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DNA Fingerprinting: A Revolution in the Field of Forensic Sciences

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Abstract

DNA profiling, commonly called DNA fingerprinting or DNA testing, has a wide range of uses, including assisting in criminal cases and wildlife conservation. The firm tissues of the teeth are resistant to external events such as decomposition, bruise, and incineration. Pulp tissue provides a rich DNA source. The review focuses on the various DNA Collection Procedures, the different techniques employed in DNA isolation and amplification. There are a numerous DNA Profiling or Typing systems such as Mitochondrial DNA (mtDNA) analysis, Restriction Fragment Length Polymorphism, Y chromosome analysis, Single Nucleotide Polymorphism (SNP) typing , Short Tandem Repeat (STR) typing, Gender typing, X-chromosome STR Typing. DNA fingerprinting has been applied to solve many cases. Although present DNA analysis techniques are fairly specialized, several upcoming simplifications may increase their simplicity of use.

Keywords: DNA Finger Printing, Forensic Dentistry, Gender Typing, Mitochondrial DNA

Introduction

Forensic odontology, often termed forensic dentistry, is a field of forensic medicine that deals with the correct investigation and handling of oral and other evidence, as well as the right presentation and interpretation of dental results in the concern of justice[1]. It is associated with the processing, obtaining, and presentation of dental evidence used to provide scientific and objective facts in judicial proceedings[2].

Forensic dentists must be knowledgeable in various fields such that the dental records acquired may be used to recognize a person or provide the necessary data by an authority to demonstrate negligence, fraud, or abuse. Traditionally, post-mortem and ante-mortem dental data have been compared for the existence of dental restorations, endodontic procedures, fixed prostheses, and other factors to know whether the two records belong to the exact person[2]. The newly evolved molecular biological techniques are more efficient than the traditional techniques used. When traditional dental recognition methods fail, this genetic information may be able to provide the missing piece of evidence to verify individuality. Dental tissues such as enamel, dentin and pulp are good sources of DNA. Forensic odontologists have employed a variety of identifying procedures for the extraction, synthesis, and analysis of DNA contained in dental pulp tissues. These techniques include rugoscopy, cheiloscopy (lip prints), imprint biopsy, Polymerase Chain Reaction (PCR) etc[2].

Forensic odontological analysis is the basic technique of recognising cases where exposure, death time, and bodily deterioration (fire, blast, etc.) have rendered these other methodologies extremely difficult. It has restrictions, however, such as when the remnants are divided and only tiny amounts of the jaw-bearing tooth can be regained. Under experimental circumstances and in ordinary forensics situations, most tooth pulp tissue could be retrieved (air accidents, putrefied bodies and burned)[3].

The standard approach in DNA analysis is to compare the DNA retrieved from an unidentified person's teeth with the DNA obtained from recognized ante-mortem materials such as a preserved cervical smear, comb, blood, clothes, a biopsy and a tooth brush, to a sibling or parent[4]. Agrippina, the mother of Roman Emperor Nero, is recognized for establishing forensic dentistry by having her opponent Lollia Paulina's discoloured front tooth removed after her death in 49 A.D[1].

Jeffery (1985) used multilocus probes to characterize hyper-variable areas of human DNA and DNA polymorphisms are used to individualize human tissues

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and plasma. DNA analysis's potential forensic applications in identifying individual remains, individualizing blood, resolving contested paternity issues, and other fluids in forensic labs were quickly identified[5].

Initially, Saiki, *et al.,* described Polymerase Chain Reaction (PCR) and later, Mullis and Faloona described automated PCR which has emerged as a powerful technology in a forensic investigation for the exponentially *in vitro* amplified involvement of small amounts of RNA and DNA, and has been rapidly implemented in forensic dentistry[5]. Schwartz, *et al.,* extracted High Molecular Weight proteins (HMW) using dentition under a variety of circumstances, including storage, temperature, humidity, PH, and so on in 1991. In 1992, Potsch, *et al.,* used DNA probe pHY 2.1 (biotinylated repetitive) to conduct genomic dot blot hybridization, and gender was accurately identified in all instances from the pulp by utilizing 50-100 ng of target DNA[5].

There are various ways of identifying individuals using dental tools. Various sorts of individual identification situations include jaw, orofacial and dentition characteristics: comparing reconstructive post-mortem, dental identification, and DNA fingerprinting[5,6].

DNA profiling is one of the most important methods to emerge from DNA and genetic science. DNA profiling, commonly called DNA fingerprinting or DNA testing, has a wide range of uses, including assisting in criminal cases and wildlife conservation. Based on the type of data you're searching for in a given sample, DNA fingerprinting may be done in a variety of ways. DNA fingerprinting is basically an identification method relying on genomic data. Each living thing, with the exception of exact (zygotic) twins, quads, and so on, is biologically different. DNA fingerprinting can be used to correlate a specimen to a known resource, to correlate two specimens to verify relatedness, to test the suitability of biologic transplants to assist in plant breeding, to recognize skeletal remnants, and to improve breeding operations in zoos and animal sanctuaries[7].

Dental Identification Using DNA[8]

The failure of standard dental identification procedures emphasized the need for biological evidence like DNA in determining identification. Because of advancements in DNA technology, forensic DNA analysis has become the gold standard for recognizing unknown residues. The purpose of DNA profiling for calamity survival recognition is to recover as much genetic information as possible from highly polluted material.

DNA Extraction[2]

As DNA's major source is blood, in some situation samples are not available for analysis then the oral cavity can be used for extraction of DNA[2]. The firm tissues of the teeth are resistant to external events such as decomposition, bruise, and incineration. Pulp tissue provides a rich DNA source. The pulp specimens are obtained in three different ways: horizontally, diagonally and vertically; by crushing, endodontic access, and tooth sectioning, other sources of tissue are more difficult to extract and evaluate than dental pulp tissue. Microorganisms or non-human DNA might possibly contaminate it. Dentin or cementum is utilized to extract DNA in these situations[3]. Sweet and Hildebrand were the first to use cryogenization to recover DNA from tooth[2].

The extraction of DNA method is divided into three phases: cell lysis (which enables the utilization of numerous methods for efficacious cellular membrane rupture), protein degradation and inhibition (by proteinases and chelating agents to deactivate components like protein molecules), and eventually DNA isolation on its own[9].

DNA Collection Methods from Dental Source[10]

- Crushing the entire set of teeth
- Endodontic treatment using conventional techniques
- Splitting vertically
- Section horizontal

DNA Collection Procedures[11]

Soft tissues or plasma, are stuck to the tooth that has to be retrieved. Scrape any debris or plaque from the teeth with instrument with small scoop and gently clean with H_2O_2 accompanied by ethanol. When all teeth are totally intact and appear to have been pulled from the bone lately, a standard dental treatment and equipment operation can be done. Splitting the teeth facilitates exposure to the pulp. While the tooth is open, the dental pulp wall should be instrumented with a

slow rotating bur or with small scoop instrument. The pulp tissue is then extracted into a sterile tube that is completely open. Dry samples of pulp could seem like mummified paper. Irrigation of chamber with saline should be done after instrumentation. The cellular material essential for testing will be removed from the fluid by ultra-filtration in the laboratory. Finally, tooth crushing may be needed.

Techniques for Isolating DNA[4]

- Organic extraction (phenol and chloroform methods)
- Extracting DNA from silica
- Chelex 100
- Commercial DNA extraction kit

The phenol-chloroform technique is an ancient method of extracting DNA from a wide range of forensic evidence utilizing a sensitive methodology. Despite the fact that it produces high-quality DNA the disadvantages are; time-consuming and labour-intensive treatment of hazardous organic solvents[4].

Extracting DNA from silica-based technologies is ideal for obtaining DNA from ancient teeth and bones (aDNA). For nuclear STR typing using deteriorated osseous specimens, the silica-based extraction method outperformed the phenol/chloroform methodology[4]. Proteinase K digestion with bone powder particles is followed by a silica-based vertebral column for direct retrieval of pure DNA. Another efficient method for separating DNA is (QIquick, QIAGEN)[4]. Ion-exchange columns were utilized to retrieve silica-based aDNA extracts, which significantly enhanced PCR amplification and might be utilized in inadequately maintained, PCR-resistant old substances[4]. When compared to the partial demineralization approach, combining the whole demineralization with ion-exchange columns recovers roughly three times more DNA from ancient bones[4].

Chelex 100 Methods for DNA Extraction from forensic-type materials and using it with the PCR were devised using Chelex 100 chelating resin[4]. Advantages: For most specimens, Simple Rapid may not require multiple tube transfers and may not require the use of organic solvents[4]. In Chelex 100, incinerated teeth are used for the extraction of DNA, amplification, and typing. Extracting DNA from tooth pulp is said to be more effective than phenol-chloroform extraction and proteinase K[4].

Commercial DNA Extraction Kits[4]

DNA extraction tool Prep Filer Forensic isolates genomic DNA from a wide range of living sources. It allows for the reversible adhesion of DNA with magnetic particulate, resulting in increased DNA recovery from specimens containing both high- and low-amounts of biological matter, such as saliva specimens, stains of semen on cotton fabric, revealed to the surroundings, specimens containing Polymerase Chain Reaction (PCR) inhibitors, cotton cloth, traces of plasma on denim, FTA paper, and touch scientific proof samples.

Methods for Amplification of DNA[4]

- Real-time PCR
- (PCR) Polymerase Chain Reaction
- AmpFlSTR MiniFiler and AmpFlSTR Identifiler PCR amplification kits

Real-time polymerase chain reaction is a highly effective method for the amplification of DNA and is a modification of the previous PCR technology. It is commonly used for Single Nucleotide Polymorphism (SNP) analysis, pathogen identification, chromosomal aberration assessment, gene expression analysis, and, more recently, protein detection.

Polymerase Chain Reaction (PCR)[4]: It is a relatively modern and widely used molecular biological method for enzymatically reproducing DNA without the need for a live creature such as yeast and Escherichia coli[12,13]. DNA which is available is amplified for evaluation using a particular DNA primer and enzyme specific to human DNA, and bacterial DNA is detected in the specimen even if findings are unchanged[12].

AmpFlSTR Identifiler PCR amplification and AmpFISTR MiniFiler l kits[4]: The PCR amplification kit AmpFlSTR MiniFiler, established and distributed by relevant bio systems, improves the AmpFlSTR identifiler PCR amplification equipment raising rates of success when analysing deteriorate or inhibitor-containing DNA.

DNA Profiling

DNA profiling has been considered the only and most dependable technique of identification. The tooth pulp contains a lot of DNA. Recognizing the dead is critical,

not only for relatives but for ethical purposes[12]. Primary goals includes; identifying the victim, placing bodily parts together, identifying criminals[12].

DNA profiling and DNA fingerprinting are both encoded collections of information that show a human's DNA profile and may be utilized to identify the individual[5]. From the interior layer of the lip and mucosal layer, epithelial cells are leached out, which is useful in the detection of DNA in saliva.

In this day and age of proteomics and genomics, forensic dentistry has evolved to encompass the use of DNA and proteins as unique recognition approaches. The forensic recognition technique requires mitochondrial and genomic DNA from the dentin, cementum and pulp of teeth, as well as desquamated cells in saliva.

DNA Typing (Profiling) Systems

- Mitochondrial DNA (mtDNA) analysis
- Restriction Fragment Length Polymorphism
- Y chromosome analysis,
- Single Nucleotide Polymorphism (SNP) typing
- Short Tandem Repeat (STR) typing,
- Gender typing.
- X-chromosome STR Typing

Mitochondrial DNA (mtDNA) Analysis[12]

Mitochondrion DNA is extracted when the sample cells lack a nucleus. In 2002, Silva and Passos claimed that mtDNA analysis may be utilized for ancient tissues such as bones, hair, and dentition when nuclear DNA analysis was not possible. Teeth, particularly in deteriorated remains, yield high molecular weight mtDNA. Analysing the mtDNA of unidentified remains is a useful technique for detecting missing people as a probable maternal relation[12]. Mitochondrial DNA sequences provide numerous characteristics for identifying human remnants[4]. Because of its maternal inheritance, high level of sequence diversity, and large replica amount, mtDNA is an effective forensic identifying method. In missing person investigations, relating the mtDNA profile of unidentifiable remnants with the portfolio of a probable familial relation might become a beneficial method[4].

Restriction Fragment Length Polymorphism (RFLP)[4]

RFLP is an ancient DNA typing technique. It's being used to assess the different lengths of DNA strands produced by processing a restriction endonuclease, which is a DNA sample along with a restriction enzyme. A restriction endonuclease recognition site is where DNA is segmented in a particular sequential arrangement.

With the advancement of the newest, more effective methods of analysing DNA, RFLP is no longer as widely utilized as it once was because it needs significantly larger quantities of DNA, cannot be conducted on samples damaged by environmental conditions, and requires more time to obtain findings.

Y-Chromosome Analysis

Y-chromosome markers are not gender marks, but rather male-specific distinguishing characteristics that allow man's DNA to be recognized in often combined female and male specimens (for example, vaginal samples after rape or finger nail swabs after such an attack). Because of their exclusive paternal inheritance, Y-chromosome markers are valuable in lineage research[14]. Human Y-chromosome DNA variations are useful for studying human migration and evolution[4] Y chromosomal is transmitted from dad to son. It is extremely helpful for tracking male connections or evaluating biological evidence including numerous male donors[4]. The result of chromosome Y provides data on every variation that happened throughout male lineages during movement and evolution.

As a result, Y-chromosomal DNA variations have mostly been utilized in studies of human evolution as well as forensic or paternity analyses[4].

Single Nucleotide Polymorphism (SNP) Typing[4]

SNPs are alterations in the segment of DNA that occur whenever a single nucleotide (C, T, G and A) in the human genome pattern is mutated[4]. SNPs for forensic assessment may be classified into 4 types: Lineage informative SNPs, ancestry informative SNPs, identity-testing SNPs, and phenotype informative SNPs are all types of SNPs[4].

Short Tandem Repeat (STR) Typing[4]

It is a popular and widely used forensic marker[4]. Microsatellites, or (SSRs) simple sequence repeats is also known as Short Tandem Repeats (STRs), are accordion-like sections of DNA that comprise core repetition components of 2 to 7 nucleotides in length that are tandem duplicated from a half dozen to many dozen times[15]. DNA is in small length which is replicated on different sites across the human genome, and this technique is utilized inside the nuclear DNA to assess particular areas (loci)[12]. Due to their higher requirements for polymorphic informational content, STRs have a great capacity for individual judgment[4]. The non-overlapping size of alleles from various donors distinguishes them. They are now identified utilizing fluorescent identification technologies such as DNA sequencers (ABI gel-based), as well as gel or capillary electrophoresis, whereas silver-stained polyacrylamide gels are used by research for detection[4]. Every individual inherits some STRs from their parents; none have STR loci that are identical to those of their parents[12]. Unique characteristics of a person's STRs are useful in forensic investigations and paternity testing and serve as a scientific identifier of identity[12]. The FBI has selected 13 distinct DNA loci, collectively known as CODIS combined DNA index system markers, and the sex-recognizing amelogenin marker (STR) to serve as its benchmark.

Gender Typing[4]

The amelogenin genes produce the enamel proteins necessary for proper tooth enamel development[4]. Gene amelogenin is a unique duplication with homologues on Yp 11.2.108 and Xp22.1-Xp22.3. The length variations with XeY similarly amelogenin genes AMELY and AMELX which are utilized to determine gender and considered an important component for the majority of multiplex tools PCR utilized for DNA analysis currently[4]. The dental pulp contains a significant amount of DNA that can be used to determine gender.

STR Specific to Chromosome X

It is utilized in the recognition and genetic research of many legal communities throughout the world[4]. X-chromosome STR alleles are small in size, typically containing 100-350 nucleotides, and they are very simple to amplify and also discover with high accuracy. X-chromosome STR markers (X-STR) are a strong supplementary approach, particularly in paternity screening for deficiency. There have been several studies on X-linked microsatellites, and more study is needed to obtain population-specific data[16].

Application of DNA Fingerprinting[17]

- To identify the living or the deceased
- Bite-mark identification, assessment, and comparison
- Lip and rugae print identification, analysis, and comparison, as well as patterned injuries
- Identification of dental specimens at crime scenes or instances of mass fatalities Age estimation
- Jaw, tooth, and oral soft tissue injuries are analysed and evaluated[18].

Evidence Based Cases

- Dental investigations in mass disaster accidents: At a distance of roughly 8 miles off the coast of California, an air accident happened, killing all of the passengers as well as the crew. Except for a handful, all of the bodies were shattered. Out of 88 casualties, 85 were recognized, and 65 were finally recognized using traditional methods. Periodic examinations, dental comparisons, tattoos, and fingerprints are examples of traditional methods. The 23 victims were recognized by DNA analysis after they had previously gone unidentified. Various bits of the corpse were discovered by fishermen, the navy, and the use of fishnets. The DNA recovered from the biopsies was matched to the segments found[19].
- Priyadarshini Mattoo Case (1996): After the case of Santosh Kumar Singh, an IPS officer's son, was acquitted of the murder and rape of law student Priyadarshini Mattoo in 1999, the case became a national phenomenon. However, the Delhi High Court found him guilty and condemned him to hang in 2006 relying on DNA proof recovered from the victim's underwear. His sentence was reduced to life in prison by the Supreme Court[19].
- In Delhi (2012), the 'Gang Rape Case', in which six guys' horrific murder and gang rape of a young woman sparked widespread condemnation, each of

the suspects was convicted to death depending upon the DNA proof and the victim's dying declaration.

- In 2014, intelligence agents stormed a residence in Mangalore's Zephyr Heights in search of accused bombers, but it was vacant. The results were confirmed by the accused after being apprehended by agents. It supported the NIA in getting all five suspects convicted[19].
- Abraham Lincoln's assassin case: John Wilkes Booth escaped imprisonment after the assassination of President Abraham Lincoln on April 14, 1865. After twelve days on the run, he was also fired and killed on April 26 by a soldier from the 16th York City Cavalry. Nevertheless, speculation persisted that John Wilkes Booth had evaded capture. Wilke's corpse was unearthed to verify his death, and his personal dentist, Dr. William Merill, recognized Booth by his atypical jawbone and the golden fillings he had done on behalf of the corpse just days earlier in the murder[20].
- Famous bite mark case - Ted Bundy, serial killer – 1978: American serial murderer Ted Bundy, who allegedly raped and murdered more than thirty young women throughout the 1970s. An eyewitness reported the murders of Lisa Levy and Martha Bowman on January 15, 1978. Both females had been brutally assaulted and died. The biological data gathered at the murder scene was inconclusive. Lisa Levy had been bitten on her left buttock and breast, which ultimately proved to be crucial evidence presented. A forensic dentist in Florida, Dr. Richard Sovran, was asked to investigate the accused and the evidence. Bundy hesitated to participate, so a court order was issued compelling him to undergo a dental checkup. From the pictures, bite data, and dental imprints that were later generated, the tooth marks on Lisa Levy's left thigh clearly matched the imprints made by the accused at Raiford Prison in Starke, Florida. Ted Bundy was sentenced to death on January 24, 1989. The Bundy case was the first in Florida history to be recorded using bite mark proof[21].

Future Scope of DNA Fingerprinting[22]

Nowadays, a range of automated or robotic devices capable of executing a wide range of activities, varying from quick extraction robots to laboratory automation workstations, have been created. Beckman Coulter Inc.'s Biomek 3000® workstation, for example, can reproducibly and precisely estimate the quantity of amplifiable individual DNA from retrieved DNA specimens.

In India, Indian Biosciences provides a wide variety of DNA analysis, paternity testing, genetic analysis, and DNA ancestry services, or DNA genealogy, to provide definitive answers to emotional concerns in a professional and discrete manner. Although present DNA analysis techniques are fairly specialized, several upcoming simplifications may increase their simplicity of use. Improved methods for preserving DNA specimens in safe locations will presumably lead to bigger advancements in the future. Trying to cure genetically predisposed illnesses would be a significant boon to many suffering people and future generations. The applications of DNA profiling are limitless. Profiling has been shown to be one of the most important assets in the history of science as well as a benefit to forensic science.

Conclusion

Forensic dentistry is important in recognizing people who cannot be identified visually or in different ways. The invention of DNA fingerprinting fundamentally transformed the concept of identity. We believe that future advancements in DNA technology may lower the cost and time required to identify unidentified remains. Meanwhile, clinical examination of relevant dental and medical patient data continues to be the top bar in forensic science.

DNA analysis has made considerable progress in identifying people and is now frequently utilized in criminal cases, family inquiries, and mass calamities. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphisms (RFLPs) on a Variable Number of Tandem Repeats (VNTRs) investigations are the two most used processes for DNA fingerprinting.

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