REVIEW ARTICLE

DNA Profiling in Forensic Dentistry: A Review

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Abstract

In forensic investigations commonly the individuals are identified using various methods, such as visual identification method by a family member, personal information, medical information, footprint records, dental records, clothing, and fingerprints. But these methods need antemortem data for comparison. Also in some circumstances, the body of the victim is damaged or disfigured. In both these situations, conventional methods of identification are not useful. DNA profiling plays a valuable role for the identification of victim in such conditions. DNA can be collected from several body tissues and organs of the body and also from human teeth. As tooth is a sealed box-like structure, DNA remains preserved for longer time even under adverse conditions. The quantity of DNA extracted from teeth depends on various factors; these factors include the type of tooth, age, and sex of an individual. This article outlines various methods of DNA profiling and also methods of DNA extraction from human teeth.

Keywords: Dentin, DNA profiling, Fingerprints, Forensic dentistry, Teeth.

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Introduction

In forensic science, commonly the individuals are identified using various methods, such as visual identification method by a family member, personal information, medical information, footprint records, dental records, clothing, and fingerprints. In these methods, the antemortem data are compared with postmortem data to confirm the identity of an individual. But in some situations, such as mass disaster, road accidents, fire explosions, where antemortem data are unavailable and body of victim is decomposed, destroyed or disfigured, making identification by these methods is unreliable, DNA profiling is the sole method in such circumstances that can confirm the exact identification of an individual.[1,2]

In this procedure, DNA extracted from unidentified person is compared with the antemortem sample available which may include stored sample of blood, parent or sibling, and individual's belongings like tooth brush, clothes, hair comb etc.[2] The prime objectives of DNA profiling are: identification of victims, related parts of body and identification of criminals in different types of crime cases.[3]

DNA remains unaffected in tooth structure and bones for very longer time period. Human teeth are most vital source to extract DNA as tooth morphology makes it closed box-like structure which preserves DNA from unfavorable situations, such as incineration, immersion, decomposition and microbial activity, except at its root apex. Dental hard tissues, such as enamel, dentin, and cementum provide an anatomical structure of great stability and durability which encloses pulp within in it.[4]

Several studies in previous history reported the extraction of DNA from the teeth. In year 1991, Schwartz et al. extracted DNA from pulp of teeth under changing environmental situations such alteration in pH, variation in humidity, altered temperature and storage conditions, etc. They concluded that alteration in environmental conditions have no effect on the isolation of DNA.[5]

Sweet and Sweet published a paper in which they presented a case of identification of human remnant which is done by using a preserved unerupted 3rd molar tooth. 1.35 µg DNA was isolated from pulpal tissue of this tooth.[1] It was also observed that single-rooted teeth can also give valuable information about the person who owned these teeth.[1]

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Historical Overview of DNA Fingerprinting

In 1953, Watson and Crick[6] invented the structure of DNA which is a double-helix consisting two complementary strands, and this DNA causes genetic inheritance. This invention brought paramount alterations in almost all aspects of science. It also led to the development of techniques for characterization of human's individuality on the basis of DNA.[6] In humans, 99.1% of genome is similar, only 0.9% DNA is variable among different individuals. This variable DNA sequence called as polymorphic markers and these markers can be used for differentiating and correlating individuals.[7]

Wyman and White[8] discovered first polymorphic locus found in the human genome. They observed higher number that is above 15 variable sizes in a modest specimen of individuals. The repeated sequences are dispersed all through the human genome with

suitable type so that it can be utilized for identification of human beings. These hypervariable loci are composed of tandem repeat of oligonucleotides sequences. Based on the size, these loci are categorized as variable number of tandem repeat (VNTR) or minisatellites consisting 9–80 base pairs and short tandem repeats (STR) or microsatellites which consist of 2–7 base pairs.

With various advancements in molecular biology, researchers are able to examine sequences of DNA. In 1978, Southern blotting was used for detecting DNA polymorphisms.[9]

In 1985, Jeffreys et al.[10] discovered radioactive molecular probes, able to recognize highly variable areas of DNA and thus determined the peculiar patterns of each human being, which were referred as DNA fingerprints. The presently performed DNA profile tests are valid and considered as legal proofs in courts for paternity determination and confirming individual identity. Various biological substances may be used for extraction of DNA, such as bone, saliva sample, stored blood, biopsy sample, hair bulb, and other tissues of the body. DNA can be extracted from all body tissues in the human body, but the quantity and quality of DNA isolated from each body tissue is variable.[11]

In the beginning, the forensic investigations used VNTR DNA testing for the recognition of humans and paternity determination. This procedure needs more quantity of material and gives low quality results. Therefore, polymerase chain reaction (PCR) was introduced which enables the amplification of small amount of DNA sample.[12] Short tandem repeats used in forensic science revolutionize human identification and paternity process. Other new DNA tools which are mitochondrial DNA and single-nucleotide polymorphism (SNP) may be used if STR tests are unable to produce an outcome or in conditions where only partial profile is achieved due to size and sample condition.[13]

Pötsch et al.[14] used genomic dot blot hybridization for sex determination. They took total genomic DNA ranged between 6 and 50 μg from a dental sample. They found that there was no difference in patterns obtained from DNA isolated from pulp and DNA extracted from blood sample or lung tissue sample.[14]

In 2002, Tsuchimochi et al.[15] isolated the DNA from the pulp of extracted teeth by exposing teeth to variable temperatures that are 100°C, 200°C, 300°C, 400°C and 500°C for 2 minutes for performing PCR test. They observed that PCR analysis failed when the samples were incinerated above 400° and samples heated upto 300°C temperature could be amplified. They used Chelex 100 chelating resin for DNA isolation and found that the use of this resin is suitable for the isolation of high-quality DNA for PCR amplification analysis.[15]

Malaver and Yunis[16] reported that DNA extracted from the pulp showed strong PCR amplification signal as compared with the DNA extracted from dentin and cementum.[16]

Remualdo,[17] in the year 2004, heated teeth to different temperatures and extracted DNA using three different methods that were organic method, ammonia acetate/isopropanol method, and silica method. They reported that isopropanol/ammonia acetate extraction technique gives decent outcomes for mitochondrial DNA isolation at high temperature.[17]

In year 2006, the microarray technology was used for single-nucleotide polymorphism genotyping for identification and paternity testing.[18] Next-generation genome sequence can be used for analyzing hundreds of loci or the whole genome.[3]

Teeth as a Source of DNA

DNA can be isolated from many sources, such as blood, body tissues, semen, organs, bones, nails, teeth, saliva, hair follicles, and body fluids. Tooth is rich with genetic information.[19] DNA extraction from the human body is a challenge and it depends on various factors and isolation methods. Studies proved that DNA remains stable in hard tissues of the body which are bones and teeth even under unfavorable conditions.

Although teeth differ from each other in morphology including shape and size; however, histologically they have same structure. Dentin forms the major structural component of tooth. In the crown part, dentin is covered with enamel which is ectodermal in origin, and is highly mineralized, acellular and avascular tissue. In the root area, cementum covers dentin which is also calcified connective tissue. Coronal and radicular pulp contains various cells, such as odontoblasts, fibroblast, undifferentiated mesenchymal cells, endothelial cells, and nucleated constituents of blood, good quantity of DNA can be isolated from these cells. Other structures used for extraction of DNA are odontoblastic process extending into dentinal tubules, soft tissue present in accessory canals, periodontal fibers, cellular cementum and associated bone.[1,20]

Factors Influencing the Quantity of DNA

Category of Teeth

Rubio et al.[21] reported that DNA content in posterior teeth that are molars and premolars was more as compared with canines and incisors. Studies found molars as preferable choice among various types of teeth. The reasons for this preference are increased cellularity due to large size pulp chamber and more size of cementum, higher stability of tooth in alveolar bone, anatomically more protected and low chances of postmortem loss or contamination of molars.[22] Corte-Real et al.[22] and Mansour et al.[23] reported that the type of tooth has no influence on the content of DNA. This may be due to the accelerated decay of pulp of teeth. Hence, the volume of pulp tissue has no impact on the content of DNA extracted from teeth. Odontoblasts rapidly degrade and disappear 5 days after the death of the individual.[23]

Age

There are two aging processes which contradict each other. These are: (1) reduced pulp volume and cellular content. (2) Increased thickness of cellular cementum with age. Mansour et al.[23] and Rubio et al.[21] reported no impact of age on DNA content, But Higgins et al.[24] observed a positive relationship of age with the amount of DNA isolated from a tooth. Deposition of cementum with age results in increased thickness of cellular cementum, thus providing more protection from microorganisms.

Sex

Various studies found no significant difference among males and females for the amount of DNA extracted from teeth. Different dental tissues may vary among males and females of different populations.[23] Martin et al. reported some differences among males and females which are associated with variability in root number and morphology of root canal.[25]

Methods of Sample Collection from Teeth

- Crushing the teeth
- Traditional endodontic access method
- Vertical splitting of teeth
- Horizontal sectioning of teeth
- Cryogenic grinding
- Orthograde entrance method

Crushing is not the commonly used method, and it is performed only as the last option. This method is not recommended as it needs multiple cycles of decalcification and purification, and this leads to complete loss of tooth morphology. Therefore, the morphology of tooth should be noted before using this method.

Sweet and Hildebrand established another method that was cryogenic grinding; this method utilized a freezer mill which is cooled with liquid nitrogen to pulverize the tooth. This procedure is sufficiently effective and prevents contamination.[26,27]

Horizontal and vertical sectioning is the method in which access to the pulp of tooth is created. Sectioning is done with carborundum discs and heavy duty gloves are used during the procedure. The tooth is cut until the pulp cavity is reached. Splitting of tooth with discs generates aerosols that can reach skin, eyes, and mouth of operator, so working under a hood is suggested. Distilled water is also used during sectioning process to prevent thermal damage due to friction between disc and tooth. After reaching the pulp cavity, splitting of tooth is done using chisel. This protects the pulp from thermal and mechanical damage which may occur with discs. After complete splitting of tooth, pulp can be scooped for further DNA isolation and analysis.[26]

Endodontic method follows the recommendations provided by the Dental Forensic Kit and the Quick Extract FFPE DNA extraction Kit. In this procedure, endodontic protocol is used to gain access to the pulp, which is called as pulp chamber perforation.[28,29] Using radiograph, the distance between the occlusal surface and pulp chamber of tooth is determined. A circular diamond bur is used to make perforation in the teeth. The diameter of bur used is 1–2 mm. With the help of endodontic file, dental pulp is removed from tooth. The holes are sealed after removal of the pulp. The collected pulp can be treated with the FFPE DNA extraction Kit. Using this method the original tooth morphological features can be preserved.[28,30]

Methods of DNA Fingerprint Analysis[1]

- Polymerase chain reaction
- Short tandem repeat (STR) typing
- Analysis of mitochondrial DNA
- Restriction fragment length polymorphism (RFLP)
- SNP typing
- Analyzing Y-chromosome
- X-chromosome STR typing

Polymerase Chain Reaction

The advancements in DNA analysis remarkably enhanced when Dr Kary Mullis found that DNA can be duplicated to great extent in laboratory as compared with in the natural world. The procedure of copying DNA is called as PCR which makes use of an enzyme named polymerase which is used to replicate DNA.[31] Polymerase chain reaction is used to amplify DNA material to get appropriate quantity of DNA for carrying out analysis. By repeating this PCR process, billions of DNA molecules can be generated from few number of DNA molecules within certain hours.[31] The standard PCR runs for 30 cycles resulting in amplified original amount of DNA to 109 times. The products obtained from the process of amplification are called as amplicons. These are detached by the process of electrophoresis.[32]

Polymerase chain reaction is used for analyzing VNTR and in cases of sexual assault, when male or female DNA is mixed in a sample, real-time PCR or quantitative PCR is emerged.[32]

Short Tandem Repeat Typing

Short tandem repeats are small lengths of DNA which present repetition at several positions all through the genome and is used to determine distinct areas.[2] Short tandem repeats are powerful to discriminate individuals as they have superior level of polymorphic informative content.[3] Every individual has STRs which are inherited from father as well mother but no individual has STRs exactly similar to their parents either mother or father. So the uniqueness of STRs of an individual gives the scientific marker of identification and very useful in forensic science investigations for human identification.[2] The non-overlapping size of the alleles coming from separate contributors serve to differentiate them from each other. Fluorescent detection methods using capillary or gel electrophoresis and ABI gel-based DNA sequencers are used for detection. Silver-stained polyacrylamide gels were used for detection in earlier works.

This method is used for the identification of victims of mass calamities and for paternity tests. It was reported that human recognition success using STR analysis is more with samples taken from dense cortical bone of weight-bearing leg bone (86.9%) and also from intact teeth (82.7%).[33]

Analysis of Mitochondrial DNA

Nuclear DNA examination in old body complicates the identification process and sometimes only teeth and bone are accessible for analysis. Teeth are very good source of high molecular weight mtDNA that help in human identification.[34] mtDNA is a valuable method for forensic human identification and it is superior when compared with nuclear genome as it is passed through maternal lineage and has 100–1000 copies of mtDNA and high degree of sequence variability.[1,2] This technique is used in tooth sample particularly dentin and cementum which possesses sufficient DNA to allow amplification of mtDNA.[16] Every child has mtDNA similar to mother as mitochondrion of new embryo comes from the egg of mother but nuclear DNA comes from the father's sperm. So identification is done by matching of mtDNA of remains of individual to be identified with a potential maternal relative.

This method is costly as it involves direct sequencing of nitrogenous bases. Also the information obtained from this method is limited due to the involvement of familial maternal relationships only.[2]

Restriction Fragment Length Polymorphism Typing

This technique involves the fragmentation of collected DNA into segments at locations which are not present within the tandem repeat sequence using restriction endonuclease enzyme. These cut segments of DNA have VNTR of variable lengths.[1] The cut fragments are separated using gel electrophoresis based on their size. Then DNA is visualized by various procedures, resulting a pattern of bands, sometimes marked as pattern same as supermarket bar code. It is easier to determine that the two samples are different, when one has a band but it is absent in other. It is more difficult to determine, based on identical banding patterns that two samples must have come from the same individual.[35] This method forms DNA fingerprint of a person by establishing a particular pattern to VNTR via detecting the repetitive sequences.

This analysis needs more quantity of DNA for performing analysis, so this analysis cannot be performed when samples are degraded. This analysis also requires longer waiting period to achieve the results.[36]

Single-nucleotide Polymorphism Typing

It is a technique which analyzes variations in genomic sequence. Single-nucleotide polymorphisms are DNA sequence alterations which occur with alteration in single nucleotide in the genome sequence. For example, SNP might alter the DNA sequence AAGGCTAA to ATGGCTAA.[2] These are detected by using real-time PCR, microarrays or next-generation sequencing technique. Single-nucleotide polymorphism is a single base substitution, but it is limited to germline DNA and must be present in at least 1% of the population.[37] Single-nucleotide polymorphisms are gaining importance as recent markers of main focus in the forensic science as they have small amplicon size which is useful in analysis of degraded samples, amenable to high throughout analysis (automation), abundant in the human genome, can provide specific information about ancestry, lineage, evolution, identity or phenotype, and also information to determine sex.[2] Single-nucleotide polymorphisms for forensic assessment may be classified into four types:

- Lineage informative SNPs
- Ancestry informative SNPs
- Identity-testing SNPs
- Phenotype informative SNPs[38]

They give valuable information regarding descent, sex, and evolution. They can be used to identify highly degraded DNA fragments.[32]

Finding a variation in single nucleotide can give important information whether it is linked to a specific disease or a pertinent phenotype. There are some SNPs which are linked with disease of heart, disorders of immune system, cancer and various other diseases. There can also be an association between SNPs and individual's response to some drugs which leads to more customized approach to drug through pharmacogenomics.[37]

Analyzing Y-chromosome

Genetic features on Y-chromosome provides a lineage marker in the form of a single haplotype passed directly from father to son.[39] The haplotype consists of a set of short tandem repeat (STR) alleles typed on a single Y-chromosome. Sometimes, in forensic work, analysis of the Y-chromosome has not been given much importance because it does not give the near certainty of recognition that can be provided by autosomal DNA.[40]

DNA polymorphisms on the human Y-chromosome are useful methods for studying human evolution, migration and also to determine relationships among male individuals.[41] Y-chromosomal DNA variation has been primarily useful in human evolution research and also used for paternity analysis.[42] As it is uniparental marker, it is a valuable tool in some conditions, such as sexual assault cases, missing individuals, identification of victims of any disaster, and for evaluating complicated kinship relationships.[40]

X-chromosome STR Typing

Along with autosomal STR, and mtDNA markers, X-STR is another complementary method which can be utilized in forensic science investigations including family relationship analysis. It is believed that X-chromosome is the stable nuclear chromosomes, with 155 million base pairs and it forms approximately 5% of the genome in human beings. This X-chromosome has some features which are absent in its counterpart that is Y-chromosome. Males have one copy of X and one of Y-chromosome, but in females two copies of X-chromosome are present.[43]

This technique is utilized for human recognition and genomic investigations of several ethnic populations in the World.[44] X-chromosome STR alleles are small in size, consisting 100–350 nucleotides, so it is comparatively easy to be amplified and its detection is highly sensitive.[45] X-chromosome STR markers are valuable addition to paternity tests and forensic investigations like complex kinship analysis. Both males and females attain one X-chromosome from mother and one from their father. Therefore, in cases of deficiency paternity, where only mother is present for typing, the possible X alleles of the putative father of child can be found and parental profile can be restored.[46] DNA testing of X-chromosomal STR polymorphisms has been the prime focus in research investigations due to its relevance in the study of population genetics by utilizing multiplex PCR.[47,48]

Conclusion

The use of DNA fingerprinting changed the identification process dramatically in forensic science. There are various methods used for DNA analysis, such as PCR, STR typing, analysis of mitochondrial DNA, RFLP, SNP, typing, analyzing Y-chromosome and X-chromosome STR typing. As DNA contains the genetic material and it is distinctive to every person, so methods of forensic identification based on DNA analysis are fully valuable and also acceptable as legal proofs in courts. Teeth are very good source of DNA even if the body of victim is damaged or disfigured under adverse conditions because pulp remain protected in teeth by hard tissues like dentin, enamel and cementum. Therefore, dental professionals working in the field of Forensic Dentistry should be aware of all these methods using DNA analysis and they should include these recent advancements in their work.

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