Review Article

DNA in Forensic Odontology: New Phase in Dental Analysis

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Abstract

In forensic identification cases, where human remains are extremely damaged or degraded by mass disaster, teeth and bones are often the only available sources of DNA. Nuclear DNA and mitochondrial DNA are the most frequently investigated types of DNA in teeth. There are various techniques including decontaminations, sampling methods, and DNA extraction methods by polymerase chain reaction. DNA content of teeth varies considerably between individual and also between teeth from the same individual. Tooth type, chronological age and dental diseases affect the DNA content of teeth. Teeth are the preferred skeletal source of DNA because they can be retrieved from human skeletal remains even after very long years after death. The comprehensive understanding of tooth structure and composition, as well as process of diagenesis in teeth, is crucial for determining the location of DNA in postmortem teeth. Targeted subsampling and careful case selection of appropriate decontamination and extraction protocols will further increase the value of teeth as a source of DNA. This method can be applied in mass disaster where the fragments of the tooth are available for disaster victim identification.

Key Words: *DNA, forensic odontology, polymerase chain reaction, tooth*

Introduction

The significance of forensic dentistry with little remaining material for identification (e.g., in fires, explosions, decomposing bodies or skeleton), has led the investigators to become familiarize with advanced molecular biology technologies.

Mass disaster identification traditionally relies on the teamwork of different experts, such as police, forensic odontologists, physicians, and pathologists. Antemortem information from the missing persons is compared with postmortem data of the deceased persons. In most cases, investigations may fail due to a lack of proper antemortem records. If such data are unavailable, then the precise identification becomes complicated, and only DNA profiling system can help in exact identification of person. The resistance of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation, and decomposition, teeth represent an excellent source of DNA material (Schwartz *et al*. 1991; Rai *et al*. 2004).[1]

DNA extracted from the teeth of an unidentified individual will be compared with DNA isolated from known antemortem samples, such as stored blood, toothbrush, hairbrush, clothing, collected buccal cells with help of DNA-SAL™, cervical smear, biopsy, or DNA of parent or sibling.[1]

History

Until the 1980s, 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 20th century by application of the ABO blood group system in evidences related to crimes or human identification. The proofs of individual identification by the 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 tests 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. Another important phase in the development of forensic sciences directed at human identification started with the publication of a study by Jeffreys, *et al.* (1985), 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 Da silva, *et al*., for resolution of an immigration problem. Year after year, these authors employed this technique to identify the rapist and murderer of two victims. Since then, criminalistic and forensic medicine have further evolved and have applied DNA fingerprint molecular typing techniques as a powerful tool for resolution of thousands of crimes and for human identification.[2]

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The first polymorphic locus in the human genome was discovered by Wyman and White (1980), using a DNA probe. In this way, more than 15 different sizes could be observed in a small sample of subjects. These repeated sequences were spread throughout the human genome and present sufficient variety to be used in human identification tests. Depending on their size, these loci were nominated as variable number of tandem repeat (VNTR) or mini-satellites, 9–80 bp, and short tandem repeats (STR) or microsatellites, 2–5 bp.

Initially, the forensic community used VNTR testing for body identification and paternity tests. However, as no quality results were obtained and the method required a large amount of material, polymerase chain reaction (PCR) technique was introduced. In addition, newer DNA tools, including mitochondrial DNA (mt DNA) and single-nucleotide polymorphism (SNP) replacements, insertions or deletions that occur at single positions, 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.[3,4]

Guidelines for Obtaining Dental DNA

Soft tissue or blood is adherent to the tooth that should be sampled.

⇓

Debride that tooth of any plaque or calculus with a curette, and wash thoroughly with hydrogen peroxide followed by ethanol.

⇓

If the tooth is intact and is supposed to have been removed from the alveolus recently, a conventional endodontic access and instrumentation can be performed. Sectioning the tooth provides greater access to pulp.

⇓

Once the tooth is opened, the walls of the pulp chamber can be curetted or instrumented with a slow rotary burr. Then, pulp tissue can be collected in a wide open sterile tube.

⇓

In dried specimens, the pulp may be mummified parchment like. After instrumentation, the chamber is best irrigated with buffer. Ultra filtration of the liquid at the lab will remove the cellular material needed for analysis.

⇓

Finally, crushing the tooth may be required.

Mitochondrial DNA

The majority of cellular DNA is present in the cell nucleus as chromosomes. A small fraction, however, is present in the mitochondria. Each mitochondrion contains 5–10 ring-shaped pieces of DNA, called mt DNA, which are important for the structure and function of the mitochondrion. A child inherits its mt DNA only from the mother. The fertilized ovary cells only contain mitochondria from the cytoplasm of the ovary cell. The mitochondria of the sperm are located in the tail, which breaks off when the head of the sperm penetrates the egg at fertilization.

The advantages of mt DNA in forensic dentistry include the following:

- Identification is possible by comparison with members of maternal origin
- Haploid, and thus easier to handle and work with
- Higher number of copies present and various mitochondria per cell
- High polymorph regions, which augments the value of the test, as these regions contain a high number of variations in the DNA sequence, and thus decrease the possibility of resemblance between the donors and volunteer.

Although changes in junk DNA usually do not result in visible consequences, they are important in forensic science. These mutations serve as pronounced variations between individuals.[5]

DNA Amplification

PCR technique allows amplification of DNA from even negligible amounts of source material. The amplified DNA is then compared with antemortem samples such as stored blood, hairbrush, clothing, cervical smear, biopsy specimens. Other methods include restriction fragment length polymorphism, Single nucleotide polymorphism based, and microassays. DNA can also help in identification of a parent or sibling. Most of these techniques involve nuclear DNA but mt DNA is more abundant, and can be identified in cases when nuclear DNA is insufficient. Dental tissues such as dentin and cementum are rich in mt DNA.[6]

DNA Research

DNA can be split by restriction enzymes, which cut DNA at certain loci. DNA ligase binds the various parts together again. DNA is first extracted from the cells obtained from saliva or teeth, and then centrifuged to separate the DNA from the other cell components. After cutting by a restriction enzyme, the fragments are separated by gel electrophoresis. DNA has a negative charge and will migrate in an electric field to the positive pool. The extent of the migration depends on the size of the particle. At the same time, the short pieces will migrate over a greater distance than the long pieces, because they have less resistance. This technique can thus be used to separate the DNA fragments according to their size.

After separation, the DNA research can be completed. Using probes, marked pieces of DNA or RNA go through a process called blotting. After denaturation, a probe with complementary segments for certain specific sequences is added. These segments bind the single-stranded chain (hybridization), and make them visible, due to the radioactive marking.[7]

DNA Teeth and Saliva

DNA can be isolated from various sources, as long as they contain nucleated cells.[8] Such sources are blood, semen, tissues, organs, bones, hair, nails, teeth, saliva, urine, and other body fluids.[9]

Collection of samples

Teeth have a rich supply of genetic information. Nucleated cells can be isolated from the surrounding bone, periodontal fibers, and blood. However, the chance of contamination or degradation is very large, except in the pulp.[10] Cellular component pulp and in root decreases with age.[11] DNA from teeth can be obtained by crushing, but it destroys the morphology of the teeth.

Conventional endodontic access

It is difficult to obtain enough DNA through conventional endodontic access. In addition, the occlusal morphology and the restorations are damaged.[10]

Vertical splitting

A lot of pulp tissue can be obtained with the vertical splitting method, although the restorations and the tooth are damaged.[11]

Horizontal section

A fourth possible approach is the horizontal section, in which the tooth is split at the cervix border. The access of the pulp and the roots is sufficient and also the crown remains intact.

Cryogenic grinding

The tooth is frozen whole using liquid nitrogen and put in an electromagnetic chamber. The tooth is ground to a fine powder by alternating magnetic fields.[10]

Crushing gives better results than sectioning of teeth as more DNA can be obtained and chance of DNA damage can be ignored considering the sensitivity of PCR. The recently developed method of cryogenic grinding is easier and very effective. It also allows obtaining DNA from endodontic treated teeth because of the presence of DNA in hard tissues.[11]

Techniques for DNA Research in Saliva

Saliva can be taken directly from the mouth or collected after deposition on the skin. Saliva can also be found on objects such as post stamps, envelopes, and cigarette ends.[12]

Single-swab method

A fresh sample from the mouth can be obtained through the collection of saliva or by a buccal swab. With a dry swab, one rubs softly over the inner surface of the cheek. This buccal swab is then dried at room temperature.[12]

Filter paper method

Saliva on the skin can be obtained by a single swab with a sterile wet tissue, by a wet filter paper placed on the skin.[13]

Double-swab technique

Skin is first gently rubbed with a wet, sterile swab, whereby a rehydration and a release of the present cells occur. Then, the cells are collected with a second dry swab. Both swabs are collected into one sample.[12]

All samples obtained with the three techniques are dried at room temperature and stored at 4°C. The use of a wet and dry double swab technique for recovery of touched evidence improves the DNA profiling results and is useful in collecting the evidence at the crime scenes. The single wet swab may not recover epithelial cells present on the surface efficiently; DNA recovered by the second swabs alone can produce DNA pro-files. Because a detectable amount of DNA could be recovered by the second dry swab from the moisture left by the first wet swab, the DNA profiling results can be improved by pooling the first wet and the second dry swabs together for extraction. When the trace amounts of DNA obtained from a tested sample are close to the limits of sensitivity of the DNA profiling methods, obtaining a useful profile will depend on how well the sample is taken.[13]

Saliva from objects

Saliva found on objects can also be collected. After drying at room temperature, the object *(*e.g., poststamp, cigarette end) is cut into pieces and put into a buffer solution, refrigerated, and transported to the laboratory.[13]

DNA Extraction

DNA extraction allows the release of cells from the substrate arriving in the lab and the liberation of DNA from the cells. DNA extraction from dental material consists of dissolving the material in a buffer solution (Guanidine-EDTA), followed by centrifugation. The white precipitate is again dissolved in buffer, Proteinase K is added, and the solution is incubated overnight. The final step consists of a second centrifugation step plus the inactivation of Proteinase K.[14]

DNA extraction from saliva can be performed by an organic method. After dissolving the material in a buffer solution, centrifugation, and Proteinase K treatment, phenol-chloroform is added. The disadvantages of this method are the use of toxic substances and the fact that it is time-consuming (IS). Instead of phenol-chloroform, the saliva samples can also be extracted by Chelex-IOO. The metal ions are then chelated and inhibited. This prevents the degradation of the samples by its warming, which is necessary for the release of the cells from the swabs. Chelex also removes the impurities present in the material. This method is simple and fast.[15]

A modified Chelex method is used, where the sample undergoes preextraction. It is first warmed to release the cells from the swabs, boiled without chelex to eliminate the nucleases, and then boiled in the presence of chelex to extract the DNA.

The advantages of the Chelex method over the organic method are that there is higher DNA recovery, no use of toxic products, and it is less time-consuming. The modified Chelex method increases the number of released cells and the amount of available DNA, but it is more time consuming than the classical Chelex method. The modified Chelex method is therefore especially used when there is little DNA present in the samples *(*e.g., dried-up saliva on the skin).[16]

Slot blot quantification

The quantity and quality of the extracted DNA is checked by slot blot.[15] DNA is denatured by the addition of NaOH, the solution is applied to a nylon membrane in a slot blot apparatus and fixation is obtained by ultraviolet light or heating. After prehybridization, a probe is added with high

repetitive, primate-specific DNA fragments. These sequences are marked and after attachment to the denatured DNA strands, are made visible by autoradiography.[10]

DNA Analysis

Restriction fragment length polymorphism

DNA strands are cut in fragments by a restriction enzyme. These fragments are electrophoresed on an agarose gel, transferred to a nylon membrane, and incubated with a radioactive probe, which contains complementary pieces of polynucleotides that hybridize with the fragments and mark the various parts.

Two kinds of genetic variation can be detected, which include:

- Mutation in the zone where the restriction enzyme attacks the strand, a place that is normally cut, is not recognized. The two pieces remain together forming a long piece that has more resistance against migration in the gel compared to the two pieces separately
- Distance between two restriction enzymes alternate through insertion, deletion, or variation in a number of repeating unit. This is particularly useful in forensic science.[12]

Teeth as source of information

Teeth are normally resistant against extreme circumstances, and therefore, their morphology is well preserved. Not only the external form is preserved, but the enamel also protects the content of the pulp chamber against external factors. Moreover, hydroxyl apatite, an important component of dental tissue, binds to DNA and stabilizes it.[15] An abundance of DNA can be extracted from teeth because of dehydration, which prevents eventual putrefaction.[4]

Saliva as source of information

Saliva is deposited on the skin by kissing, licking, sucking, biting, etc., Previously, identification tests were performed by determining blood group, antigens, proteins, or isoenzymes. However, these methods were less sensitive because of their low concentrations.[17]

Salivary DNA from human bite marks

The usual methods of analyzing human bite mark evidence involve systematic physical comparison of the pattern of the injury in life-sized photographs or tracings to models of the suspect's teeth. These comparisons are often subjective and depend on the experience and procedures used by the odontologist.[13] Saliva is normally deposited on human skin during biting, sucking, licking, and kissing, so the potential use of the DNA present in saliva stains on skin shows any role of the suspect in causing a given bite mark.

Guidelines established by the American Board of Forensic Odontology 1995 for the collection of bite mark evidence advocate swabbing of the skin to collect saliva as part of the standard operating procedure.[10] Swabs can be tested for amylase, and positive test result confirms the presence of saliva and that the observed injury is in fact a bite mark.[12]

Human saliva has been shown to be an excellent source of high-molecular-weight DNA. Saliva recovered from material at crime scenes (i.e., clothing, cigarette butts, postage stamps, envelope stamps) has been completely isolated, analyzed, and compared to reference sources obtained from suspects.

Simultaneously, the advancement of alternative light technology permits investigators to identify the location of body fluids, such as saliva, blood, and semen left on the skin of a victim or other objects at a crime scene (e.g., in association with a bitemark).[10] It is found that:

- Human saliva is indeed a useful source of forensic DNA evidence. Some samples have yielded as much as 15 ng mL-1 DNA
- Significant DNA in a deceased victim can be stable and may be recovered up to 48–60 h after deposition on the skin, depending on environmental influences
- The success of PCR amplification is independent of the time since deposition or the concentration of DNA in the saliva sample
- In cases of unwashed skin, the DNA in dried saliva may be retrievable for up to 72 h
- Contamination of the saliva with other DNA is a potential problem, i.e., blood or sloughed skin cells
- When PCR analysis method is used to test salivary evidence, two benefits are provided amplification is possible from very small amounts of DNA, which allows genetic information to be obtained from evidence samples such as a single hair, an invisible semen stain and similar minute biological samples, i.e., saliva stains amplification is possible from very old material or from partially degraded DNA
- A double-swab technique for salivary DNA is suggested from Walsh DJ.[12]

Judicial value

In the case of a judicial DNA investigation, a DNA expert is appointed by a magistrate of justice. This expert analyzes the available biological samples. If two profiles are different, a person can be excluded with certainty. If, however, two identical DNA sequences are found, a statistical approach must be made.

Ethical consideration

The controversial arguments surrounding the ethical responsibility of DNA analysis can be reduced to two issues: (i) human values (privacy, respect for the body, etc.) are threatened by constructing a DNA databank. On the other hand, human values (self-defense, lock-up of criminals, etc.) are also threatened by not making such a databank; and (ii) by maximizing the identification of criminals, one also increases the chance that an innocent person will be convicted. According to Beyleveld, considering the rights of the humans as recorded by the European Convention on Human Rights, it is worse to lock-up an innocent person than to release a criminal.[18]

Conclusion

Forensic odontology is the forensic science that is concerned with dental evidence. The role of any forensic scientist is to collect, preserve, and interpret trace evidence, then to relay the results to the judicial authority in a form of a report. 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. Teeth represent an excellent source of DNA, which is protected by epithelial, connective, muscular, and bone tissues. 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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Conflicts of interest

There are no conflicts of interest.

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