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Dental Calculus as a Vital Tool in Forensic Odontology

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ABSTRACT: Evidences are a essential tool to bring in justice to the person, but these evidences are sometimes hidden in plain sight & difficult to identify and procure because of reasons varying from storage in rugged conditions, cost and procedure involved in investigations and removal of body parts if needed for evaluation. These are the usual drawbacks that forensic specialists encounter during proof collection and presentation, but this can be easily avoided or better accepted with this evidence. Forensic odontology is primarily concerned with the use of teeth and oral structures for identification in a legal context. Various forensic odontology techniques help in the identification of the human remains in incidents such as terrorists' attacks, airplane, train and road accidents, fires, mass murders, and natural disasters such as tsunamis, earth quakes and floods, etc. (Disaster Victim Identification-DVI). Dental tooth are the hardest substances seen in the whole body and they tolerate heat and humid conditions comparatively well than other soft and hard tissues of the body. Tooth identification and the way it is placed served as a vital tool in individual identification in the past but the recent advancements paved way to be more specific to the individual identification namely m-DNA analysis. In the field of dentistry, newer methods and avenues were researched for finding evidences which will be easy to procure and provide invaluable evidence in forensics and thus dental calculus were found to be a viable option. Recently, studies reported an excellent source of nucleic acids in dental calculus as it contained a multitude of diverse biomolecules (including microbial, oral micro biome and host DNA) providing information about an individual's culture, diet, ancestry, and health as it remained intact with lesser destruction through time. Hence in this article, we highlight the importance of forensic odontology and the methods in which dental calculus will serve as reliable evidence in upholding justice.

Keywords: Forensic odontology, Dental calculus, DNA analysis, Saliva, Criminology.

INTRODUCTION

The Federation Dentaire Internationale (FDI) defines forensic odontology as that branch of dentistry which, in the interest of justice, deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings. According to the American Society of Forensic Odontology, forensic odontology is by definition, the application of dental science to the law. It is a significant outgrowth of forensics in the field of medical, dental sciences and, in the felicity of justice, pacts with the apt examination, handling and demonstration of dental evidence in the court of law and plays a pivotal role in identifying the human remains of victims, not only those of mutilated, burnt and decomposed but also victims of bioterrorism and mass disasters. The evidence that may be derived from the teeth, the age (in children) and identification of the person to whom the teeth may belong. Forensic odontology plays a crucial role in circumstances where habitual methods of identification, such as fingerprinting and visual recognition, cannot be performed, in cases of decomposed, charred or skeletonized bodies. As calculus is mineralized, it often survives well in archaeological contexts and is useful when studying the dental pathology of our ancestors (Singh and Goel 2017; Forshaw et *al.*, 2022) (Fig. 1). In human beings, DNA comparison can enable high probability matches to be made between discarded bodily substances and the person from whom those

substances originated. Bodily substances containing cellular material, such as blood, semen, seminal fluid, saliva, skin, and even hair root tissue can often be compared and matched back to its original owner with high statistical probabilities of comparison (Chen *et al.*, 2011).

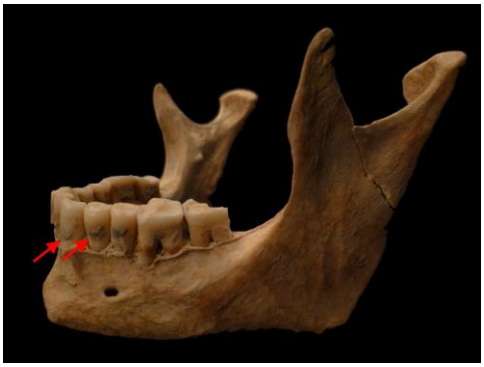


Fig. 1. Dental calculus in mandible recovered from a corpse.

Need for Forensics:

1. Criminal: Investigation on death cannot begin until the victim has been positively identified.

2. Closure: Identification of individuals missing for a longer time can bring peace and closure to family members

3. Monetary: Payment of pensions, life insurance and other benefits relies upon positive confirmation of death

4. Burial: Many religions require that positive identification be made prior to burial in geographical site

5. Marriage: Individuals from many religious backgrounds cannot remarry unless their partners are confirmed deceased (Krishan *et al.*, 2010).

Evidences Utilized in Forensic Odontology

1. Chelioscopy: Pip print studies are unique to one person like fingerprints; lip grooves are permanent and unchangeable (Fig. 2C).

2. Rugoscopy: Palatal rughae, also called plica palatinae transverse and rugae palatine refer to ridges on the anterior part of palatal mucosa, each side of the median palatal raphe and behind incisive papilla catastrophic accidents involving plane crashes, fires and explosions can destroy the fingerprints but, interestingly palatal rughae patterns are preserved (Fig. 2B).

3. Bite Marks: Used to identify as a proof in identification of sexual abuse or animal attack in both living and deceased victims. This type of evidence is usually present in the upper and lower extremities and in the private parts of the victims. (Fig 2A).

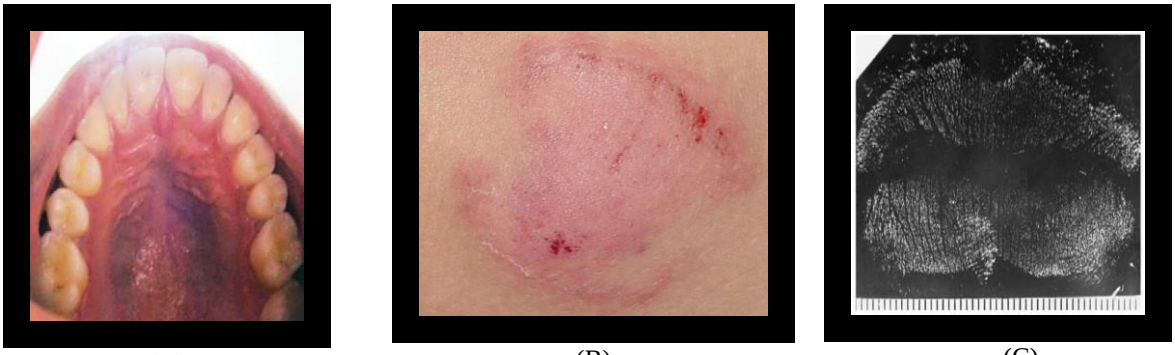
4. Biochemical Identification: Racemization Aspartic acid in root dentin.

5. Age Estimation Evaluation: Physiological (amount of secondary dentin in pulp chamber, translucency of sclerotic apical dentin, amount of secondary cementum at the apex)

6. Pathological Parameters: Presence of hypoplasias of tooth i e: amelogenesis imperfect, dentinogenesis imperfect, syphilis etc.

7. DNA: By isolation from saliva and dental calculus

8. Restorative Materials: Implants and prosthesis with unique serial numbers for easier identification.



(A) (B) (C)

Fig. 2. Evidences used in forensic odontology, namely A- Palatal rugae, B- Bite mark and C- Lip prints.

Dental Calculus:

Dental calculus is calcified dental plaque, composed primarily of calcium phosphate mineral salts deposited between and within remnants of formerly viable microorganisms. A viable dental plaque covers mineralized calculus deposits. Calculus is composed of both inorganic (mineral) and organic (cellular and extracellular matrix) components. The mineral proportion of calculus ranges from approximately 40–60%, depending on its location in the dentition, and consists primarily of calcium phosphate crystals organized into four principal mineral phases, listed here in order of decreasing ratio of phosphate to calcium. (Michael *et al.*, 2014).

— Whitlockite, $Ca_9(Mg,Fe)(PO_4)_6(PO_3OH)$
— Hydroxyapatite, $Ca_5(PO_4)_3OH$
— Octacalcium phosphate, $Ca_8H_2(PO_4)_6{\cdot}5\ H_2O$
— Brushite, $cahpo_4 \cdot 2\ H_2O$

The organic component of calculus is approximately 85% cellular and 15% extracellular matrix.

Formation of Dental Calculus:

Plaque and calculus can make your teeth appear yellow or brown, and it can be a cause of persistent bad breath. It also threatens the health of your teeth and gums.

The bacteria that form dental plaque produce acids that cause damage to your teeth and gums. As plaque calcifies and hardens into calculus, it forms a trap for new plaque and bacteria to keep forming.

Dental Calculus in Forensic Odontology:
Dental calculus is a microbial biofilm consisting of dietary components, oral microbes and host secretions such as saliva and gingival crevicular fluid. It acquires human DNA through host secretions and immunity associated process such as NETosis (Gehl & Plecas 2017; Singh and Goel 2017). Dental calculus is a cross-cultural biological matrix that is emerging as a critical source of information for anthropologists and oral health professionals.

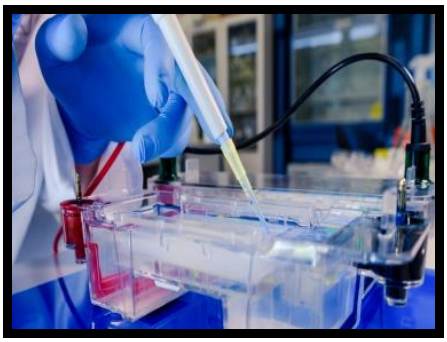
METHODOLOGY OF ANALYSIS

1. Gel Electrophoresis. Gel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis involves running a current through a gel containing the molecules of interest. Based on their size and charge, the molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another. Gels for DNA separation are often made out of a polysaccharide called agarose, which comes as dry, powdered flakes. When the agarose is heated in a buffer (water with some salts in it) and allowed to cool, it will form a solid, slightly squishy gel. At the molecular level, the gel is a matrix of agarose molecules that are held together by hydrogen bonds and form tiny pores. At one end, the gel has pocket-like indentations called wells, which are where the DNA samples will be placed. Before the DNA samples are added, the gel must be placed in a gel box. One end of the box is hooked to a positive electrode, while the other end is hooked to a negative electrode. The main body of the box, where the gel is placed, is filled with a salt-containing buffer solution that can conduct current (Fig. 3 A). Although you may not be able to see in the image above, the buffer fills the gel box to a level where it just barely covers the gel. The end of the gel with the wells is positioned towards the negative electrode. The end without wells (towards which the DNA fragments will migrate) is positioned towards the positive electrode. Once the fragments have been separated, we can examine the gel and see what sizes of bands are found on it. When a gel is stained with a DNA-binding dye and placed under UV light, the DNA fragments will glow, allowing us to see the DNA present at different locations along the length of the gel (Chen *et al.,* 2011; Lee *et al.,* 2012).

Optical Microscopy. Optical microscopy is a technique employed to closely view a sample through the magnification of a lens with visible light. This is the traditional form of microscopy, which was first invented before the 18th century and is still in use today. To view the DNA as well as a variety of other protein molecules, an electron microscope is used. Whereas the typical light microscope is only limited to a resolution of about 0.25um, the electron microscope is capable of resolutions of about 0.2 nanometers, which makes it possible to view smaller molecules. This is achieved because electron microscopes use electron beams rather than the visible light used for light microscopes (Chen *et al.,* 2011) (Fig. 3 B).

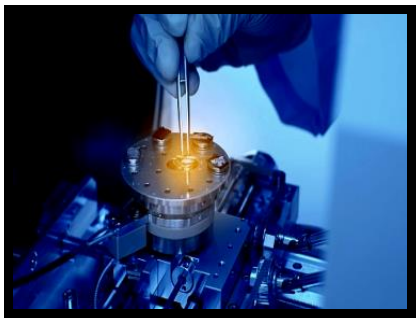
Scanning Electron Microscopy. Scanning electron microscopic analysis is an indispensable tool for high-resolution visualization of chromosomes and their ultra-structural details. It allows a three-dimensional structural approach for elucidating higher-order chromatin structure and chromosome architecture. A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons (Fig. 3 C). The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. SEM samples have to be small enough to fit on the specimen stage, and may need special preparation to increase their electrical conductivity and to stabilize them, so that they can withstand the high vacuum conditions and the high energy beam of electrons. Samples are generally mounted rigidly on a specimen holder or stub using a conductive adhesive. SEM is used extensively for defect analysis of semiconductor wafers, and manufacturers make instruments that can examine any part of a 300 mm semiconductor wafer. Many instruments have chambers that can tilt an object of that size to 45° and provide continuous 360° rotation For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum. Hard, dry materials such as wood, bone, feathers, dried insects, or shells (including egg shells) can be examined with little further treatment, but living cells and tissues and whole, soft-bodied organisms require chemical fixation to preserve and stabilize their structure (Charlier *et al.,* 2010; Choudhary *et al.,* 2017).

Ultra Performance Liquid Chromatography. Ultra-performance liquid chromatography (UPLC) is a combination of a 1.7μm reverse-phase packing material and a chromatographic system that can operate at pressures in the 6000–15 000 psi range (conventional HPLC uses 3–5 μm packing material and operates between 2000 and 4000 psi). It uses fine particles and saves time and reduces solvent consumption. This new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput found UPLC cost advantageous over HPLC. The application of UPLC results in the detection of additional drug metabolites, superior separation and improved spectral quality (Wren & Tchelitcheff 2006; Oehrle, 2008; Schummer *et al.,* 2013) (Fig. 3 D).



A. Gel electrophoresis

B. Optical microscopy



C. Scanning electron microscopy

D. Ultra performance liquid chromatography

Fig. 3. Various methods used in analysis of evidence from dental calculus.

Merits:

1. Known source of DNA which has lesser technique sensitive procedures to procure in both living and the dead
2. Can highlight the lifestyle of the individual
3. Can also hint the medical status of the individual had suffered from a chronic illness
4. Since it is a minimally invasive procedure, acceptance by the family will be better
5. Non – availability or ill – preserved samples (Masthan, 2009).

Demerits:

1. Dental remains was not available
2. Cannot be used where religious beliefs does not permit
3. Technical personals are required to process the dental calculus to identify the individual and use it as evidence.

CONCLUSIONS

The DNA samples procured from the individuals has been shown to incorporate endogenous host-DNA, microbial DNA and external environmental inclusions. Analysis of calculus may provide important insights into descendant's lifestyle, diet, and possible presence of a disease. Thus, calculus aids in approach towards evaluation of the ante mortem profile of unidentified individuals in forensics. Thus, dental calculus acts as minimally invasive, reliable and economical evidence in identification of humans hence can be used in future where it can be helpful in mass disasters or in identification in crime scenes.

FUTURE SCOPE

The use of dental calculus can serve as a vital tool in identification of forensic odontology; it can also be useful in evaluating the presence of organic and inorganic substances which can be used as a less invasive tool for evaluation of metabolic diseases too.

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