Teeth processing in human teeth bank – proposal of protocol

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Abstract

Introduction: Dentistry courses conduct preclinical laboratory trainings and research using extracted human teeth. For a safe usage of those teeth, it is necessary to subject them to cleaning, disinfection and/or sterilization and proper storage to ensure they are free of biological residues. Each of these steps should be properly described in standard operating procedures (SOPs) and in the processing protocol of the Human Tooth Bank (HTB). Objective: To create a processing protocol for Human Tooth Bank based on a literature review on cleaning, disinfection and/or sterilization and storage methods for extracted human teeth. Literature review: The hand hygiene and use of personal protective equipment are essential at all stages of processing. The previous cleaning of the tooth is essential, preferably with enzymatic detergent, and complemented by ultrasonic washing machine. The water quality should be considered in cleaning, washing, dilution of the enzymatic detergent and in the ultrasonic washing machine. Due to the prohibition of immersion use of chemical solution as sterilizing agents, saturated steam under pressure is widely used. Freezing is the recommended storage method because neither it alters dental structures nor affects research.
results. All this information should be consider for the elaboration of the processing protocol for extracted human teeth. Each action taken requires a detailed description and must be validated by the institution. **Conclusion:** The adoption of a protocol with SOPs for cleaning, disinfecting and/or sterilization and storage of human teeth in HTB standardize the processing and minimize exposure to biological agents, enabling the use of the tooth under appropriate conditions for research and laboratory preclinical training.

**Introduction**

The extracted human tooth (EHT) has been applied in the teaching, research and therapeutics of Dentistry courses. The presence of many blood pathogens within root canals, periradicular and periodontal tissues [17], and remnants of soft tissue and saliva has been considered as a contamination source [42]. The Dentistry Schools have employed EHT in preclinical laboratorial training, both in graduation/specialization courses and researches conducted in stricto sensu post-graduation courses [32, 41].

To avoid the risk of occupational exposure and cross-infection, the surfaces, instruments, dental equipments, and the tooth itself should undergo a process of cleaning, disinfection and/or sterilization [19, 33, 42].

The choice for the methods to be adopted in these stages of EHT processing should be scientifically based and attend to its requirement. All procedures involved in these stages must be written in Standard Operating Procedures (SOPs), guiding the development of techniques and operations aiming to the protection and safeness during the tooth manipulation. SOPs assure the uniformity of actions performed in services and minimize the occurrence of errors in executing routine activities [34, 39].

This study aimed to identify thorough a literature review, the methods of cleaning, sterilization, and/or disinfection and storage of EHT, seeking information to create a SOP on the processing of EHT meeting both the sanitary laws and the use of EHT in ideal conditions free of contaminants to avoid the operator exposure and occupational risks.

**Literature review**

A descriptive qualitative literature review study was performed from January 17 to March 8, 2012 on the following databases Bireme, Lilacs, Medline, SciELO and Google Scholar by using the following terms “human tooth bank”, “extracted teeth”, “sterilization of extracted human teeth”, without time limiting.

**Human tooth bank**

The concept of Human Tooth Bank (HTB) appears in 1981, through the execution of a research which required a tooth from a service that assures the quality of the dental organ. In that context, the researchers realized that a tooth bank would contribute for the elimination of the inadequate practice of acquiring teeth and could assure the safe EHT manipulation. The unknown origin of these teeth many times coming from the illegal market may pose risks during their manipulation [41].

According to the proposal of Farias [25], the goals of a HTB are: to meet the laws on the manipulation of human teeth; promote the adoption of procedures aiming to eliminate the cross-infection during the manipulation of the teeth donated; preserve the human teeth donate; fulfill the teaching requirements of the undergraduates and teachers regarding to the development of the preclinical technical-laboratorial teaching; collaborate to the banishment of the illegal practice of the human tooth market, many times the result of tomb thefts.

In a research coordinated by Zuco et al. [43] in 2006, it was found that the undergraduates from the School of Dentistry of Univille did not know the activities performed in the HTB of this university, mainly regarding to the procedures of tooth donations and the treatment adopted for their safe use.

The first Brazilian school to adopt a HTB was the School of Dentistry of the University of Sao Paulo, in the discipline of Pediatric Dentistry in 1992 [15]. Currently in Brazil, there are 60 tooth banks (not necessarily active) within public or private higher education institutions, according to the information obtained on the internet (table I). The
creation and linking of these HTBs to the graduation and post-graduation programs has demonstrated the interest on the technical-scientific knowledge during the professional formation, making viable the conscious formation during the educative and preventive practice of the undergraduate.

Table I - HTB distribution according to the Brazilian regions

<table>
<thead>
<tr>
<th>Region</th>
<th>State</th>
<th>Number of Human Tooth Bank</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Amazonas</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Amapá</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Tocantins</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Maranhão</td>
<td>1</td>
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<tr>
<td></td>
<td>Piauí</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ceará</td>
<td>1</td>
</tr>
<tr>
<td>Northeast</td>
<td>Paraíba</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pernambuco</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Alagoas</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bahia</td>
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</tr>
<tr>
<td>Midwest</td>
<td>Mato Grosso do Sul</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Goiás</td>
<td>1</td>
</tr>
<tr>
<td>Southeast</td>
<td>Minas Gerais</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Espírito Santo</td>
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</tr>
<tr>
<td></td>
<td>São Paulo</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Rio de Janeiro</td>
<td>3</td>
</tr>
<tr>
<td>South</td>
<td>Paraná</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Santa Catarina</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Rio Grande do Sul</td>
<td>7</td>
</tr>
</tbody>
</table>

Source: Google Inc. [27]

Processing of extracted human teeth

The safe manipulation of EHT from the extraction up to their usage, according to the purpose to which is intended, should follow the stages of: reception of the Free and Informed Consent Form (FICF); transportation; reception; selection; preparation, involving cleaning and scaling (removal of organic and inorganic tissues); disinfection and/or sterilization; storage; distribution, disposal or loan; disposal or return [9, 26, 32, 38, 41, 43]. These stages are equally important and demand specific procedures to assure the quality of all working develop by HTB. The preparation, disinfection and/or sterilization and storage are stages directly related to the processing of EHT, previously to the laboratorial practice [36].

To use the extracted teeth free of risks of exposure to biological material and cross-infection, it is necessary their processing by adopting hand hygiene measurements [12] and use of personal protective equipment (PPEs) for the protection of the professional [11, 17] and during the manipulation of the teeth at all processing stages.

Cleaning

The cleaning comprises the removal of blood; debris and tissues adhered to the extracted teeth [2, 17]. The cleaning efficacy is directly associated to the reduction of the microbial load on the EHT surface to be disinfected and/or sterilized, decreasing the occupational risk, that is, the contact with the patients' fluids and contamination during the manipulation [21, 37].

The manual pre-cleaning with water and soap or neutral and/or enzymatic detergent and friction with brush should be followed by automatized cleaning in ultrasonic washing machine (USWM) [22]. The ultrasound favors the removal of debris on the EHT surface by cavitation action, reached the deepest layers and sites of difficult access. The certification on the cleaning and performance of the ultrasonic activity should be periodically performed through control tests recommended by Brazilian and international guidelines [1, 7, 14, 40].

An important factor in the cleaning is the water quality, which must be potable, filtered and/or deionized as well as highly pure (reverse osmosis) [3]. The potable water can be used if it is compatible with the cleaning agent, the tooth, and the USWM [5]. The washing procedure is executed with water treated by reverse osmosis or deionization [30], to avoid that remnants are impregnated on the surface of the material to be cleaned [3]. Despite of the common use of the potable water inside USWM, the use of the demineralized and deionized water enables a better dissolution of the cleaning solutions, mainly of the detergents [45].

After the cleaning stage, both caries and defective restorations are removed with the aid of high and low speed handpieces. Also, scaling and removal of the organic tissues are executed (calculus and bone remnants).

To assure the quality of the residue removal, a visual inspection with the aid of an image intensifier magnifying glass or electronic microscope must be executed. At this stage, it is possible the detection of debris which eventually has not been removed and which can interfere in the further procedures of disinfection and/or sterilization [28].
Disinfection and sterilization

Many methods of disinfection and sterilization of EHT have been evaluated. Among them, it is cited the sterilization in autoclave, glutaraldehyde solution, sodium hypochlorite at different concentrations, formalin, chloramine, and gamma radiation [29].

The Centers for Disease Control and Prevention (CDC) and the American Dental Association (ADA), aiming to the infection control, has recommended sterilization through saturated steam under pressure, for 40 minutes, of EHT to be employed in teaching and research. This method does not alter the physical properties of the dental tissues and does not compromise the goals and/or results of the application of these teeth in teaching, research or therapeutics [2, 17].

In the study conducted by Dominici et al. [23], they inoculated ETH with *Bacillus stearothermophilus* to assess different methods of sterilization and disinfection of human teeth for dental teaching. It was verified that the exposure to 10% formalin for 7 days and autoclavage at 115°C for 40 minutes at 20 psi (1.38 bars) were the most efficient methods. The authors did not indicate the use of 2% glutaraldehyde, sodium hypochlorite at the concentrations of 5.25%, 2.6% and 1.3% and 0.28% quaternary ammonium for disinfection purposes because they were not efficient in eliminating *Bacillus stearothermophilus*. To employ 10% formalin, they recommended that the flasks containing the EHT are opened under an exhauster with the use of PPEs, followed by the washing of the teeth to avoid the exposure to the product prior to use, which can irritate the skin and eyes [23].

In 2005, a study evaluated the efficacy of the methods commonly used in the disinfection and chemical and physical sterilization of EHT for teaching purposes [31]. This study demonstrated that the sterilization through saturated steam at 121°C for 30 minutes under either 15 psi (pounds) or 1.2 bars and through immersion into 10% formalin for 7 days were the most efficient methods. The use of 2.6% sodium hypochlorite, saline solution and 3% hydrogen peroxide, with immersion for 7 days and boiling at 100°C for 20 minutes was not indicated for the disinfection because their results were unsatisfactory [31].

Pashley et al. (apud Silva et al. [36]) studied the influence of different methods of sterilization on the dentinal permeability and the bond strength to dentine, reporting that the ethylene oxide and autoclave (121°C for 30 minutes) did not alter the dentinal permeability and the results of shear bond strength. On the other hand, Kumar et al. [31] cited other studies and demonstrated that the ethylene oxide was not an efficient sterilization method of teeth by destructing from 20 to 36% of *Bacillus subtilis* spores. It is emphasized that the pulp cavity may contain many pathogens and depending on the sterilization method, these cannot be reached. Gamma radiation is efficient, yet it is not a common method used within the institutions [31].

The Consortium of Operative Dentistry Educators (CODE) conducted a searching in 2008 on the recommendations for clinical and surgical dental procedures with the enrollment of ten schools from Canada and 58 from the United States of America. Most of the respondents reported that they employed 10% formalin for 14 days and saturated steam under pressure for EHT sterilization, and they considered the latter as the safest method [20].

The tooth containing dental amalgam restorations should not be autoclaved because of the vaporization of mercury at high concentration during the process [23]. This steam can be inhaled by the operator, therefore putting the health at risk [17, 44]. In these cases, it has been recommended the previous removal of the amalgam restorations and further sterilization/disinfection by immersion into 10% formalin solution for 2 weeks, which is the period required to the solution achieves both the external and internal structures of the teeth [19]. The 2% glutaraldehyde solution was also indicated; however, it can result in resistance of rapidly growing mycobacteria so that it has not been recommended anymore [15, 29, 31].

Storage

The storage medium can contribute to the maintenance of the chemical, physical and mechanical properties of EHT tissues and influenced on the outcomes of the researches [15, 21].

The use of saline solution, water, and disinfectants are practical and saving media, recommended by CDC, but they did not disinfect safely the external surface and the internal pulp tissue [17].

In a literature review study, Silva et al. [36] critically analyzed the influence of the storage time, type of solutions employed and the methods of EHT disinfection and sterilization on bond strength tests to tooth substrate. Conclusive data showed that EHT storage in either distilled water or thymol provided the least variation in bond strength values. The best values of bond strength were obtained through freezing [36].
The revision of the papers from *Caries Research*, *Dental Materials* and *Journal of Dental Research*, during the period from 1998 to 2002, on the storage media of extracted teeth showed that formaldehyde, ethanol, chloramine, freezing, water, distilled water, saline solution and thymol have been the most used storage media. The authors reported that there was no consensus on the most adequate medium capable of assuring reliable results regarding to the enamel and dentine features, for the use of EHT in either preclinical training or researches [15].

**Sanitary consideration on disinfecting and sterilizing solutions**

The Brazilian Sanitary Regulation (RDC n. 35, of August, 16 of 2010) [13] disposes on the Technical Regulation for products with antimicrobial action for critical and semi-critical articles. At the Article 3rd, they are classified as the categories “Disinfecting solution of High Level” or “Disinfecting solution of Intermediary Level”, according to their spectrum of action. The Article 6th prohibits the register of sanitizing products as the category of “Sterilizing agent” to be applied as immersion solution, except for the cases of exclusive use in equipments properly recorded at the Brazilian Sanitary Surveillance Agency (ANVISA) which sterilize through physical-chemical action dialyzers and hemodialysis lines.

To buy such products, one should consider their concentration for dilution, exposure time, spectrum of action, water quality, toxicity, activation or inactivation in the presence of organic material, and safe conditions of use [13, 37].

The concentration, pH and other parameters indicating the effectiveness of disinfecting products must be verified at least once a day, because of the risk of incorrect dilution and multiple reuses of the solution [14].

It is necessary to select adequate chemical products of cleaning, disinfection and/or sterilization which preserve the occupational health and result in the smallest environmental impact as possible when they are discarded [8, 37].

**Standard Operating Procedures (SOPs)**

SOPs are detailed descriptions of all routine and specific operations required to perform one or more than one activities. It aims to assure the development of a safer activity free of undesirable variations, minimizing the execution errors [10, 24].

The SOP must contain: the title referencing the process to be executed; the aim of destination; the reason of its existence and importance; list of reference documents that can be consulted by whom need to use it; site of application or destination sector; identification of the acronym employed; description of all stages; identification of the executor member; inclusion, if necessary of the task’s flowchart; information on the storage and storage medium of the document; date of disclosure; frequency of updating (e.g.: annual); observation on updating; SOP’s manager (who created it) and person in charge [44].

**Discussion**

The previous cleaning of EHT is indispensable for the removal of residues on the internal and external surfaces and must be performed with preferentially enzymatic detergent and complemented by cleaning with the aid of ultrasonic washing machine because the sterilizing agent does not act on the presence of organic and/or inorganic material [4, 18].

During the stages of cleaning, washing, dilution of the enzymatic detergent and the use of USWM, it is necessary to employ water of high quality therefore avoiding the impregnation of residues on the tooth surface and promoting the action of the cleaning solution [3, 6].

The resolution of the Brazilian Sanitary Surveillance Agency regarding the method of disinfection and/or sterilization of EHT states that it is prohibited the use of chemical solutions for tooth immersion for sterilization [13, 14]; this is permitted only as disinfecting solution of high or intermediary level, according to its spectrum of action. Consequently, by considering this legal prohibition, the environmental and occupational issues, and the sanitary risk of the EHT use, the sterilization medium most commonly employed is saturated steam under pressure, which has been reported as the most favorable medium by the studies researched. Also, this is the most reliable method because it does not influence on the bond strength values of the restorative materials to the tooth structures, acts on the microbial spores in a short exposure time period, and enables the penetration of the sterilizing agent on the tooth [37]. Because this method employs only water in gaseous state, it is not toxic to the staff, tooth and environment. All these benefits qualify this method as efficient and environmental sustainable [28, 37] for the sterilization of the extracted teeth, except for those containing amalgam restorations.

There is no consensus on the storage medium for EHT, although freezing was one of the methods.
recommended because it does not offer risks for either occupational or environmental exposure. The immersion into 10% formalin is an alternative storage medium in cases of amalgam restorations.

The correct processing of EHT demands the elaboration of SOP involving all the states to which the tooth should be submitted and its availability for all collaborators. The development and execution of a SOP assures that the process is safely performed, resulting in the preservation of the tooth tissues for the different applications. Also, there are benefits regarding the staff involved in the processing because SOP aims to minimize the occupational and environmental risks.

A proposal of procedures to be adopted after the receiving, recording and bureaucratic procedures related to EHT is presented below. It is worth emphasizing that each action described in the processing protocol of extracted human teeth in HTB requires a detailed description (SOP), according to the protocols adopted and validated by the institution.

1. Hygiene of the hands;
2. Use of PPEs;
3. Choice of the water type for cleaning, washing, dilution of the enzymatic detergent and USWM;
4. Preparation of the enzymatic detergent solution, according to the manufacturer’s instructions;
5. Pre-cleaning through EHT immersion into the solution during the exposure time and temperatures determined by the manufacturer;
6. EHT cleaning through USWM after the pre-cleaning, according the exposure time and temperatures determined by the manufacturer and the institution;
7. Washing;
8. Drying;
9. Removal of caries and defective restorations with the aid of high and low speed handpiece; scaling and planing of the tooth with the aid of curettes to remove organic tissues (calculus, bone remnants);
10. Visual inspection with the aid of magnifying glass and/or electronic microscope;
11. EHT storage according to the sterilization medium adopted by the institution;
12. EHT sterilization process;
13. EHT storage.

Conclusion

The elaboration and usage of a processing protocol of extracted human teeth in HTB is unquestionable for the effective infection controlling, assuring its safe use free of risks of occupational exposure to biological material.

The adoption of standardized procedures for the cleaning, disinfection and/or sterilization and storage together with the sanitary questions standardizes the EHT processing, enabling the use of adequate conditions (maintenance of the features of tooth tissues) during the researches and preclinical laboratorial training.

It is imperative that each institution creates validated SOPs for the procedures suggested, aiming to standardize the working process and to assure the safe use of EHT in the teaching and research institutions.

References


