

Use of DNA Technology in Forensic Dentistry

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Abstract

The discovery of DNA plays vital role in Human identification is one of the major fields of study and research in forensic science. This discovery was the basis for the development of techniques that allow characterizing each person's individuality based on the DNA sequence. Individual identification of ancient human remains the most fundamental requisites for studies of paleo-population genetics, including kinship among ancient people, intra- and inter population structures in ancient times, and the origin of human populations. However, knowledge of these subjects has been based mainly on circumstantial archaeological evidence for kinship and intra population structure and on genetic studies of modern human populations. Tooth and several biological materials such as bone tissue, hair bulb, saliva, blood and other body tissues may be employed for isolation of DNA and accomplishment of laboratory tests for human identification. Here we describe individual identification of ancient humans by using short-nucleotide tandem repeats and mitochondrial DNA (mtDNA) extracted from a tooth as genetic markers. The application of this approach to kinship analysis shows clearly the presence or absence of kinship among the ancient remains examined.

Keywords: Forensic science; Human tooth; mtDNA; Kinship

Introduction

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; forensic tanatology; legal documents; forensic traumatology; image (x-ray, tomography) examinations; saliva analysis; and other aspects involving a multidisciplinary team [1].

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 [2], 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, Jeffreys et al. [3], created radioactive molecular probes 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 [4].

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 virtually all human body tissues, only with variations in the quantity and quality of the DNA extracted from each tissue. 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), has led dentists working with forensic investigation to become more familiar with the new molecular biology technologies.

Therefore, this article presents a literature review referring to the recent Brazilian works and some international studies on Forensic Dentistry that used DNA analysis for human identification, and

makes an overview of the evolution of this technology in the last years, highlighting the importance of molecular biology in cases of forensic investigation.

Literature Review

Background

Until 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 evidences related to crimes or human identification. The proofs of individual identification by use of blood group testing gained legal value in the German courts in 1920, being legally accepted in the United States only in 1935. In Brazil, these exams were given legal value with the first paternity investigation in 1948. These systems have been replaced in most centers and are rarely employed in present days [5].

Another important phase in the development of forensic sciences directed at human identification started with the publication of a study by Jeffreys et al. [3], who investigated radioactive molecular probes that could recognize certain highly sensitive regions of DNA (mini satellites 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 the same author for resolution of an immigration problem.

The first polymorphic locus in the human genome was discovered

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Received April 12, 2012; Accepted June 12, 2012; Published June 14, 2012

Citation: Shambulingappa P, Soheyl S, Rajesh G, Kaur S, Aggarwal A, et al. (2012) Use of DNA Technology in Forensic Dentistry. J Forensic Res 3:155. doi:[10.4172/2157-7145.1000155](https://doi.org/10.4172/2157-7145.1000155)

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by Wyman and White [6], using a DNA probe. In this way, 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 were constituted by tandem repeat of oligonucleotides sequences (from 2 to 80 base pairs). Depending on their size, these loci were nominated as VNTR (variable number of tandem repeat) or minisatellites, 9 to 80 bp, and STR (short tandem repeats) or microsatellites, 2 to 5 bp [7]. Initially, the forensic community used VNTR testing for body identification and paternity tests. However, as this method requires a large amount of material and has low quality results, several cases could not be solved, especially when only little biological material samples were collected in a crime scene investigation. The introduction of the polymerase chain reaction (PCR) technique, which makes possible the amplification of small DNA samples, widened the scopes in Forensic Genetics [8]. 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 [9]. Poor quality DNA can be found, for example, in mass disaster, such as the World Trade Center attacks, airplane crashes, tsunamis and decomposing bodies. DNA tests were accepted by the Brazilian legislation only after the 1990s and are still questioned by some individuals. In addition, DNA testing involves a high cost due to the scarcity of public institutes prepared for routine accomplishment of DNA examinations. However, there are already evidence that the State recognizes the efficacy of the information provided by DNA tests and the consequent need to make them available to the general public [10]. For example, the Brazilian Federal District already follows the Law no. 1097/96, which assures the accomplishment of free DNA tests for persons with proven necessity [11]. In the São Paulo State, there is a project entitled "Caminho de Volta" (Way Back) that uses molecular biology technology in the search for missing children [12]. In addition to these initiatives, further laws on this topic are currently being developed, such as the Law Project no. 6610/02 proposed by the congressman Ricardo Izar, which foresees the creation of the State Bank of DNA, with the sole purpose of performing an initial identification record of newborns, and the Law Project no. 188/99 proposed by the congressman Alberto Fraga, which establishes the criminal genetic identification, among others [13].

DNA and forensic dentistry

Forensic Dentistry has contributed remarkably to human identification processes. Protected by Law no. 5081/66, Brazilian dental professionals can carry out investigations involving biological materials derived from the human body in various conditions (quartered, dilacerated, carbonized, macerated, putrefied, in skeletonization and skeletonized), with the aim of establishing human identity [7]. Fingerprints have been historically used for identification. However, 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 analysis [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. Even though, 99% of the bodies were identified using dental records or fingerprints and only 1% of forensic identification was made by DNA profiling [14,15].

The main exogenous factors that may limit the retrieval of information from body remnants and restrict the human identification procedure are the elements present or associated with fire, such as flames, heat and explosions [16,17]. 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 structure [15].

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 [18].

Genomic and mitochondrial 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 analysis allow comparing the collected postmortem samples to known antemortem samples or parental DNA [18,19]. Mitochondrial DNA is another type of material that can be used for body identification. 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 skeletonized tissues, the likelihood of obtaining a DNA profile from mitochondrial DNA is higher than that with any marker found in genomic DNA [20]. Moreover, Silva and Passos [21], 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. 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 [22]. In a study conducted by Pötsch et al. [4], the total production of genomic DNA obtained from a dental sample ranged from 6 µg to 50 µg DNA. The results were obtained from DNA extracted from the dental pulp and did 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 [23].

Possible applications of DNA 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. [24]. The authors varied the pH (3.7 and 10.0), temperature (4°C, 25°C, 37°C 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. [25], 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 100°C, 200°C, 300°C, 400°C and 500°C. All samples incinerated at up to 300°C could be amplified and typed, whereas those incinerated at temperatures above 400°C 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 (Figure 1).

In order to evaluate the different dental tissues as DNA sources in forensic analyses, Malaver and Yunis [26], conducted a study in 2003 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). The pulp produced the strongest PCR amplification signals, while dentin and cementum signals were very similar to each other. Hanaoka et al. [27] 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. Remualdo [28], evaluated the PCR amplification of DNA retrieved from teeth subjected to heat (200°C, 400°C, 500°C and 600°C) during 60 minutes, testing 3 different extraction methods (organic; ammonia acetate/isopropanol and silica). Using the organic method for genomic DNA extraction, 50% of samples subjected to burning were amplified, but only at lower temperatures (200°C and 400°C). As a result published by Sweet and Sweet (1995), at higher temperatures (500°C and 600°C), the isopropanol/ammonia acetate extraction method yielded better results, mainly for extraction of mitochondrial DNA. This paper presents a case of human remains identification in which a victim of murder was incinerated and had her body almost completely carbonized, reduced to approximately 25% of its original size, 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 related to molecular biology is the 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 [29]. The aggressor's saliva is usually deposited on the victim's skin during biting, kissing or suction. According to Sweet (2000), it is possible to identify the aggressor's blood group by the ABO system in 90% of cases, 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. [30], this examination allows establishing DNA profile in 4 out 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 [31]. DNA can be isolated in sufficient amount for human identification by examination of chewing gums, cigarettes, bite marks in foods, among others [32].

Careful handling of samples, DNA extraction and PCR amplification

As observed, several protocols are used for DNA extraction and analysis, and there is no standard methodology. Therefore, 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 [11]. 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 [11]. Sweet [33], stated that the PCR method enables differentiation of an individual from another, with a high level of reliability and with about 1 nanogram of the target DNA.

It should be mentioned that DNA extraction is a process composed

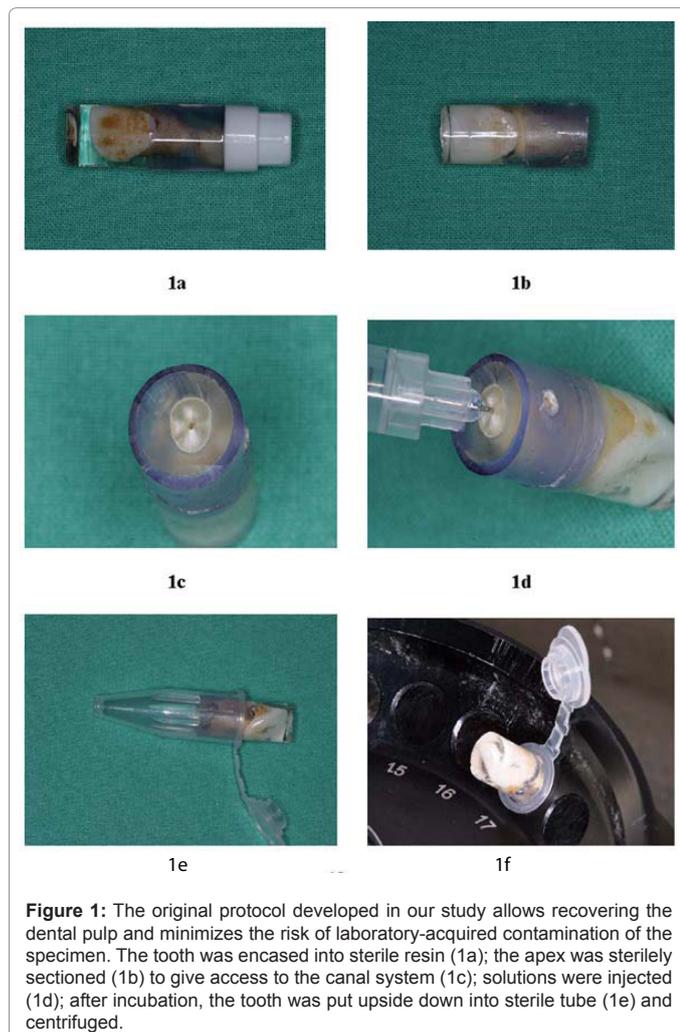


Figure 1: The original protocol developed in our study allows recovering the dental pulp and minimizes the risk of laboratory-acquired contamination of the specimen. The tooth was encased into sterile resin (1a); the apex was sterilely sectioned (1b) to give access to the canal system (1c); solutions were injected (1d); after incubation, the tooth was put upside down into sterile tube (1e) and centrifuged.

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 inactivate elements, such as proteins), and finally DNA extraction itself [34].

The techniques of DNA extraction most often employed in Forensic Dentistry are 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) [35]. An important observation is when degraded sample in the ancient DNA are the only artifacts, being necessary using techniques to overcome the problems of contamination and degradation of DNA sample. It has been reported by Rudin and Inman [36] in 2001, that 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. 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 on 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 [36].

Conclusion

Violence and crimes against human life, such as bomb explosions, wars or plane crashes, as well as cases of carbonized bodies or in advanced stage of decomposition, among other circumstances, highlight the need to employ ever faster and more accurate methods during the process of identification of victims. In such cases, 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 enamel, dentin and cementum- (hard dental tissues). Therefore, dental professionals working on the field of Forensic Dentistry should incorporate these new technologies in their work, as several methods are available for DNA extraction from biological materials, yet standardization of the protocols adopted for such purpose has not been reached so far.

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