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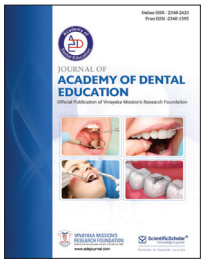

Review Article

Tooth as a vital source of DNA in forensic odontology: Recent perspective

Afziya Waseem Qadri[1], Shefali Yadav[2], Anshi Jain[3], Devi Charan Shetty[4], Nikita Gulati[5]

[1]Bachelor of Dental Surgery Final Year, I.T.S Centre for Dental Studies and Research, [2]Senior Lecturer, [3]Professor, [4]Director- Principal, Professor and Head, [5]Reader, Department of Oral Pathology, I.T.S Centre for Dental Studies and Research, Ghaziabad, Uttar Pradesh India.

***Corresponding author:**
Shefali Yadav,
Senior Lecturer, Department of Oral Pathology, I.T.S Centre for Dental Studies and Research, Ghaziabad, Uttar Pradesh, India.

shefu2593@gmail.com

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ABSTRACT

DNA has been the hereditary material of almost all organisms, including humans. In recent years, DNA fingerprinting technique has been the most renowned method used in forensics for human identification. Extracting DNA from routinely used samples becomes very challenging in degraded and fragmented human remains, i.e., in cases of incineration, mutilation, and fire. Due to the unique placement of teeth in the jaw and their composition, DNA material present gets additional protection compared to bones or other sources of DNA. Hence, it frequently becomes the only source of DNA that would be available in such situations for identification. DNA fingerprinting helps unravel the mysteries within the mouth, including the manifestations of diseases in the oral cavity. There have been highly improved methods described for analyzing various forensic scenarios and DNA extractions. Identifying the deceased victims, the missing person, the masked culprits involved in crime, the paternity issues, interpretation of genetic diseases, and determining the ancestry account for some uses of DNA fingerprinting. This review briefly summarizes recent literature and current knowledge of forensic odontology, DNA content, and distribution in teeth and its preservation. This also intends to emphasize the role of the tooth being a valuable source of DNA and thereby discusses some of the methods of recovery of DNA from teeth, ways of DNA analysis, and highlights the benefits and challenges regarding the DNA extraction protocol in the field of forensic odontology.

Keywords: Human identification, DNA fingerprinting, Forensic odontology, Short tandem repeats typing DNA extraction, Dental identification

INTRODUCTION

Molecular biology came into existence with the realization that it is the DNA that is behind all the cellular processes of any organism. DNA is the genetic material in all the cells of human body, and 99.9% of the sequences of DNA are always similar, while only the remaining 0.1% of DNA sequences are distinctive to each individual.[1] With this minute amount of uniqueness, still only a probability of 1 in 594 trillion people other than identical twins is noted to have the similar DNA profiles.[2] It was in the year 1953 that Watson and Crick discovered the DNA structure to be a double helical in form. "DNA fingerprinting" is the result of technological advances that have led to analyzing processes at the molecular level and made available powerful ways of isolating, manipulating, and analyzing nucleic acids. DNA fingerprinting, therefore, is the process by which distinguishing the individual's genetic characteristics is possible.[3]

Human identification is a multistep complex process that is dealt with not only by a single person but also by a multidisciplinary team which includes doctors, biologists, odontologists,

anthropologists, and even an investigative team.[1] The main role of forensic dentistry in human identification lies in mass disasters such as tsunamis, earthquakes, fire, decomposing bodies, and explosions.[2] Dental tissues are unsusceptible to incineration, trauma, immersion, and mutilation by virtue of which these tissues (teeth and bones) represent as excellent sources of DNA and sometimes become the only reservoir of DNA available for use. Dental comparisons have been commonly used for forensic investigations with a high success rate of identification.[4]

The DNA is of two types – (1) Genomic DNA found in the nucleus. (2) Mitochondrial DNA (mtDNA).[5] Genomic DNA is commonly used in DNA fingerprinting, while in cases of degraded or inadequate samples, mtDNA is analyzed. The dental DNA not only acts as a source of DNA samples for identification, but it also helps in age estimation, sex determination, blood grouping as well as interpretation of bite-mark injuries.

BASIS OF DNA FINGERPRINTING

DNA fingerprinting is the encrypted set of numbers that depict an individual's genetic makeup and can be used as an identifier.[6] Any DNA segment that code for protein is called a GENE. In total cellular DNA, only 2–5% accounts for coding gene, whereas the rest 95% is Junk DNA (non-coding). The function of this Junk DNA is not known and it might be present as "Spacer DNA" or as "Repetitive DNA." Out of these, the repetitive DNA is polymorphic in nature and unique to each individual.[7] The variation of mini satellite patterns and the stable inheritance in Mendelian patterns is the base for DNA fingerprinting. These polymorphisms may be used to both differentiate and correlate individuals.[8]

DENTAL IDENTIFICATION

Sometimes, due to certain circumstances of death, dental identification becomes the most general role of forensic odontologists. The deceased in forensic is usually detected by non-dental methods of visual identification or fingerprint detection, but when these techniques are not possible due to factors such as disfigurement, decomposition, and degradation, this may leave the body unrecognizable.[9]

Nowadays, there are multiple techniques available for the identification of an individual by dental means. These include – (i) comparative dental identification; (ii) reconstructive postmortem; (iii) dental profiling, and (iv) DNA profiling.[10] Table 1 discuss the disadvantages of the comparative dental identification technique. Furthermore, in cases of unknown bite marks, DNA analysis is done to differentiate human and animal bites, as the DNA will show the species differentiations. Saliva obtained from these bites will provide an adequate amount of DNA if isolated immediately.

Table 1: Disadvantages of comparative dental identification.

The discrepancy, In case the person has had any additional dental treatment between the antemortem and postmortem dental records
Antemortem records of poor quality
Inability to obtain desirable antemortem records
A diseased person from a different area
Patient treated in an emergency with no record
A huge variety of tooth nomenclature has been noted in the different regions of the globe

DISTRIBUTION OF DNA IN TEETH

For proper and optimal sampling, a detailed and comprehensive knowledge of tooth diversity and morphology is required as well as the distribution of DNA in various teeth must be known. This will help us select teeth from the alveolar bone accurately and the subsampling will be targeted. Human teeth are grossly of two components – crown, the exposed part and root, part inside the alveolar bone. The roots, composed of dentin-pulp complex and the cementum, have proven to give in a greater amount of DNA compared to the coronal portion which is predominantly composed of only enamel.[11-13] DNA from crown is 10 times less than that extracted from the root in cases of teeth with pulp present in it.[13]

Enamel

It covers the tooth crown and is the hardest tissue in humans. Being 96% mineral, acellular, and contains no DNA.[14] It acts as a barrier to protect the cells within the tooth. It has restricted permeability. Furthermore, the pore size between crystals is minute, thus blocking the entry of molecules larger than water.[15] Hence, it restricts any impurity into the tooth both in life and in the deceased.

Dentin

Normally, no nucleated cells are present, but odontoblastic cells get sometimes entrapped while the formation of tertiary dentin.[16] Dentin is densely formed by dentinal tubules – odontoblastic processes and nerve fibers. These contain mitochondria along their lengths, thus making these tubules a rich source of mtDNA.[17] Corte-Real *et al*.[18] in a study sampled 10 endodontically treated teeth and reported sufficient DNA yields for short tandem repeat (STR) profiling from 8 out of 10 teeth. They concluded that the DNA collected was obtained from dentin. Reparative dentin with cellular inclusion and dentin with odontoblastic cells imbibe into tubules with the help of hydraulic pressure, hence becoming a good source of DNA.[19]

Dentin-pulp complex

Makes up the major portion of the tooth and is highly cellular. Pulp is highly innervated and vascular-containing

numerous cells.[20] These make pulp a treasurable source of DNA. This complex connects with the periodontal tissue through the accessory canals and the apex from where the blood vessels travel.[19] Hence, pulp is the richest reservoir of DNA. However, in aged or diseased teeth, the pulp becomes limited. The dental pulp also contains the blood group antigen that may be used in forensic analysis.

Cementum

Avascular mineralized tissue covering the root of teeth. Cementum contains inorganic minerals (45–50%) – hydroxyapatite, collagen, and non-collagenous matrix. The cellular cementum has the cementocytes, hence becoming a rich source of DNA.[21] It continuously increases in thickness throughout life. This cementum is present mostly in the apical portion of roots and furcation areas in molars, while it may be absent in anterior.[22,23] Other sources of DNA here are soft-tissue inclusion, blood residue, and vessels traversing in accessory canals.

To summarize, both the pulp and the cementum are important parts in terms of being sources of nuclear DNA present inside the tooth, and dentin, along with these two, are the best sources of mtDNA. Enamel which is devoid of DNA is very important in terms of protection of dentin as well as pulp. In case enamel is sampled with these tissues, it will lead to a dilution effect, and minerals present in enamel would complicate the process of extraction, hence inhibiting the polymerase chain reaction (PCR) amplifications. Studies on a large scale have reported high success rates in DNA typing using teeth.[24-26] However, proper selection of teeth and subsampling before DNA extraction play a major role in improving DNA profiling success rates.

Sex determination can also be performed using the amelogenin gene (AMEL) gene present in enamel. Age estimation may be performed by accounting the telomere shortening in the DNA extracted from teeth.[27-29]

FACTORS AFFECTING DNA CONTENT

The DNA in teeth varies among the individuals as well as within the teeth of the same individual.[11-13] The factors that affect the DNA content include the following:

Tooth type

The best source of DNA extraction can be one with the largest pulp volume[30,31] additionally, multirooted teeth also provide a larger pulp, thus providing more cementum due to more pulp cells and hence a good source of DNA. Therefore, tooth selection for DNA extraction should be preferably started with molars that have a large pulp and are multi-rooted, in case of the absence of molars, premolars should have been considered, but canines have larger pulp volumes than premolars.[19] While selecting a tooth, it should be made sure that the tooth to be used remains in the socket so that the chances of contaminations are taken care of, hence making multirooted teeth the best choice available.[32]

Chronological age

With the increasing age of an individual multiple changes occur that causes change to DNA content in teeth. Most noticeable and negative change is decrease in the pulp volume due to continuous dentin deposition. It also decreases in cellularity.[33,34] Even after the occlusion of the dentinal tubules due to sclerotic dentin and tubular degeneration, some mtDNA remains entrapped in dentin.[17] Advancing age hence leads to a decrease in DNA content, and this is because of enamel becoming heavily mineralized over time even though it is lost due to regular wear and tear or attrition.[35] Furthermore, dentin notices a reduction in porosity due to occluding dentinal tubules. Factors such as extent of tooth wear, attrition, and abundance of cementum should be taken into consideration while selecting the sample.

Dental disease

These have an ill effect on the DNA content. Diseases such as caries or microbial diseases lead to dissolution as well as the destruction of calcified structure in teeth, which facilitates the entry of bacteria into the pulp.[36] In response, tertiary dentin deposition starts. This tertiary dentin has a less organized structure, and as the deposition is rapid, the odontoblastic cell bodies become encapsulated in mineralized tissue.[37] Caries might cause complete loss of pulp, whereas the cellular cementum is affected only if it is exposed in the oral cavity. As the cementum receives the nutrition and blood supply from periodontal tissue, not the pulp, it becomes an important source of DNA recovery in pulpless teeth.[38] Cementum gets exposed to oral cavity in case of periodontal diseases or periodontitis.[39] This indeed leads to the reduction of the availability of DNA when there is cementum loss. Dental disease not only decreases the content of DNA but also increases its potential for contamination. The factors that affect the availability of target DNA depends on various factors as listed in [Table 2].[40]

POST-MORTEM DEGRADATION

The degradation of the DNA in dental tissues is Time-dependent. However, this is affected majorly by different environmental factors.[31,41] Post-mortem degradation commences with the help of intracellular endogenous enzymes such as proteases, lipases and nucleases and is further carried on by the exogenous enzymes produced by microbial invasion.[42] The teeth, being less porous and well protected, stop the action of

Table 2: Variables affecting presence of DNA in a tooth.

Type of teeth present (incisor, canine, premolar, molar)
Condition of dentition before extraction or the degree of decay in teeth
Condition of teeth following trauma
Period from extraction to DNA isolation
Age of the individual

these organisms, but the earlier enzyme activity and process of spontaneous hydrolysis still takes place.

Rubio *et al*.[41] studies that dental tissues stored at normal room temperature for a period of 1 month–18 months have shown a halved decline in the DNA in 1st month, followed by a period of stability till 18 months. Further, at 18-month, reduction is noted, and the average yield of DNA from 18 month group was 10% of those from the fresh dental tissues. Rubio *et al*.[41] hence presumed that the stable period was because of the protection of a fraction of DNA till the structural changes occurred.

Schwartz *et al*.[40] isolated the HMW DNA from teeth at a temperature of 4°C for up to 6 weeks. At 25°C, this DNA can be isolated even 19 years later. Teeth yields DNA at 37°C following the storage period of 6 months.

Boles *et al*.[43] profitably extracted DNA from dental tissues buried over 80 years. This made it possible distinguish any deceased from other individuals with just 1 mg or lesser target DNA, whereas the DNA that is retrieved from molar tooth with a pulp volume of 0.023–0.031 is approximately 15–20 mg

Pötsch *et al*.[44] conducted a study that stated that the total manufacture of the genomic DNA through a dental sample can range from 6 mg to 50 mg. The result showed no difference in comparison between DNA from dental pulp and DNA obtained from usual blood samples and lung tissues.

Studies investigating the post-mortem tenacity of the dental pulp concluded that the pulp decomposes at slower rates as compared to other soft tissues. This is credited to its preservation from exogenous organisms by the enamel. Pulp stays biologically viable for over 12 h post-mortem and displays viable cells even 24 h after death.[45,46]

The perseverance of pulp in tooth subjects to higher temperature of up to 100°C has also been studied.[32] This resistance of pulp is underlined in a case study where an unerupted third molar from an incinerated body was taken, and it yielded nuclear DNA of enough quantity to facilitate identification and produce an STR profile.[47]

These studies and other literatures conclude that the period since death and the circumstances under which the body decomposes together account for DNA analysis and its success rate. A brief post-mortem interval as well as a dry environment, will favor the sustenance of DNA inside the pulp, whereas a longer post-mortem interval along with a wet environment will increase the dependency on hard tissue for DNA. The dry environment hence invigorates the desiccation of the pulp and protects DNA from any hydrolytic damages, while on contrary, the wet environment allows the decomposition of pulp completely.

DNA SAMPLING AND DECONTAMINATION TECHNIQUES

Selection depends on availability, logistical as well as the environmental factors. Canines and the molars are commonly used for DNA extraction. Studies have noted recovery of around 6 μg–50 μg of DNA from one tooth.[19] Before the DNA extraction, it is important to consider the potential for contamination. Although the porosities in the teeth make them highly resistant toward contamination, the contaminants from environment and the microorganisms may produce negative impact on DNA extraction, amplification, and analysis.[19]

Before DNA extraction, the tooth is decontaminated with sodium hypochlorite (0.5%) and washed with the distilled water. It is allowed to dry without tempering the temperature and then put-through the ultraviolet light for 1 h.[48] For an intact tooth recently extracted from alveolus, a regular endodontic access opening is done along with instrumentation. Pulp chamber can be curetted and the pulp collected in a sterile tube. Finally, tooth crushing may also be required so on instrumentation; the pulp chamber may be irrigated with a buffer.[49] There are further multiple methods and approaches to retrieve the DNA sample from the sterilized tooth. The Table 3 lists the various approaches for sampling dental pulp.

DNA ISOLATION METHODS

DNA fingerprinting is a multistep process involving the withdrawal of pulp, DNA isolation accompanied by DNA Analysis. Table 4 lists the different methods of evaluating the types of DNA polymorphisms.[50,51]

The different methods for DNA isolation/profiling are:

Restriction fragment length polymorphism (RELP) analysis

Most earlier method of DNA analysis. DNA collected from the tooth is treated with the restriction enzymes – "Restriction endonucleases," these enzymes divide the DNA at specific locations – recognition sites.[52] These cut segments are then separated by gel electrophoresis, and the DNA pattern obtained is transferred to nylon membrane using "Southern blotting." The DNA probes prepared are added,

Table 3: Approaches to sampling DNA from dental pulp.

Crushing entire tooth
Vertical split of entire tooth
Conventional endodontic access
Cryogenic grinding
Horizontal sectioning
Orthograde entrance technique

Table 4: Methods of evaluating different types of DNA polymorphisms.

Hybridization-based technique
• RFLP
PCR-based assays
• Manual genotyping and detection with staining
• Fluorescent non-anchored inter simple sequence repeat
• STR typing
• Time-of-flight mass spectrometry
• Automated STR genotyping
• Random amplification of polymorphic DNA (Y-STR for sex identification)
Sequence-based technology, i.e., detection of SNPs
Reverse dot blot assay
• mtDNA sequence analysis
• Single-strand conformational polymorphism
Microchip assays
• Microarray-based analysis

RFLE: Restriction fragment length polymorphism, PCR: Polymerase chain reaction, STR: Short tandem repeat, mtDNA: Mitochondrial DNA, SNPs: Single nucleotide polymorphisms

whereas the excess is washed off. X-ray exposure is allowed, and the film is developed for the pattern to be visible – autoradiography.[7,52] The major advantage of this technique is that there is no requirement of prior sequence information or any oligonucleotide synthesis. The results are majorly based on genotype characters and not the phenotypes. However, on the other hand, a major disadvantage is that we can find a relatively very low amount of polymorphism with RFLP.

PCR

PCR was introduced originally by Saiki *et al.*[53] and subsequently automated by Mullis and Faloona.[54] It allows the amplification of DNA sequencing to produce multiple numbers of copies to carry out DNA analysis. DNA sample, Primers, Taq Polymerase enzyme, and nucleotides are all added in sample test tubes and allowed to undergo thermal cycle.[6,55] Each DNA strand acts as a template for the synthesis of new strand. Depending on primer applied, we can amplify specific DNA segments also. The teeth are excellent source of both genomic and mtDNA because PCR allowed the comparison of collected post-mortem samples to the well-known antemortem samples or the parental DNA. The only limitations associated with PCR are that it is expensive and technique sensitive. Furthermore, chances of contamination by extraneous DNA is noted. Cross-contamination among samples might lead to false-positive results.[7]

Amplified fragment length polymorphism (AmpFLP) analysis

AmpFLP is a quick process that was introduced into practice in 1990s.[56] It is distinctly automated and allows the creation of phylogenetic stress caused by comparative sampling. It is mostly used in low-income countries because of its low cost as well as ease of setup and operations. It uses PCR for the DNA amplification as well. The main advantages with this technique are its genomic abundance, high level of reproducibility, more number of information bands produced per reaction but the disadvantages are not to be ignored. It requires a good quality of DNA usually, degraded DNA cannot be used. Furthermore homozygous or heterozygous individuals cannot be detected due to AmpFLP being the dominant marker.

STRs

The DNA study is preferably performed using STR. It is hypervariable region of DNA which presents successive repetitions of fragments having 2–7 base pairs.[57] It is greatly in use among forensic cases, making innovations in human identification as well as paternity tests. STR is polymorphic and it is impossible for any two individuals except the twins to the same STR. This method may not be used for cases of degraded or low DNA samples or inconsistency of number of STR markers.

mtDNA analysis

mtDNA is used to check the DNA in samples that could not be worked upon by the RELP or STR. This method is performed usually on teeth, especially on dentin and cement that contain enough sample for amplification and has some limitations associated such as time-consuming process, specialized technology, expensive nature, and availability of limited information.[58]

Limitations of tooth DNA fingerprinting

DNA generally undergoes fragmentation after death due to the activity of enzymes such as the DNAases. Furthermore, it is a task to obtain DNA from the remains as it is affected by multiple factors such as the status of the remains or the time passed since the death along with storage and transport or the contamination caused to the human remains. Another

problem is to obtain an utilizable DNA from all the DNA available in the remains.

Furthermore, before the DNA extraction, the teeth to be used are commonly decontaminated using the sodium hypochlorite so as to ensure the removal of soft-tissue remnants. This will hamper the content of the DNA present. There are high chances of contaminations during collection, storage as well as transportation. The type of teeth available, condition of the available teeth as well as the tooth structure used can affect the DNA quality and hamper the DNA fingerprinting.

CONCLUSION

DNA profiling is an essential method of human identification when the traditional methods are unavailable or impossible to perform. In severely damaged, mutilated, incinerated, decomposed remains, routine tissue is not available for DNA sampling. In all these cases, teeth become an excellent reservoir of the nuclear as well as mtDNA. Teeth are protected by the soft tissue as well as bone, thus offering better preservation. This makes them readily available DNA sources. Researches need to be concentrated on more methods to yield better and useful DNA samples from compromised remains.

Ethical approval

The Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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