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Use of DNA Technology in forensic Dentistry

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Abstract

The identification of human remains can be carried out by means of anthropological methods (1), dental structure analysis (2), and DNA-based analytical methods (3). DNA analysis remains the only method of identification mainly when there is little remaining material to perform such identification (e.g., in fires, explosions, decomposing bodies or skeletonized bodies), which has led dentists working with forensic investigation to become more familiar with the new molecular biology techniques. The currently available DNA tests have high reliable. This article presents a literature review referring to the main studies on Forensic Dentistry that involve the use of DNA for human identification, and makes an overview of the evolution of this technology in the last years, highlighting the importance of molecular biology in forensic sciences.

Key words: DNA; forensic dentistry; molecular biology

Introduction

Human identification is one of the major fields of study and research in forensic science because it deals with the human body and aims at establishing human identity. The revolution caused in 1953 by Watson and Crick[1], who discovered the double-helix structure of DNA, which is responsible for the genetic inheritance of human beings, led to important changes in nearly all fields of science. This discovery was the basis for the development of techniques that allow characterizing each person's individuality based on the DNA sequence. Three decades later, radioactive molecular probes were created by Jeffreys, et al[2], that could recognize highly variable regions of DNA and thus determine the specific patterns of each individual, which were named DNA fingerprints. The currently performed DNA profile tests are totally reliable, being accepted as legal proofs in courts, such as for investigation of paternity and human identification. Several biological materials may be employed for isolation of DNA and accomplishment of laboratory tests for human identification, including bone tissue, hair bulb, biopsy sample, saliva, blood and other body tissues. It is possible to obtain DNA from

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virtually all human body tissues, only with variations in the quantity and quality of the DNA extracted from each tissue.

Forensic Dentistry is the specialty with the goal of investigating psychological, physical, chemical and biological phenomena that can reach human beings (alive, dead or body fragments), comprehending aspects of human identification; criminal, civil, labor and administrative forensic investigation; legal documents; forensic traumatology; image (x-ray, tomography) examinations; saliva analysis; and other aspects involving a multidisciplinary team .

The established importance of Forensic Dentistry for human identification, mainly when there is little remaining material to perform such identification (e.g. in fires, explosions, decomposing bodies or skeletonized bodies), has led dentists working with forensic investigation to become more familiar with the new molecular biology technologies. Therefore, this article presents a review of literature on studies that have used DNA analysis for human identification and an overview of the evolution of this technology in the last few years, highlighting the importance of molecular biology in cases of forensic investigation.

Background

Till the 1980's the science of identification of criminal cases was based only on serological analyses of protein polymorphism, blood groups and some genetic markers. Forensic examination of biological samples started in the beginning of the twentieth century by application of the ABO blood group system in evidence related to crimes or human identification. Use of blood group testing as the proof of individual identification was legally accepted by German courts in 1920 and United States in 1935, which are rarely used nowadays.[3]

An important phase in the development of forensic sciences for human identification started with the publication of a study[2] which investigated radioactive molecular probes that could recognize certain highly sensitive regions of DNA (minisatellites in human genome) that produced a type of DNA "fingerprint". Molecular typing of the genetic material was officially employed for the first time in England by Jeffreys, et al[4].

Since then, Criminal and Forensic Medicine has further evolved and entails DNA fingerprint molecular typing techniques as a powerful tool for resolution of thousands of crimes and for human identification.[3]

The first polymorphic locus in the human genome was discovered by Wyman and White [5] (1980), using a DNA probe ,more than 15 different sizes could be observed in a small sample of subjects. These repeated sequences are spread throughout the human genome and present sufficient variety to be used in human identification tests. These hypervariable loci are constituted by tandem repeat of oligonucleotides sequences (from 2 to 80 bp). Depending on their size, these loci are nominated as VNTR (variable number of tandem repeat) or minisatellites, 9 to 80 pb, and STR (short tandem repeats) or microsatellites, 2 to 5 bp24.

Initially, the forensic experts used VNTR testing for body identification and paternity tests. However, as this method requires a large amount of material and has low quality results, resulting in failures in those cases where only little biological material samples were collected at the scene of crime investigation. The introduction of the polymerase chain reaction (PCR) technique, which makes possible the amplification of small DNA samples, widened the scope in Forensic Genetics.[6] STR testing started being used for forensic casework, making a revolution on human identification and paternity tests.

In addition, newer DNA tools, including mitochondrial DNA and SNP (single nucleotide polymorphism replacements, insertions or deletions that occur at single positions in the human genome), might be used when STR typing fails to yield a result or when only a partial profile is obtained due to the size and conditions of the sample.[7]

DNA and Forensic Dentistry

Forensic Dentistry has contributed

remarkably to the process of human identification. Fingerprints have been historically used for identification but in some situations, such as fire and skeletonization, they are easily destroyed. In addition, experts frequently need to use comparative elements of the victim produced prior to his/her death, such as the dental records, to carry on the identification. However, this documentation may be unavailable or incomplete. At present, with the application of biomolecular resources for human identification, it is possible to identify a person using small amounts of deteriorated biological material, conditions that are relatively frequent in forensic analyses.[8] This fact could be demonstrated after the South Asian Tsunami disaster on December 26th 2004, when the most varied techniques were applied for identification of thousands of victims, such as forensic pathology, forensic dentistry, DNA profiling and fingerprinting. 99% of the bodies were identified using dental records or fingerprints and only 1% of forensic identification was made by DNA profiling.[9,10]

The main exogenous factors that may limit the retrieval of information from body remnants and restrict the processes of human identification are the elements present or associated with fire, such as flames, heat and explosions.[11] In this sense, the teeth play an important role in identification and criminology, due to the uniqueness of dental characteristics in addition to the relatively high degree of physical and chemical resistance of the dental structures.[10]

Due to their capacity of enduring environmental changes, the teeth represent an excellent source of DNA because this biological material may provide the necessary relation for identification of an individual in case of failure of conventional methods for dental identification.[12]

Mitochondrial and Genomic DNA in Forensic Dentistry

The genomic DNA is found in the nucleus of each cell in the human body and represents a DNA source for most forensic applications. The teeth are an excellent source of genomic DNA because PCR analyses allow comparing the collected post-mortem samples to known ante-mortem samples or parental DNA.[12,13]

Mitochondrial DNA is another type of material that can be used for body identification, which represents 0.5 percent of the total DNA and is readily separable from the genomic DNA. Mitochondrial DNA is 16,569 nucleotide bp in length and is present in high-copy number in all cells and is more likely to survive for prolonged periods, compared to chromosomal DNA. Its main advantage is the high number of copies *per* cell (from hundreds to thousands of organelles). When the extracted DNA samples are too small or degraded, such as those obtained from skeletonised tissues, the likelihood of obtaining a DNA profile from mitochondrial DNA is higher than that with any marker found in genomic DNA.[14] Moreover, study by Silva and Passos (2002) stated that the analysis of mitochondrial DNA for forensic purposes is restricted to ancient tissues, such as bones, hair and teeth, in which the nuclear DNA cannot be analyzed, as it is inherited only from the maternal line and is the best way to test relatedness if there are several generations between ancestor and living descendant. In contrast to human genomic DNA, which codes for 100,000 genes, mitochondrial DNA encodes for only 13 different genes.

However, this examination is performed by direct sequencing of its nitrogenous bases, which is a very expensive technique because it employs a highly specialized technology. Furthermore, mitochondrial DNA is exclusively matrilineal and hence less informative. Thus, this analysis is not usual in all forensic laboratories directed at resolution of crimes and identification of persons.

The total production of genomic DNA obtained from a dental sample ranges from 6 µg to 50 µg DNA[15]. The results are obtained from DNA extracted from the dental pulp and generally do not show any difference when compared to the patterns obtained from DNA isolated from blood samples or available lung tissues.

In forensic samples, the study of DNA (genomic and mitochondrial) is usually performed by STR (short tandem repeats) analysis, which can be defined as hypervariable regions of DNA that present consecutive repetitions of fragments that have 2 to 7 base pairs (bp). The VNTR (variable number of tandem repeats) testing, which may

present short repeated sequences of intermediate size (15 to 65 base pairs), is rarely used in forensic analyses due to the poor quality DNA provided with this method. The most valuable STRs for human identification are those that present greater polymorphism (greater number of alleles), smaller size (in base pairs), higher frequency of heterozygotes (higher than 90%) and low frequency of mutations .

DNA applications in Forensic Dentistry

The environmental influence on the concentration, integrity and recovery of DNA extracted from dental pulps has been previously measured by Schwartz, et al.[16] The authors varied the pH (3.7 and 10.0), temperature (4ºC, 25ºC, 37ºC and tooth incineration), humidity (20, 66 and 98%), type of the soil in which the teeth were buried (sand, potting soil, garden soil, submersion in water and burying outdoors) and periods of inhumation (one week to six months). It was determined that the environmental conditions examined did not affect the ability to obtain high-molecular-weight human DNA from dental pulp. Tsuchimochi et al[17]tested the Chelex 100 chelating resin to extract DNA from the dental pulp for subsequent application on PCR analysis. For such purpose, the extracted teeth were incinerated for 2 minutes at temperatures of 100ºC, 200ºC, 300ºC, 400ºC and 500ºC. All samples incinerated at up to 300ºC could be amplified and typed, whereas those incinerated at temperatures above 400ºC did not produce any PCR product. The authors concluded that extraction of DNA from the dental pulp using this resin is appropriate for obtaining high-quality DNA samples for PCR amplification.

In order to evaluate the different dental tissues as DNA sources in forensic analyses, a study was conducted in which 20 teeth were obtained from unidentified bodies buried in 1995 and exhumed in 2000, providing 45 DNA samples (5 from the pulp, 20 from dentin and 20 from cementum).[18] The pulp produced the strongest PCR amplification signals, while dentin and cementum signals were very similar to each other. Hanaoka et al[19] evaluated DNA extraction from 50 teeth (pulpal and hard tissues). The DNA obtained from the dental pulps ranged from 3 to 40 µg, and no correlation was found between the storage period and the amount of DNA. The authors investigated the efficiency of DNA extraction from hard dental tissues at different concentrations of a decalcifying solution. The DNA obtained from the dental pulp was of high molecular weight, which allowed analysis by multilocus probes or PCR. On the other hand, the material obtained from the hard dental tissues showed satisfactory analysis only by the PCR technique. The PCR amplification was evaluated of DNA retrieved from teeth subjected to heat (200ºC, 400ºC, 500ºC and 600ºC) during 60 minutes, testing 3 different extraction methods (organic; ammonia acetate/isopropanol and silica)[20]. Using the organic method for genomic DNA extraction, 50% of samples subjected to burning were amplified, but only at lower temperatures (200ºC and 400ºC). At higher temperatures (500ºC and 600ºC), the isopropanol/ammonia acetate extraction method yielded better results, mainly for extraction of mitochondrial DNA.

Among the several cases described in the literature with DNA isolation from teeth, a case of human remains was published[14] for identification in which a victim's body was almost completely carbonized which then precluded DNA analysis by the usual methods. However, a preserved unerupted third molar enabled DNA extraction from the dental pulp (1.35 µg), which was an excellent source of high molecular weight genomic DNA.

In addition to human identification, another subject of study of forensic Dentistry relates to molecular biology which includes analysis of bite mark evidence. In cases of physical assault, such as sexual abuse, murders and child abuse, bite marks are frequently found on the skin.[21] The aggressor's saliva is usually deposited on the victim's skin during biting, kissing or suction, it is possible to identify the aggressor's blood group by the ABO system in 90% of cases, [20] but this method is not very informative and would not be used if DNA amplification techniques, such as STR profiling, are available. From these cells, it is

also possible to isolate DNA for identification of the aggressor.

Several studies are currently being conducted in order to optimize the methodology of DNA extraction from the saliva deposited on the skin to be used as evidence in forensic cases, such as the double-swab testing. According to Anzai et al[23] this examination allows establishing DNA profile in 4 of 5 tested samples composed of 250 µL of saliva deposited on the skin. In addition to gathering cells from the human body itself, it is also possible to retrieve cell samples from objects that had contact with the body, which are called artifacts.[25] DNA can be isolated in sufficient amount for human identification by examination of chewing gums, cigarettes, bite marks in foods, among others.[24,25]

DNA Extraction and PCR Amplification

Researchers must carefully evaluate the conditions of the material to be examined, especially when dealing with forensic cases, in which there is a greater risk of sample contamination and influence of environmental factors, in addition to a small amount of material available in most situations.

The PCR technique has been the usual choice for investigation of the frequencies of STRs. This technique allows amplification of restricted regions of the human genome, associated with genomic hybridization. Recent developments of the technique of length amplification of polymorphic fragments have enhanced the potential of analysis of forensic samples. The PCR method enables differentiation of an individual from another, with a high level of reliability and with about 1 ng (one one-billionth of a gram) of the target DNA.[26]

According to Brown,[27] those who intend to work in forensic analysis laboratories adopting DNA examination as the methodology of choice, and especially those intending to use the PCR technique as a working tool should have attention and accuracy during sample handling as well as, follow strict policies to prevent contamination. In practice, steps aiming at reliable results that might contribute to elucidate forensic cases are the adequacy of collection procedures, verification of the conditions of the collected material, choice of methodology for DNA extraction and analysis, and, finally the analysis of results.

DNA extraction is a process 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 itself. The techniques of DNA extraction most often employed in Forensic Dentistry include the organic method (composed of phenol-chloroform and used for high molecular weight DNA, with a higher likelihood of errors, given the use of multiple tubes); 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) [28] ; Silica based columns (successfully used in highly degraded samples) .

There is requirement to develop techniques to overcome the problems of contamination and degradation of DNA sample. The factors leading to the degradation of DNA include time, temperature, humidity (facilitating the growth of microorganisms), light (both sunlight and UV light) and exposure to various chemical substances[29]. Combinations of these conditions are often found in the environment and tend to degrade the samples into smaller fragments. Therefore, once a sample has been collected, it must be dried (or remain dry), depending the type of biological material. It may also be stored frozen (if necessary), although for DNA this is less important than for the conventional protein and enzyme systems. The sample should not be subjected to fluctuations in either temperature or humidity.[29]

Conclusion

The findings of the several studies reviewed in this article demonstrate that the teeth represent an excellent source of DNA, which is protected by

epithelial, connective, muscular and bone tissues in case of incineration. Additionally, the dental pulp cells are protected by hard dental tissues like enamel, dentin and cementum. New technologies should be incorporated in forensic dentistry, as several methods are available for DNA extraction from biological materials. Standardization of the protocols should be adopted, as studies on molecular biology applied to human identification will probably further enhance DNA extraction with less material available and under increasingly adverse conditions.

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