ISSN No. 2456-4400
Int J Med Lab Res 2022, 7(2):24-33

IJMLR International Journal of Medical Laboratory Research

REVIEW ARTICLE

TEETH AS A SOURCE OF DNA FOR FORENSIC INVESTIGATION - A REVIEW

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Received: 21 July, 2022 /Accepted: 07 August, 2022

ABSTRACT: Teeth are one of the most resilient structures in the human body, with respect to incineration, immersion, trauma, mutilation, and decomposition, and thus, are used in forensic investigations. Hard tissues like Teeth apart from bones are rich sources of DNA. The calcified nature of bones and teeth helps to keep them preserved when other parts of the body are destroyed or degraded in mass disasters. Teeth are frequently chosen sources of DNA because of their special makeup and location within the jawbone, which offers more protection to DNA than bones. Teeth with larger pulp and multi-root contain many pulp cells and have more tooth cementum, compared to single-root teeth. Regardless of the sort of laboratory process adopted or the time since death, studies have shown that molars and premolars are suitable candidates for obtaining DNA profiles. The aim of this article is to collate all information regarding tools and methodologies pertaining to isolating DNA from tooth samples and highlighting its importance in forensic science. This article puts forth a literature review referring to the main studies on DNA from the tooth that involve the utilization of teeth for purposes like human identification, all the while making an overview of the evolution of this technology in the last years, highlighting the importance of techniques used to isolate and analyse DNA from teeth in forensic sciences.

KEYWORDS: DNA, Tooth, Forensic Science, Extraction, DNA Profiling etc.

INTRODUCTION:

DNA profiling, a gold standard in resolving forensic cases and providing pinpointed identification of victims and suspects in some instances, remains a veritable tool in situations of multiple evidence [1]. DNA analysis may disclose a person's precise identity as well as information about their physical attributes, ethnicity, place of origin and sex. The DNA tests that are now accessible have a high level of reliability and are accepted as legal proof in courts. Recent studies have shown that effective DNA extraction can be performed using old human samples such as bones and teeth, by partial or complete decalcification [2]. Teeth are heavily mineralized, making them extremely resistant to damage. They are often the only components of DNA that can be used to identify decaying individuals or study ancient populations. Anatomically, teeth are made up of two distinct sections: the crown, which is covered with enamel,

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and the root(s), which are covered with cementum. The dentin-enamel junction separates the two at the tooth neck. The crown emerges into the oral cavity while the root remains anchored to the alveolar bone. Histologically, a tooth consists of four different types of tissue: enamel, dentine, cementum and pulp. The first three are mainly inorganic or calcified substances that surround the pulp, which contains vascular and nerve elements. Dentin is continuously and slowly formed on the dental arch by odontoblasts under physiological conditions throughout the life of the tooth. Enamel is acellular and heavily mineralized. The root surface is covered with acellular and cellular cementum [3]. The dental pulp provides a rich source of DNA for extraction purposes [4]. Liu et al. stated that an ideal method of DNA extraction is to minimize and simplify the extraction steps in order to avoid further degradation of DNA and contamination, so as to achieve a high concentration and purity of DNA [2].

This article provides a comprehensive review of the recent concepts regarding DNA extraction, isolation, amplification and DNA profiling systems.

HISTORY

The Discovery of DNA

By 1952, thanks to Alfred Hershey and Martha Chase's ground-breaking discoveries, it became evident how important DNA was as the genetic material. In 1953, the work of James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin, on X-ray diffraction patterns, significantly contributed to figuring out the double helix structure of DNA; a construct that enables it to transmit biological information from one generation to the next [1].

Forensic DNA Analysis

It has been 37 years since 'DNA fingerprinting' or DNA typing (profiling) as it is now known, was first described in 1985 by an English geneticist named Dr Alec Jeffreys. He discovered that certain regions of DNA contained DNA sequences that were repeated and could differ in each individual. Jeffreys developed a technique to examine the length variation of these DNA repeat sequences which had the ability to perform human identity tests. These DNA repeat regions are called as VNTRs (Variable number tandem repeats) and were first used to solve an English immigration case and shortly thereafter to find out the culprit in a double homicide case since then, Criminalistics and Forensic Medicine have further evolved and have applied DNA Fingerprinting techniques as a powerful tool for resolution of thousands of crimes and for human Identification [5,6].

Teeth and DNA Analysis

DNA is conserved for a long time in teeth as well as bones, making them a vital source of information. As previously stated, teeth are resistant to DNA degradation caused by humidification, high temperatures, and microbial action.

Dentin and pulp in the tooth are abundant sources of DNA that can be extracted successfully. According t o the findings of a study, enough DNA may be retrie ved from the crown body, root body, and root tip. Th e root body, however, is the area that produces the m ost DNA. The quality and purity of the DNA available for the lab are just as crucial as its availability in terms of quantity. Furthermore, an abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis [5].

Isolation of DNA from tooth samples can provide an important connection to verify identity when traditional dental identification methods fail to do so. With the introduction of Polymerase Chain Reaction (PCR), a technique that allows researchers to amplify DNA at pre-selected specific sites, the practice of isolating DNA from teeth to identify individuals has become more prevalent. Comparison of DNA extracted from teeth of an unidentified individual can be made to a known antemortem sample such as stored blood, clothing, biopsy, or even to a parent or sibling [7].

ROLE OF DENTAL DNA IN FORENSIC INVESTIGATION

Genomic DNA

The DNA source for most forensic applications is genomic DNA, which is found in the nucleus of each cell (red blood cells have no nuclei, and hence no DNA). Teeth are a tremendous source of genomic DNA. Studies have shown that even root-filled teeth provide sufficient biological material for PCR analysis. The PCR-based analysis produces a DNA profile that can be compared with known ante mortem samples or paternal DNA. Dental DNA has been used not only for identifying individuals, but the technique has allowed criminal investigators to link victims to crime scenes once the body has been removed and incinerated [7].

Mitochondrial DNA

In addition to genomic DNA, cells contain Mitochondrial DNA (mtDNA), the sequence of building blocks of which can be determined to assist in identification. The main advantage mtDNA holds is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells. This suggests that in circumstances where genomic DNA is unavailable for analysis, presumably due to degradation, mtDNA may be present in sufficient quantities. In addition to having a larger copy number, the maternal inheritance pattern ensures that siblings and all maternal relatives inherit the same mtDNA sequence, barring mutations. This has significant implications for identifying individuals for whom no antemortem comparative sample exists. Despite the fact that mitochondrial DNA is still in its infancy in forensic casework, it's a powerful tool that is expected to become more widely used in the futur[6].

DNA Isolation Methods from Tooth Samples

Forensic DNA analysis can be increasingly problematic since samples from the scene of a crime or a mass disaster may contain only a minute amount of DNA, which may include polymerase chain reaction (PCR)-inhibitors. Efficient DNA extraction procedures, as well as accurate DNA quantification methods, are critical steps involved in the process of successful DNA analysis of such samples.

Fig. 1: Flowchart of DNA Isolation from tooth samples for DNA Profiling

Organic Extraction (Phenol Chloroform Method)

Organic extraction yields high-quality, double-stranded DNA, in new as well as in old/degraded teeth and therefore can be utilized in situations where PCR typing is performed [8].

The phenol-chloroform method is a sensitive but oldest method for the extraction of DNA from a wide variety of forensic samples. Even though it yields high-quality DNA, it has many disadvantages such as being very laborious, time-consuming, and handling dangerous organic solvents which can only be done if the abundance of samples is available. Due to these setbacks, Manjunath et al. stated that the phenol/chloroform method has been superseded by many other techniques which have made it irrelevant at present [5].

Commercial DNA Extraction Kit

Biological evidence from crime scenes collected for DNA testing is frequently mixed with substances that interfere with downstream analysis steps, such as quantification and short tandem repeat (STR) amplification. Reducing the number of inhibitors present in extracted DNA can improve the quality of the generated DNA profile. The Maxwell® FSC DNA IQ™ Casework Kit is

designed for optimal DNA extraction from forensic casework samples. These samples may include soft as well as hard tissue samples like tooth and trace or "touch" DNA samples,

regularly encountered in forensic DNA analysis. The kit is optimised to produce a pure, concentrated

final DNA extract that can further be used for qualitative and quantitative analysis. The extracted DNA from each sample is quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System [10]

DNA QUANTIFICATION

PCR amplification

PCR is a three-step process that involves primer-mediated amplification of particular DNA sequences.

Denaturation— Heat the template to 94°C for 1 minute to induce single-stranded DNA production.

Annealing— The temperature is reduced sufficiently at 54°C for 2 minutes, to encourage the binding of template and primer base pairs.

Extension—The temperature is then raised to the temperature required for DNA polymerase to synthesize complementary sequences to the template, with the annealed primer serving as a starting point for the extension of a freshly synthesized single strand. A temperature of 74°C is used for the extension for 1 minute. After the reaction mixtures are in place, the apparatus is fed with this temperature programmed, and a total of 38 to 40 cycles are expected to be followed per reaction, with a ramping interval of less than a minute between the cycles. After that, the samples are kept at 4°C until they are utilized.

Real-time PCR

Real-time PCR is the most powerful instrument for DNA amplification and is an improvement of the traditional PCR technique. The original PCR method, as an analytical technique, had some serious limitations. By first amplifying the DNA sequence and then analyzing the product, quantification was exceedingly difficult since the PCR gave rise to essentially the same amount of product independently of the number of molecules of DNA template molecules that were initially present. This constraint of the traditional PCR was alleviated in 1992 with the development of Real-Time PCR by Higuchi et al. [11]. Pathogen detection, gene expression analysis, single nucleotide polymorphism (SNP) analysis, chromosome aberration analysis, and, more recently, protein detection are all common applications of real-time PCR [12].

PCR Product Analysis

On a gel documentation system (GDS), the polymerase chain's products are run on an Agarose Gel with ethidium bromide staining. The GDS is made up of a UV visualizer that is connected to a computer, which allows the results to be viewed, evaluated, and saved.

DNA PROFILING

DNA profiling uses a variety of DNA typing systems including Restriction Fragment Length Polymorphism (RFLP), Short Tandem Repeat (STR), Mitochondrial DNA (mtDNA) analysis, Single Nucleotide Polymorphism (SNP) typing and Gender typing.

Restriction Fragment Length Polymorphism (RFLP)

It is used for analysing the variable lengths of DNA fragments that result from the digestion of a DNA sample with the help of a restriction enzyme called “restriction endonuclease” or molecular scissors, which cleaves DNA at a specific sequence pattern known as a restriction endonuclease recognition site[13].

Due to the advent of newer, more efficient DNA-analysis techniques, RFLP is no longer as widely utilised as it once was since it requires relatively large amounts of DNA, and it cannot be done on samples degraded by environmental conditions, taking a long time to obtain results[13].

Short Tandem Repeats (STRs)

For PCR methodology, Short tandem repeats (STRs) or a microsatellite consisting of short repeating motifs contained within a small fragment size are, however, more suited, also shorter amplicons are feasible when there is the possibility of template DNA being compromised. STR profiling, has

become the gold standard in forensic DNA profiling[1].

This method is used to examine specific sections (loci) inside nuclear DNA. They are described as small segments of DNA that are repeated at various positions across the human genome. Each person inherits some STRs from their father and some from their mother, but no one has STRs that are identical to either parent. The uniqueness of an individual's STRs serves as a scientific marker of identity, making forensic identification and paternity testing easier. Because there are so many variants in STRs, the chances of two people matching are astronomically low[5].

Uses of Teeth in STR Analysis:

STR can also be used for the identification of bodies in mass disasters and old skeletal remains. DNA isolated from ancient skeletal remains can be subjected to STR analysis and even though the DNA present in these ancient remains appeared much degraded, it is better conserved in the tooth than in bone samples. A correct sampling of the body part is essential to obtain good quality DNA for analysis. Studies have reported that the highest success rates were observed with samples from the dense cortical bone of weight-bearing leg bones (femur 86.9%) and intact teeth also exhibited high success rates (teeth 82.7%)[5].

STR (short tandem repeats) analysis is used to exami ne DNA (genomic and mitochondrial) in forensic evi dence. STR analysis can be characterised as hyperva riable segments of DNA that provide consecutive re petitions of fragments with 2 to 7 base pairs (bp). Du e to poor quality DNA supplied by VNTR (variable number of tandem repeats) testing, which may exhibit short repetitive sequences of intermediate size (15 to 65 base pairs), it is rarely employed in forensic analysis. Greater polymorphism (number of alleles), smaller size (in base pairs), higher frequency of heterozygotes (greater than 90%), and low mutation frequency are the most valuable STRs for human identification [5].

Mitochondrial DNA (mtDNA) Analysis

Due to its large copy number, maternal inheritance, and a high degree of sequence variability, mtDNA is a valuable tool for forensic identification. Comparing the mtDNA profile of unidentified remains to the profile of a probable maternal relative can be of utmost importance in missing person investigations[14]. Moreover, Silva and Passos, 2007, stated that the analysis of mitochondrial DNA for forensic purposes is restricted to ancient tissues, such as bones, hair and teeth, in which the nuclear DNA cannot be analysed. However, this examination is performed by direct sequencing of its nitrogenous bases, which is a very expensive technique because it employs a highly specialized technology. Furthermore, since mitochondrial DNA is only passed down through matrilines, it is less informative. Thus, this analysis is not employed often in all forensic laboratories directed at the resolution of crimes and the identification of persons[6,7].

Single Nucleotide Polymorphisms (SNPs)

SNPs (single nucleotide polymorphisms) are differences in the DNA sequence that occurs when a single nucleotide (A, T, C, or G) in the genome sequence is changed. Because of their small amplicon size, which is useful in analysing degraded samples, lower mutation rate compared to STRs, amenable to throughout analysis (automation), are abundant in the human genome and can provide specific information about ancestry, lineage, evolution, or phenotype, as well as determine sex, SNPs are emerging as new markers of interest to forensic medicine [14].

Pakstis et al., 2010[15] have developed a globally applicable resource of 92 SNPs for individual identification (IISNPs) with extremely low probabilities of any two unrelated individuals from anywhere in the world having identical genotypes. SNPs have limitations, including the lack of widely known core loci and the need for massive multiplexing tests. Although efforts are being

undertaken to see if SNPs can replace STRs, SNPs are the DNA technology of the future [14].

Gender Typing

In the event of accidents, explosions of chemical and nuclear bombs, natural disasters, crime investigations, and ethnic studies, determining a person's sex becomes the first priority in the process of identifying a person by a forensic investigator. The PCR technique uses thermostable Taq DNA polymerase and sequence-specific oligonucleotide primers to amplify small amounts of relatively short target DNA sequences [22].

In a study by Tsuchimochi et al., DNA was extracted from the tooth pulp, amplified with PCR, and typed at Y chromosomal loci to find out the effects of temperature on the sex determination of the teeth [23].

Using PCR amplification of the alphoid satellite family and the amplification of X-specific (131 bp) and Y-specific (172 bp) sequences in males and Y-specific sequences in females, Hanaoka and Minaguchi conducted a study to identify sex from blood and teeth. It was demonstrated to be an effective technique for determining a person's sex [24].

Sivagami *et al*. prepared DNA from teeth by ultrasonication and subsequent PCR amplification and obtained 100% success in determining the sex of the individual [25].

Amelogenin or AMEL is a major matrix protein found in the human enamel. It has a different signature (or size and pattern of the nucleotide sequence) in males and females. The AMEL gene that encodes for female amelogenin is located on the X chromosome while the AMEL gene that encodes for male amelogenin is located on the Y chromosome. The female has two identical AMEL genes or alleles, in contrast to the male, which has two different AMEL genes. This can be used to determine the sex of the remains with minute samples of DNA [22]. This information can provide us with a strong distinction between male and female amelogenins as well as highlights the fact that females have two identical amelogenin genes present on the X-chromosome, whereas males have two different genes, present on both the sex chromosomes. This difference in male and female genotypes can be utilized as an indispensable tool having good specificity and sensitivity and is financially viable for modern forensic science [8].

In the study conducted by Praveen Kumar & Aswath[8] it was concluded that the teeth could serve as a reliable source of DNA for amplification-based on forensic methods in sex determination.

DNA DATABASE

The Combined DNA Index System (CODIS)

The introduction of amplification technology linked to the analysis of STRs led to the availability of sufficiently sensitive and robust systems for the formation of efficient and effective DNA databases[1].

The acronym for the Combined DNA Index System is CODIS. It was started and supported by the Federal Bureau of Investigation (FBI) in 1990, and it has since served as the foundation of the US DNA database. It was designed to enable open-access forensic DNA testing facilities to build searchable databases of authorised DNA profiles. The CODIS programme enables laboratories in the United States (US) to share and compare DNA data. Additionally, a central database containing all of the DNA profiles from each user laboratory is included. The likelihood of two people sharing the same 13-loci DNA profile is one in a billion. The 13 CODIS locations are TH01, TPOX, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11. The Combined DNA Index System, which had more than 60 million records as of 2007, is the world's largest DNA database that is maintained by the United States [14]. Primarily, the function of a criminal DNA database is to produce matches to STR sequences between stored DNA profiles of suspects, convicted offenders, victims and DNA pieces of evidence which are found at the crime scene as allowed by the legislation of the country [17].

RECENT ADVANCEMENT

From various tooth tissues, including pulp, dentin, and cementum, of unidentified corpses, Malaver & Yunis [16], extracted Mitochondrial DNA. They used Polymerase Chain Reaction to analyse DNA that had been extracted and found that pulp produced the strongest signal. In a different investigative process, Kaur et al. [18] used dental DNA STR analysis to identify and prove paternity in a dead cadaver that had significant craniofacial and had deteriorated into a skeleton.

As reported by Kumar et al. [10], recently, Dutra Correa et al. obtained whole DNA profiles by isolating DNA from teeth using a powder-free approach from the charred and skeletonized human body. To get enough DNA for Short Tandem Repeat (STR) analysis, Kitayama et al. used a non-powder approach to extract DNA from bones and teeth. Using non-destructive techniques, Hervella et al. retrieved DNA from teeth using three different procedures to access the dental pieces (occlusal perforation, cervical perforation, and cervical cut) to recover as many cells as possible to carry out a DNA extraction. For the purpose of isolating DNA from teeth, Gomes et. al. tested destructive (using Freezer Mill) and non-destructive extraction methods (using extraction buffer). They found that non-destructive methods produced excellent results.

DISCUSSION:

DNA is produced in varying amounts and types depending on the tooth tissue. Factors including moisture, post-mortem decay, ageing, and tooth quality have an effect on the pulp and cementum, which are the primary structural sources of DNA. Due to their unique form, they are more likely to withstand environmental changes and show less DNA damage over time than skeletal remains. The tooth, along with bone may be the sole acceptable material available for effective DNA typing in the event of significant deterioration [12].

Crushing is usually recommended in the literature DNA is produced in varying amounts and types depending on the tooth tissue. However, not only does crushing completely dismantles the tooth, preventing further radiographic, anatomic or biochemical examinations, it does not take into account the exact localization of the DNA within the tooth, adding a dilution factor to material with poor DNA content [3]. Some researchers like Garcia et al. claimed that sectioning the whole tooth provides good results and is the often method advocated if the objective is to keep tooth anatomy preserved [14].

The importance of investigations mentioned in this article suggests that teeth are a valuable source of DNA that is protected from incineration by epithelial, connective, muscular and bone tissues under such situations. The hard tooth tissues enamel, dentin, and cementum also protect the dental pulp cells [6]. There are few studies available where teeth have been used for the identification of dead bodies in forensic cases. The cellularity of pulp tissue decreases with age, as the fibrous inter-cellular elements increase [10]. A subdivision of forensic science known as "sex determination" is crucial, particularly when there is little information available about the deceased. The fundamental benefit of dental evidence is that it may be preserved even after the person has passed away. The examination of antemortem and post-mortem dental characteristics is made possible by the distinctive tooth patterns [21]. AMEL gene has served as a good marker for sex determination in the Indian population by using the AMEL gene-based primers in PCR. The PCR-based method proved to be successful for sex determination with 100% specificity and sensitivity [8].

Regarding the admissibility of mtDNA analysis in court, some questions still remain, particularly in relation to the problem of heteroplasmy and, more recently, the possibility of biparental inheritance. To guarantee the robustness of mtDNA as a significant and alternative tool in forensic investigation, significant issues that need to be addressed include the ability to fully elucidate the molecular mechanisms driving biparental inheritance of mtDNA, the capacity to identify the circumstances

where this is likely to occur, and the capacity to identify and characterize heteroplasmy with high accuracy [26]. A comprehensive understanding of tooth structure and an appreciation of the relationship between DNA and mineralized tissues in post-mortem teeth are critical for optimal sample selection (19). Even though several methods are available for DNA extraction from biological materials, standardization of the protocols adopted for such a purpose has not been reached so far. For this reason, studies on molecular biology applied to human identification will probably further enhance DNA extraction with less material available and under increasingly adverse conditions [6]. The systematic comparison and automated matching of crime scene samples and individual profiles is made possible by the centralised and computerised storing of DNA profiles in a database [1].

CONCLUSION:

With the arrival of DNA fingerprinting, the concept of identification has revolutionized. It is reasonable to expect that future developments in the arena of DNA technology will cut down the time and expense involved in identifying unidentified bodies.

Nuclear and mitochondrial DNA is not distributed evenly throughout teeth and decays at different rates in different tissues. DNA yield from these tissues is influenced by antemortem (chronological age) and post-mortem factors (for example soil temperature, time etc.) [30].

Praveen Kumar & Aswath reported that enough high-quality DNA can be obtained from tooth samples to support PCR-based diagnostic techniques [8]. Notwithstanding the subject's age, DNA could be extracted from any tooth. Pretty & Sweet emphasised that the identification of people who cannot be recognised visually or by other methods relies heavily on DNA extraction from dental samples [7]. The unique nature of our dental anatomy and the placement of custom restorations ensure accuracy when the techniques are correctly employed [7]. Recovery of STR profile from bones and teeth subjected to environmental conditions has developed into a useful technique for the location of missing people in a large-scale disaster. Apart from bones, teeth may be the only materials that can be successfully used for DNA typing in cases of extensive deterioration. However, to extract DNA from these sources, relatively specialized techniques are required. High quantities of calcium present in bone and tooth samples create a physical barrier that inhibits the entry of reagents during the extraction procedure [12]. In addition to that, decontamination with bleach has been known to reduce the yield of DNA recovered from cementum, which may have a significant effect on STR profiling success of degraded teeth [27]. Even Forensic Odontologists should be aware of the legal aspects involved in forensic investigations and report to the concerned authorities in order to obtain legal remedies for the victims involved [28].

Meanwhile, the clinical availability of medical and dental patient records remains a gold standard for forensic pathology [29].

The authors have demonstrated some of the established and cutting-edge methods in this concise review for the reader. This article presents a literature review referring to the main studies on DNA extraction from the tooth, that involve the use of DNA for human identification, and makes an overview of the evolution of this technology in the last years, highlighting the importance of molecular biology in forensic science.

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Cite of article: Nayyer S, Jaiswal AK. Teeth as a source of DNA for forensic investigation - a Review. Int. J. Med. Lab. Res. 2022; 7,2:24-33. ***http://doi.org/10.35503/IJMLR.2022.7205***

CONFLICT OF INTEREST: Authors declared no conflict of interest
SOURCE OF FINANCIAL SUPPORT: Nil