"Review of Dental Pulp- A Gem in Forensic Odontology"

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Abstract

Over the years, forensic science has exceptionally advanced due to various undesirable disasters and upsurging crimes in our society. Forensic identification is an integrative technique that relies on definite identification methods. The dental identification is of paramount significance when the dead body is burned, decomposed, dismembered or skeletonized. Forensic odontologist can resolve several crime cases by human identification and dental DNA fingerprinting. Dental pulp acts as a rich source of DNA for genetic material in forensic research since it is covered by dentin and enamel which protects the pulp. Dental pulp undergoes physiological changes as age advances. DNA, the language of life, manages all the cell activities which give unimaginable data related to health and disease. DNA fingerprinting has plod along from the conventional fingerprints and blooming in forensic science.

Keywords: forensic science, dental pulp, DNA, DNA fingerprinting, DNA extraction, DNA analysis.

Introduction

The field of forensic science is highly enigmatic which requires detailed and precise investigation for explicit identification. Forensic Odontology is a branch of forensic science that deals with the collection of dental evidence and examination of the related findings revealing human identity. Lately, in forensic odontology, the earlier methods of identification such as radiological examination and evaluation of dental prosthesis have been losing its relevance due to the newly effective advances in molecular biology and laboratory techniques.[(1)] The revolution caused in 1953 by Watson and Crick, who discovered the double-helix structure of DNA is responsible for the genetic inheritance of human beings has led to important changes in nearly all fields of science.[(2)] Several biological materials may be employed for isolation of DNA, including teeth, bone tissue, hair bulb, biopsy sample, saliva, blood and other body tissues.[(3)] The interest in using teeth as a source to extract DNA is due to its ability to resist environmental conditions such as decomposition, water, soil immersion, trauma, incineration and mutilation.[(4)] Teeth are skeletal structures that encloses pulp in the centre and better preserves DNA over time.[(5)] DNA extracted from the dental pulp can precisely establish human identity.[(3)]

Dental Pulp

The human teeth vary in form and size but have similar histological structure.[(6)] The dental pulp is mesodermal in origin and is highly vascularized, innervated connective tissue. It is well-enclosed by dentine and occupies the central part of the tooth. (Figure 1)

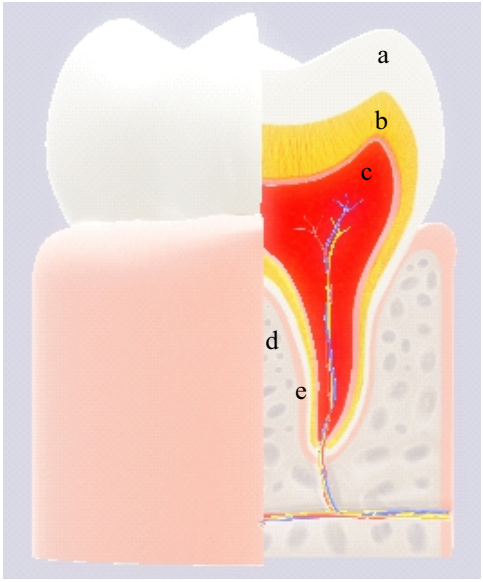

Figure 1: a-Enamel, b-Dentin, c-Pulp, d-Alveolar Bone, e- Periodontal Ligament

As the age advances, the pulp undergoes physiological changes such as decrease in cellularity, fibrosis, degeneration and calcifications.[7] Along with dental pulp, odontoblastic process in the dentinal tubules, cellular cementum, stroma within accessory canals and fibres of periodontal ligament serve as a source of DNA.[6]

In adult teeth, the average volume of dental pulp is approximately 0.02 cubic centimetres(cc). The pulp volume for the third molar ranges from 0.023 cc (maxillary molar) to 0.031 cc (mandibular molar).[8] The size of the pulp chamber generally reduces with age and irritation, due to secondary dentin deposition.[9]

Effect Of Environment On Pulp DNA

Teeth can withstand prolonged exposure to relatively high temperature, water and soil. At significantly high temperature, due to tissue autolysis, degradation and/ or fragmentation, there is a notable decrease in the amount of DNA retrieved [10] The quantity of DNA does not change with decrease in temperature and thus low temperature can be used to preserve the tooth.[11] Buried dental pulp shows specific series of histological and morphological changes up till 144 hours from burial, beyond which pulp tissue decomposes.[12] The teeth submerged in water offer poor results than that of buried in soil. This occurs due to the dilution effect of water itself, which increases the rate of DNA hydrolysis compared to incubation in air or soil.[13]

Dental pulp extirpation and storage

Teeth preserve DNA better over time. However, environmental contaminants such as microorganisms, humic acid and metals can have a negative impact on DNA extraction, amplification and analysis. The currently available decontamination techniques include physical cleaning of tooth surface, chemical destruction of contaminant, ultraviolet light irradiation of surface contaminant and combination methods. The most common physical techniques are the use of dremel, drill or shot blasting followed by scalpel blades, sandpaper and brushes. Sodium hypochlorite (bleach) or phosphate are the most commonly used chemical methods.[14]

The pulp tissue can be extracted from the teeth using various techniques:

1) Crushing entire teeth: It is simple to perform and most thorough in accessing dental DNA and provides both mitochondrial DNA and nuclear DNA.[15] However the main drawback of this technique is that it destroys the tooth completely, hampering further anatomic, radiographic and biochemical examinations.
2) Cryogenic grinding: A ferromagnetic plunger is used in freezer mill to produce oscillations which generate alternating electric current, grinding tooth into powder to increase surface area and expose trapped cells to biochemical agents that release DNA into solution. [6,16]
3) Conventional Endodontic Access: It is a well-established technique with advantageous results in terms of cost, time, quality and amount of extracted DNA. This technique leads to the disruption of the occlusal surface and restorations for further radiographic analysis.
4) Vertical Split: It involves sectioning of the tooth in longitudinal direction. This technique provides access to the entire length of the dental pulp of single rooted teeth but it limits access to the pulp in multirooted teeth. An additional drawback of this technique is the possible destruction of coronal restorations.
5) Horizontal Section: A horizontal section in the cervical area just beneath the cementoenamel junction imparts accessibility to the radicular and coronal pulp without hampering any restoration.[17]
6) Orthograde entrance technique: It proposes an entrance through the enamel surface, the most durable tissue, instead of root apex and minimizes the risk to damage the tooth during the process of taking sample.[18] Dentin rich sample can be obtained by endodontic files with coarser ridges.
7) Reverse root canal: This technique was introduced by Cobb suggesting an entrance from root apex followed by filling the interior of the tooth.[19] However, this method creates the risk of crushing the root which are already fragile.

Following the extirpation of pulp, it is placed in a sterile vial containing 1.5 – 2 ml of phosphate buffer saline (PBS) which prevents cells from rupturing or shrivelling up due to osmosis. [20] Pulp extirpation is followed by DNA isolation/ extraction and DNA analysis.

Once the pulp is extirpated, DNA extraction can be done with the following agents:

1) Detergents: Cell lysis and denaturation of the nucleoprotein layer.
2) Proteinase K: Protein digestion.
3) Chelating agents: Bind to bivalent cation and inactivates Dnase.
4) RNase A: Decontamination of RNA
5) Organic solvents like chloroform, phenol and isoamyl alcohol: Purify DNA.[21]

Dna Analysis

The various methods of DNA analysis are:

1) Polymerase chain reaction (PCR)

In 1985, Karry Mullis invented PCR and since then has revolutionized molecular biology.[22] PCR is the method used to amplify small amount of DNA sample in large quantity over a short period of time. Special enzymes and unlabelled known constant sequence of DNA primers are required to carry out the reaction. Nucleic acid denaturation is the first step of PCR in which the two strands of the DNA double helix are physically separated at a high temperature. Later, in the second step the temperature is lowered and the primers bind to the complementary sequences of DNA. The standard PCR reaction amplifies sample DNA by tens of billion copies through approximately thirty cycles in few hours.[23]

2) Restriction fragment length polymorphism (RFLP)

RFLP is a technique that distinguishes individuals by utilizing the variations in homologous DNA sequences, known as polymorphisms. Restriction enzyme fragments the DNA molecule, which is subsequently separated by length on an agarose gel electrophoresis. These separated fragments are then transferred to a membrane by the Southern blotting which creates discrete, radioactive bands [24]

Escherichia coli restriction enzyme (EcoR I) is first restriction endonuclease used in RFLP. [25,26] The chopped fragments of DNA consist of variable number tandem repeats (VNTR) of varying lengths.[27] RFLP determines the repeated sequence by representing a specific pattern to VNTR, reflecting an individual's DNA fingerprint. It requires large quantities of DNA and long waiting time to obtain results.[28]

3) Short tandem repeat (STR) analysis

The study of genomic or mitochondrial DNA in forensic samples, is usually performed by STR analysis. STR are hypervariable regions of DNA that illustrate consecutive repetitions of fragments that have 2-7 base pairs. [3] Because of the high standards of polymorphic informative content, STR have a high power of individual discernment. STR analysis can significantly identify biologically distinct individuals by determining the discrete repetitive sequence by PCR. It is used in paternity testing as each individual has some STRs inherited from father and some from the mother.[29]

4) Single nucleotide polymorphism (SNP)

SNP is automated, efficient, and inexpensive technique used for scanning and determining new polymorphism as well as the allele(s) of a known polymorphism in target molecular sequences.[30] Direct DNA sequencing or denaturing high performance liquid chromatography are used to detect SNPs.[31]

5) Mitochondrial DNA (mtDNA) analysis

Forensic cases where the sample cells lack nucleus, mitochondrial DNA plays a significant role to resolve the case.[32] It utilizes DNA extracted from mitochondrion and varies from nuclear DNA in its location, its mode of inheritance, its quantity in the cell and its sequence. The older biological samples that lack nucleated cellular material such as hair, bones and teeth cannot be analyzed with STR and RFLP, but can be analyzed with mtDNA. It is superior than nuclear genome as it is passed through maternal lineage and has 100-1000 copies of mtDNA genome.[33] The dentin and cementum contain enough mtDNA to amplify and can be used in forensic analysis.[34]

6) Amplified Fragment Length Polymorphism (AmpFLP)

AmpFLP was developed in the early 1990s by Keygene.[35] As compared to RFLP, this technique is faster and highly automated [36] Due to ease of set-up and operation and less expensiveness, AmpFLP is quite popular in developing countries.[37]

Forensic significance of Dental Pulp

1) Age Estimation

The Age of an individual can be estimated by assessing alterations in the pulp tissue such as the size of pulp chamber, decrease in cell populations, fibrosis, presence of pulp stones, calcifications and increase in dentine thickness.[38,39] The dimension of pulp cavity reduces as age advances.[40]

2) Odontoblasts in Pulp

As the age advances, the odontoblasts and the subodontogenic cells undergo morphological changes. The tall columnar, loosely packed cell transform to short, ovoid densely packed cell. There is reduction in reparative and synthetic capacity of the cell. The cells undergo apoptosis which can be studied by using apoptotic cell markers such as bcl-2. Gradually, the number of odontoblasts and subodontoblasts cell population reduces while the fibroblasts cell population remains unchanged. [41,42,43,44,45]

3) Sex Determination

PCR plays a significant role in sex determination by determining a particular sequence on sex chromosomes (X and Y). [46,47] The short or P arm of Y chromosome harbours sex determining gene (SRY gene) at p11–31 region determining the male sex. [48,49,50]

4) Barr bodies

In females, Barr bodies are present at the rim of the nucleus of

somatic cells and are absent in males. They are highly basophilic chromatin material measuring 0.8 μ ×1.1 μ. Under light microscopy these structures are spherical, biconvex, triangular and rectangular in shape. Barr bodies when viewed in an electronic microscope appear like S, V, W or X alphabets[51]. Barr bodies play a pivotal role in forensics and with 98.9% certainty can determine gender.[52]

5) Sex determining region "Y" (SRY) gene

The SRY gene is present on the short arm of Y chromosome (p11–31). A female (46 XX) consists of two X chromosomes while a male (46 XY) consists of one X and one Y chromosome. Thus, identifying SRY gene in the pulp DNA sample indicates masculine gender. [53,54]

6) Amelogenin gene

Amelogenins are the proteins formed from the amelogenin (AMEL) gene and are essential in amelogenesis i.e formation of enamel. The male DNA contains AMELY gene on the Y chromosome and the female DNA contains AMELX gene on the X chromosome. The female genome has similar AMEL genes while male has two different AMEL genes. This difference is used for gender identification [54,55]

7) Telomere Shortening

Dental pulp DNA plays a crucial role in age estimation. The end of the chromosome is known as telomere. Ageing of the dental pulp is associated with reduction in chromosomal length due to shortening of telomere. [56]

8) Blood Group Determination

The pulp is the most vascularized tissue of the tooth and harbours blood group antigens which is used to identify an individual's ABO blood group and Rh factor by slide agglutination.[57]

9) Identification in Mass Disasters

Mass disasters can involve natural (e.g. earthquakes, volcanoes, hurricanes, and tsunamis) or nonnatural catastrophes (e.g. terrorist activities, wars, or political crisis). In situations where there is extensive destruction of the soft tissue, hard tissue such as bones and teeth are used for human identification. [58,59] The Indian Ocean tsunami of 2004 hit the coasts of several countries of South and Southeast Asia in December 2004 from which 87% of the bodies brought were identified without DNA analysis.[60]

Conclusion

The field of forensic odontology comprises an integrative study and thorough investigation of the cases. With advancing era of forensic odontology, the role of dentists is emerging as an investigator in cases with minimal evidential support due to upcoming efficient molecular biology. The dental pulp is enclosed and well protected inside the tooth and serves as an excellent source of DNA for individual identification.

Source of Support : Nil

Conflict of Interest - Nil

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