

A Comparison of Different Methods for Disinfection or Sterilization of Extracted Human Teeth to be Used for Dental Education Purposes

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ABSTRACT

Objective: To determine efficacy of commonly used methods of disinfection/sterilization of extracted human teeth.

Materials and methods: A total of 140 freshly extracted human single-rooted teeth were randomly divided into 14 different groups and sterilized using different methods: Formalin, hydrogen peroxide, quaternary ammonium, glutaraldehyde, thymol, boiling, autoclaving, keeping sodium chloride as control. Following the assigned treatment procedures, teeth from each group were placed individually in separate test tubes containing 10 ml of tryptic soy broth at 37°C for 48 hours. Evidence of growth was observed after 2 days.

Results: A statistically significant difference in the outcomes was obtained when comparing the different methods of disinfection and sterilization.

Conclusion: It was concluded that immersion in 10% formalin for 1 week, 5.25% sodium hypochlorite for 1 week or autoclaving at 121°C at 20 psi for 40 minutes were significantly better than all other methods tested.

Keywords: Sterilization, Disinfection, Extracted teeth, Dental education.

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INTRODUCTION

The risk of infectious disease transmission is an inherent part of dental practice. Moreover, the curriculum of dental education requires dental students to learn technical and preclinical skills before entering the clinics to render quality treatment to the patients. Most of the preclinical procedures in operative dentistry and endodontics can be taught conceptually using manufactured instructional materials such as artificial plastic blocks and teeth on mannequins and models. However, there are some procedures in which there is no acceptable substitute for extracted teeth for examination and preparation. Extracted human teeth are also used for *in vitro* laboratory dentin bonding research, before clinical trials ultimately decide their clinical effectiveness.¹

The USAF Guidelines for Infection Control in Dentistry address disposal of extracted teeth as regulated medical

waste and use in educational settings. The guidance is based on recommendations from the centers for disease control and prevention.²

Extracted teeth used for the education of DHCWs should be considered infective and classified as clinical specimens because they contain blood. All persons who collect, transport, or manipulate extracted teeth should handle them with the same precautions as a specimen for biopsy.³ Universal precautions should be adhered to whenever extracted teeth are handled; because preclinical educational exercises simulate clinical experiences, students enrolled in dental educational programs should adhere to universal precautions in both preclinical and clinical settings. In addition, all persons who handle extracted teeth in dental educational settings should receive hepatitis B vaccine.⁴⁻⁶

Although there is common goal in infection control, there are several approaches that may be used to achieve the desired end result. Currently, there is no specific protocol for sterilization of extracted human teeth to be used for dental education purposes. The purpose of this study was to compare the efficacy of some commonly used chemicals and heat for sterilization or disinfection of extracted human teeth.

AIM OF THE STUDY

To determine efficacy of commonly used methods of disinfection/sterilization of extracted human teeth.

MATERIALS AND METHODS

A total of 140 freshly extracted human single-rooted teeth due to periodontal or orthodontic reasons were used in the study. All teeth were intact, noncarious and unrestored.

The teeth were randomly divided into following 14 groups containing 10 teeth each:

- Group 1: 10% formalin for 2 days
- Group 2: 10% formalin for 4 days
- Group 3: 10% formalin for 7 days
- Group 4: 3% hydrogen peroxide
- Group 5: 1.3% hydrogen peroxide
- Group 6: 2.6% hydrogen peroxide
- Group 7: 5.25% hydrogen peroxide
- Group 8: 0.28% quaternary ammonium

- Group 9: 2% glutaraldehyde
- Group 10: 0.1% thymol in distilled water
- Group 11: Boiling at 100°C
- Group 12: Autoclaving at 121°C for 20 minutes
- Group 13: Autoclaving at 121°C for 40 minutes
- Group 14: 0.9% sodium chloride as control group.

All teeth were immersed in separate bottles containing 10 ml of the disinfectant at 25°C.

Following the assigned treatment procedures, teeth from each group were placed individually in separate test tubes containing 10 ml of tryptic soy broth at 37°C for 48 hours. Evidence of growth was observed after 2 days. No visible growth in the broth was considered effective disinfection/sterilization.

RESULTS

Chi-square analysis of the data indicates a statistically significant difference in the outcomes when comparing the different methods of disinfection and sterilization (Table 1). The calculated Chi-square value came to 58.92 which is more than the table value at 0.01 for 13° of freedom. Immersion in 10% formalin for 1 week, 5.25% sodium hypochlorite for 1 week or autoclaving at 121°C at 20 psi for 40 minutes were significantly better than all other methods tested ($p < 0.001$).

DISCUSSION

The occupational safety and health administration (OSHA) blood-borne pathogens standard considers human teeth used for teaching and research purposes as a potential source of blood-borne pathogens. To address this concern, CDC recommends storing extracted teeth in 1:10 household bleach.⁷ However, Tate and white⁸ demonstrated that to be an ineffective disinfectant for this purpose.

Difficulties do exist in the use of extracted human teeth because:

- They are grossly contaminated,
- Difficult to sterilize because of their structure and,
- May be damaged or altered by the sterilization procedures.¹

Numerous chemicals have been tried with varying success.^{8,9} Ethylene oxide sterilization has been found to have 20 to 36% efficacy on *B. subtilis* spores in extracted teeth.¹⁰

Use of gamma radiation has also been suggested.¹¹ Pantera and Schuster found microbial growth from the canals of extracted teeth that had been autoclaved for 20 minutes, but a 40-minute cycle eliminated all microbial growth.⁹

In this study we found 10% formalin for a period of 7 days, 5.25% sodium hypochlorite for a period of 7 days and autoclaving for 40 minutes were the most effective; but previous studies recommend formalin and autoclaving because they are simple, cheap and suitable for routine use in preclinical courses, exercises and research purposes. The results obtained are in consensus with that of previous studies.^{12,13}

Safety Concerns

Formalin is hazardous, irritant and a potential carcinogen¹ but when used, it is recommended that the container holding the teeth should be opened only under a fume hood and the teeth should be rinsed prior to their use. Impervious gloves and goggles should be used to prevent skin and eye exposure.

It has been reported that sodium hypochlorite may increase enamel porosity by deproteinization and alter dentin structure, by removing or modifying the protein matrix, which could invalidate the use of teeth stored in this solution.¹⁴

Table 1: Comparison of different methods used in the study

Treatment	Concentration/temperature	Time period	No. of teeth studied	No. of teeth sterilized
Formalin	10%	2 days	10	03
Formalin	10%	4 days	10	06
Formalin	10%	7 days	10	10
Hydrogen peroxide	3%	7 days	10	06
Hydrogen peroxide	1.3%	7 days	10	02
Sodium hypochlorite	2.6%	7 days	10	07
Sodium hypochlorite	5.25%	7 days	10	10
Quaternary ammonium	0.28%	7 days	10	06
Glutaraldehyde	2%	7 days	10	03
Thymol in distilled water	0.1%	7 days	10	00
Boiling	100°C	20 minutes	10	05
Autoclaving	121°C	20 minutes	10	08
Autoclaving	121°C	40 minutes	10	10
Sodium chloride (control)	0.9%	1 week	10	00

With regard to autoclaving, there is concern about its use for sterilization of extracted teeth with amalgam restorations as it may release mercury vapors in the air through autoclave exhaust and residual mercury contamination of the autoclave.¹ It is also possible that the thermal cycling may cause teeth with amalgam restorations to fracture due to their differences in coefficient of thermal expansion.¹³

Infection control guidelines for use of extracted teeth in dental educational settings:²

Extracted teeth used for the education of dental health care workers should be considered infective and classified as clinical specimens because they contain blood.

- All persons who collect, transport or manipulate extracted teeth should handle them with the same precautions as a specimen for biopsy.
- Before extracted teeth are manipulated in dental educational exercises, the teeth first would be cleaned of adherent patient material by scrubbing with detergent and water or by using an ultrasonic cleaner.
- Teeth should then be stored, immersed in a fresh solution of sodium hypochlorite (household bleach 1:10 with tap water) or any liquid chemical germicide for clinical specimen fixation.
- Persons handling extracted teeth should wear gloves. Gloves should be disposed off properly and hands washed after completion of work activities. Additional personal protective Equipment, e.g. face shield or surgical mask and protective eyewear should be worn if mucous membrane contact with debris or spatter is anticipated when the specimen is handled, cleaned or manipulated.
- Work surfaces and equipment should be cleaned and decontaminated with an appropriate liquid chemical germicide after completion of work activities.

Sterilization of extracted teeth used in the teaching laboratory should be a concern to educators and students alike. 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. 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, researches should be carried to analyze the effects of various sterilization procedures on physical properties of tooth structure.

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