EXPLORING THE USES, STERILIZATION AND STORAGE PROTOCOLS OF EXTRACTED HUMAN TEETH FOR RESEARCH ADVANCEMENTS: A SCOPING REVIEW

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Abstract
Extracted human teeth (EHT) have long served as invaluable specimens in dental research, offering valuable insights into dental science and biomaterials. This scoping review aims to document the current uses of EHT in dental research and to identify the sterilisation and storage protocols. The study employed scoping review methods. Relevant databases (PubMed, Scopus, and Science Direct) were searched using the terms "extracted human teeth" and "*in-vitro*" to identify articles for inclusion. The most recent articles published over five years (2019-2023) and those in English were included. Two reviewers independently conducted the selection process, and the decision was consensually made. A descriptive statistical assessment was performed to analyse the studies involving EHT and the sterilising and storage solutions used. A total of 105 articles were reviewed: Endodontology (32%), Dental Materials (24%), Conservative Dentistry (20%), Prosthodontics (8%), Regenerative Therapy (6%), Oral Biology (5%), Orthodontics (4%), and Periodontology (3%). 71% of the reviewed papers did not report the sterilising and storage methods for EHT. When reported, sodium hypochlorite was most commonly used (15%). However, the concentrations and immersion time in the sterilising solutions were inconsistent. The most frequently used storage solutions to keep EHT from dehydration are saline (11%) and distilled water (10%). In conclusion, extracted human teeth continue to be extensively utilised in dental research. Nonetheless, the reporting and standardisation of sterilisation and storage methods are notably inadequate. A standardised guideline for sterilising and preserving extracted human teeth for various types of research is essential to mitigate variability and promote result uniformity. There is a stark absence of consensus regarding the optimal procedures for storing and sterilising EHT, even when used for the same laboratory investigations and different types of dental research.

Keywords: Extracted Human Teeth, *In-vitro*, Sterilization, Storage Solution

Introduction

Extracted human teeth (EHT) are widely accepted for its role in teaching and research. The first-ever reported use of extracted human teeth was as early as 1945 when Hodge et al. conducted a study to determine the adsorption of the radioactive isotope, strontium, at 40 degrees by enamel, dentin, bone, and hydroxyapatite (1). In dental education, EHTs are commonly utilised by undergraduate students for preclinical training in various specialities such as conservative dentistry and endodontics. A survey conducted among undergraduate and postgraduate students revealed that they perceived the adoption of EHT as beneficial for their hands-on and preclinical training, as it enhanced their confidence in performing treatments for the first time (2). A literature search on the PubMed database shows that for the last ten years (2013-2023), there have

been more than 400 publications on research using EHT. EHT remain the gold standard for biomaterial dental research due to its relevance to clinical applications. Human teeth offer an ideal representation of natural dentition, possessing similar physical, chemical, and biological properties. This inherent similarity enables researchers to accurately assess how dental biomaterials interact with human teeth, leading to more clinically relevant results.

For example, *in-vitro* tests are performed to determine the bond strength (3, 4), the presence of microleakage (5), and marginal gaps (6–8) in predicting the clinical performance of newly developed dentin bonding systems and pulp dressing materials. Bovine teeth have served as a substitute for human teeth in dental biomaterial research. They offer various benefits, such as its accessibility from slaughterhouses and cost-effectiveness. Moreover, they feature a more consistent composition than human teeth and are larger, making them well-suited for sample preparation, especially in laboratory tests requiring a broad, thick sample layer. However, it is important to note that bovine teeth do not fully replicate human teeth.

In recent years, the applications of EHT in dental research have expanded into diverse fields. This includes regenerative dentistry, due to the rapid progress in biomedical engineering research (9). The surge in interest in regenerative dentistry has been driven by its potential to steer personalised, minimally invasive, and biologically guided approaches in oral healthcare. Stem cell-based therapies, tissue engineering, and growth factors are pivotal in advancing regenerative dentistry. Recent research, such as the work by Tatullo et al. (9) highlights the potential of stem cells derived from dental tissues, including dental pulp and periodontal ligament, in dental tissue regeneration. Moreover, strategies involving bioactive materials and biocompatible scaffolds are revolutionising the treatment of oral and dental diseases (10).

Extracted human teeth have been discovered to harbour potential pathogens, such as Klebsiella, Enterobacter, Pseudomonas, Shigella, Proteus, and Salmonella (11). These bacteria diverge from the typical oral microbiome and suggest contamination after tooth extraction. These microorganisms remained viable on the tooth and within the storage media post-extraction. Additionally, it is worth noting that other transmissible pathogens, including human immunodeficiency virus (HIV) and hepatitis B virus (HBV), could potentially be present in the pulp or periradicular tissues associated with extracted human teeth (11).

Sterilising EHT is particularly significant in light of the ongoing Coronavirus disease (COVID-19) pandemic. Recent research has highlighted the oral cavity as a crucial site for the acquisition of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The identification of saliva as a major route of infection underscores the necessity for strict protocols when handling EHT (11). Handling non-sterilized teeth carries an elevated risk of exposure to these potentially lethal microbial communities.

Since the 1990s, the United States Occupational Safety and Health Administration (OSHA) considers extracted human teeth as potential sources of bloodborne infections (12). To address this, the Centres for Disease Control and Prevention (CDC) issued a guideline for sterilising extracted human teeth for research and teaching. According to the guidelines, teeth with amalgam restorations should be stored in 10% formalin for two weeks, whereas teeth without amalgam restorations should be autoclaved for 40 minutes at 121°C and 20 psi.

Even though the CDC has published the recommended guideline for the management of EHT to prevent cross-contamination, the literature reflects a significant array of practices concerning the storage and sterilisation of these teeth when used for dental research. Several different methods have been employed by various investigators to sterilise EHT. Broadly, they can be divided into two categories, namely physical and chemical methods. A range of physical methods, for example, boiling, microwave irradiation, gamma irradiation, and the least commonly utilised Er: Yag laser, were applied (13–16). Chemical sterilisation is preferred as they are considered cheaper and more easily accessible. The chemical agents include formalin, ethylene oxide, sodium hypochlorite, thymol, alcohol, and many more (17–22).

Before and following sterilisation, the EHT has to be stored in an appropriate manner to maintain the tooth integrity and avoid desiccation before and during the different phases of the experimental procedure. Proper storage extends the usability of extracted human teeth and enhances the reliability

of dental research outcomes. Several studies have highlighted the various practices employed for storing extracted human teeth (EHT) prior to use (23, 24). These practices include keeping EHT in a storage solution, such as saline or distilled water, before immersing them in a sterilisation solution. Alternatively, some procedures involve the immediate immersion of EHT in the sterilising solution, while others opt to keep the teeth dry and rehydrate them before use (17, 22).

An ideal sterilisation and storage process for the EHT should preserve its structural integrity, chemical composition, and inherent properties. These procedures can alter the physical, chemical, and mechanical properties of teeth. Therefore, the reliability of research findings may be compromised, limiting their applications.

This scoping review aims to document the current uses of EHT in dental research and to ascertain the sterilising and storage protocols employed. Specifically, the review objectives were to answer these research questions:

i. Is the utilisation of extracted human teeth in contemporary dental research still pertinent?
ii. Is there a prevailing standard or established protocol for sterilising and storing extracted human used in dental research?
iii. What specific types of extracted human teeth are utilised in dental research?

Materials and Methods

Study Design

This study's protocol follows the framework suggested by Peters et al. (2015), as outlined by the Joanna Briggs Institute. Reporting of this scoping review adheres to the PRISMA Extension for Scoping Reviews (PRISMA-ScR) (25). Publications written in English between the years 2019 and 2023 were included.

Search strategy

An electronic literature search was carried out using internet search engines: PubMed, Scopus, and Science Direct (Figure 1). The keywords ("Extracted human teeth" or "EHT") AND ("*in-vitro*") were used to maximise the inclusion of relevant publications in the field. Duplicate entries were eliminated, and a subset of the findings from each database was pooled. The study selection was primarily centred on *in-vitro* research conducted using extracted human teeth either as the study samples or as controls. Only publications written in English between the years 2019 and 2023 were included. Articles featuring studies using animal teeth, published in a language other than English and in non-peer-review journals such as abstracts and paper presentations without access to the full paper were rejected as the specific details of the data required were mainly found in the body of the manuscript. A supplementary manual search only produced five publications, which were not included as only the abstracts were available and not the entire manuscript.

Selection Process

Prior to the review process, two researchers (NN, JQZ) collectively screened a random 10% of selected articles and analysed the title, abstract and presence of inclusion criteria. The data extraction procedures were further refined. Discrepancies in screening of titles/abstracts and full-text articles were resolved through discussion (agreement ranged between 80%-90%). In case of disagreement, which is mainly related to the inclusion/exclusion of the articles, the opinion of a third researcher (SAB) was obtained. The same three researchers then conducted comprehensive reviews of the full articles.

Data Collection Process

An Excel spreadsheet was specially designed to record the data. The data included the following information: authors' name, publication year, publication title, the field of study, study design, aim and objectives of the study, type of EHT used, sterilisation method, storage method, laboratory investigations and study parameters. During this procedure, one of the reviewers (NN) independently extracted the data, and JQZ and SAB checked it. Any discrepancies, which are concerned with categorisation based on the study field, were resolved through discussion between the three reviewers. The objective was to reach a consensus that not only resolved any discrepancies but also ensured the accuracy of the data collection. In these discussions, the field of study is recorded based on the aim or objective of the research and the predominant theme of the manuscript.

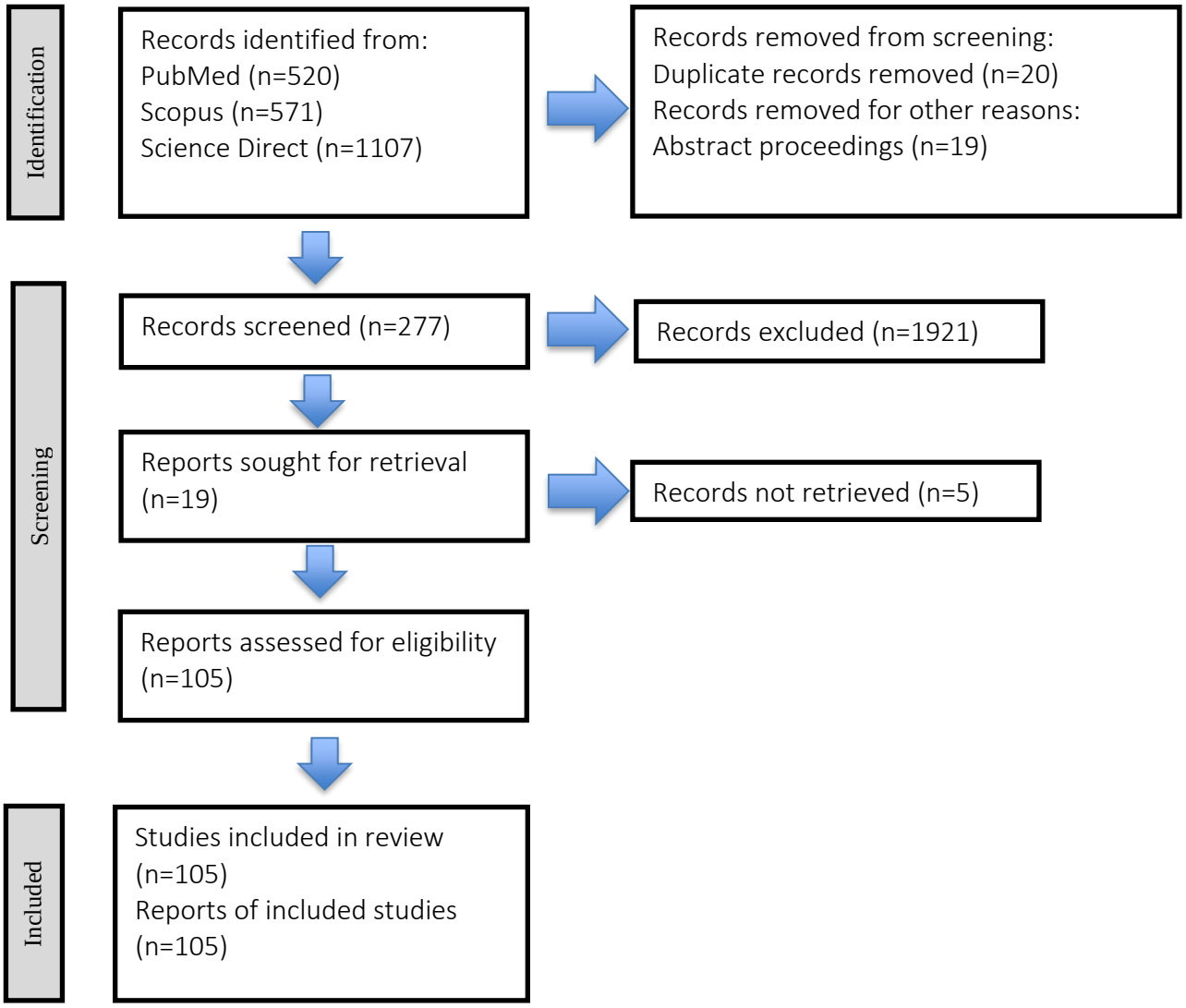

Figure 1: Flow diagram for the search and study selection in the scoping review

Results

i) Results of the research

The electronic screening process initially resulted in 2,198 articles, comprising 520 from PubMed, 571 from Scopus, and 1,107 from the Science Direct database. Subsequent removal of duplicate research narrowed down the selection to 277 articles. The reviewers then screened titles and abstracts, leading to a detailed evaluation of 123 papers. Five were excluded due to unavailable full text, inadequate abstract content, or overall irrelevance. For example, *in-vivo* and not *in-vitro* study and not using human but rather bovine teeth. Ultimately, 105 studies were chosen for qualitative examination based on the specified inclusion criteria. A visual representation of the selection process is presented in Figure 1.

From the 105 studies included in the review, four studies utilised a combination of *in-vitro* and *in-vivo* (26–29) and two other studies utilised combination of *in-vitro* and *ex-vivo* study design (30, 31). In these investigations, the authors incorporated animal teeth mice (32) and monkeys (33) as the primary intervention. However, these studies were deemed relevant for inclusion due to the use of extracted human teeth as one of the study groups in their research. Based on the examination of the PubMed, Scopus, and Science Direct databases, it was

observed that a nearly uniform distribution of research papers utilising EHT within the period from 2019 to 2023. Notably, 2022 had the highest publication count (23 articles), followed by 2021 and 2023, both recording 21 papers. Conversely, 2019 had the lowest publication count, with 20 papers. It was evident that endodontics, dental materials, and conservative dentistry were the top three areas within dentistry that prominently featured extracted human teeth in their research.

ii) Characteristics of the included studies

The data collected from the review is tabulated in Table 1. The research was categorised into eight distinct areas: (i) Endodontics, encompassing research on endodontic techniques; (ii) Dental Materials, concentrating on testing and comparing the efficacy of dental materials; (iii) Conservative Dentistry, addressing caries detection and restoration; (iv) Prosthodontics, involving tooth wear management and techniques for fixed restorations; (v) Regenerative Therapy, which delved into stem cell usage and bone regeneration; (vi) Oral Biology, examining techniques and methods to inhibit specific bacterial strains; (vii) Periodontology and finally (viii) Orthodontic which encompass research on periodontal and orthodontic treatment respectively.

iii) Field of dentistry that utilised EHT in their research

Thirty-four papers were categorised in Endodontics fields (31%), 25 papers in Dental Material (23%), 21 papers in Conservative Dentistry (20%), 8 in Prosthodontics (8%), six papers in Regenerative Therapy (6%), 5 papers in Oral Biology (5%), and 4 papers in Orthodontics (4%), and 3 papers in Periodontics (3%). Figure 2 summarises the utilisation of EHT according to the field of dentistry based on the year of publication.

iv) The type of tooth most commonly used in this review paper

There was a wide array of terminologies used to describe the types of EHT used in the reviewed studies. Most research papers failed to specify the exact tooth types used and instead provided general descriptions, for example, using terms such as “anterior or posterior teeth”, “primary teeth”, “carious teeth”, "single root”, or “curved root”.

Within the scope of this scoping review, the predominant teeth used are premolars; n=19 (18%). 15 (14%) of the reviewed publications did not include any details of the type of EHT, while 14 (13%) used anterior and posterior teeth (without indicating if they are incisors, canines, premolars, or molars). The rest of the publication included other types or descriptions of teeth, as illustrated in Figure 3. Due to the variable terminologies used to describe the teeth, the data collected were difficult to standardise, resulting in a diverse categorisation of the tooth types used in this review. Consequently, this may not accurately represent the types of teeth as the different categories may overlap.

V) Sterilization methods of EHT used in this review paper

Figure 4 shows the various sterilisation methods employed in the studies within this scoping review. A vast majority of the research papers (71%, n=75) did not provide any information on the sterilisation procedures of the EHT prior to use. The remaining 31 papers used either physical or chemical methods to sterilise EHT, with a majority (27 research papers) employing chemical sterilisation. Sodium hypochlorite (NaOCl) ranging from 0.5% to 10% appeared to be the preferred chemical sterilising solution (n=16). Other reported solutions used to sterilise EHT are alcohol (n=3), 0.1%-0.2% thymol (n=2), sodium chlorite (n=2), 0.5% chloramine-T (n=1), 10% formalin (n=1), and 1% citric acid (n=1). Among those reporting using physical sterilisation, 3 papers utilised autoclaving at 121°C, while a single paper employed gamma radiation.

Figure 5 illustrates the storage methods for EHT prior to use in the research. Notably, 51 papers (48%) did not specify the storage methods. The most prevalent approach was immersion in normal saline (n=12 papers). Other storage solutions reported are: distilled water (n=11), 0.1% to 0.2% thymol (n= 9), 0.5% to 1% chloramine-T (n=7), sterile containers (n=4), sodium chlorite (n=2), formaldehyde (n=2), water (n=1), phosphate buffered-solution (PBS) (n=1), artificial saliva (n=1), peroxide (n=1), 70% ethanol (n=1), 10% formalin (n=1) and sodium hypochlorite (n=1).

Table 1: Description of articles in the scoping review

Year of publication	Authors	Field of study	Aim of study	Type of teeth used as sample	Sterilising methods	Storage methods
2019	Al Jhany et al. (50)	Conservative	To develop a valid method for guiding dentists and dental students in estimating remaining dentin thickness (RDT) prior to caries excavation by establishing a relationship between the radiographic RDT and actual RDT.	Posterior (premolar and molar)	X	X
	Armand A. et al. (51)	Endodontic	To evaluate and compare the antibacterial effect of plasma and photodynamic therapy in root canals infected with *E. faecalis*.	Single-rooted, square root	Sodium hypochlorite (5.25%)	Physiological saline at ambient temperature
	Parirokh M. et al. (52)	Endodontic	To determine the effect of asymmetric digital subscriber line, MP4 player, cordless telephone, mobile phone and frequency modulation radio wave on an apex locator.	Single-rooted	X	X
	Adl A. et al. (53)	Dental material	To evaluate the bond strength of a resin-based sealer to root canal dentine, irrigated with the new imidazolium-based silver nanoparticle compared to sodium hypochlorite with and without the application of ethylene diamine tetraacetic acid for smear layer removal.	Single-rooted	Sodium hypochlorite (5.25%)	Normal saline
	Mohammadian F. et al. (4)	Dental material	To assess the effect of diode and Er: YAG laser irradiation of root dentin on push-out bond strength of endodontic cements such as mineral trioxide aggregate (MTA) and calcium-enriched mixture cement.	Single-rooted	Chloramine T (0.5%) at 4°C	Chloramine T (0.5%) at 4°C
	Lanteri V. et al. (54)	Dental material	To evaluate the microleakage at the interproximal horizontal margin in Class II restorations realised using four different types of composite resin.	Posterior (premolar or molar)	X	X
	Mortazavi S. et al. (55)	Conservative	To Investigate the effect of the electromagnetic field of a commercial dental light cure device and a common Global System for Mobile Communications mobile phone on microleakage of amalgam restorations.	Posterior (premolar or molar)	X	Normal saline (up to 3 months)

2019	Marotti J. et al. (56)	Prosthodontic	To assess the accuracy of ultrasound impressions taken of subgingival prepared teeth compared with digital optical impressions.	Posterior (premolar or molar)	X	X
	Sinjari. et al. (57)	Prosthodontic	To identify a clinical surface cleaning protocol after Immediate dentin sealing to achieve defect-free impression	periodontally compromised (molars, premolars, incisors)	Did not mention the method	Distilled water
	Kobayashi M. et al. (58)	Conservative	To compare six experimental caries detector dyes with different viscosities to clarify the relationship between dye viscosity and staining performance in sclerotic dentin.	Posterior (premolar and molar) with mild dentin caries	X	X
	Calvo-Guirado J. et al. (59)	Regenerative therapy	To evaluate the chemical composition of crushed, extracted human teeth and the quantity of biomaterial that can be obtained from this process.	Anterior teeth (extracted due to trauma, caries or periodontal disease)	X	Sterile crystal containers
	Farawati F. et al. (60)	Conservative	To evaluate whether tooth whitening by carbamide peroxide (20%, 35%, and 44%) after immersing the enamel in 5 solutions (wine, coffee, tea, soda, and water) for 15 days at 80°C alters the surface topography of enamel causing an increase in surface roughness that could increase susceptibility using Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy.	Not mentioned	X	X
	Kumar P. et al. (3)	Endodontic	To analyse the effect of natural antioxidants, 6.5% proanthocyanidin and 25% bamboo salt on the reversal of NaCl-induced reduced bond strength of an epoxy resin sealer to dentin.	Single-rooted, single canal extracted due to periodontal and orthodontic reasons.	X	Distilled water
	Rosentritt M. et al. (61)	Prosthodontic	To investigate the influence of material, preparation, and pre-treatment on the aging and fracture force of Computer-aided design (CAD) and Computer-aided manufacturing (CAM) CAD/CAM resin composite molar crowns.	Mandibular right first molars	X	Chloramine-T (0.5%)
	Kim D. et al. (62)	Conservative	To evaluate the incorporation of three different zwitterionic materials—2-methacryloyloxyethyl phosphorylcholine,	premolar	X	X

			carboxybetaine methacrylate and sulfobetaine methacrylate polymers—into light-curable fluoride varnish regarding the inhibition of biofilm accumulation.			
2019	Galler K. et al. (63)	Endodontic	To compare several methods of activation for endodontic irrigants, including ultrasonic, sonic, photon-induced photoacoustic streaming and shockwave enhanced emission photoacoustic streaming (SWEEPS) in their ability to penetrate dentinal tubules	Single-rooted, straight and round-shaped root canals	X	Chloramine T-hydrate 0.5%
	Kim B. et al. (64)	Conservative	The utility of comparing red fluorescence between active and inactive caries lesions and investigating whether changes in red fluorescence and fluorescence loss are influenced by lesion activity remineralisation.	Posterior (premolars and molars) with at least one caries lesion on one smooth surface	X	X
	Llena C. et al. (65)	Conservative	To evaluate the effect of casein-phosphopeptide and amorphous calcium phosphate with fluoride upon the calcium and phosphorus composition and morphology of dental enamel and dentin after the application of two bleaching agents: 37.5% hydrogen peroxide and 35% carbamide peroxide.	Maxillary incisors and canines	X	Thymol 0.5% for five days
	Katalinic I. et al. (66)	Endodontic	To evaluate the photo-thermal and photo-activated antibacterial effect of the 445/970 nm diode laser on *E. faecalis, S. aureus* and *C. albicans* mixed biofilms grown together inside root canals of human teeth and to define a potentially efficient clinical protocol for safe and predictable usage in endodontic procedures.	Single-rooted, single-canal	X	Chloramine-T (0.5%)
	Morgan A. et al. (30)	Endodontic	To establish a method using Fourier Transform Infra-Red spectroscopy to characterise the nature and depth of changes in dentinal collagen following exposure to sodium hypochlorite during root canal irrigation in an ex-vivo model.	Premolar teeth	X	Ethanol (70%) in deionised water
	Khan T. et al. (67)	Conservative	To compare the microleakage around resin-modified glass ionomer cement-	Posterior (premolars and molars)	X	Normal saline at 4°C

			based sealants and flowable resin-based sealants placed with or without ameloplasty in extracted human teeth.			
2020	Deurer N. et al. (26)	Orthodontic	To assess the sealant thickness after professional tooth cleaning *in-vitro* and *in-vivo* by optical coherence tomography.	Incisors, premolars and molars	X	X
	Zulkapli R. et al. (68)	Conservative	To evaluate the erosive activity of six probiotic drinks on tooth enamel	Free hypocalcification premolar	Autoclave	X
	Alikhani A. et al. (69)	Endodontic	To evaluate the coronal microleakage in Glass ionomer-obturated root canals in endodontically treated teeth using different thicknesses of glass ionomer.	Single-rooted	X	Saline solution (0.9%) at 4°C
	Thomas J. et al. (36)	Endodontic	To evaluate and compare the shaping ability of the Wave One Gold, Trushape 3D Conforming File, Edgecoil, and XP-3D Shaper endodontic file systems on oval-shaped canals using micro-computed tomographic technology.	Single-rooted premolar	X	Normal saline at 25°C
	Pałka Ł. Et al. (70)	Conservative	To evaluate a newly formulated desensitiser containing hydroxyapatite compared to commercially available desensitisers.	Not mentioned	X	Rinse with NaCl
	Park J. et al. (27)	Regenerative therapy	Investigated if recombinant human BMP-2 pre-treated human periodontal ligament stem cell sheets could effectively promote the regeneration of the mineralised layer with embedded periodontal ligament to promote functional human dental cementum and the periodontal complex.	Not mentioned	X	X
	Gao Y. et al. (71)	Dental material	To compare two different laser sources, a potassium-titanyl-phosphate laser with a wavelength of 532 nm and a neodymium-doped yttrium aluminium garnet (Nd:YAG) laser with a wavelength of 1064 nm, to investigate the relation between laser source and bleaching gel during laser irradiation.	Central incisors and premolars	Distilled water at 4°C containing thymol (0.2%)	X
	Guo K. et al. (32)	Regenerative therapy	To investigate the reliability of acellular decalcified teeth in developing bone scaffolds and bone regeneration in rats.	Not mentioned	X	X
	Mandal S. et al (72)	Endodontic	To evaluate the influence of different	Single-rooted	Ultrasonic	X

			vehicles on the antimicrobial efficacy of triple antibiotic paste on *E. Faecalis* infected root canals.		bath in 5% NaOCl, followed by 17% ethylene diamine tetracetic acid (5 mins each)	
2020	Jing X. et al. (28)	Regenerative therapy	To improve the biocompatibility and osteogenic activity of demineralised dentin matrix by grafting peptides on its surface.	Not mentioned	Immersed in 75% ethyl alcohol,	X
	Afshan Z. et al. (73)	Endodontic	Compare the erosive potential and smear layer removal ability of 1% Phytic acid (IP6) and 17% Ethylenediaminetetaacetic acid.	Single-rooted	X	Thymol 0.1% at room temperature
	Mutahar M. et al. (74)	Oral biology	To investigate how the composition of the acquired enamel pellicle affected a laboratory model of erosive tooth wear on human enamel by comparing whole-mouth saliva to parotid saliva.	Molar	X	Sodium hypochlorite (0.05%) at 4°C
	de Oliveira A. et al. (75)	Conservative	To investigate parameters that could predict the colour-masking effect of white spot lesions after resin infiltration.	Premolars, molars and incisors	X	Distilled water and frozen until required
	Jamadar A. et al. (5)	Dental materials	To compare and evaluate the shear bond strength of sixth- and seventh-generation bonding agents with varying pH – an *in-vitro* study	Premolar	X	X
	Tennert C. et al. (37)	Endodontic	To evaluate the effect of photoactivated chemotherapy on *E. faecalis* biofilms in root canals using a 90% isopropanol-based photosensitiser and removing excess photosensitiser before light incubation.	Single-rooted anterior and premolar	X	X
	Sainudeen S. et al. (76)	Endodontic	To evaluate the antimicrobial efficacy of Tylophora indica, Curcumin longa, and Phyllanthus amarus on *E. faecalis* biofilms formed on the tooth substrate.	Single-rooted lower premolar	X	Normal saline
	Duruk G. et al. (77)	Oral Biology	To perform an *in-vitro* assessment of the effects of radiation therapy on the morphological, mechanical, and chemical properties of primary and permanent	Primary teeth: molar Permanent teeth: 3rd molar	X	Distilled water (4°)

			teeth.			
	Galledar S. et al. (78)	Endodontic	To compare the apical microleakage AH26 and Endofill sealers using a MTA Fillapex dye, AH26 penetration method.	Single-rooted, single-canal	Sodium hypochlorite (5.25%) for an hour	X
	Timme M. et al. (38)	Endodontic	To investigate the potential of 9.4T ultrashort echo time technology visualising tooth anatomy and root canal treatment *in-vitro.*	Posterior (premolar and molar)	physiological NaCl solution at 4°C.	X
2021	Rath R. et al. (79)	Oral biology	To explore the usefulness of cellulose acetate peels in reproducing microscopic structures of teeth as seen in routine ground sections and further, if they could supplement or replace the same.	Not mentioned	X	X
	Stähli A. et al. (80)	Periodontic	To investigate how scaling affects the penetration of microorganisms into dentinal tubules, how pulpal cells seeded into the pulp cavity respond to bacterial challenge, and how penetration and inflammatory response may depend on the bacterial composition.	Posterior (premolar and maxillary 3rd molar)	NaCl (0.9%)	Chloramine (1%)
	Nasri & Afkhami (81)	Dental material	To assess the effect of adding silver Nanoparticles (AgNPs) to MTA (for use as an orifice plug) on coronal microleakage using the bacterial leakage model.	single-rooted, single canal	X	X
	Sun J. et al. (31)	Conservative	To further assess the optical properties and diagnostic accuracy of caries detection using NIR fluorescence imaging with OS750 in-vitro and ex-vivo and to analyse the therapeutic efficacy of a bisphosphonate (Etidronate) in inhibiting enamel caries progression *in-vitro.*	Posterior (premolar and molar)	X	X
	Timme M. et al. (82)	Oral biology	To investigate the potential of modern magnetic resonance imaging technology to visualise the dental pulp in direct comparison with cone beam computed tomography.	Not mentioned, indicated for extraction due to medical reason	X	Ethanol (70%)
	Polesel A. et al. (83)	Dental material	To compare the quality of the apical seal of a two-carrier-based system, Soft Core and the guttacore Pink, by measuring apical dye leakage.	Single canal	X	Stored in a sealed glass container, dipped in normal saline (0.9%) up to their use.
	Colmenar D. et al. (84)	Dental material	To compare the removability of AH Plus	Anterior single canal	X	X

			and endosequence BC sealers using in-vitro micro-computed tomography.			
2021	Leontiev W. et al. (85)	Endodontic	To determine if magnetic resonance imaging is sufficiently accurate for detecting root canals using guided endodontics.	Incisors, canines, premolar	X	Thymol (0.1%)
	Hegde N. et al. (86)	Dental material	To evaluate the bond strength and microleakage of the newer bonding agents to enamel and dentin.	Mandibular premolar	autoclave	Distilled water at room temperature
	Bhat A. et al. (87)	Dental material	To measure the release of boron, strontium, and silicon ions from surface pre-reacted glass ionomer (S-PRG) filler containing prototype endodontic sealer over a sustained period in comparison to endosequence BC sealer in a simulated clinical model using extracted human teeth *in-vitro.*	Maxillary anterior single-rooted, without caries involving root	X	X
	Karacaoglu & Orhan (88)	Periodontic	To compare the effectiveness of three different instruments on the cement loss and roughness of the root surface following scaling and root planning using micro-computerized tomography.	Anterior (single-rooted incisor and canine teeth)	Sodium hypochlorite (5.25%) for 2 hours	Distilled water
	Yazdanpanahi N. et al. (89)	Dental material	Assessed the long-term pH alterations in the periradicular area following the application of calcium hydroxide and mineral trioxide aggregate intracanal medicaments.	Maxillary central incisors	Sodium hypochlorite (2%) for 30 minutes	X
	Chaves G. et al. (90)	Endodontic	To determine the volume of dental tissue removed during endodontic access preparation with cone-beam computed tomography comparing two different software.	premolars	Sodium Hypochlorite (5%) for 30 minutes	Thymol (2%)
	Topala F. et al. (91)	Dental material	To evaluate using c-scan en-face optical coherence tomography, the optical	Monoradicular single canal, 2 canals	X	X

			opacity and the distribution inside the root canal lumen of several extracted human teeth of silver and gold nanoparticles from special irrigating solutions used in endodontic treatment	maxillary premolar, 3 canals maxillary molar, 3 canals mandibular molar		
2021	Gottenbos B. et al. (92)	Dental material	To determine whether visible light enhances hydrogen peroxide mediated in tooth bleaching using photobleaching and photon-assisted oxidation technique	premolars	X	Moist closed jar at 4°C-placed in a 0.01M phosphate-buffered saline (PBS) the solution before use and between treatments.
	Abdulrahman M. (7)	Dental material	To determine the marginal sealing ability of composite laminate veneers when employing two types of veneer techniques, direct and direct-indirect veneers, as well as two types of composite resin, nanohybrid and microfilled composite resin restorations, using the dye penetration method.	Maxillary first premolar	X	Normal saline
	Abdul-Ameer Z. et al. (93)	Endodontic	To evaluate the effect of ultrasonic agitation for retrograde bioceramic root repair, MTA and biodentine filling materials on push-out bond strength to dentine walls.	Single-rooted maxillary incisors	autoclave	Thymol (0.2%)
2022	Uppalapati V. et al. (94)	Endodontic	To evaluate canal transportation of the different file systems in various combinations of the glide path by cone-beam computed tomography.	Single canal	X	X
	Raji Z. et al. (95)	Dental material	To assess the micro-shear bond strength of composite to deep dentin by using mild and ultra-mild universal adhesives.	3rd molars	Thymol solutions (0.1%)	X
	Binanzan & Alsalleeh (96)	Endodontic	To determine the cytokine expression by human gingival fibroblasts in response to different calcium hydroxide dilutions and test the effectiveness of these dilutions in root canal dentin infected with E. Faecalis.	Single-rooted	X	Normal saline
	Hoshika S.* et al. (33)	Dental material	To morphologically evaluate the interface between a conventional glass-ionomer cement and dentin one day after placement and the changes at the interface after one year of ageing/functioning in monkey teeth.	3rd molars (as control group in-vitro)	X	Distilled water

	Padmakumar I et al. (97)	Endodontic	To compare and evaluate the effects of different irrigating solutions using 5.25% sodium hypochlorite, ozonated olive oil, silver citrate, or distilled water on the chemical structure of root canal dentin in extracted human teeth.	Single-rooted mandibular premolar	X	Chloramine-T
	Shimada Y. el al. (98)	Conservative	To evaluate the diagnostic accuracy of 3D swept-source optical coherence tomography for enamel caries at smooth tooth surface if the lesion was with remineralisation.	Molars	X	Water at 4°C
	Altufayli M. et al. (35)	Endodontic	To compare the shaping ability of two heat-treated nickel-titanium single file systems using reciprocation motion and rotary motion in curved canals.	Curved root 25-26°C	X	X
2022	Arvelaiz C. et al. (99)	Dental material	To compare the bioceramic materials: MTA and EndoSequence BC root repair material (RRM)-fast set putty capacity to prevent microleakage of E. Faecalis over time.	Single-rooted, single canal	Sodium hypochlorite (5%) for 15 minutes	Phosphate-buffered solution (PBS)
	Tisler C. et al. (100)	Prosthodontic	To investigate the capacity of photodynamic therapy to increase the bond strength of full ceramic restorations.	Incisors, premolar and molar	X	Artificial saliva
	Blanchard A. et al. (101)	Orthodontic	To investigate the antibacterial efficacy and mechanical properties of experimental orthodontic adhesives containing newly synthesized antibacterial dimethacrylate monomers using a series of compound monomers: doubly charged 1,4-diazabicyclo[2.2.2]octane (DABCO) group (dication).	Mandibular incisors	X	Formaldehyde aqueous solution (10%)
	Vula V. et al. (102)	Dental material	To evaluate apical leakage after crown-down preparation and root canal obturation with Endomethasone N, glass ionomer cement, and endorse sealers.	Maxillary central incisor	Sodium hypochlorite (1%)	Normal saline
	Kreher D. et al. (103)	Conservative	To assess carious lesions on root surfaces using quantitative light-induced fluorescence and to compare the readings with axial lesion depth on microcomputed tomography (microCT or μCT).	All type	X	Sodium chloride (0.9%) for one week
2022	Shirur K. et al. (104)	Endodontic	To develop the nano-liposomal Chlorhexidine digluconate and to	Mandibular single-rooted premolar	X	Peroxide solutions

			characterise (size, zeta potential, polydispersity index, and surface topography) and evaluate them concerning the drug release study, cytotoxicity in L929 mouse fibroblasts, antibacterial efficacy against *Fusobacerium nucleatum, Staphylococcus aureus*, and *Streptococcus mutans*, and the depth of penetration into dentinal tubules.			
	Kolsuz M. et al. (105)	Endodontic	To evaluate the diagnostic accuracy of CBCT images of chemically induced external root resorptions on extracted human teeth taken in different voxel sizes.	Incisor teeth	X	X
	Matsuda Y. et al. (106)	Conservative	To evaluate the effectiveness of sealing materials in inhibiting demineralisation and increasing fluorine (F) uptake by acid-treated root surfaces.	3rd molars	X	X
	Engeler O. et al. (107)	Orthodontic	To evaluate the use of fluorescence inducing light to aid the clean-up of tooth surfaces after bracket removal when using buccal or lingual orthodontic appliances.	All type	X	Chloramine-T (0.5%)
	Ormiga F. et al. (108)	Endodontic	To obtain ionic quantification in periradicular medium after diffusion tests of the solution used Inside root canals during electrochemical dissolution of NiTi file fragments and the same solution containing the dissolution product via the apical foramen of extracted human teeth.	Single-rooted	Formalin 10%	Formalin 10%
	Maladkar S. et al. (109)	Conservative	To analyse the erosive potential of nine different acidic beverages: commercially available orange juice, lemon juice, pineapple juice, Coca-Cola, Mountain Dew, Red Bull, apple cider, vinegar, and tomato ketchup.	All type	X	X
2022	Jones N. et al. (110)	Conservative	To evaluate the *in-vitro* diagnostic accuracy of fluorescent cationic starch nanoparticles in detecting occlusal caries compared to histologic reference standards.	All type (sound tooth)	X	X
	Parirokh M. et al. (111)	Endodontic	To evaluate the effect of long service life on the accuracy of the Dentaport Root ZX	Single-rooted, single-canal	X	X

			electronic apex locator.			
	Zeng L. et al. (112)	Conservative	To investigate the effects of amino sugars N-acetylglucosamine (GlcNAc) metabolism on the genomics and biochemistry of a saliva-derived microbial community and the surface integrity of human teeth and restorative surfaces.	All types (anterior and posterior)	Sodium hypochlorite (10%)	X
	Al-Asady N. et al. (113)	Dental material	Evaluated the efficacy of a new gel containing carbopol, anionic polyacrylamide, APAM polyelectrolyte, and carbamide peroxide CP 16% in the whitening of discoloured teeth.	Not mentioned	X	Formaldehyde
2023	Jabbour Z. et al. (114)	Conservative	Compares the changes in the radiopacity of carious lesions after SDF application, potassium iodide application, and water rinse.	Any teeth with large carious lesions and no previous restoration	X	X
	Thienngern P. et al. (115)	Endodontic	To test the efficacy of chitosan paste against E. faecalis and Candida albicans biofilm microorganisms compared with calcium hydroxide [Ca(OH)2] in root canals of extracted human teeth.	Premolars and straight single-canal maxillary incisors	X	Thymol (0.1%)
	Albar&Khayat (116)	Dental material	To compare the fracture strength of direct non-reinforced class II composite resin restorations and polyethene fibre-reinforced restorations and also to investigate the influence of the locations of polyethene fibres within the cavity on the fracture strength	Posterior (premolar and molar)	Sodium hypochlorite (0.5%) for 15minutes	Distilled water
	Bhavan Ram U. et al. (117)	Dental material	To evaluate the dentinal tubule occlusion, depth of penetration, and dentin permeability of oyster shell-derived nanohydroxyapatite with and without 15% proanthocyanidin pretreatment.	Molar	X	Thymol (0.1%)
2023	Potewiratnanond P. et al. (118)	Prosthodontic	To compare the surface wear rate between polymethyl methacrylate-based occlusal splints and opposing dentin-exposed teeth in bruxism-simulating models.	Premolar	X	X
	Hassan & Roshdy (119)	Endodontic	To evaluate the effect of various irrigation protocols on the penetration depth of a calcium silicate–based sealer into dentinal tubules using confocal laser scanning	Single-rooted premolar	X	Normal saline containing 0.1% sodium azide

		microscopy.			
Qutieshat A. et al. (120)	Prosthodontic	To demonstrate the potential of ostrich eggshell compared to human enamel in evaluating the efficacy of a preventive agent in protecting against dental erosion, using an artificial mouth model.	Molar	X	X
Gündüz & Özlek(121)	Endodontic	To evaluate the smear and debris removal efficiency of laser and ultrasonic irrigation activation methods in traditional and conservative endodontic access cavity preparations.	Mandibular molar	X	X
Kalaoglu E et al. (122)	Endodontic	To compare the three biomaterials used in the apexification treatment of immature molar teeth in terms of the time spent, the quality of the canal filling and the number of X-rays taken to complete the process.	Molar	Sodium hypochlorite (5.25%	Distilled water
Ahmed B. et al. (10)	Regenerative therapy	To study the odontogenic potential of dental pulp stem cells (DPSCs) after induction with three different bioactive materials: activa bioactive (base/liner), theracal LC, and mineral trioxide aggregate (MTA), when combined with two different types of scaffolds	Not mentioned	X	stored in sterile falcon tubes containing storage medium composed of Dulbecco's phosphate-buffered saline
Todorova M. et al. (123)	Endodontic	To investigate the temperature changes on the outer root surface of extracted human teeth during irrigation with different volumes of solutions at different temperatures.	premolar	Sodium hypochlorite (5%) for 30mnts	Distilled water
Natsir N. et al. (124)	Dental material	To evaluate the effectiveness of moringa (Moringa oleifera) leaves decoction for removing a smear layer compared to sodium hypochlorite and ethylenediaminetetraacetic acid and its antimicrobial activities against *E faecalis.*	Single-rooted premolar	Ethanol (70%) for 1 hour	Normal saline
Laky M. et al. (125)	Dental material	To evaluate the in-vitro efficacy of a carbon dioxide (CO2) laser, a tetracalcium phosphate/dicalcium phosphate anhydrate desensitiser and the combination of the desensitiser and additional CO2 laser irradiation as a treatment modality for cervical dentin	Posterior (premolar and molar)	Citric acid solution (1%) for 30sc	X

			hypersensitivity			
2023	Piesiak-Panczyszyn D. et al. (126)	Dental material	To evaluate the dynamics of in-vitro fluoride ion release from first- (Duraphat) and second-generation (MI Varnish and Embrace Varnish) fluoride varnishes and the impact of the type of varnish, the time from its application and the pH of the environment on this process.	Not mentioned	X	X
	Snigdha N. et al. (8)	Dental material	To compare the sealing ability and marginal adaptation of three calcium silicate-based cement (Biodentine, Pro root MTA, MTA Angelus) using a bacterial leakage model and scanning electron microscope.	Single-rooted mandibular first premolar	sodium hypochlorite (2.5%) for 24 hours	X
	Sultan & Jayash* (29)	Regenerative therapy	To assess the cytotoxicity and osteogenic potential of demineralized dentin matrix compared to hydroxyapatite (HA) nanoparticles on bone marrow mesenchymal stem cells using a hydrogel formulation.	premolar	X	Subjected to infection control standards approved by their ethical committee
	Monteiro L. et al. (127)	Endodontic	To evaluate the effectiveness of passive ultrasonic irrigation compared to mechanical activation with Easy Clean in removing organic tissue from simulated areas of internal root resorption.	Single-rooted	X	X
	Shaaban S. et al. (128)	Oral biology	To assess the antibacterial activity of multi-strain probiotics supernatants, L. plantarum, L. rhamnosus, and L. acidophilus as an intracanal medication on *E. faecalis* biofilm in a tooth model.	Single-rooted, single canal	Sodium hypochlorite 5.25% for 30mnts	X
	Hurtado A. et al. (129)	Dental material	To evaluate the microtensile bond strength and nanoleakage immediately and after 6-month ageing, and in situ degree of conversion of two universal adhesives applied with etch-and-rinse and self-etch strategies, in comparison with a two-step self-etch adhesive.	3rd molar	X	Thymol (0.1%) at 4°C
	Chang N. et al. (130)	Conservative	To employ the methods of using shortwave-infrared and thermal imaging during dehydration with forced air to measure the rate of fluid loss from lesions during dehydration with forced air to	Not mentioned	Gamma radiation	Thymol (0.1%)

			assess lesion activity.			
2023	Baraba A. et al. (131)	Endodontic	To investigate the effectiveness of Shock Wave-Enhanced Emission Photoacoustic Streaming in removing epoxy-resin-based and calcium-silicate-containing sealers when combined with single-cone and core-carrier techniques.	Single-rooted, single-canal	X	X
	El-Farag S. et al. (6)	Prosthodontic	To assess and compare the impact of various computer-aided design/manufacturing materials on internal and marginal discrepancies, fracture resistance, and failure probability of Endocrown restorations with 3D Finite Element analysis.	Maxillary first molar	Sodium hypochlorite (5.25%)	Distilled water
	Dong X. et al. (132)	Dental material	To evaluate the sealing ability of combined application of iroot BP Plus Root Repair Material and iroot SP Injectable Root Canal Sealer for root-end filling.	Maxillary 2nd premolar	X	X
	Marković L. et al. (133)	Endodontic	To determine the influence of preparation techniques on marginal adaptation and sealing of Biodentine™ and totalfill® RRM bioceramic retrograde fillings.	Single-rooted	X	X

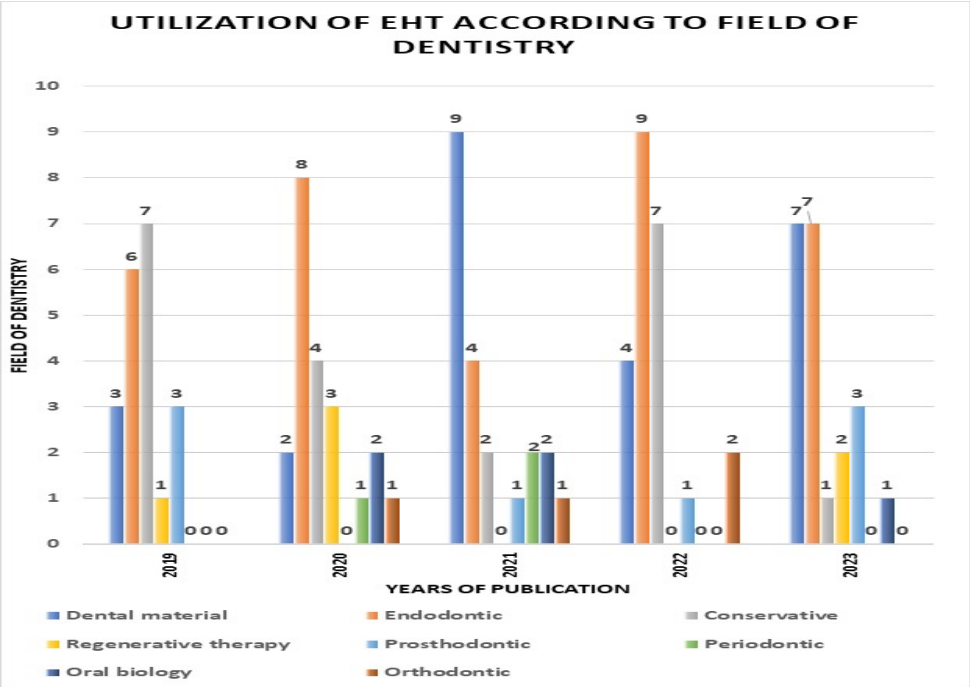

Figure 2: Utilisation of EHT according to the field of dentistry

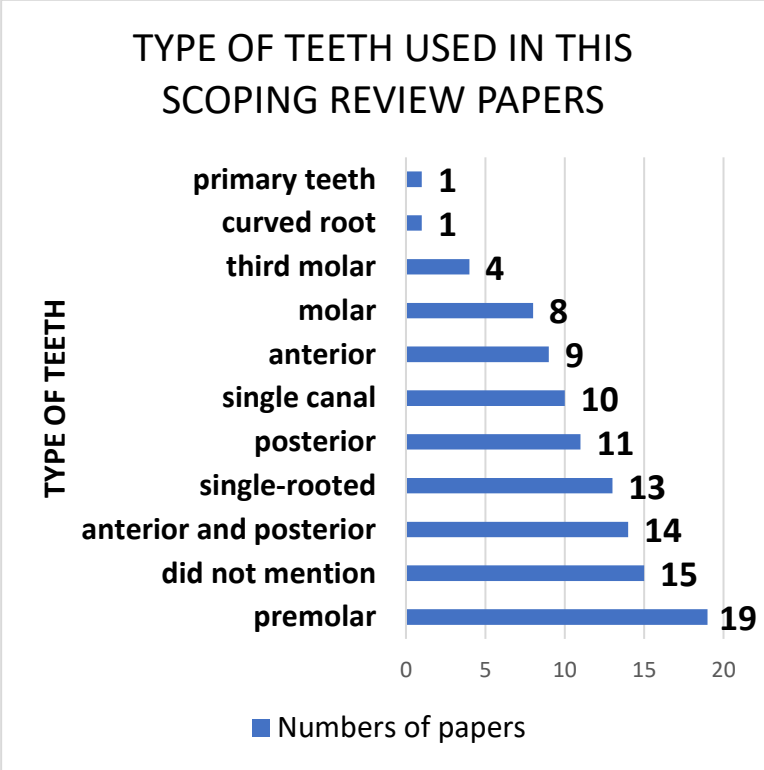

Figure 3: Type of teeth used in this scoping review papers

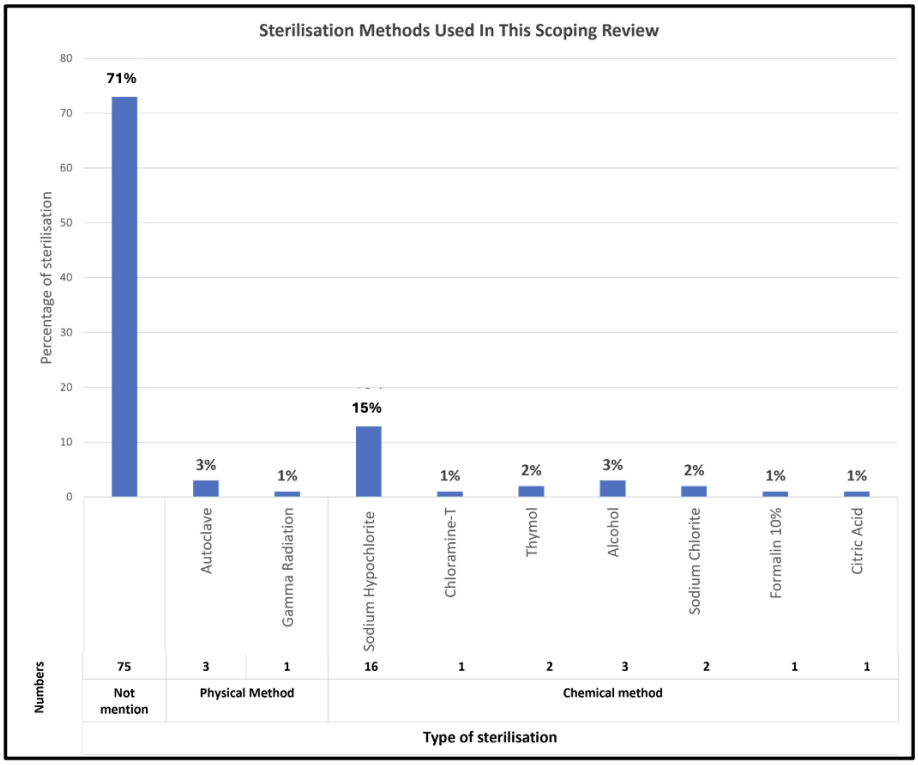

Figure 4: Percentage of sterilising methods used in this scoping review

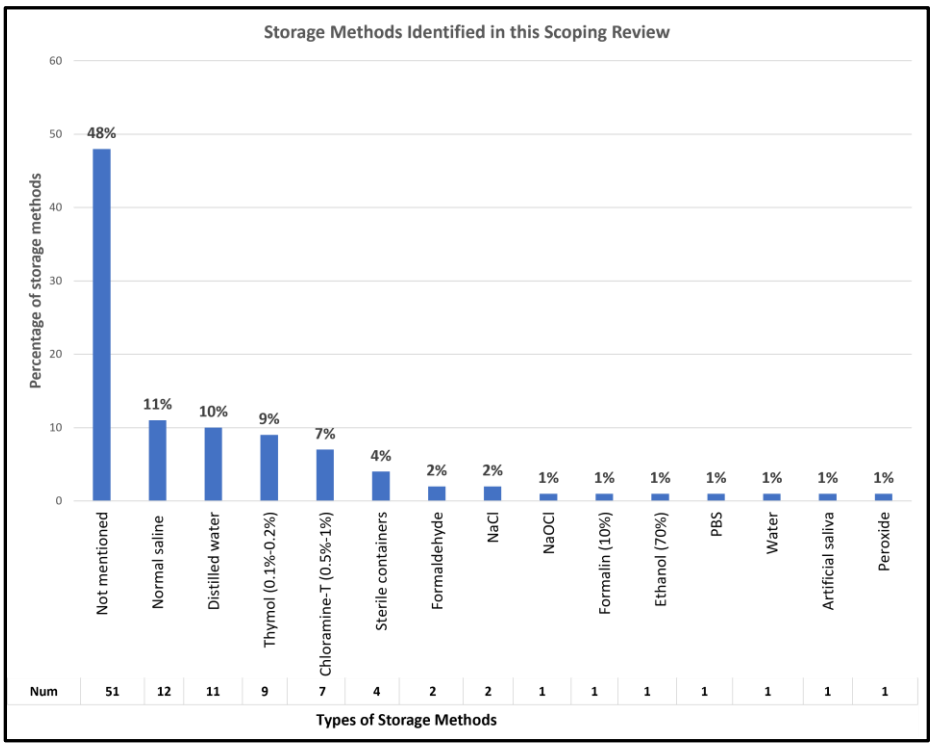

Figure 5: Percentage of storage methods used in this scoping review

Discussion

i) Use of EHT in dental research.

This study aims to assess the contemporary relevance of extracted human teeth (EHT) in dental research. Data on the type of tooth utilised, the field of research, and the approach for sterilising and storing the EHT were analysed in this study. Only studies employing an *in-vitro* design, published from January 2019 to October 2023, written in English with full paper access, were reviewed.

A fairly consistent number of *in-vitro* studies using EHT is published yearly over the five-year span (2019-2023), ranging from 20 to 23 papers per year. Most of the studies using EHT are in the field of endodontics, conservative dentistry, and dental materials. Natural teeth are invaluable in endodontic research due to its unique and complex root canal anatomy that closely mimics the clinical setting. Its use allows researchers to investigate various aspects of endodontics, including instrumentation techniques, obturation materials, and treatment outcomes (30, 34–38). Similarly, natural teeth serve as a realistic and clinically relevant model for analysing the durability, biocompatibility, and aesthetics of various restorative materials in conservative dentistry and dental materials research. These include tests on bond strength (25–29), fracture strength of newly developed composite resin (30), microleakage (31–33), marginal seal (34–36), and apical seal (37, 38). In endodontics, studies were related to newly developed intracanal medicament and improved cements for obturation (39–48).

A recent study conducted by a research team at Hong Kong University introduced a novel dentin analogue material designed for laboratory-based mechanical and fatigue tests (39). Although the initial results appear promising, the researchers acknowledged that dentin analogues may not fully replicate the intricate microstructure and bonding mechanisms found in natural dentin. Consequently, until a more precise tooth analogue is developed, EHT will continue to play a significant role in various dental research.

In recent years, the scope for the application of EHT has further diversified. Driven by the rapid advancements in biomedical engineering, EHT is utilised for regenerative dental research including stem cells and bone regeneration. One example is breakthrough research centred on the development of new cementum and periodontal complex tissues that can potentially reduce patients' tooth movement (49). Achieving these outcomes necessitates the use of natural teeth and its adjacent tissues. In the area of bone regeneration, research has demonstrated that EHT can be employed to create a demineralised dentin matrix hydrogel (DDMH). Importantly, this material has been found to be non-toxic to bone marrow mesenchymal stem cells (BMMSCs) and possesses osteogenic potential. This suggests that DDMH from EHT has the ability to promote the growth of new bone (50). As the boundaries of dental research continue to expand, the role of EHT remains pivotal in enhancing our understanding of dental sciences.

Type of teeth used in in-vitro studies

Various types of teeth were used in this study. This review revealed that premolar teeth were the most commonly used natural tooth for *in-vitro* studies, likely due to their accessibility, often being extracted before orthodontic treatment. This was consistent with research conducted at the Universidade Estadual do Rio de Janeiro Orthodontic Clinic, which found that over a 31-year period (1980 to 2011), first premolars were the most commonly extracted (40). As most research centres are often located in dental teaching institutions, extracted premolar teeth for orthodontic reasons are readily accessible and would fit the general requirement for selection of EHT for research, which is sound tooth (not diseased).

All the reviewed studies imposed specific inclusion criteria for EHT, which were tailored to their research objectives. For instance, studies exploring caries detection (65) or potential biofilm-inhibiting materials for caries prevention (66) typically necessitated the use of carious EHT or sound EHT on which carious lesion will be simulated. In endodontic research, teeth samples with a specified degree of root curvature are often chosen as it results in narrow root canals that are well-suited for testing new rotary files (19). Assessing the flexibility of the newly developed rotary instrument on curved canals is crucial to prevent common clinical issues such as instrument separation or canal transportation.

The preponderance of using a particular type of tooth, in this instance, the premolar tooth, may limit the applications of the study results. Generally, human teeth exhibit natural variability in anatomy,

morphology, and mineralisation. Using specific types of extracted teeth may introduce biases, as these variations might not fully represent the diversity present in living populations.

ii) Sterilizing and storage methods of EHT

A significant portion (71%, 74 studies) of the reviewed papers did not provide any information regarding the sterilisation methods for the EHT or whether sterilisation had been carried out. Additionally, 51 studies (47%) failed to state the type of storage medium employed to maintain tooth integrity and prevent dehydration.

The Centers for Disease Control and Prevention (CDC) have long recommended two EHT sterilisation methods: immersion in a 10% formalin solution or autoclaving at 121°C for 40 minutes (67). However, among the 74 studies that discussed EHT sterilisation as part of their study protocol, only three papers favoured autoclaving (28, 57, 68), and only one paper immersed the EHT in a 10% formalin solution (55). Gamma radiation was used in one study (69), although this method is less common as it is generally considered impractical, expensive, and time-consuming for sterilising EHT (70) and is primarily employed in the packaged food industry. The two sterilisation methods recommended by the CDC are not without any drawbacks. Autoclaving has been found to significantly reduce dentin microhardness (71), which could potentially affect the outcome of any research that relies on the mechanical properties of teeth.

The high pressure and temperature (121°C for 40 minutes) used during autoclaving may cause denaturation of the organic dentin component (41) negatively impacting the tooth's mechanical properties. For example, autoclaving has been shown to cause a significant reduction in the microhardness of dentin (41), elastic modulus and, to a varying degree, microleakage (42). Therefore, changes in the properties of autoclaved EHT may impact the findings from preliminary research, including bonding, shear strength, compressive strength, hardness, and fracture toughness. Formalin is known for its cytotoxic and genotoxic properties and potential risks to the skin, eyes, and respiratory system (67). Although numerous studies have shown that 10% formalin is highly effective at sterilising all samples (19, 20, 43–47), it is crucial to keep in mind that this chemical has significant hazards and should be handled with extreme caution. Therefore, it should only be opened under a fume hood and kept in firmly closed containers. Researchers handling formalin solutions must employ comprehensive personal protective equipment and fume hoods to mitigate potential health hazards during sterilisation (10, 72, 73).

In this review, the predominant chemical sterilising method used for disinfecting EHT is sodium hypochlorite solution, with a variable range of concentrations. Concentrations from as low as 0.5% (30), 1% (38), 2% (42), 2.5% (35), 5.25% (25, 43, 62, 74-77) to the highest concentration of 10% (78) has been reported. The most commonly employed sodium hypochlorite solution is at 5.25% concentration. The widespread use of sodium hypochlorite (NaOCl) can be attributed to its accessibility, ease of use, and cost-effectiveness. However, it is essential to note that sodium hypochlorite is a highly alkaline solution with a pH exceeding 11 and reacts chemically with organic tissues (79). Consequently, the likelihood of inducing structural changes in EHT, including a reduction in root dentin mechanical strength, increases with higher concentrations of this solution (80). Alterations in the chemical and mechanical properties of teeth through these sterilising procedures can compromise the accuracy of research findings. This review highlights the absence of standardised sterilisation solutions, with reported options including alcohol, 0.1%-0.2% thymol, sodium chlorite, 0.5% chloramine-T, 10% formalin, and 1% citric acid. Furthermore, the concentration chosen for a particular sterilising solution varies between different investigators.

In addition to sterilisation, proper storage is crucial for preserving the properties of EHT until they are prepared for the experimental study (81). Selecting a suitable storage medium is paramount to maintain tooth integrity (82, 83). Within the scope of this review, the most frequently reported storage solutions are normal saline, distilled water, and thymol solution. It is worth noting that three studies employed unique storage containers without offering a clear rationale for their choice. These unconventional methods included storing extracted human teeth in sterile falcon tubes with Dulbecco's phosphate-buffered saline (84), using sealed glass containers immersed in normal saline (0.9%) (37), and opting for a sealed, humid container for storage (84). In a previous study, it was observed that

sodium azide promoted loss of tooth structure from enamel specimens in the time interval of 15 days and hardened these structures after 30 days of storage (48). Using 0.1% thymol and distilled water as storage solution promoted significant loss of enamel structure (48, 49).

There is currently no consensus or set of guidelines concerning handling EHT prior to usage. Several reasons could account for this. Firstly, research objectives and the intended use of EHT can vary widely, leading to the adoption of different sterilisation methods and concentrations tailored to specific study requirements. Additionally, the availability and accessibility of sterilisation agents, as well as institutional preferences, play a role in the lack of uniformity. Lastly, the absence of comprehensive guidelines or widely accepted best practices has perpetuated this variability, leaving researchers to make individual decisions based on their needs and available resources. This diversity highlights the need for EHT sterilisation and storage standardisation to ensure both scientific rigour and the preservation of tooth properties. The issue of standardising the sterilising and storage protocol of EHT used for dental research is crucial because variability in storage methods across different studies may introduce confounding factors, affecting the validity of comparisons.

This study also highlights the need for a standardised guideline for reporting studies utilising EHT. Particularly, in regard to the tooth type used and the justification for inclusion, sterilisation (material, concentration, duration) and storage (material and duration) methods employed, which improves study reproducibility. This consistency allows for meaningful comparisons between different studies, promoting a more robust and reliable body of scientific knowledge. Furthermore, standardised guidelines contribute to quality control by providing a framework for researchers to follow. This ensures that the sterilisation and storage processes are conducted in a controlled and systematic manner, reducing the risk of procedural errors that could compromise the integrity of the study.

Given the lack of ideal sterilising and storage solutions currently available, to ensure reproducibility and minimise confounding factors in research using EHT, researchers from various institutions should try to adhere closely to the recommendations by CDC until alternative methods which can sterilise teeth without affecting their properties are developed. Further research is necessary to ascertain the sterilising solutions' optimal concentrations and immersion times and their efficacy in preventing microbial growth without causing structural changes to the tooth.

In this paper, the scoping review method is used to document information available in the literature regarding the current uses of EHT in dental research and to identify sterilisation and storage protocols. While scoping reviews are useful for mapping the breadth of current literature on a given topic, they frequently lack the depth of analysis found in systematic reviews. Furthermore, relying on a broader search method might include studies with varying quality, compromising the synthesis. Nonetheless, the scoping review is an appropriate strategy for this topic because as provides a comprehensive overview of existing literature, helping researchers identify existing trends, research areas and current protocols related to EHT use.

Conclusion

In conclusion, EHT maintains its significance in dental research, as evidenced by the sustained annual publication rate from diverse research fields observed during this study.

Secondly, there is a notable absence of a prevailing standard or established protocol for sterilising and storing EHT used in dental research, highlighting the lack of consensus in this critical area. Moreover, there is poor reporting regarding tooth selection criteria, sterilisation and storage protocol. Lastly, a variety of teeth are used depending on the research objectives and protocol. Premolars are the most commonly utilised teeth for dental research, probably because they are the most frequently extracted sound teeth.

The findings of this review call for two urgent actions. Firstly, a standardised reporting guideline for research using EHT should be drafted, particularly with regard to the tooth type and the justification for selection, sterilisation (material, concentration, duration) and storage (material and duration) methods employed. Secondly, a standard protocol for EHT sterilisation and storage tailored for specific laboratory investigations should be prepared. This will ensure consistency and reproducibility of the research and allow for

comparison of research findings across different studies, advancing the collective knowledge base in dental research.

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Competing interests

The authors declare that they have no competing interests.

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