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The Role of DNA in Forensic Odontology: Part II

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Abstract: During the last years, DNA analysis methods are applied to forensic cases. Also, forensic dental record comparison has been used for human identification in cases where destruction of bodily tissues or prolonged exposure to the environment has made other means of identification impractical, i.e., after fire exposure, aircraft inflammation or mass disasters. Teeth represent an excellent source of genomic DNA. The interest in using dental tissues as a DNA-source of individual identification falls within the particular character of resistance of this organ towards physical or chemical exterior aggressions. Because of their resistant nature to environmental assaults such as incineration, immersion, trauma, multilation and decomposition, teeth represent an excellent source of DNA material. When conventional dental identification methods fail, this biological material can provide the necessary link to prove identity. Even root-filled teeth supply sufficient biological material for PCR analysis in order to be compared with known antemortem samples or paternal DNA. DNA can be used for determination of the found remains' identity. The identification of individuals is not the only use for dental DNA. The technique has allowed criminal investigators to link victims to crime scenes once the body has been removed and incinerated. Therefore, it is prudent for the forensic odontologist to become familiar with the fundamentals for obtaining and analyzing DNA from the oral and dental tissues. The purpose of the Part II of this report is to review of the application of the DNA technology to forensic odontology cases, the responsibilities of the odontologist and the importance of DNA extracted from oral and dental tissues and saliva.

Key words: Dental DNA, DNA analysis, forensic odontology, bite marks, salivary DNA, human identification

INTRODUCTION

Using the methods described in Part I, DNA testing can be a powerful method of human identification (Smith *et al*., 1995). The DNA testing can provide information about:

- The source of a biological specimen found at the scene of a crime (suspect or victim): If >50 nanograms of high molecular weight DNA is present, the RFLP testing can be performed. If degradation has occurred as is commonly found in cadaver tissue, AmplitypeR HLA DQalpha and PolyMarker and/or AmpFLP testing can be performs. If extensive degradation and/or limited quantities of DNA were recovered, mtDNA testing may be the only viable alternative
- The individual: If immediate relatives (mother, father, spouse and/or children) are available, nuclear methods can be used, otherwise or when the quantity and/or quality of the DNA extract is poor mtDNA sequence analysis of maternal relatives can be performed

Holland *et al*. (1993) reported the successful use of mitochondrial DNA sequence analysis on human skeletal remains from MIAs in the Vietnam conflict when attempts at using nuclear genomic DNA (HLA-DQ alpha and VNTR locus D1S80) were unsuccessful using the PCR method.

DNA analysis can and will be an important tool for establishing the identity of a suspect, victim or parent or even an historic person (Vernesi *et al*., 2001).

RESPONSIBILITIES OF THE ODONTOLOGIST

- Collaborating with the laboratory and their capabilities, techniques and experiences
- Becoming familiar with the contact individuals and DNA procedures
- Consulting on all matters of dental and salivary evidence
- Examining the evidence (tooth or salivary sample) thoroughly and documenting the circumstances under which the sample was obtained

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- The documentation should include photographs and/or radiographs where appropriate. These may be supplemented by sketches and a thorough written description of the location and identifying features of the evidence, temperature, humidity and potential sources of contamination should be noted as well (Smith *et al*., 1995)

The most frequent contaminating material will be blood or saliva but may include bacterial sources such as feces, decaying tissue, vomit or animal hair which may act as inhibitors to procedural steps such as restriction enzyme activity and PCR or may also represent unintended sources of DNA as well (Smith *et al*., 1995). All material should be labeled and managed according to the local procedures for maintaining the chain of custody. DNA must be relatively undamaged in order to be useful for forensic analysis.

Generally, DNA is a hardy molecule but there are specific environmental conditions that put its forensic usefulness at risk. DNA may become denatured at high temperatures (i.e., in a fire exposure or aircraft fall) or extremes in ph <4 or >13 (i.e., after remaining in sea water for more than 5 days or an acid attack) (Smith *et al*., 1995; Stavrianos, 2009). Denatured DNA poses no problem when PCR is used but can prohibit the use of RFLP (Vernesi *et al*., 2001). Wet or humid surroundings foster degradation (breaking apart) by nucleases and exposure to ultraviolet light or other radiation can cause damage to the DNA molecule as well. Specimens are ideally preserved in a freezer at -20°C or in a cool, dry environment until freezer storage is accessible (Smith *et al*., 1995).

DNA FROM ORAL AND DENTAL TISSUES

The interest in using dental tissues as a DNA-source of individual identification falls within the particular character of resistance of this organ towards physical or chemical exterior aggressions (Gaillard *et al*., 1994). Also, because of their resistant nature to environmental assaults such as incineration, immersion, trauma, multilation and decomposition, teeth represent an excellent source of DNA material (Smith *et al*., 1995; Pretty and Sweet, 2001). When conventional dental identification methods fail, this biological material can provide the necessary link to prove identity (Pretty and Sweet, 2001; Schwartz *et al*., 1991).

Dentin and enamel provide a protective enclosure for genomic and mitochondrial DNA as well as providing the basis for radiographic, biochemical and ultrastructural forensic studies (Smith *et al*., 1993). Soft tissue (gingivae, tongue, follicular tissue, blood clot) and salivary evidence (Berlin and Kazazian, 1992) can be transported to the laboratory after complete documentation. However, in cases where DNA is to be extracted from dental hard tissue, the odontologist may be asked for an opinion concerning how best to access the DNA (Smith *et al*., 1995). Because teeth possess identification value beyond that of DNA analysis alone (radiographic, ultrastructural and biochemical studies), the tooth should not be arbitrarily destroyed. If other studies are to be conducted, such decisions must occur before the tooth is manipulated (Smith *et al*., 1995).

If the hard tissue (Smith *et al*., 1995; Avitabile *et al*., 1993) is to be retained for further analysis, then a conservative technique is mandatory. However, if documentation is complete and no further need of the intact tooth is anticipated, a more aggressive technique can be employed (Smith *et al*., 1995).

With the advent of the Polymerase Chain Reaction (PCR) (Fig. 1), the dental DNA evidence is becoming increasingly popular with investigators. Comparison of DNA preserved in and extracted from the teeth of an unidentified individual can be made to a known antemortem sample (stored blood, hairbrush, clothing, cervical smear, biopsy, etc.) or to a parent or sibling (Pretty and Sweet, 2001). Yamamoto (1996) underlines the importance of DNA extracted from dental pulp for DNA fingerprinting with Y-specific probe. DNA typing is especially useful for sex determination, allowing valid determination from teeth extracted up to 21 months before the examination. A 3-cycle repetition of PCR provided an accurate sex determination from a considerably degraded DNA specimen, comparable to one freshly sampled.

Generally, the most DNA-rich site will be the dental pulp (Takasaki *et al*., 2003; Duffy *et al*., 1991) which is enclosed by the coronal pulp chamber, root canals and

Fig. 1: Applied biosystems, Step Ove Plus, TM real time PCR system

accessory canals. Some DNA may be recovered from the dentin or cementum but none should be expected within the enamel (Smith *et al*., 1993). Molar teeth are generally better protected due to their location and tend to have larger pulp chambers, if not deeply restored (Smith *et al*., 1993, 1995). On the basis of gross volume, the coronal pulp chamber and radicular canals are the basic targets for DNA sampling but also odontoblastic processes, accessory canals and cellular cementum contain DNA too (Smith *et al*., 1993) (Laboratory of Experimental Pharmacology, Medical School, Aristotle University of Thessaloniki).

When body tissues have decomposed, the structures of the enamel, dentine and pulp complex persist (Smith *et al*., 1993). It is necessary to extract the DNA from the calcified tissues. In some laboratories the cryogenic grinding method is employed (Pretty and Sweet, 2001; Sweet and Hildebrand, 1998). Teeth represent an excellent source of genomic DNA. Pretty and Sweet also have found that even root-filled teeth supply sufficient biological material for PCR analysis (Sweet and Hildebrand, 1998). PCR-based analysis produces a DNA profile that can be compared with known antemortem samples or paternal DNA (DNA can be used for determination of the found remains' identity) (Pretty and Sweet, 2001). The identification of individuals is not the only use for dental DNA. The technique has allowed criminal investigators to link victims to crime scenes once the body has been removed and incinerated (Pretty and Sweet, 2001; Sweet and Sweet, 1995).

Cryogenic grinding (Pretty and Sweet, 2001) is used to extract DNA from calcified tissues such as teeth. In a freezer mill a ferromagnetic plunger is oscillated back-and-forth in alternating electric current. Liquid nitrogen is used to cool the sample which results in marking it extremely brittle and also protects DNA from heat degradation. The tooth is reduced to a power to increase surface area and expose trapped cells to biochemical agents that release DNA into solution.

All procedures should be conducted under sterile conditions to protect the evidence from contamination and the operator from infection (Smith *et al*., 1995). Gloves, mask, eye protection and a clean working environment are mandatory.

GUIDELINES FOR OBTAINING DENTAL DNA

- Determine if there is any soft tissue or blood adherent to the tooth that should be sampled
- Debride the tooth of any plaque or calculus with a curette and wash thoroughly with hydrogen peroxide followed by ethanol
- If the tooth is intact (unrestored, non-carious, unbroken) and is believed to have been removed from the alveolus recently, a conventional endodontic access and instrumentation can be conducted
- Sectioning the tooth provides a greater access to the pulp chamber (vertical axis sectioning)
- Once the tooth is opened, the walls of the pulp chamber can be curetted or instrumented with a slow-speed rotary bur. Pulp tissue and powder can be collected over a wide-mouthed sterile container. In dried specimens, the pulp may be mummified, parchment-like or consist of wispy strands of tissue contracted against the chamber wall. After instrumentation, the chamber is best irrigated with TE buffer. Subsequent ultrafiltration of the liquid at the laboratory will remove the cellular material needed for analysis
- Finally, crushing the tooth may be necessary (Smith *et al*., 1995).
- The odontologist has to report to the idence contributor the procedures that he performed and the findings he obtained.

Based on the micro-anatomy of the human tooth and the location of cells that harbor potentially useful DNA, five techniques were considered for study:

- Crush entire tooth
- Conventional endodontic access
- Horizontal section of tooth with partial extirpation of coronal and radicular half of tooth
- Horizontal section of tooth with aggressive extirpation and apicectomy
- Horizontal section of tooth with aggressive pulpectomy and crushing of radicular half of tooth (Smith *et al*., 1993)

An interesting experiment about sampling of dental DNA was occurred by Smith *et al*. (1993). Ten pairs of maxillary right and left third molars were sampled for DNA following storage for 18 weeks at ambient temperature and humidity. Right 3rd molars were crushed whereas the left molars were sectioned conservatively prior to sampling DNA. The quantity and quality of human DNA obtained from each tooth was compared as well as the radiographic appearance of remaining hard tissue and the overall simplicity of each approach. DNA typing was performed, both sequence and length based analyses, comparing teeth from the same individual and teeth from different donors. The results of this study suggest that the odontologist will maximize the dental DNA yield by crushing the entire specimen but that substantial yields of human DNA can be obtained by using a conservative

technique that preserves the tooth structure. Concluding, the method of sampling does not affect the ability to perform DNA typing analyses.

On December 26, 2004, a 9.4 Richter scale earthquake occurred north of Sumatra Island and a forensic investigation was required for identification of tsunami disaster victims in order to identify the victim, to determine the time and place of death along with the cause and manner of death (Balwant and Anand, 2007). The main purpose of forensic investigation was the identification of victims. The deceased bodies were recovered and transferred to morgues by rescue teams. Without any refrigerated container nor method to preserve the bodies, the forensic teams had to examine the bodies quickly as before the corpses decomposed. The forensic teams recorded external appearances, personal belongings and specific marks on the deceased following their protocols. Photographs were taken in almost every case with digital cameras. For identification different methods were used for identification such as DNA (Dental tissue, rib, femur), dental identification. DVI identification process (Disaster Victim Identification) was consisted of four main steps i.e., body tagging and bagging, finger printing, forensic pathology and forensic dentistry. Forensic dentistry team was divided into two parts i.e., dental examination and dental radiology. Facial bilateral dissection was performed to examine the maxilla and mandible. The teeth examination was denoted on pink DVI form Interpol charting system (World Dental Federation Tooth Numbering). Two untreated teeth with large pulp were selected for DNA profiling. If teeth were not available then femur shaft or rib was selected. Two bitewing radiographs were taken and labeled, each exposed film with body number. The radiograph was recorded with pink DVI form. Teeth were sent for DNA profiling.

About 7 months after the disaster, Thai tsunami victim identification has identified 2010 victims with over 1800 cadavers remaining unidentified. About 61% of victims were identified using dental examinations, 19% using finger print records, 1.3% using DNA analysis, 0.3% using physical evidence and 18% of cases >1 type of evidence. There are 2315 bodies left waiting to be identified. In conclusion, forensic identification techniques such as dental, fingerprint and DNA analysis are effective because they can identify decomposed or damaged bodies.

Few countries have the capacity for DNA collection and analysis following large natural disaster. DNA identification is expensive, technically demanding and logistically difficult to implement on large scale (Balwant and Anand, 2007). In case of the tsunami in Thailand, it proved to be a relatively unimportant method of identification. DNA identification should not be considered as a first live method of identification but rather should only be implemented when physical, fingerprint and dental methods have been unsuccessful. Among the victims, a high number of relatives are expected be found as well as entire families were died without any family reference to compared with.

The situation can also be further complicated by rate and speed of body recovery from the sea, affecting DNA integrity in some cases. All these challenges require an approach to identification process of the tsunami victims as an integral forensic science identification effort, based not only upon DNA data but also on forensic anthropology, fingerprinting, odontology and radiology.

SALIVARY DNA RECOVERED FROM HUMAN BITE MARKS

The usual methods of analyzing human bite mark evidence involve systematic physical comparison of the pattern of the injury in life-sized photographs or tracings to models of the suspect's teeth (Sweet *et al*., 1997a; Stavrianos, 2009). These comparisons are often subjective and depend on the experience and procedures used by the odontologist (Sweet *et al*., 1997b).

Saliva is normally deposited on human skin during biting, sucking, licking and kissing, so the potential use of the DNA present in saliva stains on skin shows any role of the suspect in causing a given bite mark (Sweet *et al*., 1997a, b). Guidelines established by the American Board of Forensic Odontology or ABFO, 1995 for the collection of bite mark evidence advocate swabbing of the skin to collect saliva as part of the standard operating procedure. These swabs can be tested for amylase, a component of saliva. A positive test result confirms the presence of saliva and that the observed injury is in fact a bite mark (Sweet *et al*., 1997a, b).

Human saliva (Smith *et al*., 1995) has been shown to be an excellent source of high molecular weight DNA. Saliva recovered from material at crime scenes (i.e., clothing, cigarette butts, postage stamps, envelope stamps) has been completely isolated, analyzed and compared to reference sources obtained from suspects (Hochmeister *et al*., 1991).

Simultaneous with these successful case findings has been the advancement of alternative light technology which permits investigators to identify the location of body fluids, such as saliva, blood and semen left on the skin of a victim or other objects at a crime scene (for example in association with a bitemark) (Smith *et al*., 1995). It is worth mentioning that:

- Human saliva is indeed a useful source of forensic DNA evidence. Some samples have yielded as much as 15 ng mL^{-1} DNA (Ohhashi *et al*., 1993)
- Significant DNA in a deceased victim can be stable and may be recovered up to 48-60 h after deposition on the skin, depending upon environmental influences (contamination, degradation and putrefaction) (Sweet *et al*., 1997a, b). The success of PCR amplification (polymerase chain reaction) is independent of the time since deposition or the concentration of DNA in the saliva sample
- In cases of unwashed skin, the DNA in dried saliva may be retrievable for up to 72 h
- Contamination of the saliva with other DNA is a potential problem i.e., blood or sloughed skin cells (Smith *et al*., 1995; Sweet *et al*., 1997a, b)
- When PCR analysis method is used to test salivary evidence, two benefits are provided) amplification is possible from very small amounts of DNA which allows genetic information to be obtained from evidence samples such as a single hair, an invisible semen stain and similar minute biological samples i.e., saliva stains) amplification is possible from very old material or from partially degraded DNA (Sweet *et al*., 1997a, b)
- A double swab technique for salivary DNA is suggested from the Dr. Sweet (Smith *et al*., 1995)

Several techniques were evaluated to determine the best method of recovering saliva from human skin before extracting genomic DNA from the collection substrate. (Sweet *et al*., 1997a, b) studied of three techniques. A classical stain recovery technique using a wet cotton swab was tested against one utilizing a wet filter paper. Additionally, a new method, referred to as the double swab technique, using a wet cotton swab followed by a dry cotton swab was also evaluated. After recovering a dried saliva stain, DNA was extracted using the modified Chelex method, quantified using the slot-blot procedure and amplified at three polymorphic loci. The double swab technique showed the highest percentage recovery of saliva from human skin among the three methods studied. This technique is suggested as an improvement over the classical single wet cotton swab technique.

CONCLUSION

The application of this DNA technology to forensic odontology cases is already a fact. DNA has been isolated and characterized from the dental pulp and saliva. This success provides a basis for reassociation of body parts that might not be otherwise possible because of decomposition. There is particular interest in mitochondrial DNA analysis because of its availability, especially in skeletal material remains and its successful characterization by PCR method. Using the DNA analysis' methods, DNA testing can be a powerful method of human identification.

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