Evaluation of methods for maintaining sterility of the extracted human dental organ during storage in a tooth bank

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ABSTRACT

The importance of a human teeth bank in an institution is justified and it is advantageous, since it allows the maximum approximation of reality when working with the extracted organ. This study aimed to evaluate methods for maintaining the sterility of extracted human teeth during storage. A total of 72 human incisors extracted in the undergraduate and graduate clinics of Universidade Positivo (Curitiba, PR, Brazil) were used in this study. The teeth were provided by patients, who signed a donation form. After all teeth were subjected to cleaning and disinfection procedures, 36 teeth were autoclaved as well. Teeth were then stored in various solutions for periods of 15 and 120 days. Microbiological tests were conducted to determine which method or storage promoted maintenance of sterility. Better results were obtained for teeth - including autoclaved teeth - stored in Incidin Extra N®, formaldehyde, and alcohol. The solutions analyzed over the proposed time periods have been shown to prevent microbial growth. Results of this study will aid in developing a protocol of processing for extracted human dental teeth to be stored in a tooth bank.

Descriptors: Dentistry; Biosecurity; Sterilization; Tooth; Microbiological techniques

1 INTRODUCTION

An human tooth bank (HTB) is very important to the higher education institution (HEI), while being able to manage the extracted human dental organ (EHDO), commonly used in preclinical laboratory procedures and in research in various areas of dentistry[1-3]. There is an important need to legally obtain structurally sound teeth and to handle them in a safe manner[2]. In order to be used in all conditions, it is important that the integrity of the teeth be preserved and that they retain maximum *in vivo*

structural features[4,5].

The entire manipulation process must ensure that the physical, chemical, and mechanical properties of the teeth are maintained, ensuring the preservation of tissue shape and composition, to avoid a negative influence on studies and research[4,6]. The storage medium should ensure not only sterility over a defined period of time but should also ensure preservation of the structural properties of the EHDO.

The indiscriminate handling of teeth is associated with risks to the operator or occupational exposure, in addition to cross infection[1,6]. The presence of periradicular and periodontal pathogens, cellular debris, and dirtiness should not be ignored[7,8].

In order to standardize the procedures for manipulation and use of EHDOs in an HTB, HEIs should follow a standard operational protocol (SOP). The SOP should guide donation (in terms of written consent from the patient)[9], through the handling, storage, and distribution of those teeth. The EHDO should be freely donated and consented to by the patient, respecting ethical principles[10]. It is also necessary to standardize transportation, previous preparation, disinfection and/or sterilization, storage, loans, and the devolution or disposal of used teeth[1-3,7,11].

In preparing the SOP, it is necessary to clearly determine each stage, based on legal and scientific questions[2]. The storage stage has been well studied; however, there are no studies in the literature regarding maintenance of sterility of EHDOs during the storage period. Existing research generally evaluates disinfection and autoclave sterilization of teeth prior to storage[7,11-13]. Campregher et al.[4] observed that there is no consensus as to the best method or storage medium. The most commonly used storage media are distilled water[6,7], saline[7,14], artificial saliva[14], thymol[6,15], formalin[12], sodium hypochlorite[13], alcohol, and chloramine solutions. Freezing and cryopreservation methods are also used[16,17]. All storage protocols must consider the possible structural alterations of the dental tissues that may be induced by the solutions or methods used[5,15,17].

Once an SOP is established, the HEI will certify that the EHDO is clean, sterile, and in good condition for study or laboratory practice. Adherence to an SOP will prevent students from facing difficulties or following potentially illegal practices[3,18,19].

Based on the above, this study aimed to evaluate the methods for maintaining sterility of the extracted human dental organ during storage.

2 MATERIAL AND METHODS

This study was approved by the Research Ethics Committee of Universidade Positivo under registration number 58075516. 4.0000.0093. Seventy-two human incisor teeth extracted at the undergraduate and postgraduate clinics of the Universidade Positivo (Curitiba, PR, Brazil) were used in this study. Donors of the extracted teeth signed EHDO donation forms.

The teeth were first cleaned according to guidelines of the Centers for Disease Control and Prevention (CDC)[7] and the American Dental Association[11]. This entailed using a brush and soap detergent enzyme (ASPER, São Caetano do Sul, Brazil), followed by rinsing in potable water for one minute. Dirtiness, carious tissue, and defects or amalgam restorations were then removed with a high- and low-speed diamond spherical drill (KG Sorensen®, Cotia, Brazil) under water cooling. In addition, root scaling with periodontal curettes (Golgran®, São Caetano do Sul, Brazil) was performed to remove periradicular calculus or residues to ensure a clean and smooth surface without possible retention factors or niches.

A flowchart of this method is presented in figure 1. To evaluate the methods and solutions for storage of the teeth, the teeth were randomly divided into two experimental groups: group 1 and group 2. In group 1, teeth were autoclaved at 121 ºC for 40 minutes (n=36). In group 2, teeth were cleaned but not autoclaved (n=36). The groups were each subdivided into eight subgroups of four teeth (n=4). Eight more teeth were stored - four autoclaved and four nonautoclaved - because frozen teeth were deposited directly onto Petri dishes for tests of that subgroup. More teeth were needed from each group and subgroup for analysis at 120 days.

The subgroups were divided into the following subgroups: A, Peresal® (Profilática, Araucária, Brazil); B, Incidin Extra N® (Ecolab Deutschland GmbH, Düsseldorf, Germany); C, chloramine T (Merck Millipore, Darmstadt, Germany); D, distilled water; E, saline solution; F, formaldehyde; G, alcohol 70%; H, frozen teeth. Subgroup H was stored in a freezer at an average temperature of -6 °C.

Peresal®, Incidin Extra N®, and chloramine T solutions were prepared following the manufacturers' instructions.

Each tooth was stored in a sterile test tube submerged in 5 mL of the solution of choice, the amount needed for complete immersion of the sample. Samples to be frozen were each inserted into a test tube with no solution.

The samples were stored for an initial period of 15 days and subsequently evaluated by visual inspection. The test tubes containing teeth were carefully shaken, and the tubes that showed visible cloudiness of the solution in which the tooth was submerged were recorded as having a positive result - that is, they showed growth of microorganisms. In contrast, samples that did not present turbidity were registered as having a negative result - that is, no bacterial growth. The group of frozen teeth (subgroup H) was not evaluated in this first analysis, due to the absence of liquid storage medium.

Subsequently, a 0.1 ml aliquot of each assay was removed and transferred to identified Petri dishes divided into four parts. The plates contained sterile culture medium (blood agar). This sample was sown in a quarter of the surface of the plaque with the aid of a bacteriological loop. This test was performed to confirm bacterial growth in tubes in which turbidity of the storage solution was seen and to verify that there was no bacterial growth in the tubes that were not cloudy. Subgroup H (frozen) samples were inserted directly onto the plate in the determined space.

All samples were stored in an oven at 37 °C for 48 hours. Further analysis was performed by visual inspection. Plates that showed growth of colonies of microorganisms were recorded as positive (figure 2A), while those without growth were recorded as having a negative result (figure 2B).

The tests and analyses were repeated after 120 days of storage, using the same procedures.

3 RESULTS

Results of the analysis performed at 15 days are shown in tables 1 and 2.

Visual inspection of the test tubes showed a positive result for group 1 (autoclaved teeth) in the following subgroups: chloramine T in three samples and distilled water in one sample. The other subgroups were negative for all samples.

In group 2 (nonautoclaved teeth), turbidity results were positive for the subgroups: Peresal® in three samples and chloramine T, distilled water, and saline in all samples. The other subgroups were negative for Incidin Extra N®, formaldehyde, and alcohol in all samples. The subgroup of frozen teeth was not analyzed at this time because those teeth were not inserted in a liquid storage medium.

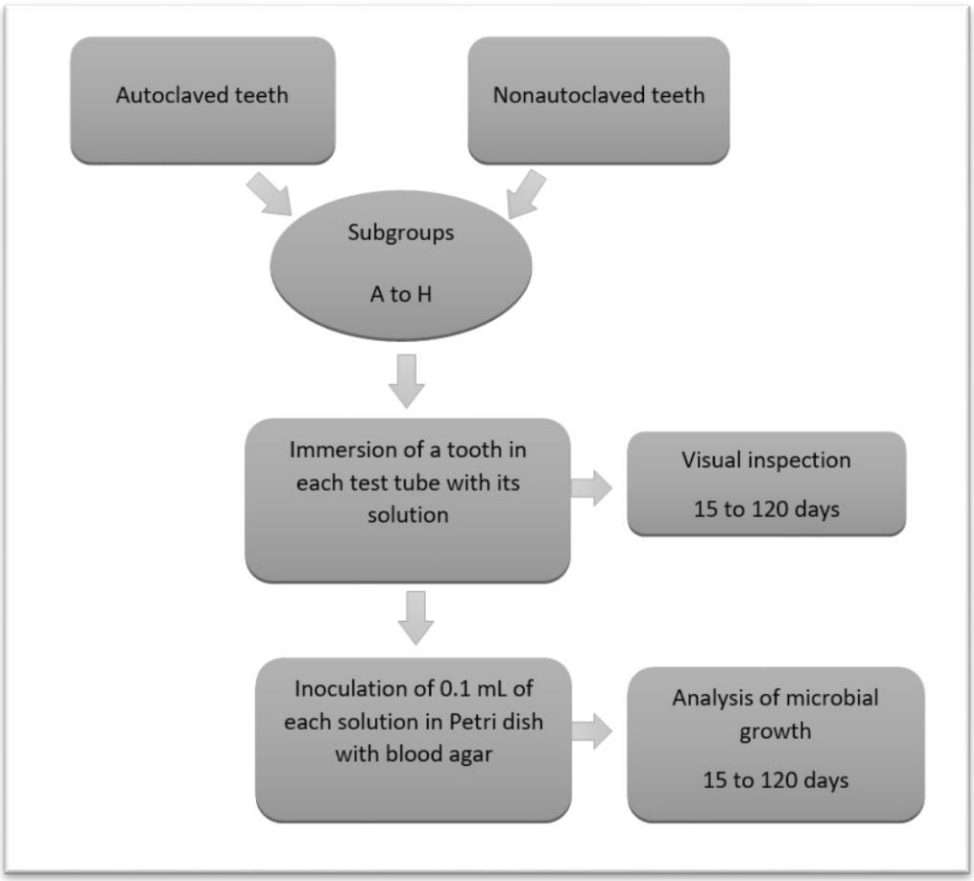

Figure 1. Flowchart of the laboratory method.

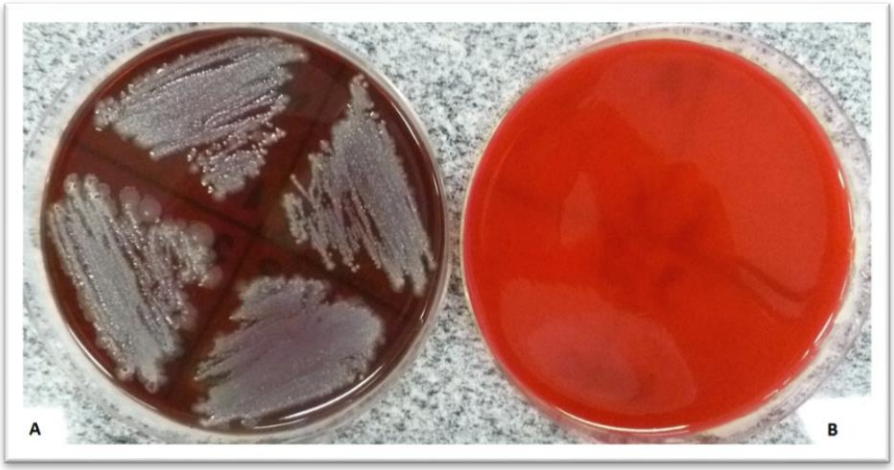

Figure 2. (A) Microbial growth on blood agar, positive result. (B) Absence of microbial growth on blood agar, negative result.

The second analysis, in Petri dishes, showed positive results for group 1 (autoclaved teeth) as follows: one positive chloramine T sample and all four distilled water samples. The other subgroups - Peresal®, Incidin Extra N®, saline, formaldehyde, alcohol, and frozen teeth - presented negative results in all samples. Group 2 (nonautoclaved teeth) presented positive results as follows: Peresal in three samples and in the subgroups, chloramine T, distilled water, saline solution, and frozen teeth in all samples. Incidin Extra N®, formaldehyde, and alcohol subgroups were negative for all samples.

The results of the 120 days storage analysis are shown in tables 3 and 4.

Table 1. Efficacy of solutions used for the storage of autoclaved teeth in maintaining sterility: analysis at 15 days.

	Autoclaved teeth group	
Subgroups	**Visual*/ Total**	**Culture**/ Total**
Peresal®	0/4	0/4
Incidin Extra N®	0/4	0/4
Chloramine T	3/4	4/4
Distilled water	1/4	1/4
Saline solution	0/4	0/4
formaldehyde	0/4	0/4
Alcohol 70%	0/4	0/4
Frozen	X	0/4

* Visual inspection of microbial growth in test tubes. ** Confirmation of microbial growth on blood agar culture plate.

Table 2. Efficacy of solutions used for the storage of nonautoclaved teeth in maintaining sterility: analysis at 15 days.

	Nonautoclaved teeth group	
Subgroups	**Visual*/ Total**	**Culture**/ Total**
Peresal®	3/4	3/4
Incidin Extra N®	0/4	0/4
Chloramine T	4/4	4/4
Distilled water	4/4	4/4
Saline solution	4/4	4/4
formaldehyde	0/4	0/4
Alcohol 70%	0/4	0/4
Frozen	X	4/4

* Visual inspection of microbial growth in test tubes. ** Confirmation of microbial growth on blood agar culture plate.

Table 3. Efficacy of solutions used for the storage of autoclaved teeth in maintaining sterility: analysis at 120 days.

	Autoclaved teeth group	
Subgroups	**Visual*/ Total**	**Culture**/ Total**
Peresal®	0/4	0/4
Incidin Extra N®	0/4	0/4
Chloramine T	4/4	4/4
Distilled water	4/4	4/4
Saline solution	1/4	4/4
formaldehyde	0/4	0/4
Alcohol 70%	0/4	0/4
Frozen	X	0/4

* Visual inspection of microbial growth in test tubes. ** Confirmation of microbial growth on blood agar culture plate.

Table 4. Efficacy of solutions used for the storage of autoclaved teeth in maintaining sterility: analysis at 120 days.

	Nonautoclaved teeth group	
Subgroups	**Visual*/ Total**	**Culture**/ Total**
Peresal®	4/4	4/4
Incidin Extra N®	0/4	0/4
Chloramine T	4/4	4/4
Distilled water	4/4	4/4
Saline solution	4/4	4/4
formaldehyde	0/4	0/4
Alcohol 70%	0/4	0/4
Frozen	X	4/4

* Visual inspection of microbial growth in test tubes. ** Confirmation of microbial growth on blood agar culture plate.

The turbidity analysis yielded the same results for group 1 Peresal®, Incidin Extra N®, formaldehyde, and alcohol subgroups at 15 and 120 days. However, positive results were obtained in all samples of chloramine T and distilled water. In the saline subgroup, one sample was positive at 120 days. In group 2 (nonautoclaved teeth), the results with 120 days of storage were the same as with 15 days for the Incidin Extra N®, chloramine T, distilled water, saline, formaldehyde, and alcohol subgroups. Only the Peresal® subgroup presented all positive samples.

In the agar analysis, autoclaved subgroups maintained the same results as at 15 days, except for the distilled water and saline subgroups, which presented growth in all the samples. For the group of nonautoclaved teeth, the Peresal subgroup was the only one that yielded a different result from the 15-day result, showing all samples with growth in 120 days.

4 DISCUSSION

Several studies have been carried out to determine the efficacy of solutions, methods of cleaning, and disinfecting and/or sterilizing

procedures for extracted teeth, as well as to find the ideal method of storing them in an HTB[4-6,12,14,20]. However, the ability to maintain EHDO sterility during storage has been ignored.

In order to eliminate, or at least reduce, the risk of contamination during tooth handling in laboratory and research procedures, it is important to establish a cleaning or disinfection routine and/or sterilization protocol[1]. Effective methods and solutions to achieve optimal cleaning and sterilization[8] must be established so as not to compromise the structure of the dental tissues[5, 21].

The methods or solutions used for EHDO disinfection and sterilization must ensure a high level of biocidal action, that is, capable of eliminating microorganisms[12,20].

In the present study, cleaning, disinfection, and sterilization were performed according to literature reviews[7,11]. It was decided to sterilize the teeth in an autoclave prior to storage, due to Resolution of the Collegiate Board of Directors (RDC) of the National Health Surveillance Agency, no. 35 (August 16, 2010), which prevents the use of disinfectant solutions for the sterilization of critical and semicritical articles[22].

In addition to manual cleaning and disinfection, it is possible to use automated cleaning in the form of an ultrasonic machine. According to DeWald[23], the use of ultrasound favors the removal of debris in deeper layers, promoting more effective cleaning in regions difficult to access. Due to the need to develop a processing protocol for the HTB possible for implementation, we opted for practicality of technique and a reduction in steps. Thus, only manual cleaning and disinfection of the samples was carried out.

Alcohol 70% yielded good results, even in nonautoclaved teeth. However, because it is volatile, evaporation of the solution was observed over the period of storage, especially at 120 days. Replacement of the solution over the storage period becomes necessary, thus requiring reintervention by an operator and new risk of contamination[24]. In addition, alcohol may dehydrate dental tissues, and storage media should be used that prevent the dehydration of specimens, in addition to controlling changes in pH and preventing microbial growth[25].

Formaldehyde also yielded satisfactory results in nonautoclaved samples. However, due to the high toxicity risk to the operator[26,27]. Longitudinal studies of formaldehyde exposure have shown evidence of an increased incidence of nasopharyngeal cancer[26] and a causal association with myeloid leukemia and nasosinusal adenocarcinoma[27]. For safety, formaldehyde solutions should only be handled within a laminar flow environment, which may not be available, thereby exposing the operator to risk of contamination.

Freezing is a well-studied method for storage of EHDOs[16,17]. Despite being considered easy, practical, and low cost, the results in the present study were positive only in autoclaved teeth. In the nonautoclaved samples, the growth of colonies was observed in all cases, which makes previous autoclaving sterilization indispensable. The advantage of this method is the lower risk of exposure to the sample, since replacement of solution is not required. In addition, freezing may occupy less storage space, which may be an important consideration for the organization and processing of the teeth that will be borrowed in the future [17].

Regarding autoclave sterilization, studies have been carried out to determine the influence on dental tissues. According to Pashley et al.[21], the sterilization of teeth in an autoclave does not promote significant alterations, when analyzed for dentin permeability. In contrast, Parsell et al.[28], analyzed the effects of autoclaving on extracted teeth by microhardness test and

perceived cutting. The authors observed changes in autoclaved samples in enamel microhardness tests. Findings from Salem-Milani et al.[29] corroborate these results concerning the analysis of dentin microhardness. Based on the current study, wich followed CDC protocol, formalin immersion at 10% was recommended.

Distilled water was used as a control group in the present study. It was also decided that only half of the samples would be sterilized, with the intention of obtaining a positive and a negative control. This is justified by the fact that autoclave sterilization has the power - or not - to significantly alter the properties of dental tissues, and its use is controversial[19,28].

In addition to keeping teeth sterile for the specified storage period, the solutions or methods of choice must ensure maintenance of the physicochemical properties of the EHDO. The permeability of the tissues, enamel hardness, and dentin adhesion should be considered. According to Silva et al.[6], investigations or laboratory situations should simulate the conditions of the buccal environment. If the method of sterilization, or even storage of the EHDO, changes such properties, the research becomes impracticable.

In 1985, Williams and Svare [30] performed a survey of teeth stored in distilled water and thymol at 4 °C for 24 hours, three months, and five years. After these periods, the teeth were stored for one week in distilled water and submitted to the shear test. The results did not show statistically significant differences between the resistance values. Likewise, Oh et al.[17] performed tests of hardness on frozen teeth and did not find significant differences between the control group and the group of frozen teeth.

The results obtained by Goodis et al.[15,31] revealed significant differences in dentin permeability and adhesive strength of stored teeth. After storage in 70% alcohol, 10% formalin, distilled water with thymol, and saline with thymol at periods of one, two, four, eight, 15, and 22 days, it was observed that the permeability was lower for the teeth stored in alcohol or formalin. In the second study, the influence of five solutions was evaluated: alcohol 70%, formalin 10%, distilled water with 0.02% thymol, distilled water, and saline solution. Adhesive strength tests were performed at eight, 15, and 15 days. Adhesive strength values showed significant changes in the samples stored in saline solution[15].

Laboratory tests to evaluate the physicochemical properties of EHDOs stored for this study should be performed after the period of time determined for the different storage methods. Thus, one can determine the method that presents the best performance, both in maintaining sterility and in the integrity of dental tissues. It should not be forgotten that the practicality, cost, and ease of obtaining and using the products must be considered.

In this study, we opted not to use thymol, a solution used to store EHDO already reported on in the literature[6,15.] Existing norms for the purchase of thymol in specific commercial establishments (manipulation pharmacies) prevent large-scale acquisition without prior written authorization and the request of a research laboratory technician[32]. This makes it difficult to use the product and renders it impractical for use in an HTB processing protocol; this, coupled with the high cost of the product when employed on a large scale, is not feasible.

It is worth mentioning that the storage of EHDOs in an HEI can be a simple and safe step regarding the ease of handling, maintenance of sample sterility, and integrity of dental tissues, guaranteeing the future use of teeth in studies and research. The structuring of an HTB in an HEI in an organized way and that fulfills its main

functions is fundamental. In addition to the students' ability to acquire ideal study models for laboratory practices, there is a marked increase in learning quality, since practical activities with EHDOs simulate reality[3,18,19].

Despite the great importance and benefits, many HEIs do not yet have an HTB or, even if they do, they have not consolidated an appropriate SOP. In addition to this, more information about the donation and effective actions in the HEIs themselves should be contributed by the students, teachers, and coordinators of courses for the advancement of dentistry.

5 CONCLUSION

Incidin Extra N, formaldehyde, and alcohol yielded the best results, preventing microbial growth even in nonautoclaved teeth after storage periods of 15 and 120 days.

RESUMO

Avaliação de métodos de manutenção da esterilidade do órgão dental humano extraído para armazenamento em banco de dentes

A importância de um banco de dentes humanos em uma instituição de se justifica e se mostra vantajosa, uma vez que permite a máxima aproximação da realidade ao se trabalhar com o órgão extraído. Este estudo teve o propósito de avaliar métodos de manutenção da esterilidade do órgão dental humano extraído armazenado. Foram utilizados 72 dentes incisivos humanos extraídos, obtidos em clínicas de graduação e de pós-graduação da Universidade Positivo, Curitiba, PR, Brasil. Os elementos dentários foram cedidos pelos pacientes, por meio de termo de doação devidamente assinado. Após os procedimentos de limpeza e de desinfecção, 36 dentes foram esterilizados em autoclave e 36 foram somente limpos. Os dentes foram, então, armazenados em recipientes contendo o método (autoclavagem ou limpeza) ou a solução de escolha, por um período de 15 e 120 dias. Testes microbiológicos foram realizados a fim de determinar qual método ou solução de armazenamento promove a manutenção da esterilidade no tempo determinado. Melhores desempenhos foram observados quando as amostras foram armazenadas em Incidin Extra N®, formol e álcool, mesmo para os dentes não autoclavados. As substâncias em análise nos períodos propostos se mostraram capazes de impedir o crescimento microbiano. Este experimento poderá auxiliar o desenvolvimento de um protocolo de processamento e de administração do órgão dental humano extraído em um banco de dentes.

Descritores: Odontologia. Biossegurança. Esterilização. Dente. Técnicas microbiológicas.

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