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Tooth -an important source of DNA in forensic investigation

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Abstract

Forensic odontology has become an integral part of forensic investigation ,since teeth withstand in mass disasters and can serve as the basis for identification of the victim ,dead or an unkown human being. Among all the methods of identification in forensic odontology ,DNA profiling is evoloving immensely in this era of globalization. Teeth provide sufficient amount of DNA especially the pulp tissue which can be extracted easily and with minimum efforts. Further DNA is isolated from the extracted pulp tissue by various methods and compared with ante mortem records. This DNA profiling using teeth thus serves as the basis of identification when other body parts are destroyed completely.

Keywords : Teeth ,DNA profiling ,Pulp tissue .Extraction

Introduction

Forensic dentistry is an important sub specialty of forensic medicine which is contributing immensely in solving difficult criminal cases and in identification of individuals in mass disasters⁽¹⁾. Dental professionals are increasingly being employed in expert assessment and testimony in legal proceedings. As boundary between forensic science and forensic medicine blur in part due to technological advances, dentists are impelled to expand their frontiers of knowledge and practice to confront challenging forensic situations^(2,3). Teeth are the hardest structures in human body and can withstand even the mass disasters(fire explosions, air accidents) and will be available when other body parts are destroyed. This is the basic reason that the branch of forensic odontology is getting adequate attention now a days. Forensic dentistry is evolving in many aspects from lip prints ,bite mark investigation(main identification basis in Nirbhaya case, Delhi), tongue prints to different age estimation methods, sex determination methods, etc. The field of forensic medicine has undergone revolution by the fast technological advancement in DNA research⁽¹⁾. The following work is undertaken to elucidate the importance of DNA profiling and availability of DNA from tooth in forensic investigations. Traditionally,

forensic odontological investigations are based on comparing prothesis, restorations, any developmental anomaly of teeth with previous dental records such as dental casts, radiographs, other clinical records of the dead so that the numerous signs can be obtained to identify the unknown⁽⁴⁾. In cases, where the ante mortem records are not available or can not be obtained then the exact identification becomes difficult and there DNA profiling plays a crucial role in revealing the identity of the victim. Because of the resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation and decomposition, teeth represent an excellent source of DNA material^(5,6). When the conventional dental identification methods fail, this biological material can provide the necessary link to prove identity^(7,8). Matching of the DNA extracted from the teeth of an unidentified individual with DNA isolated from known ante mortem samples such as stored blood, tooth brush, hairbrush, clothing, cervical smear, biopsy, to a parent or sibling is the usual procedure in DNA analysis⁽¹⁾. Since the early 1990s, DNA technology has been utilized as a powerful tool for identification, paternity testing, criminal investigation, and other forensic investigations⁽⁹⁾ 11). As the genetic material in soft tissues rapidly degrades post-mortem, teeth and bones are of interest to yield sufficient DNA for identification^(12,13).

Distribution of DNA within teeth

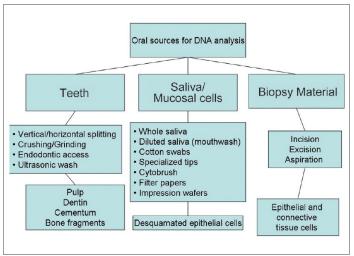


Figure 1: Distribution of DNA within the oral cavity⁽²⁶⁾.

The teeth differ in form and size but have similar histological structure. Enamel is acellular in nature mainly consisting of 96% of inorganic material and 4% organic components such as proteins like Amelogenin, ameloblastin, Enameline and Tuftelin in very very small amount and water by weight. Enamel does not contain much DNA, because the ameloblasts; the cells which produce and transport the enamel proteins and form the mineral deposits, self-destruct after they do their job. They undergo **programmed** cell death which essentially destroys the DNA. DNA can only be found in nuclei, not in the the hydroxyapatite crystals which are the main component of enamel. More suitable elements for this purpose is the dental pulp, which is a connective tissue particularly rich in cells (odontoblasts, fibroblasts, undifferentiated mesenchymal cells, macrophages, and lymphocytes), and the cellular cementum, so called because it contains nucleated cells with numerous branching canaliculi. The cellular cementum covers the most apical portion of the root surface, characterized by the presence of numerous lacunae spaces (cementum corpuscles) filled with cementocytes, which, because of their anatomic location, are least exposed to external

insults, especially chemical processes such as oxidation and bacterial degradation⁽⁴⁾.

Anatomic location⁽¹⁵⁾ of DNA in tooth

- Soft tissue within coronal and radicular pulp chamber consisting of odontoblast, fibroblast, endothelial cells, peripheral nerve cells, undifferentiated mesenchymal cells, nucleated components of blood
- Odontoblastic processes that extend into dentinal tubules.
- 3. Accessory canals.
- 4. Cellular cementum.
- 5. Less stable sites: adherent bone, periodontal ligament.

Types of DNA

- Genomic DNA Teeth provide a good source of genomic DNA⁽¹⁶⁾. They are within the nucleus of the cell.
- Mitochondrial DNA Used when DNA sample obtained is insufficient or degraded⁽¹⁷⁾. mtDNA is a powerful tool for forensic identification as it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring have the same mitochondrial DNA as their mothers since the mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by father's sperm⁽¹⁾.

Factors affecting the availability of DNA

Amount of DNA available in tooth depends on individual factors and external factors (4,20).

Individual Factors such as-

- 1. Type of tooth: incisor, canine, premolar or molar.
- 2. Condition of teeth prior to extraction (degree of decay).
- 3. Condition of tooth following trauma
- 4. Pathologic conditions.
- 5. Pulp weight/volume Among the individual factors, special attention has been drawn to the volume of the pulp chamber, which varies according to the type of tooth. In

adult teeth, the average volume of the pulp chamber is ~ 0.020 cc, the highest value being reached in the third molars: 0.023 cc for maxillary molars and 0.031 cc for mandibular molars⁽¹⁵⁾.

- 6. Previous therapeutic treatments
- 7. Age of the individual.

External Factors such as-

- 1. Storage temperature
- 2. Degree of humidity
- 3. Time between death and examination.

The process to identify a victim by DNA includes:

- 1. Collection of best possible AM samples.
- 2. The choice of PM samples in the best condition possible.
- 3. DNA analysis, matching of AM and PM data, and statistical weighting of the match. Data is best handled electronically.
- 4. Quality must be prioritized throughout the process⁽²¹⁾. Removal of dental pulp to further isolate DNA^(1,20):

Crushing the entire tooth/Cryogenic grinding - Crushing of the teeth or cryogenic grinding is another method which also yields sufficient amount of the genetic material where a freezer mill is used to pulverise teeth under frozen preparation in liquid nitrogen under sterile conditions. The drawback of this technique is that it can result in the total destruction of the tooth sample⁽²²⁾.

Conventional endodontic access - Recently conservative methods are gaining importance rather than grinding the whole tooth⁽¹⁾. The pulp chamber can be accessed through conventional access cavity preparation and dental pulp can be retrieved. The advantages of this technique are its simplicity, relatively low cost and preservation of the tooth integrity which can be considered in forensic investigations^(15,23).

Vertical split- A section through vertical axis of tooth allows conventional access to entire length of pulp

chamber.



Figure 2: Splitting of tooth⁽²⁶⁾

Horizontal sectioning - In a modified horizontal sectioning procedure, the tooth is circumferentially scored 1 mm below the cemento-enamel junction with a longshanked round bur leaving a 2-3 mm wide isthmus of intact tooth structure on the facial surface. The crown is then manually separated from the root. A large round bur is then used to remove as much coronal and root dentin as possible. Restoring the shape of the tooth back to its presampled state is possible, by re-approximating the crown and root portions of the tooth at the isthmus and can be returned to the surviving family members after completion of the analytical procedures and publishing of the results. This method has many advantages like simplicity, ease of access, preservation of crown as well as root structure, and the ability to restore the tooth very close to its pre-sampled state⁽²⁴⁾.

DNA can also be extracted by horizontal sectioning of the tooth with extirpation of the dental pulp from the chamber and grinding the remaining root to a fine powder saving the crowns of the teeth. The dentine cement powder and dental pulp can be used for DNA extraction separately⁽²⁵⁾.

Crushing with mortar and pestle.

Conclusion

The oral region is the best source for simple and non-invasive methods of DNA sampling. Teeth provide an excellent source of quality DNA compared to other parts of the body and has to be considered in all the forensic investigations. The arrival of DNA finger printing has revolutionized the concept of identification. It is reasonable to anticipate that future advances in DNA technology will reduce the time and cost factor for identification of unknown diseases⁽²⁰⁾. DNA identification is one discipline that is able to help relatives repatriate their loved ones and to proceed with the legal aspects concerning the deceased⁽²¹⁾.

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