Teeth: An Informative Tool!!!

Abstract: In human body, tooth is the hardest known structure which is impervious to adverse conditions like incineration, immersion, trauma, mutilation, decomposition etc and hence, can be used in forensic investigations.Blood, hair, teeth, and various body fluids can be used as samples for DNA analysis.As compared to other samples which may get destroyed or degraded over time, teeth are more resistant and can be used as a valuable source of DNA. The DNA print is specific to an individual.By analyzing the DNA recovered from the teeth,the identification of human remains may be possible.DNA profiling can help in providing the exact identification of the human remains in mass disasters, in the identification of culprits in crime scene investigations and also in solving paternity issues. It can also provide information regarding the physical characteristics, ethnicity and gender determination of the individual to be identified. Dental tissue can be considered for DNA analysis as they are a rich source of quality DNA and hence can be utilized in forensic investigations. Recent advances in DNA profiling have high reliability and are accepted as legal proof in courts.

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Introduction

In forensic science, identification of an individual is one of the foremost fields of study and research. Forensic Dentistryis the specialty which aims at investigating psychological, physical, chemical and biological pheno-mena that provides information about human beings (alive, dead or body fragments) focusing onaspects of human identification, criminal, civil, labor and administrative forensic investigations, legal documentations and other aspects that incorporate a multidisciplinary team.[1]Forensic dentistry plays a major role in identification of humans in adverse conditions like incineration, immersion, trauma, mutilation, decomposition etc.[2] The identification of the deceased victims dependson the comparison between ante-mortem information from the missing persons and the post mortem data of the dead person. The unavailability of ante-mortem data makes the identification of an individual difficult.In such circumstances, only the DNA profiling system helps in revealing the exact identity of a person.[3]

With the advent of polymerase chain reaction (PCR), amplification of DNA hasnow become a powerfultool in forensics.[4]DNA analysis involves matching DNA from extracted teeth of an unidentified individual to the DNA isolated from a known ante-mortem sample. DNAcan be retrieved from the blood, hair brush, tooth brush, clothing, cervical smear as well as biopsy tissue of a parent or sibling which is used in the usual procedure of DNA analysis.[1]Various methods of DNA profiling include Restriction fragment length polymorphism (RFLP), mitochondrial DNA (mtDNA) analysis, single nucleotide polymorphism-based (SNP) etc.

DNA

DNA is the fundamental building block of an individual's entire genetic makeup.[5]Each person's individuality is characterized by DNA sequences. DNA molecule is responsible for the formation of genes. Genes carry genomic information that determines theinheritanceof characteristics from our parents.[6] In forensic science, two types of DNA are used: Genomic DNA and Mitochondrial DNA.[3] Nucleus of all cells of human body contains double stranded genomic DNAwhereas the mitochondria, the 'powerhouse' of a cell, contains mitochondrial DNA (mtDNA) molecules.[7]Whenanalysing samples which lack sufficient amount of nuclear DNA, mtDNA analysis can be useful. This is because mtDNA is found in large numbersand its location aids inpreserving it in highly degraded tissues.[8] However, the mtDNA analysis is limited to ancient tissues like bones, teeth and hair as nuclear DNA cannot be examined in such tissues.[1] Moreover the mtDNA is entirely matrilinealand hence provides less information.[9]Both the types of DNA can be efficiently retrieved from human skeletal remains even hundreds ofyears after death.[8]

Sources of DNA within teeth

Human teeth prove to be a preferred source of DNA for various reasons.[10]The human teeth differ in form and size but they maintain same histological structure.They are located within the jawbones and remain protected from the various external environmental and physical assaults that can lead to post-mortem decomposition process thereby resulting in decaying of DNA.[11]Therefore, the DNA obtained from human teeth is of superior quality and there are less chances of its contamination as compared to the DNA that is recovered from bones.[10]Presecki Z et al, Pretty IA et al and Higgins D et al have conducted studies that support the reliability of teeth as a source of DNA.[6,7,8]

The result of genetic analysis of teeth relies on the DNA quality,itsdegradation leveland the proficiency of DNA sampling methods as well as the various methods involved in DNA extraction.[8]In human dentition,the crown is exposed to the oral cavity whereas the root is encased by alveolar bone. The dentin is a connective tissue that forms themain structural portion of the tooth and remains barely exposed to the oral environment.[4]Cementum, a type of calcified connective tissue covers the radicular dentin.[12]The soft tissue present in both the coronal andradicular pulp chamber consists of fibroblasts,odontoblasts, endothelial cells, undifferentiated mesenchymal cells, peripheral nerve and nucleated components of blood which are rich sources of DNA.[13]Adental sample yields about 6µg to 50µg of genomic DNA. They extracted DNA from the dental pulp chamber and concluded that there was no significant difference seen when compared to the DNA obtained from the blood samples or other available tissues.[1]

Factors affecting DNA content

a) Tooth type
It has been shown that multi-rooted teeth exhibit more cellular cementum than single-rooted teeth. Tooth selection should preferably target teeth with larger pulp volume and root surface area, with molars being the prime source.[14] Tooth retention in socket should also be considered as seen in multi-rooted teeth which are less likely to be lost during post-mortem breakdown.[15]

b) Chronological age
With increasing age, the volume of dentin increases and it also becomes sclerotic progressively. Some amount of mtDNA remains trapped in the dentinal tubules.[16]a However, DNA content is believed to decrease with advancing age.[17]a

c) Dental pathologies
The onset of dental diseases reduces the amount of DNA available as well as increases the potential for contamination.[12]Diseased teeth and restored teeth is said toyield sufficient DNA for extraction and amplification.[8]

d) Post-mortem degradation
The process of DNA degradation is initiated by the release of endogenous intracellular enzymes and ends up producing exogenous enzymes by the invading microorganisms.[18] Pulp remains biologically viable for more than [12]hours post-mortem. A short post-mortem interval and a dry environment favours the preservation of DNA.[19]

Sampling &Extraction of DNA from teeth

Successful DNA analysis of sample depends on two critical steps: Efficient DNA extraction and accurate DNA quantification.[20] Anumber of sampling methods have been reported for DNA extraction from tooth like horizontal sectioning of teeth at cemento-enamel junction or vertical split extending till the root tip, scraping or drilling and aspiration. Various other methods available are crushing teeth, cryogenic grinding of teeth, conventional access cavity preparation and retrieval of pulp.[21] The latter method is most considered in forensic odontology because of its simplicity,low cost and preservation of

tooth integrity.[2]Prior to extraction, decalcification of the mineralised tissues is done by soaking it in EDTA. The tissues can be soaked for [8] min extending to several days as reported by various studies. The value of demineralisation is based on the premise that the DNA is tightly bound in dense crystalline aggregates and without demineralisation, it will not be released into solution.[8]Some studies report a reduction in DNA quantity following decalcification whilst other studies have reported significant increase.[22] DNA extraction process consists of three stages[2]:

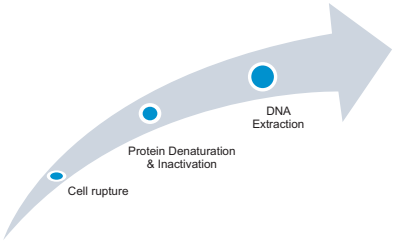


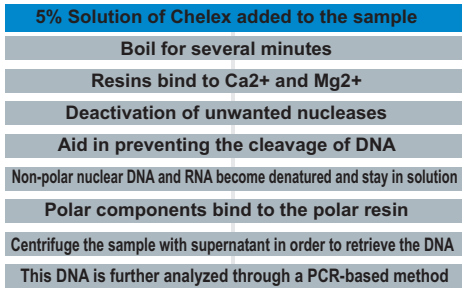
Fig. 1 DNA Extraction Process[2]

Out of all the other methods available, the most preferredDNA extraction techniques in forensic odontology are[8]:

1. Organic methods (that comprises of phenol/chloroform and silica binding extraction methods).
2. Chelex 100 (most rapid method and has less chances of contamination but it is expensive)
3. FTA Paper (it contains absorbent cellulose paper containing chemical substances)
4. Isopropyl Alcohol(it contains ammonium isopropanol and is a less expensive method)

The organic extraction methods use SDS and proteinase K to breakdown the cell membrane and causes proteolytic digestion. The addition of proteinase K rapidly inactivates nucleases that may otherwise degrade DNA during extraction. After lysing, the DNA sample is purified. This is done by mixing it with phenol-chloroform solution, centrifuging it and then the DNA is precipitated using ethanol. Then it is re-suspended in a low-salt buffer.[5]The phenol-chloroform method is considered as the most effective method as it extracts high molecular weight DNA.[12]

Another methodof DNA extractionis the use of chelating resins that are based on an ion-exchange approach.[5]



Chelex 100 method of DNA extraction.[23]

However, the purity of the DNA is not good as there areincreased number of PCR inhibitory components present. These PCR inhibitors obstruct subsequent quantification and short tandem repeat (STR) reactionsthat areused in DNA profiling.[24]

When the sample used is blood or saliva, we prefer the Fast Technology for Analysis of nucleic acids (FTA) for DNA extraction.[2]FTA reduces extraction time and provides a way to store samples at optimum temperature. It can also be incorporated in automated system where the cellulose-based matrix is treated with a chelating agent, weak base, a detergent or anionic surfactant and a urate salt or uric acid. The cells getlysed by the chemicals and the DNA gets immobilized simultaneously.[5]

DNA Profiling In Forensic Odontology

1. Restriction Fragment Length Polymorphism (RFLP)
   This is done by analysing the different lengths of the DNA fragments that result from digesting a DNA sample. It uses a special kind of restriction enzyme called 'restriction endonuclease,' which sections the DNA at a specific sequence pattern, known as a restriction endonuclease recognition site.[2,4] This is difficult in samples that are degraded by environmental factors, thereby resulting in a longer duration to get the results.[3]
2. Short tandem repeat (STR) analysis
   The PCR technique is done to amplify STR typing with highly polymorphic DNA sequences of repeating [2-7] base pairs. These STR loci are polymorphic and are considered unique to each individual.[1]Significantly, [5-10] alleles of particular STRs are often the focus for forensic profiling. The amplification of STR, via PCR, occurs by targeting the loci with sequence-specific primers. The method of electrophoresis is done to separate the DNA fragments. [25]The STR markers used in human identification, exhibit highest variability amongst individualsand they are marked by the lengths of the different alleles. STRs are classified by the length of their repeat: mono-, di-, tri-, tetra-, penta- and hexa- nucleotides.[5]Samples from dense cortical bone of our weight-bearing leg bones (femur 86.9%) showed highest success rates for human identification using STR analysis.Intact teeth also exhibit high success rates (teeth 82.7%).[26]Based on the STR analysis, Combined DNA Index System CODIS was recognized by the Federal Bureau of Investigation (FBI). It was developed particularly to help public forensic DNA laboratories to create DNA databases of authorized DNA profiles that could be easily searched.[27]
3. mtDNA analysis
   The long span between the occurrence of death and examination of tissues complicate the genetic identification process with nuclear DNA. Sometimes only bone and teeth are available for such analysis.[2] Teeth provide an excellent source for high molecular weight mtDNA; hence, offer several unique advantages for the identification of human remains. The mtDNAis different from nuclear DNA in various aspects like; its location, its quantity in the cell, its mode of inheritance and its sequence.[13]mtDNA can analyse older biological samples collected such as-hair, bones and teeth that cannot be analyzed with STR and RFLP as they lack nucleated cellular material.[4]For long term unsolved mysterious cases, mtDNA is extremely valuable for investigation purpose. It is considered better thanthe nuclear genome as it passesthrough maternal lineage and has 100-1000 copies of mtDNA genome.[28]Silva et al., in 2007 stated that the analysis of mtDNA for forensic purposes is unique to ancient tissues, such as bones, hair and teeth as nuclear DNA cannot be analysed from such tissues.[1,29]
4. Single nucleotide polymorphism (SNP) analysis:SNPs are advantageous over STRs as even heavilydegraded DNA fragments can be analyzed with SNPs.[3]The SNPs are actually basesubstitutions, insertions or deletions and occur particularly at one position of a genome. The bi-allelic nature of SNPsproves to be the sole factor that can help in DNA profiling.But such nature also makes them less informative per locus than STR and therefore gets difficult in identification when working with DNA mixtures. Increasing the number of SNP markers analyzed overcomes the less informative aspect of single SNPs when compared to STR's.[5]Thisvaried heterozygosity level of the genome provides as the most unique characteristic of SNPs.[30]

Conclusion

The introduction of DNA finger printing has revolutionized the concept of human identification.Teeth have summed up to be an excellent source of both nuclear and mitochondrial DNA, and have successfully been used in the forensic identification of compromised human skeletal remains. It is important to have a clearunderstanding of tooth structure and composition, as well as the process of diagenesisin teeth, for determining the location of DNA in post-mortemteeth. This aids in more appropriate selection of samples and sampling techniques. Targeted sub-sampling and appropriate extraction protocols further increases the value of teeth as a critical source of DNA for the use in human identification.

References

References are available on request at editor@healtalkht.com