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A modified DNA extraction method for bone and teeth

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Three different DNA extraction methods for bone and teeth were compared. The amount extracted DNA, time consumption and contamination were taken into account. Teeth from modern humans and a cow were used for DNA extraction and there was evidence that the phosphate-buffer extraction method had the highest potential in all these respects. More experiments on both contemporary and ancient material are however warranted.

Introduction

Although the first scientific publications on DNA in ancient bones appeared almost seven years ago (Hagelberg et al. 1989; Horai et al. 1989), we still struggle to refine the extraction methods. In order to use bone DNA it has to be extracted from the bone matrix, it has to be clean and it has to have a quality good enough to serve as template for PCR reactions. Today, there is no single extraction method that fulfils all these demands. The purpose of this paper is thus to evaluate some of the existing methods in order to create a new extraction protocol where DNA yield is optimized and where contamination is minimized.

Hydroxyapatite is one of the major constituents ofbone and teeth. DNA is attached on the six surrounding sides of the hydroxyapatite crystal where the adsorption sites are scattered. The affinity of any molecule to the hydroxyapatite crystal is determined by how well the carboxyl or the phosphate groups in the specific molecule fits the adsorption sites on the surface of the crystal, i.e. phosphate groups for DNA (Kawasaki et al. 1985; Kawasaki et al. 1990). Hence, one way to elute DNA from the hydroxyapatite is by the addition of competing phosphate ions, e.g. by adding a phosphate buffer. This is important when working with bone-DNA since the skeleton consists of both inorganic and organic material, 2:1, and the inorganic part mainly consists of hydroxyapatite.

Present DNA extraction methods can be divided into three major groups where one group focuses on the binding capacity of DNA to hydroxyapatite. The bone is here decalcified by incubation in an EDTA buffer and DNA is extracted from that buffer (Hagelberg et al. 1991). Another group concentrates on purifying DNA, where DNA is extracted from the bone by incubation with a caothropic salt, often guanidium thiocyanate. The salt binds nucleic acids to silica (SiO_2), hence other substances can be washed away (Boom et al. 1990; Höss & Pääbo 1993). None of these methods are however optimal, because DNA is never completely eluted and also, some of it is lost during the washing process. To overcome these problems Persson (1992) experimented with an extraction method based on the experiences from 40 years of hydroxyapatite chromatography. He used a phosphate buffer to elute DNA from hydroxyapatite in the bone matrix (Persson 1992). His protocol however, became complicated since he was unable to purify the DNA from the attached phosphate ions, or from other substances, in an easy way.

Although the use of extraction methods based on a phosphate buffer are fairly uncommon today, such a buffer will be the central part in the method suggested in this paper.

Material and methods

DNA was extracted from six modern (i.e. less than three weeks old) human teeth representing two individuals (1–6), using three different extraction methods (A, B, C), two teeth for each method.

A. The silica method described in detail by Boom et al. (1990) and Höss & Pääbo (1993). Here the protocol given by Höss & Pääbo (1993) was followed for sample 3

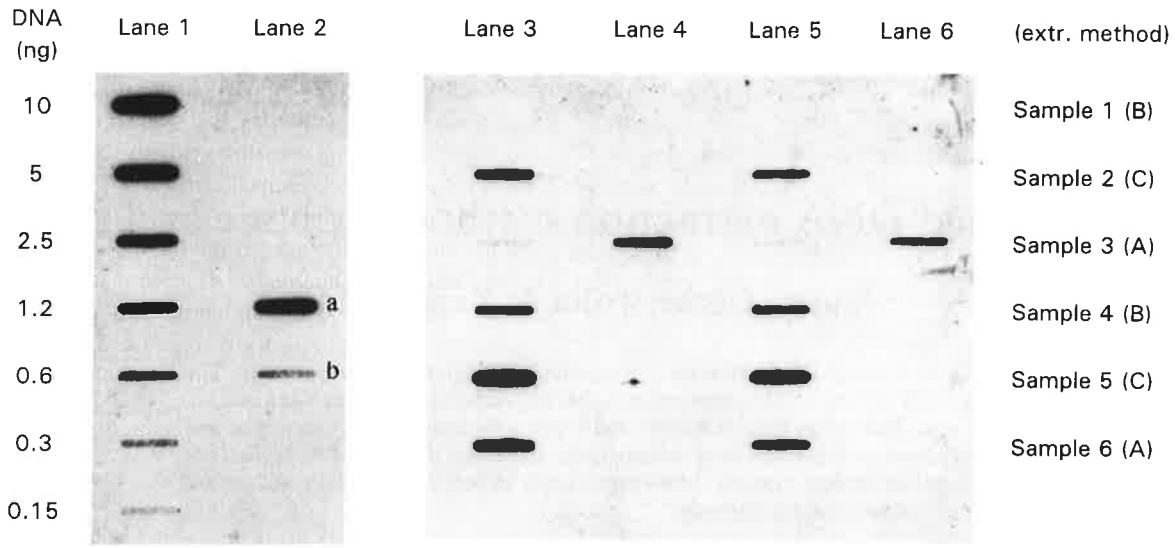

Figure 1. Quantification with QuantiBlot™, where the amount of DNA is indicated by intensity. Lane 1 is a standard with known DNA content; lane 2 is a calibration sample (a=5 ng); b=0.3 ng). Lane 3 and 5 are the six extracted samples with twice as much extract added to lane 3 compared to lane 5. Lane 4 and 6 contain the re-extractions, where twice as much extract was added to lane 4 compared to lane 6 (samples 2 and 5 are re-extracted by method A; samples 3 and 6 re-extracted by method C).

and 6, although slightly modified. In the elution step, silica was suspended in 130 µl TE, of which 115 µl was eluted and used as extract.

B. The EDTA method based on decalcification (Hagelberg et al. 1991). The tooth powder from sample 1 and 4 was incubated with 1 ml 0.5 M EDTA and agitated for one week. The supernatant was collected after centrifugation for 3 min at 10,000 rpm. DNA was extracted from the supernatant with two phenol/chloroform (1:1) purifications and one *n*-butanol purification. The extract was concentrated to 115 µl with a Centricon™ 30 concentration step and a TE dilution.

C. The phosphate method based on a phosphate buffer (Persson 1992). The tooth powder from sample 2 and 5 was incubated with 1 ml collagenase buffer at 37°C and agitated for 12 hours. The sample was centrifuged at 10,000 rpm for 3 min and the supernatant disposed. A phosphate buffer, 0.5 ml, was added to the tooth powder and then incubated at 60°C for 30 min. The sample was centrifuged at 10,000 rpm for 3 min and the supernatant collected. This step was repeated once. The extract was washed four times with TE buffer in a Centricon™ 30 to purify the extract from the phosphate buffer. Each time the 40 µl that remained after centrifugation was diluted to 2 ml with TE buffer. The final extract was diluted to 115 µl with TE buffer.

The human teeth (1–6) were split in two with a nail and the pulps removed. They were washed with 0.4 M HCl for 30 s and then treated with UV light to remove surface contamination and leftover from the pulp. DNA was later also extracted from the pulp and used in an STR system as control. Sodium-hypochlorite bleached mortars were used to grind the teeth to powder. Approximately 0.5 g tooth powder was used in each extraction. In order to exclude inter sample variation, two additional DNA extractions were carried out on one single modern tooth from a cow (7–8). The extraction methods used were A and C, with the modification that the Centricon™ 30 centrifugation was excluded.

Quantification

The result of the extracted DNA was visualized by a quantification based on a hybridization to a primate-specific probe (the α-satellite on the 17th chromosome), and was detected by chemoluminescence and an X-ray film (fig. 1). By comparing the extracted samples to a sample of known DNA content, DNA in the extracted samples was quantified using QuantiBlot™ (Perkin Elmer). DNA extracted from the cow tooth was quantified using a spectrophotometer. Further, the human tooth powder that was used in the phosphate extraction (C) was additionally treated with the silica method (A), and the tooth powder that was used for the silica extraction (A) was additionally treated with the phosphate method (C), in order to see if any DNA remained and could be extracted by an additional method after the first treatment. The same procedure was followed for the cow tooth extractions.

Results

The strongest indication of DNA in the human teeth is given by sample 5, a phosphate extraction (C)(fig. 1), where the extracted amount of DNA was 0.5 ng/µl (table 1). The other phosphate extraction, sample 2, also gives a strong indication, 0.125 ng/l. One of the silica extractions (A), sample 6, gives a good indication, 0.125 ng/µl,

Table 1. Extracted amount of DNA, time consumption for DNA extraction, number of tube openings and amount of DNA remaining in the tooth after extraction. Sample 1–6=human teeth; sample 7–8=cow tooth.

	Sample no.	Extracted DNA from the two samples (ng/µl)	Time consumption (days)	Number of tube openings	Remaining DNA (ng/µl)/re-extraction method
Silica extraction (A)	3 6	0.0157 0.125	1	8	0.125/C 0.0/C
EDTA extraction (B)	1 4	0.0 0.125	7	6	—
Phosphate extraction (C)	2 5	0.125 0.5	2	5	0.0/A 0.0/A
Silica extraction (A) Phosphate extraction (C)	7 8	0.5–0.6 0.65	1 2	8 5	0.2/C 0.0/A

while the other, sample 3, is barely detectable, 0.0157 ng/µl. The EDTA digestions (B), sample 1 and 4, provide weak indications in both cases, 0 ng/µl and 0.0125 ng/µl. It was apparent that additional DNA could be extracted from the tooth powder treated with silica (A), using the phosphate buffer (C), whereas the opposite did not work.

Quantification of DNA with spectrophotometer is sometimes troublesome, because absorption of light can be affected by products other than DNA remaining in the solution. However, the quantification provided information that the phosphate method (C) eluted the highest amount of DNA in the cow tooth, 0.65 ng/µl, as compared to the silica method (A), 0.5–0.6 ng/µl. The phosphate method (C) was also able to elute 0.2 ng/l DNA from the tooth powder already extracted using the silica method (A), while the opposite did not elute any DNA.

The risk of contamination must be considered when amplifying degraded DNA. One way of minimizing contamination is to minimize the number of tube openings. This and time consumption for DNA extraction are listed in Table 1.

Discussion

The experiments described above provide information that the phosphate method has a larger ability to extract DNA from teeth than the EDTA- and silica-based methods. This might be due to the fact that collagen, the major protein constituent in bone and teeth, is first broken down by collagenase in the phosphate method. This seems to facilitate the use of the competing phosphate ions as an eluent of DNA. Also, the lower number of tube openings in the phosphate method is advantageous taking contamination into account, as is the fast extraction.

Six different human teeth were used for the extractions and it might be possible that they contained different amounts of DNA. The DNA in the different teeth might also have been of different quality. The conclusion that the phosphate method is superior is however supported by the additional extractions on the cow tooth. The experiment must however be repeated both on a more homogeneous material, as in the cow tooth, and also on bones from different periods and environments.

The phosphate extraction could also be simplified. In additional experiments a gene clean kit replaced the Centricon™ 30 wash to purify the extract from the phosphate buffer (Geneclean®, Bio 101 Inc.).

To conclude, a phosphate buffer seems to have the highest potential to extract DNA from teeth and bone powder, although more studies are warranted before it is apparent how the final extraction method should be designed.

Buffers

Collagenase buffer. 10 mM Tris-HCl pH 7.5, 2 mM EDTA, 10 mM NaCl, 2.5 mg collagenase/ml

Phosphorus buffer. 2.5 M K_2HPO_4 neutralized to pH 7 with phosphoric acid

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