	10%
1% NaOCl 1 wk	10	0
10% Formalin 2 d	10	90%
10% Formalin 4 d	10	80%
10% Formalin 1 wk	10	100%
Quaternary Am. 1 wk	10	30%
2% Glutaraldehyde	10	50%
Autoclave 20 min	10	90%
Autoclave 40 min	10	100%
Saline control 1 wk	10	0%

cent growth. Chi-square analysis of the data indicates a statistically significant difference in the outcomes when comparing the different methods of disinfection and sterilization. Immersion in 10 percent formalin for one week or autoclaving at 240°F at 20 psi for forty minutes were significantly better than all other methods tested ($p < 0.001$).

Discussion/Conclusions

Sterilization of extracted teeth used in the teaching laboratory should be a concern to educators and students alike. Potentially pathogenic microorganisms have been recovered after drilling on extracted teeth in the dental technique laboratory.⁶ The results of this study indicate that extracted teeth inoculated with *B. stearothermophilus* then autoclaved at 240°F for forty minutes or soaked in 10 percent formalin for seven days did not have spore growth. All the other methods tested were less effective in preventing spore growth. *B. stearothermophilus* is a biologic testing standard for evaluating sterilization. This microorganism is very resistant to steam sterilization. Also, teeth can be particularly difficult to sterilize. The requirement for a longer autoclave cycle (forty minutes vs. twenty minutes) confirmed the difficulty in sterilizing extracted teeth. The difference in positive growth with various time periods in formalin may have occurred because the formalin did not adequately penetrate through the tooth into the pulp space. Dentinal debris from the root canal instrumentation may have occluded the tubules. Also, an intact cemental surface may provide a barrier to diminish the penetration of some of the treatment agents.⁷ Some of the solutions tested have not been recommended for use against spores. These treatment solutions were tested because they are common disinfectants found in the dental office and are solutions in which extracted teeth have been stored by students. These solutions (5.25 percent NaOCl, 2.6 percent

NaOCl, 1.3 percent NaOCl, 0.28 percent quaternary ammonium and 2 percent glutaraldehyde) should not be relied on to disinfect teeth for laboratory/research use.

Infection control measures to protect students and faculty are not limited just to the disinfection/sterilization of extracted teeth. Instrument sterilization as well as the use of gloves, eye protection, and masks should also be used in the preclinical laboratory. If formalin is chosen for treatment of teeth for preclinical laboratory use, the container holding the teeth should be opened only under a fume hood, and the teeth should be rinsed prior to their use. Impermeable gloves and goggles should be used to prevent skin and eye exposure.⁵

This study demonstrates the effectiveness of one week immersion in 10 percent formalin or a forty minute autoclave cycle at 240°F and 20 psi. The sterilization procedures should not affect physical properties of the dentin and enamel to the extent that the “feel” and cutting characteristics are noticeably different from the clinical situation, as this is one of the major advantages in using extracted teeth. Further studies on the physical properties of sterilized extracted teeth are in progress.

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