FORENSIC IDENTIFICATION BASED ON TOOTH MATERIAL

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ABSTRACT

Human teeth are the most robust and stable parts of the body, providing biological clue material for forensic purposes even when most of the other means of identification have been seriously affected by adverse environmental conditions. In particular blood grouping, isozymes, serum proteins and DNA polymorphisms can be detected from teeth that protect these identification markers in addition to the traditional dental records. While in general the value of traditional dental records in the forensic work is decreasing eg due to improved dental care, the newer means of identification from tooth material provide considerable promise for effective identification in difficult cases.

The DNA analysis from tooth material has been shown to be a viable route in forensic analysis, when other material for such an analysis is unusable. However, in most cases useful biological material other than teeth is available, and then DNA analysis can be made from other tissue with less effort than by using teeth. Also, in cases with lacking other tissue, blood grouping, isozymes and serum proteins may provide cheaper and more widely available means of analysis for forensic purposes. Since multiple and independently inherited combinations of blood grouping, isozymes and serum proteins can be treated similarly to polymorphic DNA loci as independent markers, their identification power can be increased far beyond that of eg blood grouping alone. The probabilities of identification can be managed if the false positives and negatives in analysis can be minimised, and the corresponding frequencies of occurrence are known.

It was the purpose of the present work to review the methods of forensic identification from tooth material, based on analysis of blood grouping, isozymes and serum proteins. It appears that such a combined analysis, provides a robust method for forensic purposes. Nevertheless, for efficient identification it is recommended that as many (multiple) forensic methods as possible are combined, so that faster and cheaper methods such as imminent medical forensics are used first, and more thorough analysis is used to support and complement these methods.

INTRODUCTION
Identification of victims and assailants of crime as well as clarification of familial relationships remain prime objects for
forensic analysis. Since the turn of the century, the forensic science has provided many tools that rely on human features that are more or less bound to each individual. Such classic tools include, e.g., visualization of fingerprints, typing of blood group antigens, and comparison with dental record.

However, the traditional forensic methods tend to be limited in scope, required records and sampling, as well as reliability of the results. The recent advances in genotypic identification have made it possible to use DNA analysis for such purposes (for reviews see 1, 2, 3, 4, 5). The present trend is clearly increasing use of forensic DNA analysis. Since the technique is relatively new, it is however not widely utilised everywhere, nor are the methods in the DNA analysis and its application developed to a stage of internationally standardised procedures. Although a breakthrough has already happened to that DNA genotyping has during the recent years become a regular forensic tool in the industrialised countries, much remains to be done in simplifying the techniques for faster results and in decreasing the cost from the present (1995) level of about 800 to 2500 USD (in the US for one analysis with 3 to 5 markers).

The recent scientific and legal debate 2, 4, 6 does no more question the general validity of DNA analysis in forensic applications but the ideal methods of analysis and interpretation 6. In this context, the technical difficulties are mainly related to the fact unlike in traditional biochemical forensic analysis (using, e.g., blood grouping), DNA testing involves also aspects of population genomics.

Of the 3.3 billion (3.3 \times 10^9) base pairs that make up the human genome, only about 1/1000 or some 3 million differ between any two individuals of the world population 4. To see the difference between individuals, most of the DNA is useless and only the small differentiating fraction targeted for DNA genotyping. Never-theless, using these DNA loci as markers, a very wide selection is available for comparisons. The genetic material is highly polymorphic and therefore the genotype of any individual represents a unique identifier for that person, except for identical twins. In practice it is at present not possible to use all the differentiating loci in DNA analysis. However, by using multiple DNA markers that have been selected to represent highly polymorphic regions of the DNA, the expected probabilities of positive identification (and positive exclusion of the alternatives) are generally much higher than for most traditional forensic methods. Also noncoding regions (which represents 99% of all genome) are useful, since these regions, particularly repetitive sequences of DNA called satellites that for humans comprise of 30% of the genome, are abundant and highly polymorphic 12. DNA genotyping (= DNA analysis = DNA fingerprinting = DNA profiling) has become a routine toll for human identification, but not yet the option for first-aid use because of relatively tedious, slow and costly processes involved, and because the method is not yet as widely penetrated in forensic analysis as the traditional methods.

In principle all other classical and newer biochemical forensic tools continue to exist as viable alternatives and complements to the DNA analysis. Whether these other methods also are cost effective and realistic alternatives, apparently can depend on the practicalities of local availability for each technique. Certain
main principles, however, are the same for any method that is used for forensic identification:
- for good identification power, unique (variable or polymorphic) yet in the same time as common markers (but with many rare variants) are needed; and
- the size and stability of the samples must fulfill the basic practical requirements for an analysis with unambiguous results.

The enamel of the tooth is the hardest substance in the human body, and in general the outer layers of the tooth protect it both mechanically and chemically from the environmental impact better than most - if any - other parts of the body. Consequently, the teeth are one primary object available for forensic analysis, including difficult cases where sometimes the teeth are the only remains left for identification. The classical forensic odontologic analysis is based on comparison of the observed dentition and available dental records. This is however often impossible when the previous dental records do not exist, or when the remaining number of teeth is not sufficient. It is the purpose of the present work to review the methods performance of forensic identification from tooth material.

DNA ANALYSIS FROM TOOTH MATERIAL

Methods of DNA Analysis

The forensic DNA analysis by sample collection. A sufficient (recommended) normal sample size is described in Table 1, but with methods like PCR (polymerase chain reaction) the sample size is decreased substantially (1,7), ideally even to a single cell.

Table 1. Recommended sample sizes for DNA analysis (1), when PCR amplification is not used. Note that any other nucleated cells from eg bones or teeth are also acceptable.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Quantity (μl)</th>
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<tbody>
<tr>
<td>Blood</td>
<td>50 1)</td>
</tr>
<tr>
<td>Semen</td>
<td>10 1)</td>
</tr>
<tr>
<td>Liver, kidney or skin</td>
<td>15</td>
</tr>
<tr>
<td>Muscle</td>
<td>25</td>
</tr>
<tr>
<td>Tooth</td>
<td>(one tooth)</td>
</tr>
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</table>

1) As stains preferably larger than 18 mm in diameter.

The collected samples should be carefully protected from contamination by foreign DNA. For example, the sample collecting personnel should wear gloves, and the samples placed in a paper rather than a plastic bag to avoid DNA-destroying mold infections. Fortunately, the DNA is usually quite robust material, and it has been obtained from blood and semen stains more than 10 years old. However, fresh-frozen tissue or ethanol-fixed samples are preferable if tissue samples are available from eg autopsy; also tissues fixed in buffered formaldehyde can be used.

The dry samples should be stored in a cool place with desiccant or in a frozen state. Non-dry tissue (like autopsy) samples should be wrapped in aluminum foil and placed in a plastic bag for immediate freezing at -20 to -80 °C. It is important to document the samples and any potential foreign DNA contamination, the storage temperature and other information that may be significant in future.
Classic restriction fragment length polymorphisms (RFLP) are due to single base pair changes in the DNA. A base change causes the loss or addition of a restriction enzyme site and therefore a longer or a shorter restriction fragment, respectively. The polymorphism in size of such a known DNA marker can be used as a fingerprint of the DNA. In practice, DNA is isolated from any nucleated cells (also the maternally inherited DNA of the mitochondria can be used), yielding DNA that can be cleaved by commercially available restriction enzymes. Usually, enzymes with four base pair recognition sites (HinfI, MboI, AluI, HaeIII) are preferably used for DNA fingerprinting since they produce a DNA fragment length distribution (typically 1.5 - 30 kb) that is suitable for separation by conventional agarose (0.7%) gel electrophoresis.

The DNA fingerprint is shown through band pattern classification of fragment sizes in standard electrophoresis, often transferred to immobilizing membranes (Southern blots). Even simple DNA analysis with single-locus DNA markers can be very powerful for identification in multiallele systems (Fig 1 to 3). Multi-locus analysis is still more effective (Fig 4).

Knowledge of the frequencies of the polymorphisms in a population permits quantitative assessment of probabilities for or against identity or familial relationships. To obtain high probability of identification, highly polymorphic loci with known frequencies in the population should be selected as markers. Also, as many genetic markers as possible should be used; for example using three variable number (short) tandem repeat (VNTR) loci can give an average individualization potential of 1 in 500 persons. With six loci the same may be 1 in 200 000, and with 12 loci about 1 in 90 000 000.

Amplification of minute samples of DNA by the PCR has greatly improved the sensitivity of DNA analysis. The PCR has been facilitated by the use of the heat-stable enzyme Taq polymerase from Thermophilus aquaticus and automated temperature-cycling equipment. Particular care is needed when very small samples and PCR are used, because then extremely small contamination with foreign DNA can lead to major errors. For the purpose of DNA amplification via PCR, destruction of the cell nuclei and boiling of the sample is usually sufficient. About 25-1000 ng of human DNA are used for each PCR reaction, which then can produce practically unlimited numbers of the target sequences.

Reliability of DNA analysis

The DNA analysis is only reliable if the molecular genetics based procedures involved can accurately disclose DNA patterns that reflect DNA differences between human individuals. At present the validity of the DNA analysis approach is no more seriously questioned regarding basic principles (4). However, this does not mean that any particular analysis result could not be in error, ie the question of quality always remains an issue like in any other forensic technique. Typical possible error sources for DNA analysis are:

- contamination of the sample by foreign DNA
- destruction of DNA in storage or preparation
- errors in statistical comparison of the background population and
establishment of probability of identification 2,3,5,6

Of these, the two first error sources can be avoided through applying carefully the appropriate procedures for sampling, storage and preparation (see 2,1). The last one is usually only a relative uncertainty, which cannot totally remove the value of DNA analysis in identification. Nevertheless, it is important that actual probabilities are known reasonably well.

Fig 1. Inheritance of single-locus alleles, summarizing results in the corresponding Southern blot; a) two-allele system with both parents heterozygous for A and B alleles; here the pattern shown is observed if the B allele was associated with an additional restriction site that was not present in the A allele, resulting in cutting into two smaller B fragments (B' and B''); b) multiallele system with results characteristic to variable number tandem repeat (VNTR) alleles; sizes of the four bands (A to D) differ according to the number of repeat units in each fragment. Note that multiallele systems tend to be more powerful in identification than two-allele systems (1).

Fig 2. Example of single-locus, multi-allele system identification of a crime suspect. The DNA pattern from evidence (E) is compared with patterns from three suspects S1, S2 and S3, and from the victim (V). It is seen that the evidence is not from the victim or from suspects S1 and S3. However, suspect S2 shares both alleles with the evidence and cannot be excluded as the possible assailant (1).

Fig 3. Single-locus multiallele probing for clarification of paternity. The child (C) shares one allele with the mother. The child's nonmaternal allele is observed in alleged father 1 (AF1), who cannot be excluded as biological father. The alleged father 2 (AF2)
can be excluded because neither of his alleles in seen in the child's DNA. Multi-locus fingerprinting would improve probability of identification; for example, if 25 maternal-specific DNA markers were used, probability of sharing the bands in the mother and the child by chance is only about $10^{-13}$.

![Fig. 4. Two-locus example (with mini-satellite probes λMS1 and λ MS31) of Southern blots showing sample DNA analysis of two rape-murder victims (1983 and 1986) and a suspect from Narborough Village, England. The nonvictim alleles in semen samples (lanes 2, 5 and 6) collected from the victim are identical, showing that both girls were raped by the same assailant. The DNA pattern of the suspect differs from the samples obtained from the victims, showing that the initial suspect was innocent to the rapes, although he had already confessed. The real rapist and murderer was found and convicted in 1988, also based on DNA matching. This case was the first murder case solved by evidence from forensic DNA analysis.](image)

In addition, depending on the particular method selected for DNA analysis, sub optimal results are possible regarding the resolving power of the analysis. Such variation is likely to decrease in time, as more standardised methods and procedures gain acceptance. As a natural consequence of the fact that the methodology is so new, relatively little is standardised in the field of DNA analysis or its forensic applications.

The applicability of DNA analysis can be enhanced considerably in many crime cases, if prior data of the suspect DNA exists. Since much of serious violent crime is committed by repeat offenders, DNA data banking is emerging as a complementary tool to traditional data of criminal identification. The necessary legislation or other infrastructure is not yet in place in many countries, but is likely to spread quickly together with increasingly popular DNA analysis. Such DNA data banking is probably only cost-effective when limited to violent crime offenders, because other crime such as non violent felony and property crime are not usually associated with biological evidence (5).

Quality control in crime-related DNA analysis and DNA data banking is very important. False negatives steer the investigations away from the real perpetrators, and on the other hand sloppiness in creating the data bank profiles results in imprecision that increases the number of individuals unnecessarily targeted for further investigations- ie for drawing more blood. This is because initial profiles are typically made with only few DNA probes, and later confirmed with larger
number of DNA loci

PROTEIN AND ENZYME ANALYSIS FROM TOOTH MATERIAL

Methods of protein and enzyme analysis

To date, the blood grouping (ABO), polymorphic serum proteins (Gm, Km, GC) and polymorphic enzymes (phosphoglukomutase PGM1, 6-phos-phogluconate dehydrogenase 6-PGD, adenosine deaminase ADA, adenylate kinase AK, esterase D or ESD, erythrocyte acid phosphatase EAP, glyoxalase GLO-I, glucose-6-phosphate dehydrogenase G-6-PD, carbonic anhydrase II CA-II, FUC and DIA3) as well as transferring (TF) have been identified from tooth material.

Generally, the likelihood of achieving a definite result in analysis of any of the above compounds from tooth material depends on amount, age and condition of the sample, and also on the part of the tooth that is the sample origin. Obviously, too small, contaminated or decayed samples can make analysis difficult. Contamination by common dental decay by oral bacteria may be sufficient to render analysis inconclusive. Furthermore, the more extensively calcified part of the tooth is used for analysis, the larger sample is needed to provide a sufficient amount of tissue (organic material).

The sufficient sample size in case of analysis of blood substances such as red antigens (e.g. ABO), red cell enzymes, serum proteins and white cell antigens, is generally of the order of 1.1 mg of dental pulp. This amount, while considerably larger than the minimum required for DNA analysis, is usually nevertheless obtained from a single tooth except for some lower incisors. The sample size as the reliability of the result appears to be much affected by the method of analysis.

DISCUSSION

Comparison of DNA and protein polymorphic techniques

Since DNA fingerprinting provides a rapidly expanding and increasingly popular tool for forensic purposes, it is worthwhile to compare it with more traditional approach.

Of these alternative methods, eyewitness testimony is notoriously unreliable and frequently unavailable. Visualized fingerprints can work well but are less robust than DNA, and in case of eg professional criminals fingerprints are not likely to be found. Typing of human leucocyte antigen markers, blood group (ABO) antigens and other protein markers may be useful but require again larger and relatively fresh samples to be reliable. This is why they cannot be used for eg identification of seriously decomposed or disintegrated victims of crime or accidents.

The DNA typing has clear advantage over these methods, since
- DNA analysis is more robust and requires smaller and less well preserved samples than the classic methods; and
- because DNA marker are more effectively discriminating, it is easier to clear wrongly accused persons in court cases: in the USA results in exclusion of the primary suspect.

The main disadvantages related to DNA analysis are at present higher cost and locally more limited availability of equipment and expertise than for classical methods. Both disadvantages are likely to
fade as the now relatively new DNA typing techniques continue to develop and become more popular worldwide.

**Comparison of dental and other data**

Dental forensic methods are among the most common and easiest methods of identification, even for relatively badly decomposed or desintegrated bodies. However, there are clear advantages in DNA analysis over dental (or other similar medical) forensic identification:

- DNA analysis can be made of nearly any sample containing DNA and is hence much less prone to misinterpretation due to tissue destruction or objects limited to very small non-dental samples, and
- through kinship comparisons, positive identification by DNA analysis is often possible even in cases of no previous records of the subject, while using dental records relies on the more uncertain existence and availability of such records. As in comparison with physical and protein polymorphic techniques, the DNA analysis also has disadvantages:
  - equipment and professionals for appropriate DNA analysis are generally not yet available everywhere; and
  - cost of DNA analysis is presently clearly higher and the procedures slower than with traditional dental forensics.

A recommended procedure to achieve efficient forensic identification would typically combine the available methods, so that faster and cheaper methods such as dental (and other imminent medical) forensics are used first, and only difficult cases are subjected to DNA analysis either alone or to support other forensics.

**SUMMARY**

The teeth comprise the most stable biological clue material for forensic purposes when other means of identification have been destroyed by adverse environmental conditions leading to extensive decomposition of charring. In particular blood grouping, isozymes, serum proteins and DNA polymorphisms can be detected from teeth that protect their characteristic features of identification. In comparison, the value of the traditional dental records in the forensic work is decreasing eg due to improved dental care and expansion in DNA data banks relying mostly on information obtained from other tissue than teeth. Identification from tooth material analysis provides considerable promise in difficult cases where other tissue is not available.

The DNA analysis from tooth material has been shown to be a viable route in forensic analysis, when other material for such an analysis is unusable. However, in most cases such severe lack of biological material other than teeth does not occur, and then DNA analysis can be made from other tissue with less effort than by using teeth. Also, in cases with lacking other tissue, blood grouping, isozymes and serum proteins may provide cheaper and more widely available means of analysis than DNA analysis for forensic purposes. Since multiple and independently inherited combinations of blood grouping, isozymes and serum proteins can be treated similarly to polymorphic DNA loci as independent markers, their identification power can be increased well beyond that of blood grouping alone. The probabilities of identification can be
managed if the false calls in analysis can be minimised, and the corresponding frequencies of occurrence are known.

It was the purpose of the present work to review the methods of identification from tooth material, based on analysis of blood grouping, isozymes and serum proteins. It appears that such a combined analysis provides a robust method for forensic purposes. Nevertheless, for efficient identification it is recommended that as many (multiple) forensic methods as possible are combined, so that faster and cheaper methods such as imminent medical forensics are used first, and more thorough analysis is used to support and complement these methods.

LITERATURE