

DNA Profiling in Forensic Dentistry

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Abstract

The recent advances in molecular biology have revolutionized all aspects of dentistry. Deoxyribose Nucleic Acid (DNA), the language of life yields information beyond our imagination with respect to our body health conditions. DNA is present in chromosomes and is responsible for storing all the genetic material and they are not the same for all individuals. Forensic dental comparison has been used for identification of humans in cases where body tissues are damaged or prolonged exposure to the environment has made the identification impractical. The use of DNA profile tests in forensic dentistry provides a new perspective in human identification. This article summarizes the recent literature on use of this technique in identification of unidentified human remains, highlighting the importance of DNA in the cases of forensic investigation.

Key Words: DNA profiling; forensic dentistry; human identification; molecular biology.

Introduction

The discipline of Forensic Dentistry was introduced to the curriculum of the Brazilian Dental Schools after issuing of Decree no. 19852, amended in 1931. Since then, this specialty has continued to evolve, showing remarkable scientific and professional maturity lately¹. The role of dental restorations, and radiological identification among forensic odontology has declined, whereas the molecular biology and laboratory procedures are increasing at a fast rate in efficiency and availability². The Deoxyribose Nucleic Acid (DNA) extracted from the teeth of an unknown individual is matched with DNA isolated from known ante mortem samples such as stored blood, tooth brush, hairbrush, cervical smear, biopsy, to a parent or sibling. This is the usual procedure in DNA analysis³.

Several biological materials are used for isolation of DNA and accomplish the laboratory tests for human identification, including bone tissue, hair bulb, biopsy sample, saliva, blood, etc. It is possible to obtain DNA from all human body tissues but only with variations in the quality and quantity of the DNA extracted from each tissue. Teeth play an important role as the subject of DNA studies as the dental hard tissue physically encloses the pulp and offers an anatomical configuration of great durability⁴. When morphologically evaluated, even a single tooth provides valuable information regarding the individual to whom the tooth belongs^{5,6,7}. The importance of Forensic Dentistry in the field of human identification, mainly when there is little remaining material to perform these identifications has brought dentists to work with forensic investigation and make them more familiar with the new molecular biology technologies. Previously our team had conducted numerous original studies⁸⁻¹⁴ and surveys¹⁵⁻²² over the past 5 years. Now we are focusing on applying this knowledge to write the review on new advancements in various fields. This article summarizes the recent literature on use of this technique in identification of unidentified human remains, highlighting the importance of DNA in the cases of forensic investigation.

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BASIS OF DNA FINGERPRINTING

Each individual has a unique DNA sequence. The DNA is the same for every cell in a person. Every person has a different DNA fingerprint which will identify only that individual. The knowledge of DNA science is the only way to identify a particular individual. DNA fingerprinting or DNA profile are the only codes that reflect a person's DNA makeup, which can also be used to identify individuals. The repetitive sequence is highly polymorphic and unique to each individual. It is generally seen as long tandem repeats (mini satellites), short tandem repeats (STR; mini satellites) and distributes repetitive sequences. A gene particularly codes a separate protein which is found in a segment of DNA. The function of the remaining 95% or more of the DNA is not known and is called non-coding DNA or junk DNA²³. Variations in DNA sequence called polymorphisms can be used both to differentiate and to correlate individuals²⁴. A variable number tandem repeat is a location in a genome where an organized tandem repeat is from a short nucleotide sequence. These are commonly found on many chromosomes, and often show variations in length (number of repeats) among persons. Each variant acts as an allele which is inherited, allowing them to be used for identification of a person or a parent. Their analysis is useful in genetics, forensics, and DNA fingerprinting.

DNA AND FORENSIC DENTISTRY

The main factors which are exogenous may limit the retrieval of information from body remnants and stops the processes of human identification are the elements present or associated with heat and its impacts²⁵. In this sense, the teeth play an important role in identification and criminology, due to the high uniqueness of dental characteristics in addition to the relatively high degree of physical and chemical resistance of the dental structure²⁶. Due to the resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation, decomposition and microbial action, teeth represent an excellent source of DNA material. In a tooth, dentin and pulp are rich sources of DNA which can be successfully extracted where pulp is vascular and dentin is avascular²⁷. Total production of genomic DNA obtained from dental samples may range from 6 µg to 50 µg DNA²⁸. A study stated that the polymerase

chain reaction method enables the differentiation of an individual from another, with a high level of reliability and with about 1 ng of the target DNA. Thus, abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis²⁹. DNA is preserved in the teeth and bones for a very long period and thus is a valuable source of information. Ancient DNA (aDNA) analysis can be carried out in samples that are hundreds to tens of thousands of years old³⁰. Even though 99% of the bodies were identified using dental records or fingerprints and only 1% of forensic identification was made by DNA profiling³¹.

SOURCES OF DNA IN TOOTH

The teeth differ in shape and size (dimensions) but have similar histological structure. The dentin is a connective tissue that forms the major structural axis of the tooth. The dentin on the crown of the tooth is covered by enamel. The tooth is attached to the bone through periodontal ligament. The enamel origin is ectoderm and is a highly mineralized tissue. Furthermore, it is an acellular and avascular structure without nerves. Soft tissue within the coronal and radicular pulp chamber consists of odontoblasts, fibroblasts, endothelial cells, peripheral nerve, undifferentiated mesenchymal cells and nucleated components of blood which are rich sources of DNA. Odontoblastic processes that extend into dentinal tubules, soft tissue within accessory canals, cellular cementum, adherent bone and periodontal ligament fibres are less frequently used in anatomical locations of DNA. Pulp tissue is most commonly used because it is usually abundant and has blood supply and has least chance of contamination by nonhuman DNA. Sampling of the pulp tissue is done in three ways: crushing, vertical or horizontal splitting, and by endodontic access.

DNA EXTRACTION

DNA extraction process is composed of 3 different stages: cell rupture or lysis (which allows use of several techniques for effective rupture of the cell membranes), protein denaturation and inactivation (by chelating agents and proteinases in order to inactive elements, such as proteins), and finally DNA extraction itself³². The techniques of DNA extraction most often employed in Forensic Dentistry are the live and natural method (composed of phenol-chloroform and used for high molecular weight DNA, laborious, time consuming, with

a higher likelihood of errors, given the use of multiple tubes and can only be done if abundance of sample is available); Chelex 100 (the fastest with the lowest risk of contamination, yet very expensive); FTA Paper (composed of absorbent cellulose paper with chemical substances, which speed up its use); and isopropyl alcohol (containing ammonium and isopropanol, which is less expensive and also an alternative to the organic method)³³.

TYPES OF DNA

Genomic and mitochondrial are two types of DNA which are used in forensic sciences. The genomic DNA is found in the nucleus of each and every cell in the human body and represents a DNA source for most forensic applications. The teeth are an excellent source of genomic DNA. Mitochondrial DNA (mtDNA) is another type of material that can be used when the extracted DNA samples are too small or degraded, such as those obtained from skeletonized tissues, the likelihood of obtaining a DNA profile from mtDNA is higher than that with any marker found in genomic DNA³⁴. Various biological samples such as hair and teeth that lack nucleated cellular material can be analyzed with mtDNA.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

RFLP is used for analyzing the variable lengths of DNA fragments that result from digesting a DNA sample with “restriction endonuclease” which is a special kind of restriction enzyme. The separate sections of DNA at a specific sequence pattern is known as a “restriction endonuclease recognition site”. RFLP requires relatively large amounts of DNA. Hence, cannot be performed with the samples degraded by environmental factors and also takes longer time to get the results³⁵.

PCR

Polymerase Chain Reaction (PCR) is used to amplify the amount of DNA material available, so DNA analysis is carried out by sufficient quantity. To proceed the special enzymes and DNA primers are required. These primers resemble the probes with known constant sections of DNA but they are unlabeled. They are designed to know constant sections of DNA at the

ends of variable regions to be amplified. The principle of PCR is that the DNA has a special feature that it can duplicate itself. This is usually done by unwinding the DNA strands and each strand helps in synthesis of new strands. By PCR technique, amplification of specific DNA segments dependent on the employed primer is done. The standard PCR reaction runs through 30 cycles in a couple of hours which results in amplification of original DNA by over 109 times³⁶.

The DNA found can be of two types, they are genomic DNA and mtDNA. The teeth are excellent in PCR analyses and allows comparing the collected postmortem samples to known antemortem samples or parental DNA. Main advantage of mtDNA is the high number of copies per cell (from hundreds to thousands of organelles). PCR was performed in a thermal cycler. More recently, application has also been found in molecular biology, where different methods are used in optimizing PCR.

STRs TYPING

In forensic samples taken from humans, the study of DNA is usually performed by Short Tandem Repeat (STR) analysis, which can be defined as hypervariable regions of DNA that present consecutive repetitions of fragments that have 2-7 bp³⁷. The Federal Bureau of Investigation has chosen 13 specific STR loci to serve as the standard for the Combined DNA index system³⁸. STR was used on 45 DNA samples from teeth obtained from unidentified bodies buried in 1995 and exhumed in 2000, and pulp showed the strongest PCR amplification signals³⁹. STR testing is being used for forensic identifications, making a revolution on human identification and paternity tests.

mtDNA ANALYSIS

mtDNA is the strongest tool in forensic identification since it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring has the same mtDNA as their mothers since the mitochondrion of each new embryo comes from the mother’s egg cell and the nuclear DNA is contributed by the father’s sperm. It is used in finding missing persons, comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique³⁵. However, mtDNA analysis is a

very costly technique and is less informative. Thus, this analysis is not used in all forensic laboratories directed at resolution of crimes.

Y - CHROMOSOME ANALYSIS

DNA-polymorphisms on the human Y chromosome are valuable tools for understanding human evolution, migration and for tracing relationships among males⁴⁰. Most of the length of the Y chromosome is inherited as a single block in linkage from father to male offspring as a haploid body. Hence Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis⁴¹.

X - CHROMOSOME STR

Chromosome X specific STR is used in the identification and the genomic studies of various ethnic groups in the World⁴². Since the size of X-chromosome STR alleles is small, generally including 100-350 nucleotides, it is relatively easy to be amplified and detected with high sensitivity⁴³. X-chromosome STR (X-STR) markers are a powerful complimentary system especially in deficiency paternity testing.

SNPs

Single Nucleotide Polymorphism (SNP) detection technologies are used in scanning the new polymorphisms and helps in determination of the allele(s) of a known polymorphism in target sequences⁴⁴. SNP detection technologies have evolved from labor intensive, time consuming, and expensive processes to some of the most highly automated, efficient, and some inexpensive methods. Local, target, SNP discovery relies mostly on direct DNA sequencing or on denaturing high performance liquid chromatography⁴⁵.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (Amp FLP)

AmpFLP, amplified fragment length polymorphism was also put into practice during the early 1990s⁴⁶. This technique is faster than RFLP analysis and used in PCR to amplify the DNA samples. AmpFLP analysis can be highly automated, and allows in creating easy phylogenetic trees based on comparing individual samples of DNA. Owing to its relatively low cost and ease of set-up and operation, AmpFLP remains popular

in lower income countries⁴⁷.

Conclusion

The finding of DNA fingerprinting has revolutionised the concept of identification. It is sensible to anticipate that future advances in DNA technology will reduce the time and cost factor for identification of unknown deceased. It has also revolutionised the forensic identification procedures. Therefore, dental professionals who are working on the field of Forensic Dentistry should include these new technologies in their work, as several methods are available for DNA extraction from biological materials, yet similarity of the protocols adopted for such purpose has not been reached so far. The field is developing to find out many riddles in the human genome.

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