Sterilization of extracted human teeth: A comparative analysis

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ABSTRACT

Background: Extracted human teeth are regularly used for practice and educational purposes in dental institutions at undergraduate and postgraduate levels. Different materials and methods are used for sterilizing extracted teeth to avoid infection from them.

Aims: The present study was done to determine the efficacy of some frequently used methods for disinfection/sterilization of extracted human teeth.

Materials and methods: A total of 120 intact, non-carious teeth extracted due to periodontal or orthodontic purpose were divided randomly into 8 groups consisting of 15 teeth in each group. Agents used for sterilization included 10% formalin, 0.1% thymol in distilled water, 5.25% sodium hypochlorite, 2% glutaraldehyde, 3% hydrogen peroxide, boiled in water (100 °C), autoclave (121 °C, 15 lbs psi), normal saline. Samples were collected with the help of inoculating loop and then streaked over the surface of Mc-Conkey agar medium and Blood agar medium. The media were then incubated at 37 °C for 24 h. No visible growth in the culture medium was considered as the method of effective sterilization.

Results: 10% formalin, autoclaving, 5.25% sodium hypochlorite could be efficiently used for sterilization and disinfection of extracted human teeth.

Conclusion: Extracted teeth should be handled with extreme care as these are potential source of infection and need to be disinfected before they are used in the laboratories.

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INTRODUCTION

Intact extracted human teeth are used by undergraduate and postgraduate dental students to develop and sharpen their mechanical and clinical skills for treating patients. Extracted human teeth are used for various purposes such as preparing ground sections to study histology of teeth, for learning endodontic procedures such as cavity preparation, root canal treatment, developing and testing various restorative materials, *in vitro* laboratory dentin bonding

research (bond strength measurements, microleakage evaluation and marginal gap measurements), for learning crown preparation, a dental restorative material—the ‘Natural Inlay’.¹ Nowadays typodonts are available as educational tool for dental and hygienist students, allowing them to practice certain dental procedures on the plastic teeth of a model before actually performing the procedures on live patients. In third world countries like India, per capita income is less, so extracted teeth serve a low budget substitute to typodont. There are instances where even typodont

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cannot replace extracted teeth. These include preparing the ground sections and *in vitro* dentin bonding research.² Thus, there is no suitable alternative for extracted teeth for evaluation, preparation or research.

Teeth extracted for various purposes are possible cause for cross-contamination to various laboratory equipments and workers so extracted teeth must be decontaminated to prevent spread of infection. Various methods of sterilization are used for instruments, surgical gloves and other items that come in direct contact with the blood stream to avoid infections such as human immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV). It can be achieved by high-pressure steam (autoclave), dry heat (oven), chemical sterilants (glutaraldehydes or formaldehyde solutions) or physical agents (radiation). Because sterilization is a process, not a single event, all components must be carried out correctly for sterilization to occur. The effectiveness of any method of sterilization is dependent upon time, contact, temperature with steam sterilization, high pressure, type of microorganism present, number of microorganisms, amount and type of organic material that protects the microorganisms, number of cracks and crevices on an instrument that might harbor microorganisms.

Extracted human teeth are difficult to sterilize because of their structure as they may be spoiled or changed by the sterilization measures.^{3,4} Various methods have been tried for disinfection/sterilization of extracted human teeth with variable results. These agents include formalin, sodium hypochlorite, glutaraldehyde and thymol. The Centres for Disease Control (CDC), USA recommends storing extracted teeth in 1:10 household bleach.³ However, Tate and White showed household bleach to be a poor disinfectant for this purpose.⁴ At present, there is no precise proposal for sterilization of extracted human teeth used in dentistry. Ethylene oxide can also be used as sterilizing agent. Its efficacy has been found to be 20–36% on *Bacillus subtilis* spores in extracted teeth.⁵ Various new methods of sterilization have been introduced with minimal effect on the tooth structure and more efficient sterilization. Gamma radiation sterilizes without high temperature, high pressure, chemicals or gases. They have no effect on the nano-mechanical properties of teeth.⁶ However, such a set-up is lacking in various institutions.^{7,8} NaOCl subsonic agitation causes more effective reduction in bacterial load in the root canal as compared to NaOCl irrigation alone.

Preferably, a scheme of sterilizing teeth should be developed that not only renders them pathogen-free but also does not modify the enamel or dentinal structures of teeth. Since we are in an educational set-up in which extracted teeth are used in pre-clinics for number of

procedures, a need was felt to find the best method of sterilizing extracted teeth.

The present study has been conducted with view to determine efficacy of frequently used methods of disinfection/sterilization of extracted human teeth and to assess the effects of disinfection methods on recently extracted teeth.

MATERIALS AND METHODS

A total of 120 freshly extracted human teeth were obtained from the Department of Oral and Maxillofacial Surgery of our institute. The teeth included were intact, non-carious and extracted due to periodontal disease or orthodontic purpose. These were stored in sterile saline till tested and were randomly divided into one of the following 8 groups consisting of 15 teeth in each group.

Group 1: Teeth were immersed in 20 ml of 5.25% sodium hypochlorite (Novo Dental Products, Mumbai, India) at 37 °C for 5 days. Group 2: Teeth were immersed in 20 ml of 0.1% thymol (Nice chemicals Pvt Ltd, Cochin) in distilled water at 37 °C for 5 days. Group 3: Teeth in this group were autoclaved at 121 °C at 15 lbs psi for 20 min. Group 4: Teeth were boiled in water for 20 min. Group 5: Teeth were immersed in 20 ml of 3% Hydrogen Peroxide (Qualikems fine chemicals Pvt Ltd, New Delhi) at 37 °C for 5 days. Group 6: Teeth were immersed in 20 ml of 2% glutaraldehyde (Qualikems fine chemicals Pvt Ltd, New Delhi) at 37 °C for 5 days. Group 7: Teeth in group 1 were immersed in 20 ml of 10% formalin (Merck Limited, Mumbai, India) at 37 °C for 5 days. Group 8: This includes a control group, in which teeth were immersed in normal saline. All teeth were immersed in separate bottles.

Collection of sample

In order to obtain samples metal transfer loop was first flamed till it was red-hot then allowed to cool. The loop was dipped in a container containing teeth. The samples were then streaked over the surface of Mc-Conkey agar medium and Blood agar medium. Both media were incubated at 37 °C for 24 h.

Determination of sterilization

Sterilization was determined based on the growth of microorganisms on culture medium. Evidence of growth was observed after 24 h of inoculation. Absence of any visible growth in the media was indicator of an effective sterilization.

RESULTS

Before sterilization lactose fermenters were observed on Mc-Conkey agar medium with few non-lactose fermenters (Fig. 1). Pinhead sized and pinpoint colonies were observed on Blood agar medium (Fig. 2). It was observed that no growth on culture media after sterilization was seen with teeth immersed in 10% formalin for 5 days, 5.25% sodium hypochlorite for 5 days and autoclaved at 121 °C at 15 lbs psi pressure for 20 min, thus indicating complete sterilization (Fig. 3). These agents showed 100% efficacy in sterilizing extracted teeth as compared to other methods. The least successful method among various chemicals used was 0.1% thymol in distilled water (13.3% efficacy) (Table 1).

DISCUSSION

Sterilization is defined as the process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.⁹ As extracted teeth serve as an invaluable educational tool in dental institutes for various pre-clinical courses, sterilizing them is mandatory. It is evident that many blood borne pathogens including HIV, HBV, HCV and bacterial pathogens may exist in pulp, radicular and periradicular tissue of extracted human teeth,^{10,11} so sterilizing agent should be highly effective with high penetration ability. Since tooth preparation in technique laboratories is usually done without a liquid coolant, hence there is a greater chance of contact to pathogenic organism in the laboratory and the danger exists for the spread of infection, both, through aerosol

Fig. 2 Growth of micro-organisms on Blood Agar.

and the unintentional injuries that might occur with dental instruments during practice.

In our study we found that immersion in 10% formalin for 5 days, 5.25% sodium hypochlorite for 5 days and autoclaving at 121 °C, 15 lbs psi for 20 min were successful methods in disinfecting/sterilizing the extracted human teeth. Our results are in accordance with observations made by Kumar et al,¹² Lolayekar et al¹⁰ and Dominici et al,¹³ who also observed 10% formalin, 5.25% sodium hypochlorite and autoclaving were the best methods of sterilization. These procedures did not seem to affect the “feel” and cutting characteristics of the teeth.^{13,14} However,

Fig. 1 Growth of micro-organisms on Mc-conkey Agar.

Fig. 3 No growth seen on division 1,2 and 6 of culture media, indicating complete sterilization by 10% formalin, autoclaving, 5.25% sodium hypochlorite.

Table 1 Comparison of efficacy of different methods used and teeth disinfected/sterilized.

Type of sterilizer/disinfectant used	Duration	No. of teeth studied	No. of teeth disinfected/sterilized	% age efficacy of different disinfectant solutions
5.25 % Sodium hypochlorite	5 days	15	15	100
0.1% Thymol in distilled water	5 days	15	2	13.33
Autoclaved (121 °C, 15 lbs psi)	20 min	15	15	100
Boiled in water	20 min	15	9	60
3% Hydrogen peroxide	5 days	15	10	66.66
2% Glutaraldehyde	5 days	15	11	73.33
10% Formalin	5 days	15	15	100
Normal saline	5 days	15	0	0

a study by Abdul-Rahman et al¹⁵ showed that the immersion of the extracted teeth for 7 days in 2.5% sodium hypochlorite, autoclaving at 121 °C, 15 lbs for 15 min and the use of the microwave for 6 or 3 min were effective in disinfecting the extracted human teeth. In the present study, formalin, 5.25% sodium hypochlorite and autoclaving were more effective than other chemical and physical methods for sterilization and disinfection.

Many new methods of disinfection and sterilization have been introduced. Disinfection methods include a persistent antimicrobial coating that can be applied to inanimate and animate objects (Surfacine), a high-level disinfectant with reduced exposure time (orthophthalaldehyde) and an antimicrobial agent that can be applied to animate and inanimate objects (superoxidized water).¹⁶ Under the sterilization various methods are chemical sterilization process for endoscopes that integrates cleaning (Endo-clens), a rapid (4 h) readout biological indicator for ethylene oxide sterilization (Attest), and a hydrogen peroxide plasma sterilizer that has a shorter cycle time and improved efficacy (Sterrad 50).¹⁶ Effective method of sterilization should render teeth pathogen-free, cause minimum distortion of teeth structure and have high penetration ability so that no pathogens remain viable in pulpal space. Recently, Pasqualini et al concluded that 30 s of NaOCl subsonic agitation with EndoActivator appeared to be slightly more effective in reducing bacterial load in the root canal compared with NaOCl irrigation alone.¹⁷ The difference in the effectiveness of the methods could be due to poor infiltration of the agents into the pulp space because of integral cemental surface and inactivation of the disinfectants by the organic substances present in the teeth.^{1,12}

But there are certain drawbacks linked with the sterilizing methods used in the present study. Formalin is dangerous, irritant and a probable carcinogen. So its 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 worn to prevent skin and eye exposure.^{13,18} Autoclave requires a continuous source of heat in the form of electricity and strict adherence to time, temperature and pressure settings. It has been reported that sodium hypochlorite may increase enamel porosity by deproteinization and alter dentin structure, by removing or modifying the proteic matrix, which could nullify the use of teeth stored in this solution.^{19,20} Brauer et al studied effect of sterilization by gamma radiation on nano-mechanical properties of teeth and found no significant dose—response relationship in elastic modulus or hardness in either dentin or enamel.⁶ Lee et al evaluated the effect of storage medium and sterilization on dentin bond strengths and found that storing teeth in 5.25% sodium hypochlorite may negatively affect composite-to-dentin bond strengths. Sterilization with the autoclave negatively affected the bond strength of teeth stored initially in distilled H₂O or 10% formalin, while sterilization with formalin alone had no significant effect on bond strengths.^{21,22} A study by Attam et al, showed that the microleakage of extracted teeth stored in the formalin was considerably lower than teeth sterilized by autoclaving.²³ Baba et al evaluated the effects of disinfectants on the bond strength of resin to dentine and found that highest bond strengths was obtained with 5% glutaraldehyde after 168 h of exposure.²⁴

Our study is a pilot endeavor. Further studies on a larger sample and including more sterilizing agents are required to be undertaken to substantiate the more effective method of sterilization. The extracted teeth serve as a source of potential infection when used in dental set-up necessitating a need for astringent infection control measures (Table 2). The present study documented the role of various disinfectants and concluded that 10% formalin, autoclaving, 5.25% sodium hypochlorite are the most efficient methods of sterilization/disinfection of extracted teeth. The chemicals are simple, cheap and suitable for regular use in pre-clinical courses, training and research purposes. Despite various

Table 2 Infection control measures.²⁵

Extracted teeth used for teaching dental health care workers should be considered infective and classified as clinical specimens as they contain blood.
All persons who collect, transport or manage extracted teeth should handle them with the similar precautions as a sample for biopsy.
Before extracted teeth are manipulated in dental educational training, the teeth first would be cleared 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 make contact with debris or spatter is expected when the specimen is handled, cleaned or manipulated.
Work surfaces and instruments should be cleaned and decontaminated with a suitable liquid sterilizer after completion of work activities.

disadvantages associated with different methods, autoclave is the most commonly used, effective method of sterilization and sterilization cycle time is shorter than with dry heat or chemical sterilants.

CONCLUSION

Based upon the current research findings above mentioned chemical and heat sterilization methods are recommended for preventing cross-contamination as per requirement to use the extracted human teeth. Concluding from the observations and facts of the present study, it should be kept in mind that extracted human teeth should be handled with extreme care.

CONFLICTS OF INTEREST

All authors have none to declare.

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