Molecular Advancements in Forensic Odontology

A Babu RS, D Rose

ABSTRACT

Forensic odontology explores the field of human identification through dental tissues in cases where there is the destruction of body tissues in criminal investigations and mass disasters. Forensic odontology involves dentists participating in legal and criminal issues. Parameters such as age and gender identification are important in identifying the person or persons. Over the last two decades, the molecular aspect of forensic sciences has increased, and these molecular techniques now provide a novel approach to forensic odontology. Molecular advancements in science like DNA analysis have extended the range of forensic dentistry, as teeth possess the character of resistance toward physical or chemical aggressions. Teeth provide the abundant space for DNA, and hence teeth represent an excellent source of genomic DNA. The present paper focusses on molecular advancements in the field of forensic odontology.

Keywords: DNA, forensic odontology, mitochondrial DNA, polymerase chain reaction, short tandem repeat

Avances moleculares en la odontología forense

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RESUMEN

La odontología forense explora el campo de la identificación humana a través de los tejidos dentarios en casos donde hay destrucción de tejidos en investigaciones criminales y desastres masivos. La odontología forense es el campo de dentistas que participan en cuestiones jurídicas y penales. Parámetros tales como la identificación de la edad y el sexo son importantes en la identificación de la persona o personas. En las últimas dos décadas, ha aumentado el aspecto molecular de las ciencias forenses, y estas técnicas moleculares ofrecen ahora un nuevo enfoque de la odontología forense. Los avances moleculares en la ciencia, como el análisis de ADN, han ampliado el campo de acción de la odontología forense, ya que los dientes poseen se caracterizan por su resistencia a las agresiones físicas o químicas. Los dientes proporcionan abundante espacio para el ADN, y por lo tanto representan una excelente fuente de ADN genómico. El presente trabajo se centra en los

Palabras claves: ADN, odontología forense, ADN mitocondrial, reacción en cadena de la polimerasa, repeticiones cortas en tándem

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INTRODUCTION

Forensic odontology explores the field of human identification in cases where the body tissues are destroyed in the crime investigation and mass disasters (1). The established importance of forensic dentistry for human identification is mainly strengthened when there is little remaining material to perform such identification. The use of DNA analysis has, in recent times, expanded the scope of forensic dentistry.

DNA profiling or fingerprinting is useful because of the fact that although all humans display some genetic commonality, each person has unique genetic identifiers. In fact, with the exception of identical siblings, the DNA of every single individual is different. DNA identification tests use sophisticated techniques of molecular biology to compare samples taken from various sources such as: blood, semen,

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tissues, organs, bones, hairs, nails, teeth, saliva, urine and other body fluids. DNA can be isolated, as long as the sample contain nucleated cells, since every somatic cell contains the whole genome (2). The teeth are unique in that they are resistant to physical or chemical aggressions such as incineration, trauma and decomposition (3). DNA can be split by restriction enzymes, which cut DNA at certain *loci*, and can be bound together by DNA ligase. This has paved the way for the use of DNA techniques in the field of forensic odontology for identification (4). This article reviews the techniques for collection, extraction and analysis of DNA from teeth and the judicial importance of DNA research in forensic odontology.

The DNA molecule

The word genome stands for the complete set of genes. The structural and functional unit of the genome is deoxyribonucleic acid (DNA), which is the basis for all inheritance of character. The genetic information is coded within the chemical structure of the DNA molecule. Adenine (A), cytosine (C), guanine (G) and thymine (T) are the bases that make up the gene. These molecules pair with each other in a complementary fashion *ie* A with T, C with G and *vice versa*, and they determine the DNA sequence. These strands serve as identification tools in forensic investigation (5).

DNA and forensic odontology

The use of DNA technology is a molecular method that can be used as evidence in a forensic investigation. The DNA molecule can withstand adverse conditions such as temperature, pH, salt and other factors that usually destroy the classical serological markers. The molecular techniques help in hybridizing the obtained DNA to check the identity (6). The three basic steps for DNA in forensic research are extraction, quantitation, and analysis and interpretation (Fig. 1).

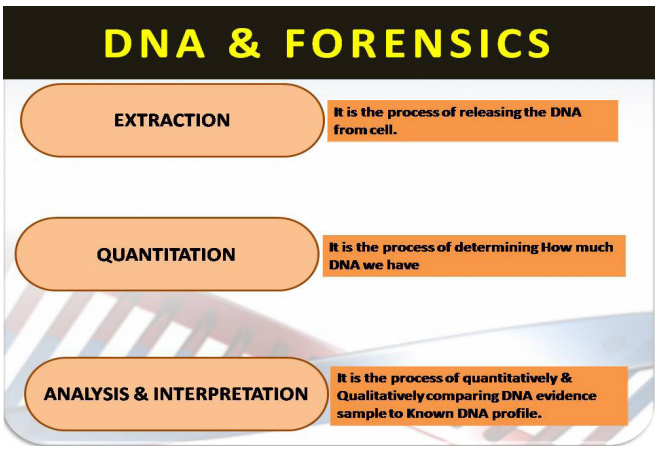

Fig. 1: Three basic steps for DNA research in forensics.

Techniques for collection of DNA

DNA can be obtained from various sources such as (cells that contain nucleus) blood, semen, tissues, bones, hair, nails, teeth, saliva, body fluids *etc*. The pulpal tissues in the teeth are rich in genetic information due to the availability of a higher number of nucleated cells. Nucleated cells can be isolated from the surrounding bone, periodontal fibres and blood. However, teeth are uniquely resistant to physical and/or chemical aggression such as incineration, trauma and decomposition (7). The chance of contamination or degradation of DNA is large with any tissue sample except the tooth. During the forensic odontology investigations, DNA can be extracted from cells obtained from saliva and/or tooth. Stravinos *et al* proposed guidelines to obtain dental DNA (8) [Table 1].

Table 1 : Guidelines to obtain dental DNA

1	Determine if there is any soft tissue or blood adherent to the tooth that should be sampled.
2	Debride the tooth of any plaque or calculus with hydrogen peroxide.
3	If tooth is intact, a conventional endodontic access can be conducted.
4	Sectioning the tooth provides a greater access to the pulp chamber (vertical).
5	Once the tooth is opened, the walls of pulp chamber can be curetted. Pulp tissue and powder can be collected over a wide mouth sterile container
6	Tooth crushing may be performed.

DNA can be isolated and tested from virtually any post mortem tissue, although the tissue undergoes progressive fragmentation after death. DNA is generally degraded into fragments through autolytic and bacterial enzymatic action. However, the sequence information is still present within the DNA fragments and therefore the information is not completely lost. Excessive DNA fragmentation during the decomposition process may result in the loss of genetic information and thus, becomes less useful in the forensic investigation (9). It is noteworthy to mention that not all DNA testing methods are appropriate for the sample tissue containing degraded DNA material. The type of DNA test is determined based on the status of the DNA material in the sample tissue (Table 2).

Table 2 : DNA test based on status of DNA material

Restricted fragment length polymorphism (RFLP) requires non degraded DNA.
Polymerase chain reaction (PCR) based analysis can be performed on degraded samples.
Mitochondrial DNA can be performed when nuclear DNA cannot be obtained.

Techniques to obtain DNA from teeth

Teeth posses rich genetic information in the nucleated cells such as odontoblasts, fibroblasts, endothelial cells, nervous tissue, blood cells and undifferentiated mesenchymal cells. All these cells are present in the pulpal tissue. The cells can also be obtained from periodontal fibres, blood and bone. The tooth provides sufficient space for pulpal tissue which is protected by the hard tissues such as enamel, dentin and cementum. Due to the presence of these hard tissues, the pulpal tissue is protected from physical and chemical aggressions. However, the dental DNA from the pupal cavity can be

obtained from many methods such as crushing, conventional endodontic access, splitting (vertical/horizontal) and cryogenic grinding (9). Crushing is a method of obtaining DNA by grinding complete teeth. Although DNA is obtained by this procedure, morphological/anatomical information of tooth is lost. The morphological/anatomical information may sometimes provide information such as developmental pathology/abnormalities of the teeth (10). In order to restore the tooth, dental DNA is also obtained from conventional endodontic access opening procedure. However, it is difficult to obtain a sufficient amount of DNA from this procedure. Furthermore, occlusal patterns and morphology are damaged with this procedure (7). Splitting is a procedure of cutting the teeth into two equal halves either vertically or horizontally. A larger quantity of pulpal tissue may be obtained by vertical or horizontal splitting. The vertical splitting method may have challenges due to varied root anatomy such as curvature and dilacerations, whereas horizontal splitting gives sufficient access for pulp excavation and the crown remains intact (10). Cryogenic grinding is a procedure of grinding of tooth by liquid nitrogen (11). Among all the above-mentioned methods for collecting DNA from the tooth, the best and most reliable procedures are cryogenic grinding and horizontal splitting. The steps to be followed are DNA extraction and DNA quantitation, DNA analysis and interpretation.

Step for DNA Research in Forensics

DNA extraction

This step allows for the release of DNA from cells. DNA can be extracted from tooth material by dissolving the material in a buffer solution such as guanidine EDTA. Later, this step is followed by centrifugation. The white precipitate obtained is again dissolved in buffer – proteinase K – and incubated overnight. Centrifugation is repeated following the inactivation of proteinase K (12–14).

DNA quantitation

The DNA obtained is quantified and qualified by the slot blot test. The extracted DNA is then denatured by the addition of sodium hydroxide solution. This solution is applied to a nylon membrane in a slot blot equipment and fixation is obtained by ultraviolet light or heating. The last step allows for binding of the DNA molecule to a positively charged membrane. The hybridized complex is then made visible by the procedures such as autoradiography and chemilumiscence (15).

DNA analysis and interpretation

This area of DNA research is tremendously increasing due to the high sensitivity of the procedures. The sensitization of this step will improve the legal value of the DNA technology. Although DNA reports were criticized based on the technical aspects of the procedure, DNA analysis has provided a significant advance in identification in criminal records and mass disasters (2). Sample DNA can be compared with a databank for identification. There are many DNA analysing tests. However, not all are used to test every sample. In the majority of times, the selection of the test is based on the status of the DNA – whether it is degraded or not, as it is clearly understood that DNA will undergo progressive fragmentation after death (9). The sequence of information will still be present within the DNA fragments and therefore it is not completely lost. Sometimes nuclear material may be lost and in such instances, mitochondrial DNA can be used for analysis (Table 2).

The conclusion that is drawn is that although the DNA molecule is common to all humans, the information on DNA is valid and reliable to differentiate from person to person, with the exception of identical siblings. Thus, it can be suggested that DNA of every individual is unique and stands as a means of profiling or DNA fingerprinting. Recent developments in molecular biology have shown that many sophisticated techniques are available for DNA analysis. Restricted fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) are two very important methods. Mitochondrial DNA and short/long tandem repeat (STR/LTR) are considered to be important methods in the little or degraded DNA samples (Fig. 2).

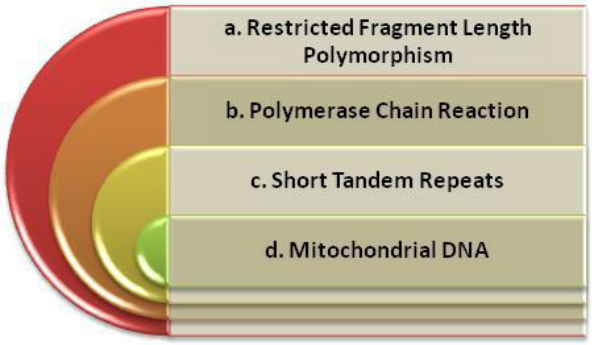

Fig. 2: Molecular techniques and molecular sources in forensic odontology research.

Molecular techniques in DNA analysis

Restricted fragment length polymorphism

In this method, the DNA strands are cut in fragments with a restriction enzyme. The fragmented DNA strands are then electrophoresed on an agarose gel and then transferred to a nylon membrane. Incubation with a radioactive probe, which consists of complementary pieces of polynucleotides that can move and hybridize with DNA fragments, is the final step (7) [Fig. 3].

Polymerase chain reaction

Polymerase chain reaction is a highly sensitive molecular technique which is a valuable molecular tool in forensic DNA research. This technique can be adapted into different ways such as amplification/multiplication of DNA strands and determination of the variation in the DNA sequence. This technique possesses the advantage of being quicker and less labour intensive. The potential cross-contamination is the only disadvantage of this procedure (9).

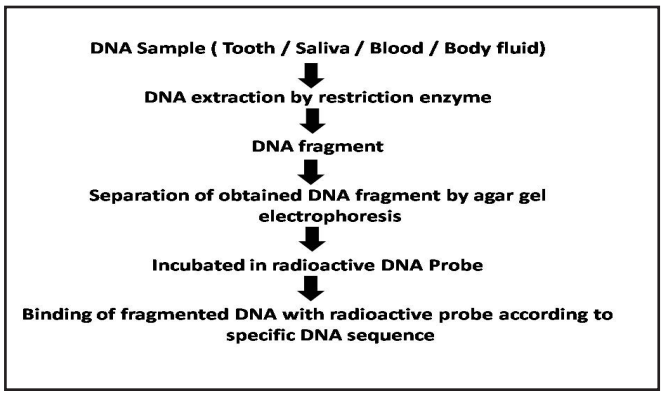

Fig. 3: Schematic representation of restricted fragment length polymorphism procedure.

The PCR technique requires DNA template and primers. The five main steps in PCR are initiation, denaturation, annealing, elongation and extension. The DNA obtained from the extension method are then transferred to the gel electrophoresis for analysis and interpretation. Initiation is performed by heating the sample tissue at 96 °C for five minutes. This is done to ensure the DNA strands and primers are melted. Denaturation subjects the sample to heat treatment at 96 °C for 30 seconds. This step is done to ensure the breakdown of the hydrogen bonds in the nucleic acids. Annealing is achieved by treatment of the sample at 68 °C for 30 seconds. At this temperature, the primers will jiggle around; ionic bonds will be constantly formed and broken between the single-stranded primer and the single-stranded template. This step will help in the formation of the stable bond between the single-stranded primer and template, thus resulting in the double-stranded DNA. The polymerase enzyme can now attach and start copying the template and thereby form a new DNA copy. Elongation is performed by treatment of the sample at 72 °C for 45 seconds. The technique helps in lengthening the double-stranded DNA. This is the ideal working temperature for the polymerase enzyme. The primers have a stronger ionic attraction to the template; however, greater force can break these bonds [Fig. 4] (9, 16, 17).

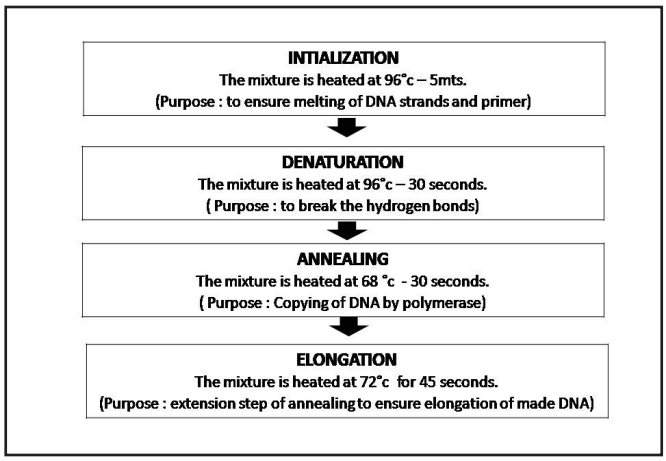

Fig. 4: Steps in polymerase chain reaction.

Short tandem repeats

Genomic regions with core repeat sequences of approximately three to seven base pairs are known as short tandem repeat (STR) or microsatellite. Core repeat sequences greater than seven base pairs have been called long tandem repeat (LTR) or mini satellite. Dinucleotide repeats are not generally used in forensic science laboratories due to the artificial production of shadow and stutter bands. The STR/LTR are typed by both RFLP and PCR methods. Shorter STR fragments are generally preferable for a variety of technical reasons (2). Corte Real *et al* in 2006, suggested that despite some adverse forensic conditions such as degraded human body remains, the dentine holds its integrity. This study revealed that dentine provided a genetic profile of mitochondrial DNA and STR in the study sample (18).

Mitochondrial DNA

Mitochondria produce cellular energy as a result of the oxidative phosphorylation process. The proteins involved in these processes are encoded from both mitochondrial and nuclear DNA. The human mitochondrial DNA (Mt DNA) exists as a double-stranded circle containing 16.6 kb DNA. It can be concluded that DNA is not only in the nuclei of cells, but also located in the mitochondria of cells. Since many mitochondria are present within a single cell, each mitochondrion may contain several DNA particles. It means a cell which contains only one copy of nuclear DNA has thousands of copies of mitochondrial DNA. It can be suggested that Mt DNA can be considered in investigation, if the nuclear DNA is not available (19). Pfeiffer *et al* in 1998, suggested a technique to extract Mt DNA that includes 700 µL lysis buffer, 10 mM Tris, pH 8.0, 100 mM NaCl, 50 mM, EDTA, pH 8.0, 0.5% SDS pH 8.0 and 20 µL proteinases K added to 0.2 g sample and incubated at 56 °C overnight. Later, 720 µL of extraction buffer will be added to the sample followed by centrifugation for two minutes at 15 000 rpm (revolution per minute). The upper aqueous layer will be transferred to a sterile microcentrifuge tube. Later, 720 µL of isobutanol will be added to the aqueous layer and centrifuged at 15 000 rpm for two minutes. At this stage, the lower aqueous layer will be used and transferred to the reservoir column. Then 1 mL of the Tris EDTA buffer for washing is added in the same reservoir column, and then followed by 3000 rpm for 20 minutes. Finally, the sample in the collection column which contains Mt DNA will be transferred to a sterile microcentrifuge tube and stored at -20 °C (20).

A unique feature of Mt DNA is its mode of inheritance, where Mt DNA is inherited in a strictly mother-to-child manner, there is no paternal contribution. Due to the fact that there is no recombination and since only a single copy is present in the cell, an exact sequence match is anticipated. Mitochondrial DNA can be traced through a family *via* maternal lineages for many generations. Mitochondrial DNA sequencing has great application to severely decomposed remains (19, 21).

Molecular Advancements in Age Estimation

Telomere shortening

The terminal end of human chromosomes is termed the telomere. It consists of repeating sequences of TTAGGG. After every cell division, telomere shortening occurs. Telomere shortening occurs during the ageing process in many cells. Using DNA technology, age estimation can be done from dental pulp DNA based on telomere length shortening (22). Takasaki *et al* suggested an age estimation in dental pulp DNA based on human telomere shortening in the Japanese population. His study results showed the calculated age from the telomere shortening was ± 7.52 of the actual age (23).

Amino acid racemization

This is a chemical reaction in which Levo - (L) amino acid is transformed into Dextro - (D) amino acids or *vice versa*. These biochemical changes will often detect slow metabolic activity. This method is accurate and estimates within ± 3 years of actual age. Racemization of L- to D-forms correlates highly with age. With advances in age, the L-form converts to D-form [predominantly aspartic acid] (24).

^{14}C Levels

This method looks at the amount of carbon 14 isotope in enamel and compares it to recent atmospheric levels. The estimated age from this technique is around ± 1.6 years of actual age (25).

Molecular Advancement in Gender Determination

AMEL gene

AMEL gene codes for a highly conservative protein called "amelogenin". This gene is located on the X and Y chromosomes in human allosomes. The two alleles are similar in exonic sequence, but differ in intronic sequence. Female chromosome (XX) consists of two identical genes (AMEL), whereas male chromosomes (XY) have two unidentical genes [AMEL] (25). Thus, genetic material possesses the gender relevant in determining the sex.

Merits, demerits, judicial values of DNA technology and test results

The major advantage of the PCR technique is that it is quicker, and can be performed on degraded samples which can yield highly sensitive results. The disadvantage of this technique is the potential for cross-contamination. Mitochondrial DNA procedures can be performed on samples where the availability of the nuclear DNA is minimial. Although amelogenin helps in gender identification, the major disadvantage is the deterioration of the nuclear material with the passage of time (26).

General acceptance and the relevance of DNA test results has broad support. If two profiles are different, a person can be excluded with certainty. If two identical DNA sequences are found, a statistical approach has to be made to determine the frequency of a particular sequence at a specific locus in that population. However, the most frequent issues that are raised during the legal conversations are 1) quality assurance of testing procedures, 2) adequate genetic interpretations, and the interference of unfairness to defendants. Criticism of DNA technology/DNA fingerprinting was first directed to the procedures in the laboratory but after improvement of these procedures, it is now directed more to the statistical approach (27, 28).

Medico-legal and Ethical Considerations of DNA Technology

The data on personal information can be obtained from various agencies, which also includes the health services. The database system may store the genetic detail of the person, which may be obtained by a free donation of a sample from individuals in the population. The information present in the database system must be retained in high confidential note. A future implication is that access to input the medical, dental and genetic information into the database system should be given to medical/dental practioners. However, the access to review the information passed by the medical/dental practioners should be given only to the crime investigators and expert witness. This will allow the confidentiality of the information present in the database system. The data would play a significant role in expert evidence in courtroom cases (29). The acceptable use of DNA in legal system includes: 1) consent to the DNA sample being taken, 2) DNA sample retained for a defined period, 3) DNA sample taken for violent crimes, 4) DNA sample used to cross-match only, 5) DNA profile used to identify a suspect, 6) DNA following a chain of custody and 7) authorized access to the DNA data bank. The debatable/unacceptable uses of DNA in the legal system are: 1) DNA sample taken by force/taken on arrest, 2) DNA sample taken for minor offences, 3) DNA profile used for familial searching, 4) DNA interpretation of degraded sample, 5) unauthorized access to DNA data bank and 6) involvment of politicians in the scientific process (30). The principles for presenting evidence in court are that dentists, when presenting themselves as the expert witness, must attend the court punctually and must be familiar with the details of the case. Scientific language and long discussions must be avoided in the courtroom. Text book or journal references can be produced as a part of evidence support (31). Expert witnes must provide a signed and notarized ethics statement. Expert witnes should maintain professional competency through existing programmes of continuing education (32).

CONCLUSION

The use of DNA technology in forensic sciences is established and the particular interest of providing DNA from the hard tissue remains of the body is extended to the tooth in forensic odontology. The tooth is considered a storehouse of DNA, hence, exploring the procedures of extracting DNA from teeth will extend the reach of molecular advances in forensic sciences and enhance the view of DNA as "a molecular signature in forensic odontology".

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