

Genetics & Molecular Biology in Forensic Odontology

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ABSTRACT

This research review concisely and systematically discusses various aspects of genetics & molecular biology in forensic odontology. It compiles the importance of different parts of teeth, dentine, pulp and cementum for DNA extraction and its application in forensic identification supported by various forensic studies done throughout the world till date. Furthermore amasses different cases reported around the globe incorporating teeth DNA for identification and forensic analysis. The review emphasizes on the fact that all the different parts of tooth are significantly important for DNA retrieval and even small amount of tooth fragment can be beneficial for forensic analysis. Based on proven facts and numerous research studies, paper emphasize on the importance of genetic and molecular aspects of forensic odontology and recommends strongly for the collection of whatever available tooth sample from crime scene, mass disaster, accidents and skeletonized remains for forensic analysis.

Keywords: Forensic, Personal identification, Odontology, MtDNA, STR, VNTR, amelogenin.

INTRODUCTION

The identification of humans is one of the major fields of study and research in forensic science and forensic dentistry has contributed significantly to this process. The most common role of the forensic dentist is the identification of deceased individuals. Owing to the high uniqueness of dental characteristics in addition to the relatively high degree of physical and chemical resistance of the dental structure teeth play an important role in personal identification.¹

Teeth represent an excellent source of DNA due to their capacity of enduring environmental changes and are therefore a valuable potential source of forensic DNA evidence, using PCR analyses that allow comparison of the collected postmortem samples to known ante mortem samples or parental DNA.¹ The recovery of DNA from teeth by cryogenic grinding has been shown to be

successful though is a very slow and labour intensive process. Collecting pulp or cementum from teeth on the other hand is an easy and quick alternative and provides a method for cases where large numbers of teeth need to be analyzed.

Previous studies have evaluated the different dental tissues as DNA sources in forensic analyses but have shown variable results for the quantity of DNA obtained from pulp, dentin and cementum but only scarce information is present on the DNA quality and quantity of DNA in diseased and degraded teeth where pulp tissue is reduced or absent and therefore the likelihood of retrieving DNA from pulp and dentine is reduced.² It's been proven that teeth survive most postmortem events and changes, and are valuable potential sources of forensic DNA evidence. Recovery of tooth DNA from recently extracted teeth by cryogenic grinding has been shown to be successful. Cases where comparison of antemortem and postmortem dental records is not possible due to lack of data can be solved by comparing DNA extracted from the teeth found in the remains to a DNA reference sample.³ Despite teeth providing such a valuable source of DNA from very ancient and/or degraded remains little is known about the quality and quantity of DNA extracted from teeth of deceased person. Study examining the post-mortem persistence

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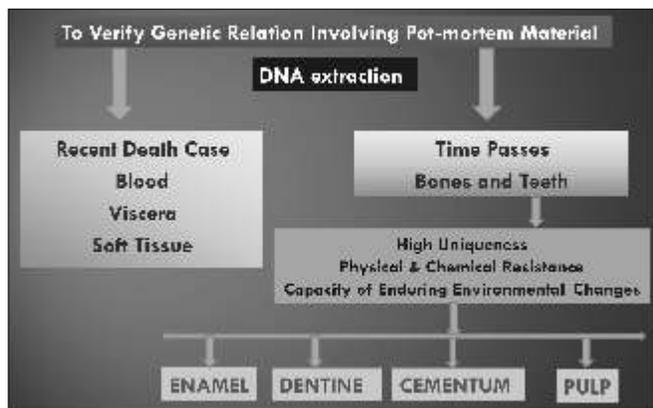


Fig. 1: Flow chart summarizing significance of different sections of tooth for DNA extraction and amplification.

of pulp has shown pulpal cells viable for more than 12 h post-mortem till over 24 h after death.⁴

The application of tooth DNA technology to forensic odontology cases is already a fact. DNA has been isolated and characterized from dentine, pulp and cementum. This accomplishment provides a basis for re-association of body parts that might not be otherwise possible because of decomposition. This review systematically compiles importance of different parts of teeth, dentine, pulp and cementum for DNA extraction and its application in forensic identification. Furthermore various aspects of genetics & molecular biology in forensic odontology is been discussed, supported by cases reported all over the world (Fig. 1).

Genetics

'DNA fingerprinting' or DNA typing (profiling) as it is now known, was first described in 1985 by an English geneticist named Alec Jeffreys. He found that certain regions of DNA contained DNA sequences that were repeated over and over again next to each other. He also discovered that the number of repeated sections present in a sample could differ from individual to individual. By developing a technique to examine the length variation of these DNA repeat sequences, Dr. Jeffreys created the ability to perform human identity tests.⁵

Genomic DNA

Teeth represent an excellent source of genomic DNA. It is found in each cell nucleus and is a reliable source for most forensic applications. After decomposition of body tissues, the calcified tissues structures like the enamel, dentine and pulp complex persist and can be used to

extract the DNA. It is also been reported that root-filled teeth can provide sufficient DNA for PCR analysis.⁶

Mitochondrial DNA (mtDNA)

0.5 % of the total DNA embodies mtDNA and it can be separated with ease from the genomic DNA. mtDNA exists in high-copy number in all cells and have more probability to survive for extended periods, as compared to chromosomal, genomic DNA. Human DNA usually encodes 100000 genes, mtDNA is 16569 nucleotide bp in length, and encodes 13 different genes.⁷ Furthermore, when the extracted DNA samples for forensic analysis acquired from skeletonized tissues are either too small or degraded, then the likelihood of procurement of DNA profile from mitochondrial DNA is higher as compared with any other marker found in genomic DNA.⁸

Additional advantages of mtDNA is that it is inherited only from the maternal line, and is the finest way to test relation if there are several generations between ancestor and living descendant. Whereas disadvantage is, it is exclusively matrilineal henceforth not as much informative for analysis directed at tenacity of crimes and identification of persons.^{1,9}

Molecular Biology

At the present time, forensic odontology has molecular biology as a powerful akin, mainly in species, sex and age investigation. Determination of species using tooth samples has been done searching for exclusive human molecular indicators. These indicators are polymorphic and easily detectable in the population.¹

In cases where insufficient amount or when partially degraded DNA is available short tandem repeats (STRs) or microsatellites specimens are potentially informative as they are amongst the most polymorphic markers reported till date. STR are also referred to as simple sequence repeats (SSRs), consist of tandemly repeated DNA sequences with a core repeat of 1–5 base pairs (bp).¹⁰ Attractiveness of STR typing includes its simplicity, rapidity, amenability to automation and capability for testing very small quantities of DNA.¹¹

Variable number of tandem repeat (VNTR) are variable noncoding DNA stretches in the human genome composed of core units of a fixed nucleotide sequence that are repeated between 2 and 10000 times, depending on the type of polymorphism. The core units of these

repeats are composed of hundreds of nucleotides which can be repeated hundred times.¹²

Application of Different Dental Tissues For Forensic Analysis:

It's been established in literature through several research studies that different dental tissues like enamel, dentine, pulp and cementum even in minimum quantity and degraded quality can be used as source of DNA for

human identification in forensic cases. Based on availability of whole tooth or precisely any particular part of the tooth from crime scene, mass disaster, burn, skeletonized remains, severe trauma and accidents, this review discusses in concise the description of these tissues, when to consider them based on their availability and how sampling of these tissues can be done (Table 1).¹³⁻¹⁸

Table 1
Precise description of different dental tissues, considering various circumstances to contemplate them and respective method of their sampling

Dental tissue	Consideration	Sampling
Enamel	Through molecule analysis of teeth it is possible to determine sex or gender of a deceased. Amelogenin is a protein found in the dental enamel and the responsible gene for analysing the sex (indicators in Y chromosomes). The amelogenin gene is found in chromosome X and Y and variations in both genes pattern and different size are used as reliable indicators to determine sex, even when there is tiny amount of DNA.	Grinding
Dentine	? Odontoblastic processes undergo degeneration with age and remains of the mtDNA trapped in dentine is a good source DNA in cases with severe degradation. ? Tooth devoid of the pulp or endodontically filled ? Contaminated by micro-organisms	? Grinding the root dentin - cementum powder ? Solubilized and stored in vials ? Similar procedure -DNA extraction as for pulp
Pulp	? Most commonly used ? Usually abundant ? Least chance of contamination by nonhuman DNA	? Crushing ? Vertical or horizontal splitting ? Endodontic access
Cementum	The possibility of encountering non human DNA is lesser in calcified tissues, except in cases of carious lesions. The organic material being calcified, there is more probability of recovering intact DNA. ? Pulp tissues become Non -Viable within a relatively short period of time. ? Teeth- Undergone Root Canal Therapy: contain no pulpal tissues ? In an ideal dry postmortem environment pulp may mummify & persist for extended periods but in a moist environment putrefaction rapidly leads to complete destruction ? Cementum is an easily accessible source of nuclear DNA from teeth ? It can be a preferred source of DNA where large number of individuals needs to be sampled quickly (for example, mass disaster victim identification). ? It can be obtained without the need for specialist equipment. ? It can be extracted from diseased and degraded teeth, where pulp is absent.	? Grinding the root dentin - cementum powder ? Solubilized and stored in vials ? Similar procedure- DNA extraction as for pulp

Research Studies Relating DNA Extraction From Dental Tissues & Forensic Analysis:

Selected research work from past two decades have been reviewed and tabulated to comprehend the significance of tooth DNA (Table 2) for forensic purposes.¹⁹⁻³²

Table 2
Tooth DNA extraction and amplification studies documented in literature

AUTHOR	SOURCE OF DNA	CONDITIONS CREATED	RESULT DNA AMPLIFICATION
Schwartz et al. 1991	Pulp	Environmental conditions Varied pH (3.7 & 10.0) ,Temperature: (4°C, 25°C, 37°C & tooth incineration) Humidity (20, 66 & 98%) Type of the soil teeth were buried (sand, potting soil, garden soil, submersion in water and burying outdoors) Periods of inhumation (1 week to 6 months)	Amplified
TC Boles et al. 1995	Teeth	Buried up to 80 years	Amplified
Lessing et al. 1995	Pulp	Different conditions Teeth extracted: person alive and after death At room temperature 12 and 6 months respectively	Amplified
Pfeiffer et al. 1999	Pulp	Environment influence Teeth Underneath Soil 1yr Opened pulp exposed to external agents	mtDNA amplification Significant degradation in 18 weeks
Hanaoka et al. 1995	Teeth (pulpal & hard tissues)	Different concentrations of a decalcifying solution	Pulp DNA: high molecular weight analysis by multilocus probes or PCR Hard dental tissues showed satisfactory analysis only by the PCR technique
Tsachimochi et al. 2002	Pulp	Teeth incinerated: 2 min Temperature: 100°C, 200°C, 300°C, 400°C & 500°C.	300°C amplified At 400°C PCR product didn't amplify
Malaver&Yunis 2003	Pulp Dentine Cementum	Unidentified bodies buried in 1995 and exhumed in 2000	Pulp strongest amplification Dentine & Cementum equally amplified
Remualdo et al. 2004	Teeth	Heat: 200°C, 400°C, 500°C & 600°C Time: 60 minutes Testing: 3 extraction methods (organic; ammonia acetate/isopropanol & silica)	Organic method at 200°C & 400°C Isopropanol/ammonia acetate at 500°C & 600°C for mDNA
K Sowmya et al. 2013	Teeth	Decalcification acid tested - Concentrated hydrochloric acid, nitric acid, and sulfuric acid Time- DNA was extracted on an hourly basis using phenol-chloroform method.	Sufficient quantity ofDNA was obtainable till the first 2 hours of acid immersion
Sara C. Zapico 2013	dentin and pulp	Determine the sex: amelogenin gene	DNA yield depended on the type of tooth and was lowest in the smallest teeth correctly identify a person's sex
ASSD Teradaetal. 2014	intact teeth isolated dental pulp tissue	Immersion of the biological sample - Tissue Reagent (Qiagen, Hilden, North Rhine-Westphalia, Germany) Temperature - 4 to 8°C Room temperature - 24 to 37°C Time- 180 days, 30 days, 7 days, 1 day	Tissue reagent showed a significant stabilizing DNA in samples of intact human teeth stored at room temperature for 30 and 180 days
R Pandey et al. 2014	Dental Tissue	Samples tested: under prolonged formalin fixation	Proved these dental tissue samples can be used for forensic odontology
D Higgins et al. 2015	coronal dentine, root dentine, pulp cementum	Examine- Nuclear DNA and mitochondrial DNA Based on - Post-mortem interval and soil temperature.	cementum from teeth buried for up to 16 months can provide a reliable source of nuclear DNA
J A Garriga 2016	Teeth	Temperatures: between 100 and 700 °C Time: duration of 1–15 min	Silica-based methodology. Burnt teeth reached chalky white appearance at 400 °C 5min Fractures observed from 300 °C 10min.

Emphasising Cases Reported in Literature Resolved by Means of Dental DNA:

For forensic investigations an individual's identity can be accomplished by collecting rightfully all possible samples such as: dead; recent or past death; the body is complete, in parts or decomposed left in the crime scene. Circumstances wherein, obtained material does not show adequate characteristics of quality or quantity to be compared, genetic profile analysis can be executed. For doing so even small fragment of tooth or any part of dental tissue can be very beneficial to obtain desired results, if samples are collected in accurate manner. This has been proven in various cases solved around the globe using dental DNA received from varied circumstances.

Cases reported here emphasis on the fact that after evidences being appropriately collected, suspected samples are compared to materials where origin is previously known or proven, that is, standard material that can have biological nature or records (medical, dental or photographic).³³

Sweet et al. 1995, revealed an identification of a homicide victim that had been charred with fuel. DNA was obtained from dental pulp (1.35 µg) extracted from intra osseous third molar.⁸ In 1999 again Sweet et al. presented an identification of human parts from woman that had been disappeared for 3 years. Presumed victim had 3 smear cell laminae in the laboratory files. DNA was extracted and compared to genetic profile obtained from the dental sample of the found corpse. The result was positive, showing coincidence in 8 of the 8 examined loci, including amelogenin.³ Meyer et al. in 2000, using old bones ageing 4000 and 7000 years compared the morphologic sex with amelogenin analysis results from bone and tooth materials, they found an amplification index of this gene greater than 90%.³⁴ In 2003 Bilge et al. identified corpse whose head was found 6 months after the body was found. Sex was determined by amelogenin analysis. DNA extracted from pulp was compared to genetic profile of the victim's presumed daughter and wife. Fatherhood indication was verified in 11 examined loci.³⁵ In 2005 Andelinovic et al. in mass disaster situations, showed that DNA analysis from tooth material allowed identity of 109 victims of 12 year war in the former Yugoslavia.³⁶

Furthermore, there were more than 20,000 specimens of human remains recovered after the 9/11 attacks. Only about half of the victims were identified by 2005 and the

rest were considered unidentifiable. But in 2007, the processes were restarted as an advanced STR-based technology became available and even tiny and degraded samples of teeth and bones became adequate for identification.³⁷

Recently in 2012, Raimann P E et al. solved 26 questioned samples, DNA from molar & pre-molar teeth from 26 cadavers with post-mortem intervals from 2 months to 12 years. They suggested the high potential of tooth samples as source for DNA typing independently of the decomposed corpse's time or laboratory procedures.³⁸

CONCLUSION

Crimes and vicious events against human life, such as murder, aeroplane crashes, bomb explosions in wars and terrorist attacks, as well as cases of carbonized bodies or in advanced stage of putrefaction, among other conditions, highlight the need to practice ever faster and more precise approaches during the process of identification of victims.

In such scenario, the findings of the several research studies and cases reviewed in this article determine that the teeth represent an excellent source of DNA, which is protected by epithelial, connective, muscular and bone tissues. Moreover, the dental pulp is protected by enamel, dentin and cementum, all hard dental tissues. Hence, dental experts working in the field of forensic odontology should integrate these new methods of molecular & genetic biology for forensic analysis.

Forensic Odontology have advanced in present times, but there are still some limitations that has to be taken care off while answering the enquiries in the court of law while prosecuting a suspect because an inappropriate decision can change and shatter the dreams and lives of alleged accused too!!

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