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Original communication

Comparison of three different DNA extraction methods from a highly degraded biological material

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ABSTRACT

The identification of unknown victims is one of the most challenging tasks faced by forensic medicine. This is due to the rapid decomposition of tissues, beginning at the moment of death and caused by released enzymes and microbial activity. Decay is directly associated with the decomposition of soft tissues and also the degradation of genetic material inside cells. Decomposition rates vary depending on a number of environmental factors, including temperature, humidity, season, and soil properties. Decomposition also differs between bodies left in the open air or buried. To date, forensic medicine has identified mainly people who were the victims of various types of criminal offences. However, with advances in identification methods, increasingly frequent attempts are made to identify the victims of armed conflicts, crimes of totalitarian regimes, or genocide. The aim of the study was to compare three different methods for the extraction of nuclear DNA from material considered in forensic medicine as difficult to handle, i.e. fragments of bones and teeth, and to determine the performance of these methods and their suitability for identification procedures.

1. Introduction

The identification of unknown victims is one of the most challenging tasks faced by forensic medicine. This is due to the rapid decomposition of tissues, beginning at the moment of death and caused by released enzymes and microbial activity. Decay is directly associated with the decomposition of soft tissues and also the degradation of genetic material inside cells.[1]

Decomposition rates vary depending on a number of environmental factors, including temperature, humidity, season, and soil properties. Decomposition also differs between bodies left in the open air or buried.[1]

To date, forensic medicine has mainly identified people who were the victims of various types of criminal offences. However, with advances in identification methods, increasingly frequent attempts are made to identify the victims of armed conflicts, crimes of totalitarian regimes, or genocide.

Most recovered human remains are strongly decomposed or completely skeletonized, which makes their direct identification difficult. In many cases, human remains are dismembered (e.g. victims of mass accidents or terrorist attacks) or decomposed in a way hindering their anthropological analysis.[2]

Decomposition of soft tissues is also associated with the degradation of genetic material inside cells, which is digested into increasingly small fragments by released enzymes.[1]

Although nuclear DNA degrades rapidly, advanced molecular techniques increase the possibility of obtaining full genetic profiles used for the identification of individuals. Over the years many researchers have investigated the progressive degradation of genetic material extracted from various types of osseous tissue.[3–5] Comprehensive studies carried out by the team from the Department of Forensic Medicine PMU in Szczecin discovered that the increased degradation of genetic material is associated with the decreased amplificability of longer STR markers used in commercially available kits such as NGM from Applied Biosystems. The best results were obtained for compact osseous tissue and teeth, where no additional alleles indicative of contamination or significant degradation of biological material were found in the STR analysis. The amplificability of markers also determines the concentration of extracted DNA. A significant increase in the amplificability of STR was reported for increased DNA concentrations.[6] Therefore, it is very important to consider pre-extraction procedures, such as the preservation of material or the choice of isolation technique, when analyzing degraded bone material.

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2. Aim

The aim of the study was to compare three different methods for the extraction of nuclear DNA from material considered in forensic medicine as difficult to handle, i.e. fragments of bones and teeth, and to determine the performance of these methods and their suitability for identification procedures.

3. Materials and methods

Bone fragments used in the study were collected from human cadavers during medicolegal autopsy, from human remains being evidence material in criminal cases conducted by the Department of Forensic Medicine, PMU in Szczecin, as well as from the exhumed remains of soldiers killed during World War II (Table 1).

4. Preparation of bone material

Complete teeth and phalanges, and skull and rib fragments obtained at medicolegal autopsy were used for the analysis. An oscillating mechanical saw was used to cut out 2 × 2 cm fragments from the shafts of long bones (humerus and femur), each time with a new, sterile cutting blade.

First, bone samples were mechanically cleaned with a sterile surgical blade, then washed with 5% sodium hypochlorite solution and distilled water. Cleaned bone material was irradiated with UV rays for 30 min (bone fragments were turned over to expose the whole surface area).

Every bone fragment was placed in a separate, sterile grinding chamber and placed inside a Freezer Mill 6770 Spex SamplePrep for grinding.

5. DNA extraction

DNA from all powdered bone samples was extracted using two methods: organic (phenol/chloroform), and a PrepFiler® Forensic DNA Extraction Kit from Applied Biosystems. An QIAamp®DNA Investigator Kit from QIAGEN was used to additionally extract DNA from 10 out of 12 samples collected during medicolegal autopsy and criminal cases, and from 14 out of 31 bone samples from exhumation (each type of soil).

6. DNA extraction with the organic method

Samples of bone powder (0.5–0.8 g) were weighed and digested. For that purpose 2.5 ml EDTA buffer and 20 μl proteinase K were added to each sample placed in a sterile 15 ml Falcon tube. Samples were digested overnight at 56 °C.[7–10]

After completed digestion samples were centrifuged for 2 min at 10,000 rpm, and the supernatant was transferred to new sterile 2 ml test tubes. Phenol reagent was added (phenol: chloroform: isoamyl alcohol, 25: 24: 1, pH 8.0) in a ratio of 1:1, and samples were centrifuged for 1 min at 10,000 rpm. This stage was repeated 3 times.[7–10]

The upper fraction containing DNA was transferred into new sterile 2 ml test tubes, chloroform reagent was added (chloroform: isoamyl alcohol, 24:1, pH 8.0) in a ratio of 1:1, and then samples were centrifuged for 1 min at 10,000 rpm. 400 μl of supernatant obtained in this procedure was purified with a QIAquick PCR Purification Kit from QiaGen.[11]

1 ml of phosphate buffer (PB) was added to the supernatant and mixed. 750 μl of the obtained solution was loaded onto the silica columns provided with the kit and incubated at room temperature for 5 min. Next, the columns were centrifuged for 1 min at 10,000 rpm, the filtrate was poured out, and the loading procedure was repeated until the whole solution was used.

Then, the columns were washed with 750 μl PE wash buffer and centrifuged for 1 min at 10,000 rpm, and again for 1 min at 13,000 rpm to dry the filter.

The dried columns were transferred to new sterile 1.5 ml test tubes, filled with 200 μl elution buffer EB, incubated at room temperature for 10 min, and centrifuged for 1 min at 12,000 rpm.

200 μl of extract was purified again, repeating the steps from adding PB buffer onwards. Finally, 50 μl of EB buffer with suspended DNA was obtained.

7. Extraction with a PrepFiler® forensic DNA extraction kit

Samples of bone powder for extraction (0.05 g) were weighed and digested, following the manufacturer's recommendations.[12] Bone powder samples were placed in sterile 1.5 ml test tubes, and 220 μl BTA buffer, 3 μl DDT reagent and 7 μl proteinase K were added. The samples were then digested in a thermocycler for 2 h at 56 °C and 900 rpm.

Table 1
Characteristics of the biological material used in the study.

Material	Sample name	Time passed after death	Material condition
Fragment of a skull	1	About 5 years	Found in water
Fragment of a humerus	2	About 2 years	Decay, partly buried
Fragment of a femur	3	About 1 year	Decay
Tooth	4	A few months	Strong decay
Fragment of a rib	5	A few months	Found in a salt water
Tooth	6	About 1 year	Strong decay
Tooth	7	About 2 years	Skeletonized, buried in the ground
Fragment of a femur	8	About 1 year	Skeletonized
Tooth	9	About 1 year	Found in water
Phalange	N1	About 70 years	Skeletonized, exhumed from the ground
Phalange	N2	About 70 years	Skeletonized, exhumed from the ground
Phalange	N3	About 70 years	Skeletonized, exhumed from the ground
Phalange	N4	About 70 years	Skeletonized, exhumed from the ground
Tooth	N5	About 70 years	Skeletonized, exhumed from clay
Tooth	N6	About 70 years	Skeletonized, exhumed from clay
Phalange	N7	About 70 years	Skeletonized, exhumed from clay
Tooth	N8	About 70 years	Skeletonized, exhumed from sand
Tooth	N9	About 70 years	Skeletonized, exhumed from sand
Tooth	N10	About 70 years	Skeletonized, exhumed from sand
Tooth	N11	About 70 years	Skeletonized, exhumed from sand