ORIGINAL ARTICLE

Vinegar as a disinfectant of extracted human teeth for dental educational use

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ABSTRACT

Context: Extracted human teeth are routinely used in dentistry to learn technical and preclinical skills. Centers for Disease Control and Prevention (CDC) has adopted guidelines for infection control of extracted teeth used for research and teaching, requiring that teeth be sterilized before use. Many of the proposed disinfection methods starting from use of formalin, sodium hypochlorite and to autoclaving have their own drawbacks and may not be practical. **Aim:** To assess the effectiveness of vinegar for disinfection/sterilization of extracted human teeth. **Materials and Methods:** A total of 80 extracted non-carious human teeth were kept in seven disinfectant media—10% formalin, 3% hydrogen peroxide, 5.25% sodium hypochlorite, 70% alcohol, vinegar, neem extract and normal saline. Ten samples were placed in each disinfectant individually for a period of 7 days, at room temperature. In all, 10 teeth were treated with microwave irradiation at 650 W for 3 min. Later, teeth from each group were placed individually in separate test tubes containing 10 ml of tryptic soy broth at 37°C for 48 h to observe the evidence of growth of microorganisms. Semiquantitative analysis of all the samples was done in Clade agar at 37°C for 48 h. **Statistical Analysis Used:** The number of teeth disinfected in each group was compared using Chi square test. **Results:** 10% formalin, 3% hydrogen peroxide and vinegar were totally effective. The result was statistically significant with a Chi square value of 61.414 and $P < 0.001$. **Conclusions:** Vinegar can be used as an effective disinfectant medium for extracted human teeth.

Key words: Disinfection, extracted teeth, formalin, vinegar

INTRODUCTION

Extracted human teeth are routinely used in dental institutions to train and acquaint dental students about various procedures before they do it on patients.[1,2] Dental students practice their preclinical skills on artificial tooth models, typhodont teeth, extracted teeth and so forth to learn numerous dental procedures. Whereas artificial models and teeth pose no hazard, many of the dental procedures are best learnt on extracted human teeth, as these best simulate the clinical situations. This exposes dental operators to the risk of cross infections from pathogens associated with the extracted human teeth, if appropriate infection control measures are not followed. Cross-infection control has been a cause of concern in the practice of dentistry.

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Universal precautions, as applied in the clinical setting, require that all body fluids and tissues be treated as if known to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) or other blood borne pathogens. The Occupational Safety and Health Administration (OSHA) blood borne pathogens standard considers human teeth used for research and teaching purposes as a potential source of blood borne pathogens.[3] The Centers for Disease Control and Prevention (CDC) has adopted guidelines for infection control of extracted teeth used for research and teaching, requiring that teeth be sterilized before use, to minimize the risk of transmission of blood borne pathogens.[4,5] Infection control concerns regarding the handling of teeth for research purposes have prompted investigators to evaluate the effects of disinfection/sterilization on extracted teeth.[6]

Several chemicals have been tried for disinfection/sterilization of extracted teeth, with various success rates.[2,3] Chemical heat, microwave radiation and autoclave sterilization methods

are recommended for preventing cross-contamination during *in vitro* dentin bonding research.[6,7] Though these methods are effective, they are not practical for students to use routinely. Moreover, they are laborious and time-consuming. Extracted teeth with amalgam restorations should not be autoclaved because of release of mercury vapor in the air through autoclave. For students to disinfect extracted teeth in educational settings, a solution that can be used to immerse the samples for disinfection would be more practical.

Investigators have found formalin storage to be effective for infection control purposes. Most of the studies including the recent ones report that immersing extracted teeth in 10% formalin can disinfect the tooth sample in 7 days.[1,2,8,9] Formalin may be the most effective disinfectant, but it is a hazardous material and a potential carcinogen.[9,10] In a recent study, 5% Virkon and Gigasept PA that proved effective against the laboratory model of disinfection was carried forward to challenge freshly extracted human teeth. Gigasept PA was the only disinfectant that sterilized 100% of the tooth samples. Hence, Gigasept PA could be considered a safer and effective alternative to formalin for the sterilization of extracted teeth destined for teaching purposes.[10] This product though effective is a high level hospital disinfectant that is used on medical instruments. Hence, a suitable alternative to formalin for storage and disinfection of extracted human teeth is essential. Studies done in the past as well as the recent ones have assessed the disinfection of various chemicals—formalin, sodium hypochlorite, hydrogen peroxide, thymol, gluteraldehyde and so forth—apart from autoclaving and microwave radiation techniques.[1-3,8,10,11] Many studies have been conducted recently, but there is no evidence to suggest a suitable alternative to formalin for disinfection of extracted teeth. So far, none of the studies have assessed the effectiveness of vinegar as a disinfectant of extracted teeth. Vinegar is a household commodity in most of the kitchens and if effective could be a better alternative to formalin for storage and disinfection of extracted human teeth. The present study aimed to determine if vinegar could be used as an alternative to formalin for disinfection/sterilization of extracted human teeth.

MATERIALS AND METHODS

Ethical approval to conduct the study was obtained from the institutional ethical committee of the college. Eighty non-carious, unrestored and intact freshly extracted human teeth were used in the present study. In all, 10 teeth were stored individually in closed containers in one of the seven disinfectant media [Figure 1]:

- 10% Formalin (Fisher Scientific, Mumbai, India)—served as a positive control. It is commonly found in most of the laboratories and is a frequently used fixative, preserving biologic specimens for pathologic and histologic examination.
- 3% Hydrogen Peroxide (Bhandari Labs, Ujjain, India)—easily available in most of the dental clinics, has antibacterial action due to its oxidative property.
- 5.25% Sodium Hypochlorite (Dentpro, Mumbai, India)—commonly used as a root canal irrigant and has antibacterial property.
- 70% Alcohol (Fisher Scientific, Mumbai, India)—commonly used as a chemical disinfectant in most of the laboratories and used in tissue processing.
- Vinegar (Tops, India)—a common household commodity and is very economical. Its uses include preservation and disinfection.
- Neem extract—a herbal antiseptic product, safer compared to other chemicals agents.
- Normal saline—served as negative control.

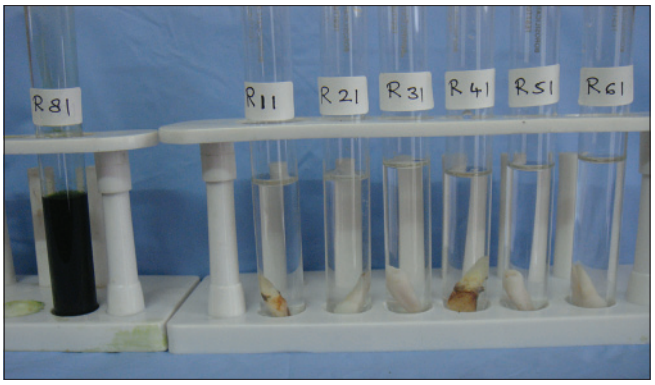

Figure 1: Extracted teeth immersed in different disinfectant media

All the teeth were kept immersed separately in 10 ml of the media for 7 days at 25°C. A total of 10 teeth were treated with microwave irradiation at 650 W for 3 min.

100 ml of neem extract was prepared by shade drying 1 kg of neem leaves for a period of 7 days. Then the leaves were powdered and an aqueous extract of the same was prepared by using a "Soxhlet extractor." The powdered neem leaves were tightly packed in a muslin cloth bag and loaded into the main chamber of the Soxhlet extractor. The extraction solvent was taken into a distillation flask and the Soxhlet extractor was now placed onto this flask. The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The solvent vapor travelled up a distillation arm and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapor that cooled, dripped back down into the chamber housing the solid material.

This cycle was repeated continuously for 50 times. During each cycle, a portion of the nonvolatile compound dissolved in the solvent. After 50 cycles the desired compound was concentrated in the distillation flask. After extraction the solvent was removed, by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remaining in the thimble was discarded. Neem extract was tried in the present study so that if found effective could be used as a natural/herbal alternative to formalin.

After the disinfection process, the solution was discarded and all the tooth samples were vortexed on a vortex shaker (Acumen Labware, Ambala, Haryana) in sterile saline for 60 s. The saline was discarded. A nutrient medium (tryptic soy broth) was prepared and autoclaved. Then, teeth from each group were incubated individually in separate test tubes containing 10 ml of tryptic soy broth at 37°C for 48 h. Evidence of growth was observed after 2 days. Evidence of microbial growth in the broth was visible as turbidity in the sample. Semi-quantitative analysis of all the samples was further done in Clade agar at 37°C for 48 h. No visible growth in the broth was considered effective disinfection. Statistical significance of the results was assessed using 'Chi square test'.

RESULTS

The nutrient broth was observed for all the eight groups at the end of a 48-h period. Evidence of turbidity in the broth indicated microbial growth and hence ineffective sterilization. Figure 2 shows the clear and the turbid samples. Nutrient broth in samples on the left side is clear and hence indicates effective sterilization. The other samples on the right side are turbid indicating ineffective sterilization/disinfection. Aliquots from all the samples were further streaked on to Clade agar and incubated at 37°C for 48 h. Microbial colonies were counted on the positive samples. All the turbid samples exhibited microbial growth of more than 10^5 colony-forming unit (CFU)/ml as depicted in Figure 3.

The results have been displayed in Table 1. No turbidity of tryptic soy broth was observed for three groups in the present study—10% formalin, 3% hydrogen peroxide and vinegar. All the samples in the normal saline and microwave radiation group exhibited turbidity in the nutrient broth. Of the eight disinfectants used in the present study, 10% formalin, 3% hydrogen peroxide and vinegar disinfected all the teeth. The results were statistically significant with a X^2 value of 61.414 and $P < 0.001$.

DISCUSSION

Dental educators and students need to exercise adequate care while handling extracted human teeth. Because these teeth can harbor pathogens that may be viable in the root canals for extended periods of time, persons handling them are at risk during tooth preparation procedures.[1] 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.[12] Since tooth preparation in laboratories is usually done without a liquid coolant, 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 and the unintentional injuries that might occur with dental instruments during practice.[11]

Disinfection refers to an action that reduces the microbial load present on the surface of an object, whereas sterility refers to an object without a detectable microbial load. By this definition, it is possible to disinfect an object to the point at which it becomes sterile.[10] CDC recommends storing extracted teeth in 1:10 household bleach, which has been proved to be unsuccessful.[1,13,14] 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.[15] Various new methods of sterilization have been introduced with minimal effect on the tooth structure and more efficient sterilization.

Table 1: Disinfection of extracted teeth in all the eight groups

Disinfectant	Duration (days)	Number of teeth disinfected
Normal saline	7	0
3% Hydrogenperoxide	7	10
70% Alcohol	7	3
10% Formalin	7	10
5.25% Sodiumhypochlorite	7	1
Vinegar	7	10
Microwave radiation	160 W, 3 min	0
Neem extract	7	2

χ^2=61.414, $P \leq 0.001$

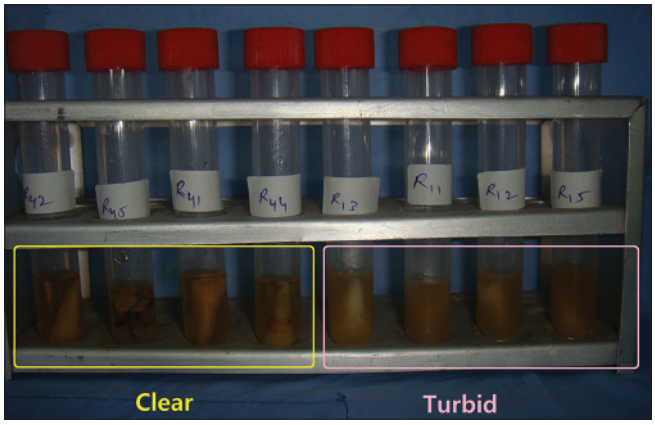

Figure 2: Extracted teeth samples after 48 h of incubation in tryptic soy broth

Figure 3: Microbial colonies on Clade agar observed with ineffectively disinfected samples

Gamma radiation sterilizes without high temperature, high pressure, chemicals or gases. They have no effect on the nanomechanical properties of teeth.[16]

Autoclaving for 30-40 min at 240°F and 15-20 psi, soaking in 10% formalin for 1 week and 5.25% sodium hypochlorite have demonstrated effective disinfection.[1-3,8,11,17] Abdul-Rahman *et al*., conducted a study which showed that the immersion of 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.[18] Out of the chemicals used in a study, such as 5.25% hypochlorite sodium, 5% microten, 5% deconex, 2% glutaraldehyde and 10% formalin, only 10% formalin was effective in sterilizing/disinfecting all the extracted teeth.[19] Chemicals such as 2.6% sodium hypochlorite and 3% hydrogen peroxide were not effective in disinfecting all the teeth in a previous study.[2]

In a study by Hope CK *et al*., biofilms of oral bacteria were grown on previously autoclaved extracted human teeth. These biofilm-laden teeth were then screened against a range of disinfectants for an exposure time of 7 days in a laboratory refrigerator. Culture methods were used to validate the sterility of the tooth samples. A total of 5% Virkon and Gigasept PA proved effective against the laboratory model of disinfection and were carried forward to challenge freshly extracted human teeth. Gigasept PA was the only disinfectant that sterilized 100% of the tooth samples. They considered Gigasept PA to be a safer and effective alternative to formalin for the sterilization of extracted teeth destined for teaching purposes.[10] Gigasept PA is a high level hospital disinfectant that is used on medical instruments and is not easily available.

In the present study 10% formalin, 3% hydrogen peroxide and vinegar were 100% effective in disinfecting/sterilizing all the extracted teeth when immersed for a period of 7 days.

Chemicals used in assessing the disinfection of the extracted teeth should not alter the surface integrity of the teeth post-disinfection. Studies have shown autoclaving for 40 min to be an effective method of disinfection/sterilization of extracted human teeth. This may not be used if the teeth are having amalgam restorations because of mercury vapor released in the air through autoclave exhaust and residual mercury contamination of the autoclave.[1,3] Formalin may be the most effective disinfectant, as repeatedly proved in previous studies,[1-3] but it is a hazardous material and a potential carcinogen.[7] Formalin is found to be effective for infection control purposes, but is not recommended as a storage medium for dentin bonding studies due to the variability in dentin bond strengths resulting from its use.[6] 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 nullify the use of teeth stored in this solution.[20,21] Hence, an appropriate disinfectant that overcomes these limitations is required, as extracted human teeth are commonly used in dental institutions.

In all, 10 ml of vinegar was able to disinfect an extracted tooth sample in 1 week in the present study. Vinegar, a household disinfectant that has not been tried earlier, was assessed in the present study. Vinegar is a common household commodity and is easily available. It is simple to use, as the teeth are just required to be kept immersed in the solution in a closed container. Based on the findings in the present study, vinegar could be used as a storage and disinfectant medium for extracted human teeth. It should be kept in mind that the extracted human teeth should be handled with extreme care apart from following the CDC-recommended guidelines.[22]

Infection control measures:

- 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 and 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. Additional personal protective equipment, e.g. face shield or surgical mask and protective eyewear should be worn if our mucous membrane makes 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.

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