Review Article

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DNA from Tooth: Practicalities and Feasibilities

Abhishek Banerjee*, V.V. Kamath**

*Postgraduate Student **Professor and Head, Department of Oral and Maxillofacial Pathology, Dr Syamala Reddy Dental College, Hospital and Research Centre, Bangalore, India.

Abstract

Background: The structure of the human tooth is a great boon in forensics where its seeming invincibility makes it an ideal material for evidence. The structure of the tooth is both an advantage and a disadvantage in the extraction of DNA for forensic examination. While the soft tissue pulp and cellular dentine make ideal reservoirs for the DNA extraction, hard tissue acellular cementum and enamel deny the obtainment of the same. *Aims:* The present review aims to highlight, analyze and collate the anatomical and histological areas in a tooth that render DNA obtainment possible and also discusses the various procedures and techniques available to carry out the same. *Materials and Methods:* The literature was reviewed for data relating to the different dental tissues which can yield qualitative DNA material with relation to forensics and odontology. The data were analyzed in and the shortcomings of the various sources will be discussed. In addition the various DNA extraction methodologies will be compared. *Results:* The paper highlights the facts that the best available sources of DNA in a tooth are the pulp, dentine and to a lesser extent the cellular cemetum. Enamel is useless for DNA extraction. The procedures needed to contain DNA material, avoid contamination and optimally extract DNA are explained. Target tissue and techniques for use of the DNA in forensic odontology is also explained.

Keywords: DNA; Forensic Odontology; PCR.

Introduction

In forensic cases, where human remnants are extremely destroyed and degraded, teeth may be the only available source to divulge the identity of an individual. Human identification is a complex issue that is usually addressed by forensic experts, biologists, odontologists and anthropologists. Use of teeth in the investigations is favored generally to maximize the scope for identification. Radiocarbon dating of the enamel of the tooth can indicate the year of birth and death since unlike bone it does not undergo continual remodeling [12]. Odontological investigations like the morphometrics of teeth, chelioscopy, rugoscopy are studied vividly, but they are transient in nature and are subjected to variations over a period of time. A successful reliable alternative to these methods is the use of DNA technology for forensic identification. Compared to the DNA in soft tissue, the DNA within the tooth is well encased and protected by the virtue of its durable structure; hence it holds a better chance to stay viable and can be utilized. DNA profiling reflects individual's genetic setup and is unique. Variations in DNA sequence called polymorphisms can be used to differentiate or correlate individuals [3]. Tooth serves as an undoubtable source of DNA. Almost every part of a tooth renders its DNA, but the quantity of the DNA will depend on its location within the tooth [4,5]. The studies on the DNA extraction methods have established the fact that DNA retrieval from the pulp, dentin and cementum is possible. Pulp serves as an excellent source of DNA based on its histological characteristics. Cementoblasts and odontoblasts embedded in the mineralized matrix of the tooth structure that are protected from the environmental degradation forces act as an challenge for DNA extraction, but they hold a strong promise that even

Corresponding Author: Dr. V.V. Kamath, Professor and Head, Department of Oral and Maxillofacial Pathology, Dr Syamala Reddy Dental College, Hospital and Research Centre, Bangalore, India.
E-mail: kamathvv2003@yahoo.com

after long periods of death or extreme environmental conditions the DNA extraction is feasible [6].

There are currently no standardized accepted protocols in the scenario of the forensic odontology handling, sampling and DNA extraction from the teeth that showcase and reflect the unique features of the dental tissues. The outcome of genetic analysis is purely dependent on the quantity of DNA sampling and the various extraction methods used. This paper offers a reflection on the optimization of the various DNA extraction techniques, feasibilities in isolation of DNA from different dental tissues and their applications in forensic odontology.

Mapping of the DNA within Dental Tissues

Before laying the criteria for the use of DNA technology in forensic odontology, it is very important to know the biological significance of the dental tissues. This understanding helps in isolating a better quality of DNA which can be easily used for profiling and phylogenetic studies. The whole tooth consists of two principle parts i.e.crown and the root. The crown of the tooth comprises of pulp, dentine and predominantly enamel which yields comparatively less DNA material than the root portion which comprises of pulp, dentine and cementum [7,8,9].

Enamel is the hardest tissue in the body and is the outermost layer of the crown, acts as a means for protection. It has the highest mineral content (96%) among the other dental tissues. It is highly acellular and does not yield any DNA content.

Dentine is composed of 65 % mineral (by weight). Dentine has an intricate structure making it unique. The dentine is densely structured by dentinal tubules which host the odontoblastic process. These odontoblastic cell processes has mitochondrion along the length of the nerve fibres and hence makes it a rich source of mitochondrial DNA (mtDNA). Dentine is generally a poor source of nuclear DNA [10,11], but in a study of DNA extraction from pulpless endodontically treated teeth , nuclear DNA was isolated from dentine. The presence of extractable DNA was attributed to the hydraulic pressure generated during endodontic therapy that probably displaced the odontoblast from the dentine pulp junction to the dentinal tubules [12].

Dentine-pulp complex includes odontoblasts, fibroblasts, plasma cells, nerve cells and undifferentiated cells. The odontoblasts are situated directed towards the pulp and makes it main bulk along with fibroblasts. Among the other dental tissues, pulp tissue provides the richest source of DNA because of its rich cellularity.

Cementum is a hard, mineralized non-vascular tissue that covers the roots of the teeth and is composed of 45-50 % of inorganic mineral hydroxyapatite crystals, collagenous as well as non-collagenous matrix proteins. Cellular cementum acts as a source of DNA because of the presence of cementocytes within the lacunae in a protected environment [13,14] within the extracellular matrix. DNA can also be retrieved from the soft tissue inclusions within the canaliculi, blood vessels traversing in accessory canals and adherent periodontal tissues to the roots.

Hence it can clearly be summarized that pulp and cementum are a good source of nuclear DNA and mitochondrial DNA can be better derived from dentine.

Types of Deoxyribonucleic Acid

The nuclear DNA and mitochondrial DNA are the two main types of DNA which are used in the forensic identification. The nuclear DNA otherwise known as the genomic DNA is derived from the nucleus of the cell which is abundantly derived from the various portions of the teeth other than enamel [11,39]. Nuclear DNA form also the major backbone of the eukarytotic genome which is adhered to the principles of the Mendelian inheretence with the genetic information obtained from the parents, whereas the mitochondrial DNA is mostly derived from the maternal lineage. The chances of reconstructing an identity profile from mitochondrial DNA is higher compared to nuclear DNA [40,45]. The mitochondrial DNA depicts a complex structure, (FIG 1) [17] where multiple variations between the indivizuals are demonstrated within 1.1kb fragment that contains two regions within the D-loop [15,16]. Forensic identifications can be established by analyzing and detecting the amplified hypervariable structures(HV1 and HV2) [17].

Basic Protocol for DNA Investigation

Decontamination of the Specimen

DNA examination is a highly technique sensitive procedure and it needs utmost care and contamination becomes the potential hindrance. Contamination with the DNA of exogenous bacteria always leads to a negative impact on the DNA amplification and often it causes interference in genetic profiling. Various surface decontamination methods have been utilised like physical abrasion [18] On the surface of the tooth, ultraviolet ray

exposure, sodium hypochlorite bleach etc. Though bleach is one of the widely accepted technique [19] because of its serious effect on the exogenous unwanted DNA, bleach may have an effect over the DNA from the cementum. It is potentially better to avoid the use of bleach to minimize the chance of DNA wastage and less abrasive techniques like simple cleaning or light scrapping must be encouraged (Figure 2).

Sampling Techniques

Sampling is aimed at sourcing the tissue from which DNA can be possibly retrieved. Many techniques have been used to obtain the tissue causing less damage to it (Figure 3). The yield of DNA purely depends on the sampling techniques. Traditional methods of retrieving the tissue involved grinding and crushing, but it considerably increases the chances of contamination. The heat produced during the grinding with a dental handpiece can cause degradation of the DNA. Horizontal sectioning [20] of tooth is a simple, easy technique, that has the advantage of preservation of crown and root structure. Endodontic access[21] causes less damage to the tooth structure simultaneously increasing the chance of better yield of genetic material. Cryogenic grinding [22,23] is one the best and most commonly used methods used for DNA sampling but the whole tooth structure gets destroyed which is a major disadvantage of this procedure. Nondestructive techniques like the use of guanidine thiocyanate (GuSCN) on the teeth of museum animals have successfully yielded DNA from the buffer obtained after soaking these teeth in the solution [24]. Dentine is a rich source of mtDNA and can be isolated from crown and root, so judiciously the source should be used. It is recommended to sub-sample the tooth, targeting the exact tissue of interest without much loss of tooth structure and also an emphasis on the preservation of the sample for future investigations.

Dna Extraction Methods

There are different methods and techniques which are available to recover genomic DNA. Various traditional methods gave successful results in regard with isolation of DNA but they usually lack standardization and therefore it affects the yield. In general all the DNA isolation methods i.e. separation of the DNA from the cellular components involves disruption and lysis of the starting materials followed by removal of proteins and contaminants and finally recovery of the DNA.

Preparation of Crude Lysates

In this particular technique, the cell lysates are incubated at 90^0C for 20 minutes, or proteinase-K digestion is performed over cell lysates. The treated lysates are often not at optimal pH and contain contaminants which can lead to negative results and high failure rates [25,26].

Salting out Methods

It is a conventional technique where salts like sodium chloride, EDTA potassium acetate, ammonium acetate are used to precipitate the proteins and contaminants from the cell lysates. The precipitates are removed by centrifugation and the DNA is isolated by alcohol precipitation. The removal of contaminants using this particular method is not very efficient and often required repeated alcohol precipitation for gaining a proper yield [26,27].

Organic Extraction Methods

This technique uses organic solvents like phenol, chloroform and isoamyl alcohol to extract out the contaminants from the cell lysates. The correct salt concentration and pH must be used during the procedure to ensure that the contaminants are separated and DNA remains in the aqueous phase and after that by alcohol precipitation the DNA is extracted. It is highly impossible to automate this procedure and it generally generates toxic components as waste and hence hazardous to environment [26-29].

Cesium Chloride Density Gradients

This is a method of genomic DNA purification through the cesium chloride density gradient. The lysates are alcohol precipitated; the resuspended DNA is mixed with cesium chloride and ethidium bromide and centrifuged. The DNA band is collected from the centrifuge tube, extracted and precipitated with ethanol to recover the crude DNA. This technique is expensive, time consuming and technique sensitive but yield a very high quality of DNA [26,30].

Anion-Exchange Methods

The principle behind this technique is based on the interaction between the negatively charged phosphates of the nucleic acid and positively charged surface molecules on the substrate. Solid phase anion exchange chromatography is used in this procedure. DNA gets bound to the substrate under low salt

conditions and the impurities and other metabolites are washed away using medium salt buffers and simultaneously the DNA is eluted using high salt buffers. This DNA is finally subjected to all applications. The main advantage lies in the fact that it completely avoids the use of toxic substances [26,30-32].

Silica based Extraction Methods

This technique uses the silica gel membrane to selectively adsorp the nucleic acids in the presence of salts. [26,34] Optimized buffers ensures only DNA is adsorbed and other impurities are washed away. This is comparatively more effective and efficient than others. DNA is obtained from the silica gel membrane using low salt buffer. There is no precipitation of alcohol is required and DNA resuspension is not required like other extraction methods. Various readymade kits are commercially available for rapid isolation of DNA from a variety of sample sources. It is suited in various applications like polymerase chain reaction techniques, restriction fragment length polymorphism etc. analysis [35].

Chelating Resin Based Extraction

It is also known as chelex-based DNA extraction. DNA that is extracted using this technique can be amplified by using PCR. In this method there is a lesser chance of clinician introduced contamination of exogenous DNA into the sample mix and hence the yield of quality DNA is more [36].

Applications of DNA Technology in Forensic Odontology

Short Tandem Repeat Typing

Short tandem repeats (STRs) are identified as mini chains of DNA that are repeatedly seen at various region in the DNA chain and the specific loci within which the DNA are identified. STRs can be used to predict the disease links in the family , retrieve the familial identity and also be helpful in anthropological studies. These STRs are derived from parents and hence they are unique in their charecteristics.[37] A study in Serbian population shows that specific autosomal loci (STRs) from teeth was found to be useful in revealing identities of the individual from a particular population [5]. Even though the DNA is degraded in the bones of the ancient remnants, it is well conserved within the tooth. Combined DNA index system (CODIS) was established by Federal Bureau of Investigation (FBI-USA) on the basis of STRs and helped in creating a large DNA based database [38].

Restriction Fragment Length Polymorphism (RFLP)

It is an application by which the differences in the DNA sequences which are homologous in nature and can be detected by the identification of fragments at different length. The DNA is broken down into fragments by the cleavage enzyme known as restriction endonuclease. Most RFLP are highly locus specific. It signifies a labeled DNA sequence that gets hybridized with more than DNA fragments. Finally it is subjected to the gel electrophoresis and a unique blotting is revealed which denotes a definite genotype at a specific loci. There are different types of RFLP probes like short, single or low copy genomic DNA or complementary DNA. The application of these varies from gene mapping, genomic studies, phylogenetic tracing, diagnosis etc. Extraction of sufficient DNA for RFLP analysis is time bound and technique sensitive. However, PCR helps in amplifying very small amounts of DNA to the quantity required for RFLP analysis. Therefore, more samples can be examined in a shorter span. An alternative terminology for the technique is Cleaved Amplified Polymorphic Sequence (CAPS) assay [39-41]. DNA from ancient bones and teeth were extracted analyzed for RFLP, it provided a good alternative to sequence the PCR products, further it helped in differentiating species even in cases of destroyed DNA template or minimal quantities of DNA availability [42].

X and Y Chromosome Short Tandem Repeats

DNA polymorphisms are valuable tools for tracing out human evolution, migration and familial relationships. The Y chromosome is inherited from father to male offspring as a haploid [43]. The size of the X chromosome short tandem repeats alleles are small and hence they are relatively easy to amplify and study [44].

Single Nucleotide Polymorphism (SNPs)

They have emerged as markers of interest because of its small size .The most primary fact is that SNP need a smaller target and a single nucleotide need to be investigated rather than numerous nucleotides. It is useful in analysis of DNA from degraded tissue samples and are better than STRs. In a particular study genetic disturbances associated with MSX1 and PAX9 genes were studied using SNP and hence play a definite role in tracing a race or a group of endangered population and also phylogenetic studies [45]. The challenges which exist in SNPs is that it needs multiple detection levels and the standardization of the markers and still the application of STR versus SNP is debatable [46].

Human identification using the morphometric features of the teeth have been used since ages. The use of tooth DNA has added an advantage to the existing identification modalities. Minimal quantities of DNA retrieved from site of investigation also hold promise to render accurate results. The extraction of mitochondrial DNA has always been more advantageous as it is more stable with lesser variations when compared to DNA derived from nucleus. Hence will yield better results in solving forensic investigations [47].

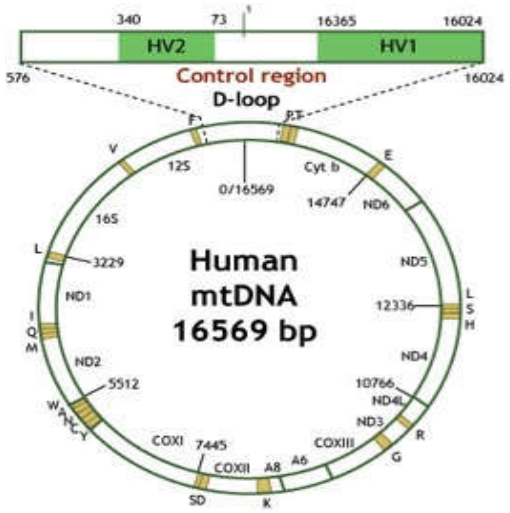

Fig. 1: Diagrammatic representation of the mt DNA to identify the D-loop region used for identification of DNA.(available on:http://projects.nfstc.org/pdi/Subject09/pdi_s09_m02_01_a.htm.)

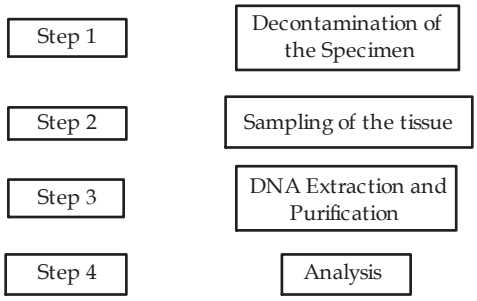

Fig. 2: Protocol to be followed for the preparation of a tooth specimen during DNA identification procedures

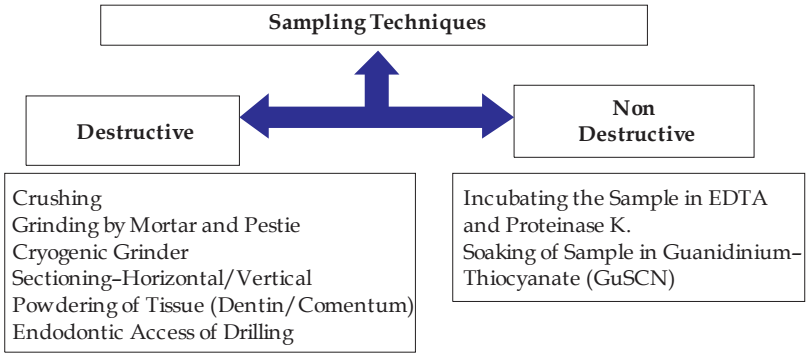

Fig. 3: An overview of the sampling techniques utilized during preparation of the specimens

Conclusion

Tooth is a rich source of DNA, and can be used for various forensic investigations. The most common source of DNA from tooth is pulp as it is highly cellular and also very easily extractable from the tooth complex for DNA analysis. Attempts are been made to extract DNA from dentin and cementum in cases of teeth with infected pulp. The use of STRs and detection of SNP has significantly improved the chances in ruling out phylogenetic cases. A protocol of DNA sampling from various tooth tissues and also the procedure of analysis should be folowed so that it will be helpful for forensic odontologist in tracing the identity and also to render justice to the victims.

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