Extracted human teeth and their utility in dental research. Recommendations on proper preservation: A literature review

Zastosowanie usuniętych zębów ludzkich w badaniach naukowych. Wytyczne dotyczące przechowywania próbek – przegląd piśmiennictwa

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Abstract

Laboratory research in dentistry and dental education use extracted human teeth as a model for simulation for ex vivo procedures. Human-borne tissues are the first choice of material for bond strength assessment. To obtain comparable results in dental material tests and to ensure microbiological safety, specimens must be stored under specific, uniform conditions. The aim of this paper was to present the contemporary view and recommendations on preserving extracted human teeth. The antimicrobial properties of the storage medium are a crucial aspect, as extracted teeth pose a risk of cross-infection. A classification of different methods (using solutions and otherwise) is presented and their sterilizing efficiency is compared based on the literature. The emphasis is put on the interaction between the storage conditions and the substrate. Tooth specimens should be biologically safe and have normal mechanical properties. The sterilizing process must be neutral for the enamel and dentin microstructure, because even a minor change can affect the adhesive bonding. Autoclave sterilization and storage in 10% formalin solution are widespread and reliable methods, although they do have their disadvantages. There is a need for further investigation in order to establish uniform recommendations on preparing and preserving extracted human teeth used for research purposes.

Key words: sterilization, extracted teeth, formalin, autoclave, adhesion

Słowa kluczowe: sterylizacja, usunięte zęby, formalina, autoklaw, adhezja

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Introduction

Extracted human teeth are a valuable source of biological material and are indispensable for research purposes. Numerous ex vivo dentistry studies are conducted to assess the physical, chemical and biological features of various materials in order to implement the results in dental practice.^{1–5} In order to obtain reliable results in such research, the tested materials or tooth samples should be properly prepared and stored.

One particularly important aspect in material science is the measurement of bond strength. Restorations bonded to extracted teeth can be tested by applying a shear load. The amount of force that leads to a fracture defines the adhesion between the 2 materials (shear bond strength test). A method which is widely used in endodontic research is the push-out test. Enough force is applied to cause the extrusion of material from the root canal.⁶ Researchers can also assess the marginal adaptation by means of a dye penetration test.⁷ A tooth sample is stored in a staining solution. The dye can penetrate through gaps and empty spaces between the tooth and the restoration device, indicating microleakage.⁷ Vertical, horizontal and cross-sections are investigated with microscopic techniques in histological studies.⁸ The crystalline structure of the teeth is revealed by observing non-decalcified sections under polarized light, and then the sample is decalcified to analyze the soft tissue component. Samples used in immunohistochemistry studies must be precisely prepared; sections can be obtained through cryomicrotomy (a freezing microtome).⁸ These microscopic observations are a source of knowledge about tissue composition, developmental changes and the demineralization process, among other things.

Extracted human teeth are the material of choice for conducting laboratory tests. The proper preservation of teeth is mandatory because even slight differences in microstructure or composition can adversely affect the results. Specimens should be biologically safe with unaffected mechanical properties. The chemical composition should remain unchanged and should reflect the condition of the tooth as observed in the natural environment of the oral cavity.

Sterilization of extracted human teeth

The usage of human teeth is not without disadvantages. The biological material poses a risk of cross-infection (the transfer of pathogenic microorganisms). Hepatitis viruses (HBV and HCV) and the human immunodeficiency virus (HIV) are particularly dangerous. Students, dental practitioners and researchers who come into contact with specimens should follow safety precautions and use personal protective equipment. The Occupational Safety and Health Administration (OSHA) of the USA considers extracted human teeth to be a potential source of blood-borne pathogens.⁹ In order

to ensure epidemiological safety, materials must be sterilized or disinfected before use.^{10,11} The aim of disinfection is to kill pathogens by means of chemical solutions or miscellaneous inactivating agents.¹² Sterilization is a process that eradicates not only all living forms of microorganisms but their heat-resistant vegetable forms (bacterial spores) as well.¹³ Upon sterilization, a specimen is free of potentially pathogenic bacteria, viruses and fungi. However, the sterilizing agent cannot interfere with dental materials or rearrange the composition of dental tissues. Centers for Disease Control and Prevention (CDC) put forward recommendations on the proper methods for storing and preparing extracted human teeth for ex vivo purposes.^{12–14} Firstly, the Centers proposed the use of a standard 1:10 household bleach.^{11,15} This is usually a solution of sodium hypochlorite or hydrogen peroxide. These chemical agents exert a bactericidal and virucidal action based on oxidation. However, microbiological tests revealed that not all pathogens are eliminated by them.¹¹ Researchers have tested various sterilizing agents to find the best measure for preserving extracted teeth. The most common methods are presented in Table 1.^{8,11–23}

Dominici et al. compared the effectiveness of 5 different methods of sterilization/disinfection of extracted human teeth.¹¹ Antimicrobial eradication was determined by the absence of *Bacillus stearothermophilus* (heat-resistant bacteria used to validate sterilization). The results showed that preserving extracted teeth in a 10% formalin solution for 1 week or autoclave sterilization are the best antimicrobial methods (Table 2).^{10,11}

Similar conclusions were reached by Sandhu et al.¹⁰ when preserving samples for 5 days. In the first stage of their experiment, different sterilizing agents were used; the samples were then incubated on agar medium. The specimens treated with formalin, sodium hypochlorite and an autoclave did not show any signs of bacterial growth. Attam et al. described formalin as the most effective disinfectant.²⁴ Autoclave and formalin are the methods recommended by the CDC.¹⁴

An interesting alternative for formalin seems to be a household vinegar solution.²¹ The liquid mainly contains 5% acetic acid in water. The greatest advantage is its accessibility and the ease of use. Teeth immersed in the solution for 1 week were as free of microorganisms as the group stored in 10% formalin and 3% hydrogen peroxide.^{21,23}

Table 1. The most frequent methods tested for sterilizing/disinfecting extracted human teeth^{10–21,23,25,27,29,30}

Non-solution methods	Solution methods
Steam autoclave ^{10–14,17,25}	Ethanol ^{21,23}
YAG laser ^{16,29}	NaOCl ^{10,11,17,19,21}
Microwaves ^{20,21}	Hydrogen peroxide ^{10,15,18, 21,23}
Gamma radiation ^{16,27}	Thymol ¹⁰
Ethylene oxide ^{27,30}	Formalin ^{10–14,19,27}
	Glutaraldehyde ^{10,11}
	Vinegar ²¹
	Quaternary ammonium compounds ^{11,19}

Table 2. The antimicrobial effectiveness of different methods^{10,11}

Substance	Concentration/conditions	Effectiveness [%]	
		Dominici et al. ¹¹	Sandhu et al. ¹⁰
NaOCl	5.25%	60	100
Formalin	10%	100	100
Quaternary ammonium compounds	0.28%	30	not tested
Glutaraldehyde	2%	50	73.33
Thymol	0.1%	not tested	13.33
Hydrogen peroxide	3%	not tested	66.66
Autoclave	20 min	90% (20 psi, 115.6°C)	100% (15 psi, 120.6°C)
Autoclave	40 min	100% (20 psi, 115.6°C)	not tested

However, this method requires further investigation in order to establish the proper concentration and its influence on tooth structure.

Another proposed substitute to formalin which maintains 100% antimicrobial effectiveness is Gigasept PA solution (Schülke & Mayr GmbH, Norderstedt, Germany). The formula, based on peracetic acid, is commonly used as a safe disinfectant for medical instruments. Freshly extracted teeth immersed in a solution for 7 days at 4°C are devoid of bacteria.²⁶ However, the influence of Gigasept PA on dental tissues has not been tested and is still not recommended before ex vivo tests.

The other substances presented in Table 1 did not demonstrate sufficient antimicrobial properties. In some studies, the sterilizing efficiency of NaOCl depended on the concentration.¹⁹ Although some researchers achieved disinfection using 2.5% NaOCl,¹⁹ Tijare et al.²¹ reported that only 1 tooth out of a group of 10 samples was disinfected after immersion in a 5.25% solution for 1 week. That result corresponds with a study performed by Dominici et al.¹¹ (Table 2). The chemical instability of NaOCl is also important – exposure to air and high temperatures can impair its biocidal potency.²²

Thymol, an aromatic oil derived from plants, has antiseptic properties and can palliate the inflammatory process. However, the effectiveness of 0.1% thymol as a disinfectant is poor, ranging from 0% to 13.3%.^{10,18,26,27}

According to the literature, immersion in 70% ethanol disinfected only 20–30% of samples.^{21,23} Quaternary ammonium compounds, although they are widely used as surface disinfectants, exhibit insufficient antimicrobial action for sterilizing extracted human teeth. Only 30–60% of samples in a solution of quaternary ammonium compounds were free from bacteria (Table 2).^{11,15} A 2% solution of glutaraldehyde displays enhanced effectiveness. In the medical industry, higher concentrations of this compound are used as a “cold sterilization method” to prepare equipment that cannot be exposed to the heat of an autoclave.¹⁴ The 2% solution is also used to eradicate bacteria from infected dental canals during endodontic treatment. There are inconsistent results regarding its ability to sterilize extracted teeth. In some studies, more than half of the samples were successfully disinfected,^{10,11} while other researchers obtained only

20% of bacteria-free specimens.¹⁸ To recommend this solution as a storage medium, it should be able to successfully disinfect all tested samples; glutaraldehyde does not meet that requirement. Additionally, the compound is highly toxic and even medical gloves may be an insufficient measure for protecting skin from irritation.¹⁴

Hydrogen peroxide (H₂O₂) is described as a high-level disinfectant.¹⁴ The most common concentration used in research is a 3% solution. The sterilizing effect reported in different studies is compared in Table 3.

It has been claimed that 3% hydrogen peroxide led to similar levels of sterilization as 10% formalin, though its effectiveness in ex vivo studies remains controversial.²¹ It is a strong oxidant that can dissolve organic matter.

Among non-solution methods, a steam autoclave is the gold standard in sterilizing extracted teeth and numerous studies have provided justification for this recommendation.^{5,9,12–14} Heat is a well-known bactericidal agent causing the denaturation of bacterial enzymes and proteins. High temperatures – 121°C or 132°C – along with steam pressure kill microorganisms effectively.¹³

The antimicrobial mechanism of an Er:Yag laser has been thoroughly described, especially in reference to endodontics and periodontology. However, the application of an Er:Yag laser for disinfecting extracted teeth was unsuccessful because of damage to the superficial tissues.¹⁶ The denaturation of dentine collagen can decrease the ability of the dentin to form a firm connection with the adhesive resin. Thus, lower shear bond strength can result not only from the properties of the tested material, but also from the impaired quality of the tooth sample. The obtained result can lead to erroneous conclusions.^{25,28,29}

Table 3. The effectiveness of 3% hydrogen peroxide in sterilizing extracted teeth^{10,15,18,21,23}

Study	Period	Effectiveness
Chandki et al. 2013 ¹⁵	7 days	60%
Tijare et al. 2014 ²¹	7 days	100%
Sandhu et al. 2012 ¹⁰	5 days	66.6%
Gogineni et al. 2016 ²³	7 days	100%
Lolayekar et al. 2007 ¹⁸	7 days	70%

The effectiveness of ethylene oxide to sterilize extracted human teeth has also been tested.^{30,31} Its ability to penetrate into internal layers and to eliminate resistant bacterial strains is insufficient, so this method is not recommended.

Microwave irradiation has been investigated to assess its sterilizing efficiency and its influence on enamel microhardness.²⁰ Although the mechanism is not fully understood, the method eliminates a wide spectrum of pathogens. It probably combines thermal and electromagnetic effects and leads to molecular changes in the bacterial cell.²⁰ Bovine enamel sections were exposed to 650 W microwaves for 3 min, which resulted in the eradication of pathogens without impairing the micromechanical properties of tissues.²⁰ The application of 350 W and 600 W microwaves eradicated 100% of bacteria.¹⁹ However, these results were not supported by Tijare et al.²¹ Therefore, the influence of microwaves on the mechanochemical properties of dental tissues remains unresolved.

The introduction of gamma radiation has provided promising results, but requires carefully adjusted parameters to obtain full eradication without disarranging the sterilized material. The method has been applied in the food industry because the results are satisfactory and features of the product remain unchanged.¹⁶ However, high costs of the equipment mean that it can only be used for large-scale sterilization, not in small laboratories.

Specific issues in laboratory practice

In laboratory practice, the chemical, mechanical and tribological characterization of human teeth and dental materials is crucial. Even a minor change in composition can be statistically significant. The storage methods which ensure 100% microbiological safety (autoclave, gamma radiation and formalin solution) require further consideration. There are some problems and limitations according to their usefulness in research.

Autoclave

The antimicrobial effect of high temperature and steam pressure does not considerably change the mechanical properties of teeth, so they can still be used for dental training. However, the conditions of *ex vivo* research are rigorously applied. Changes at the microscopic level can decrease the adhesion strength between dental tissues and the material being tested. The ability to create a sound connection with the bonding material is widely described and studied in terms of permeability of the dentin.^{31–33} Although Pashley et al.³² did not report any adverse effects of autoclave sterilization, another study³³ using a modified method led to the opposite conclusion. Before sterilization, the teeth were etched with 35% orthophosphoric acid.³³ That procedure removed the smear layer and partially demineralized the intertubu-

lar dentin. The organic components responsible for creating a hybrid layer (collagen network) were exposed. Due to the temperature, collagen fibers denatured and disintegrated and the ability to form a chemical connection with the bonding resin was limited.³³

Sound teeth can be effectively sterilized at 115.6°C at 20 psi for 40 min. Conversely, teeth with amalgam restorations may pose problems. The high temperature and pressure lead to the release of toxic mercury vapor from amalgam alloy. The tooth and amalgam exposed to heat simultaneously exhibit dimensional changes to different extents. Changes in the size of tissues and restoration apparatus may lead to fractures in enamel and dentine. For restored teeth, an alternative method must be implemented – storage in a 10% formalin solution for 1 week is recommended.¹¹ The behavior of other dental materials (such as composites or glass ionomers) under pressurized steam has not been tested and requires further investigation.

Gamma radiation

The influence of radiation on adhesion strength is ambiguous.^{10,34,35} White et al. did not prove any adverse effect of gamma radiation on dentin properties while assessing its optical properties and specular reflectance with Fourier-transform infrared spectroscopy (FTIR).¹⁶ Compared to other methods, tissue changes were more extensive when ethylene oxide, dry heat or an autoclave was used. A dose of 0.173×10^6 rads (a unit of absorbed radiation dose) was applied. However, a dose of 2.5×10^6 rads (used for instrument sterilization) decreased the adhesion in a shear bond test³⁵; therefore, further investigation is recommended in order to establish the precise conditions of sterilization.¹⁶

Chemical sterilization with reference to adhesion strength tests

Adhesion is a crucial factor that determines the longevity of dental restorations. It is a physicochemical process which depends on the microstructure of the surfaces that are in contact. Due to this fact, adhesion is susceptible to changes in the composition of substrates. A Shear Bond Strength test (SBS) is a universal method for *ex vivo* research to evaluate the connection between a tooth and dental material. The storage environment can decrease adhesion with composite resins, which has been widely proven.^{17,24,34,35} Solutions used to store extracted teeth contain active agents. The ability to deteriorate the organic compounds of bacterial cells is not specific, and these substances interfere with elements forming dental tissues.

The influence of different methods of sterilization on SBS test results has been evaluated.¹⁷ The largest reduction in adhesive properties was observed for a 5.25% solution of NaO-

CI. This may result from enamel deproteinization and alterations of the dentin matrix.¹⁰ Moreover, adhesion can be also altered by residual chlorine.¹⁷ For these reasons, compounded by its insufficient antimicrobial effect, NaOCl is not recommended as a storage solution. In contrast, formalin was the only solution found to be neutral to the adhesion process.¹⁷ The 10% concentration provides both desirable properties: the previously mentioned 100% effective sterilization and a neutral adhesion process.¹⁰ In the second phase of this study,¹⁷ teeth from each group were randomly selected to introduce additional sterilization. One-half of the specimens in each subgroup were autoclaved for 40 min while the second half of the specimens were immersed in 10% formalin for 14 days. Interestingly, after autoclaving SBS decreased in the control group (stored for 60 days in distilled water). On the other hand, after the previous SBS reduction in the NaOCl group, subsequent autoclaving led to an improvement in this parameter. Shear Bond Strength test performed after 60 days of storage in sodium hypochlorite yielded significantly better results after 40 min of autoclaving (almost 200%). The storage in 10% formalin also improved the previously decreased SBS, but to a lesser extent. With regard to this observation, an important conclusion can be drawn: the 2 recommended sterilizing methods – autoclave and 10% formalin – have an “equalizing effect”. This means that the reduction of SBS which occurs after storage in an inappropriate medium can be balanced by a subsequent proper sterilization method.

Although another study³⁵ showed unpromising results in testing two-step self-etching bonding systems on teeth preserved in 10% formalin for 3 months, the adhesion obtained with an etch-and-rinse system did not change. Moreover, the special preserving properties of formalin were assessed spectroscopically. The components of formalin (formaldehyde, methyl alcohol and sodium acetate) interact with organic compounds such as proteins, glycoproteins and carbohydrates. The ability to fasten the proteins can prevent a collagen network from collapsing after etching.^{24,36} Improper adhesion can also, indirectly, be defined by microleakage. Research based on the dye penetration method revealed that a sound connection between hard tissues and a restoration device was maintained when the tested samples were preserved in 10% formalin.²⁴

In comparison to gamma radiation, autoclave and formalin are methods that can be successfully implemented due to their accessibility and low cost. However, the main disadvantage of formalin is the toxicity of formaldehyde (a potential carcinogen). The harmful mechanism is based on an interaction with molecules on the cell membranes, nucleic acid destruction and protein precipitation. Exposure to high concentrations of formalin vapors may lead to immediate local irritation of the eyes, nose and throat. Absorption through the respiratory epithelium is rapid. Working with teeth preserved in formalin solution requires safety precautions. A container with extracted teeth should be opened in a safe, well-ventilated area. Moreover, working under a fume hood is recommended.³⁷

One scanning electron microscopy (SEM) study did not indicate an ideal method for sterilizing extracted teeth.²² The 3 common sterilizing agents (autoclave, sodium hypochlorite and vinegar) caused changes in enamel microstructure, increasing its roughness and porosity. The dentin morphology was also altered.

Thus, a dual approach is called for. Firstly, a universal storage solution for extracted teeth should be established. The second, parallel direction in research should be focused on introducing the best alternative for human tissues for ex vivo purposes.

Substitutes for extracted human teeth

One important aspect is the quality of material used in dental research. According to reports and clinical practice, the main cause of dental extractions is still caries and its complications.³⁸ The extraction of non-carious teeth due to periodontal or orthodontic indications is infrequent. Ongoing decay deteriorates the mineral and organic composition, affecting the usefulness of samples. Moreover, researchers often cannot define the source of material, so the age of the donor remains unknown. Thus, age-dependent changes in the tissues should be taken into consideration in research which includes an analysis of mineral and organic composition.³⁹ The size of a sample can also be problematic. A relatively small and curved surface can be a hindrance in some laboratory tests for which a flat, thick layer is required.

Taking into consideration the epidemiological safety issues in preclinical dentistry, human teeth are being replaced by typodont artificial dentition. However, models made from plastics are expensive and do not reflect the mechanical properties of natural enamel and dentin.

Whereas acrylic substitutes can be used in dental education, natural tissues are irreplaceable in laboratory tests of dental materials. Bovine teeth are widely used as an alternative substrate, and they do have many advantages. Yassen et al.²⁸ presented a detailed meta-analysis which included the methods of material testing used over the past 6 decades (1953–2010). Bovine teeth are easily accessible and have a more homogeneous composition. They provide a broad, flat surface without carious lesions. However, the microstructure of animal tissue is quite similar but not identical to human enamel. The lack of thorough analyses and unambiguous results comparing human and bovine material in SBS testing do not exclude animal tissues for ex vivo tests,²⁸ but the minimal differences should be considered when scientific conclusions are drawn. Thus, human-derived material remains the gold standard.

The last point to be raised is agreement with ethical principles. The issue of specific consent to use extracted teeth in research (human, biological material) remains unresolved. Medical experimentation requires the approval of a bioethics committee, though informed consent for tooth donation is not necessary.²⁸

Conclusions

Autoclave sterilization is a widespread and reliable method that can be used to prepare extracted teeth for educational purposes and laboratory research, excluding adhesion testing. Pressurized steam is low-cost and innocuous; moreover, its microcidal and sporicidal activity is rapid. Human-borne tissues are the first choice of material for bond strength assessment. The material should be stored in a solution that is neutral towards enamel and dentin microstructure. Numerous studies have suggested 10% formalin, though its potential health risk must be taken into account. There is a need for further investigation in order to establish uniform recommendations for preparing and preserving extracted human teeth used for research purposes.

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