Review Article

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Applications of DNA Profiling in Dental Forensics - Part I Background & Overview

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Abstract

Teeth are a valuable source of DNA and resistant to adverse conditions such as incineration, immersion, trauma, mutilation, decompositionwhen compared to other parts of the body that are destroyed or degraded in mass disasters, so they are highly useful in forensic investigations. DNA profiling gives an exactidentification of culprits in crime scene investigations, identification of an individual in mass disastersas well as solving paternity issues. It also provides information regarding the physical characteristics, ethnicity and sex determination. The recent advancements in the technology of DNA research have revolutionized the field of forensic odontology and this paper provides an insight in to the recent concepts of DNA profiling in forensic dentistry.

Keywords: DNA profiling; mtDNA; Dental DNA; DNA analysis.

Introduction

The world has experienced many mass disasters like acts ofterrorism, bombings, earthquakes,tsunami and transportation mishaps in therecent times[1]. Disastervictim identification relies on the efforts ofpolice, dentists and pathologists[2-4]. Because of the resistant nature of dentaltissues to environmental assaults, such as incineration, immersion,trauma, mutilation and decomposition, teeth represent anexcellent source of DNA material[5-7]. When the conventional dentalidentification methods fail, this biological material can provide thenecessary link to prove identity[8-10].

By drawing together the current knowledge of tooth structure and post-mortem diagnosis of DNA in tooth tissues, this paper offers optimization of tooth selection and targeted sampling to maximize successful outcomes of DNA extraction and profiling.

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Role of DNA in Identification

Any type of organism can be identified by examination of DNAsequences unique to that species. Every cell of an individual carriesa copy of the DNA[11]. This uniqueness is due totheintron regions of DNA which contain sequences that are 20e100bp in length that are repeated at different locations (loci) along thechromosome like AGACTAGACATT— AGATTAGGCATT which arecalled sequence polymorphism[12]. The length polymorphism like (-AATG)(AATG)(AATG) (3 repeats) and (AATG)(AATG) (2 repeats) aretermed as Short tandem repeats (STRs) which are used in forensicidentification[16,17].

History

The Discovery of DNA

DNA, a double helix molecule carrying genetic material which determines the makeup of all living cells from onegeneration to the other was discovered by James Watson and Francis Crick in 1953[18]. They presented the structure ofthe DNAhelixfor the first time and shared the Nobel Prize with Maurice Wilkins, nine years later in 1962, for solving one of the mostim portant biological riddles[19].

Forensic DNA Analysis

In 1985 an English geneticist named Dr. Alec Jeffreysfirst described'DNA fingerprinting' or now known as DNA typing (profiling)[20]. He discovered that DNA sequence in certain regions were repeated and were different in each individual[21]. Jeffreys developeda technique to examine the length variation of these DNA repeatsequences which had the ability to perform human identity tests[22].These DNA repeat regions are called as VNTRs (variable numbertandem repeats) and was first used to solve an English immigrationcase and shortly thereafter to find out the culprit in a doublehomicide case and since then, human identification using DNAprofiling methods has been widespread [23].

Evett and Buckletonadvocated a change to DNA profiling that has been largelyaccepted. DNA profiling made the system more sensitive, morereproducible, amenable to computer databasing, and soon becamethe standard forensic DNA system used in criminal case work aswell as paternity testing worldwide[24].

Guidelines for Obtaining Dental DNA

Teeth and bones are the only sources of DNA material which is available for the identification of fragmented or degraded human body parts. The location of teeth in the jawbone and their unique composition provide additional protection to DNA as compared to bones making them a superior source of DNA in many cases.

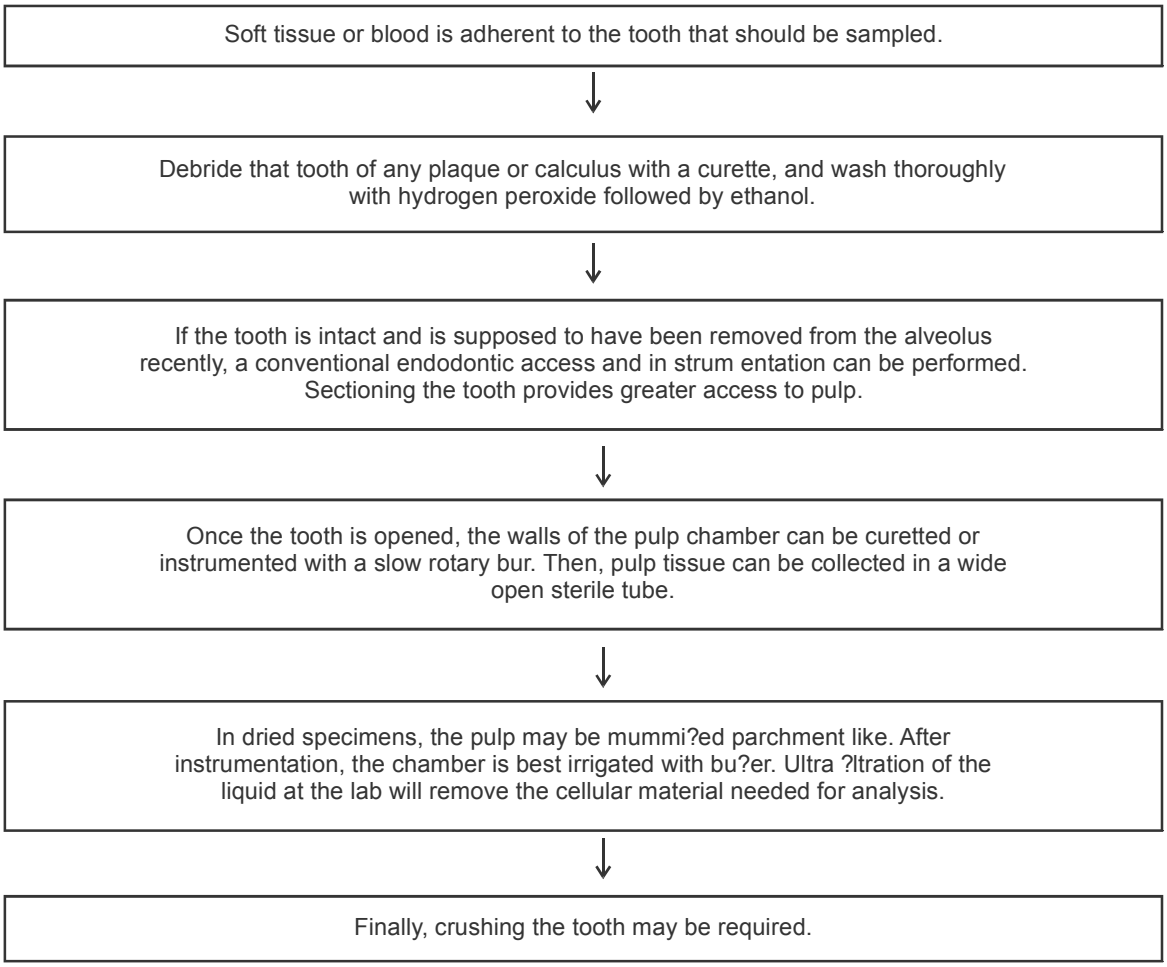

Fig. 1: Guidelines for Obtaining Dental DNA

DNA Teeth and Saliva

DNA can be isolated from various sources, as long as they contain nucleated cells.

Collection of Samples

Teeth are the good source of genetic information. Nucleated cells can be isolated from the surrounding bone, periodontal fibers, and blood. However, the

chance of contamination or degradation is very large, except in the pulp.DNA material from teeth can be achieved by crushing.

Conventional Endodontic Access

It is difficult to obtain enough DNA through conventional endodontic access. In addition, the occlusal morphology and the restorations are damaged[13].

Vertical Splitting

A lot of pulp tissue can be obtained with the vertical splitting method, although the restorations and the tooth are damaged[14].

Horizontal Section

It is the technique by which the tooth is cut at the cervix region. As the crown of teeth remains intact, the access of the pulp and the roots is sufficient.

Cryogenic Grinding

The tooth is kept in an electromagnetic chamber after freezing it in liquid nitrogen. By alternating the magnetic fields, the tooth is grinded into a fine powder[15].

More amount of DNA can be obtained by crushing method rather than just sectioning of teeth as the chance of DNA damage can be minimized considering the sensitivity of PCR[11]. (Figure.2)

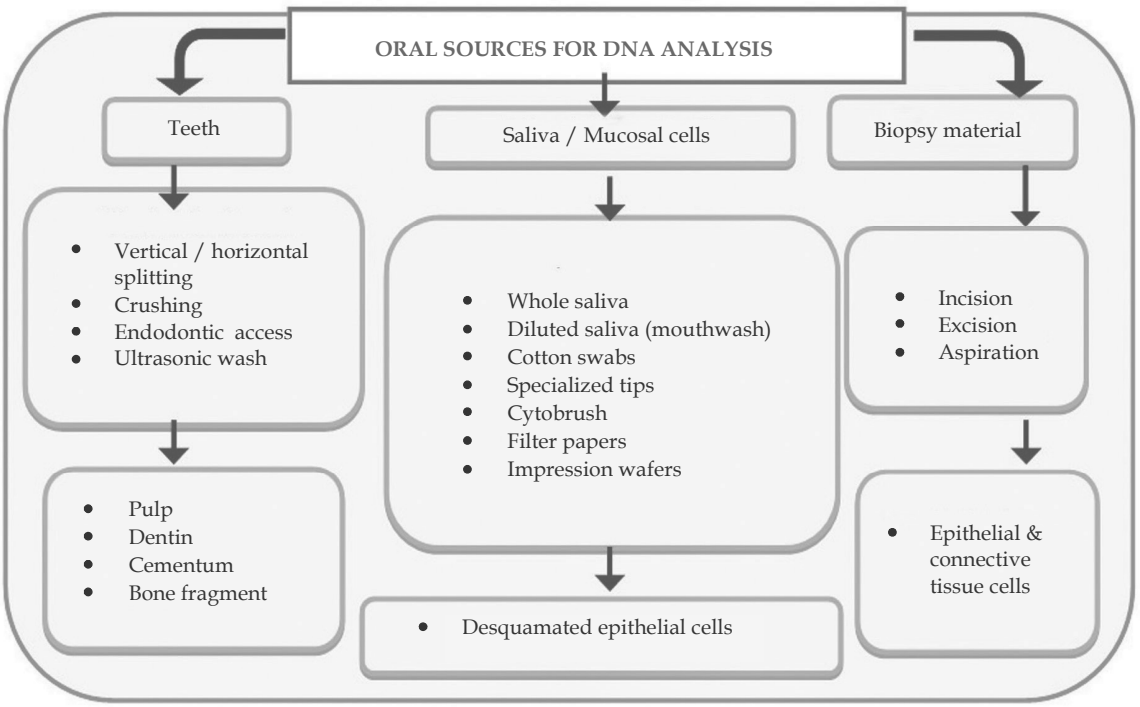

Fig. 2: Oral Sources for DNA Analysis

Factors Influencing DNA Content

Some of the factors indicated to have an effect on DNA content include tooth type, chronological age of the donor, and health status of the tooth. Each of these factors will influence the relative proportions of DNA present in the crown and root, and in the pulp, dentine and cementum.

Tooth Type

It has also been shown that more DNA is retrieved from multi-rooted teeth than single rooted teeth. In the absence of molars, premolars would be expected to have more cellular cementum than anterior teeth, but canines would have a larger pulp volume.

Chronological Age

Advancing age leads to a decrease in DNA content and a change in the distribution of DNA throughout the tooth. Whilst in mature individuals molars would still be the teeth of choice, factors such as the extent of tooth wear and the abundance of cementum should be considered when selecting a sample. Sample choice may be limited in older individuals due to increased exposure to dental disease, and dental treatment and increased tooth loss. Dental diseases not only reduce the amount of DNA available but also increase the potential for contamination.

Teeth and DNA Analysis

Ancient DNA (aDNA)analysis can be carried out through extraction of the tiny amountsof DNA remaining in samples that are hundreds to tens of thousandsof year's old[25]. Teeth are resistant to adverse conditionsdegrading the DNA, such as humidity, high temperature, and themicrobial action[26,28]. In the tooth, dentin and pulp are a richsources of DNA which can be successfully extracted[28,29]. Results ofa study demonstrated that sufficient quantity of DNA can beextracted from the crown body, root body, and root tip. However,the root body is the region which yields highest quantities ofDNA[30]. Not only the quantity of DNA available for the laboratory isimportant, but also the quality and purity. Furthermore, an abundanceof quality DNA can be extracted from a tooth which is animportant advantage in DNA analysis[31,32].

There are various steps in DNA analysis- Extraction of the dental DNA, quantitation followed by DNA amplification and separation and finally DNA analysis and interpretation. (Figure.3)

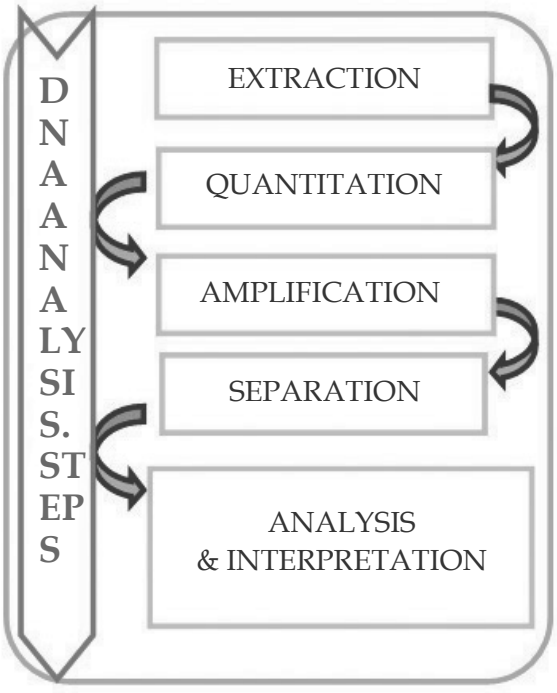

Fig. 3: Basic Steps in DNA Analysis

DNA Isolation Methods

Forensic DNA analysis can be increasingly problematic sincesamples from the scene of crime or a mass disaster may containonly minute amounts of DNA, which may includepolymerase chainreaction (PCR)-inhibitors. Efficient DNA extraction procedures aswell as accurate DNA quantification methods are critical stepsinvolved in the process of successful DNA analysis of suchsamples[37].

Organic Extraction (Phenol-Chloroform Method)

This method of isolation yields high quality DNA, it has many disadvantageslike it is very laborious, time consuming, handling of dangerousorganic solvents and can only be done if abundance of sample isavailable. Due to these setbacks, the phenol/chloroform method hasbeen superseded by many other techniqueswhich have made itirrelevant at present[37,38].

Silica Based DNA Extraction Methods

Silica based methods are used for isolation of DNA fromancient bones and teeth (aDNA). The silica-based extractionmethod showed better results in nuclear STR typing from degradedbone samples than a commonly used phenol/chloroform method[39]. Digesting of the bone powders with proteinase K, and thenextracting purified DNA directly using silica-based spin columns(QIAquick, QIAGEN) is also an efficient method of DNA isolation[40]. Recovery of PCR-amplifiable DNA from ancient bone and teethspecimens can be maximised by a combination of DNA extractionfrom bone powder usinga buffer consisting solely of EDTA andproteinase K, and purification of the DNA by binding to silica[41]. Silica-based aDNA extracts using ion-exchange columnsconsiderably improved PCR amplification and can be useful inpoorly preserved, PCR-resistant, ancient samples[42]. Acombinationof total demineralization and ion-exchange columns increasesapproximately three times higher DNA recovery from old bonecompared to incomplete demineralization method[43,44].

Chelex 100

The procedures are simple, rapid, involve noorganic solvents and do not require multiple tube transfers for mosttypes of samples[45]. The extraction of DNA from dental pulp usingthis method is reported to be efficient compared to proteinase Kand phenol-chloroform extraction. Chelex 100-based DNA extraction,amplification, and typing are possible in incinerated teeth[46].

Commercial DNA Extraction Kits

The PrepFiler Forensic DNA extraction kit enables isolation ofgenomic DNA from a variety of biological samples. The kit facilitatesreversible binding of DNA with magnetic particles resulting in highDNA recovery from samples with very low and high quantities ofbiological materials example

saliva on swabs[47,48].

DNA Amplification Methods

The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA and amplify it to a large enough amount to study in detail[49]. Polymerase Chain Reaction (PCR) was developed in the year 1983 by Kary Mullis for which hewas awarded Nobel prize in chemistry in 1993[50,51]. The drawbacksof PCR is that Quantification is exceedingly difficult[52].

Real Time Polymerase Chain Reaction

Real time PCR is refinement of the old PCR technique and is themost powerful tool for DNA amplification. Some of the limitationsof the old PCR were resolved in 1992 by the developmentof real-time PCR. Itwas developed by Higuchi et al [53]. Typical uses ofrealtime PCR includes pathogen detection, gene expressionanalysis, single nucleotide polymorphism (SNP) analysis, analysis ofchromosome aberrations, and most recently the protein detectionas well[52-55].

AmpFlSTRMiniFiler and AmpFlSTRIdentifiler PCR AmplificationKits

The AmpFlSTRMiniFiler polymerase chain reactionamplification kit, developed and supplied by applied biosystems,compliments the AmpFlSTRidentifiler polymerase chain reactionamplification kit[56].

Conclusion

Teeth are an excellent source of both nuclear and mitochondrial DNA, and have been successfully used in the forensic identification of compromised human skeletal remains.A comprehensive understanding of tooth structure and composition, as well as the process of diagenesis in teeth, is crucial for determining the location of DNA in post-mortem teeth.A detailed description of DNA profiling and its various methods will be laid out in the next part of this paper.

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