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SHORT COMMUNICATION

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The Kaiser's tooth

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Abstract The recovery of DNA from teeth is usually associated with the destruction of the evidential tooth using, for instance a special grinding mill. In some cases, however, a minimal invasive method of DNA retrieval with a high recovery is required particularly when historical material has to be investigated. A tooth attributed to the German Emperor Wilhelm II was the only source of DNA in an analysis of a possible paternity and the DNA had to be extracted without destroying the appearance of the tooth. Here, the results of the DNA analysis are presented.

Keywords Short tandem repeat systems · Mitochondrial DNA · Teeth · Ancient DNA · Kaiser Wilhelm II

Introduction

A tooth attributed to the German Emperor, Kaiser Wilhelm II (1859–1941) and stored in the museum Huis Doorn, The Netherlands, where the Kaiser lived in exile from 1918 until his death, had to be investigated for two objectives: firstly, possible paternity in relation to a woman who died in 1952 and secondly, to verify it's authenticity. Thus, without causing visible damage a sufficient amount of DNA had to be retrieved from the tooth.

In paternity cases the evidential value of short tandem repeat systems (STRs) is usually extremely high (Brinkmann et al. 2001) and teeth can be used as an excellent source of the DNA necessary (Pfeiffer et al. 1999). Among old and putrefied or degraded tissues, DNA in teeth seems to show the best preservation (Schwartz et al. 1991). The pulp is well protected by the dental hard tissues and contains different kinds of cells (Alvarez Garcia et al. 1996). The dental cement covering the dentin of the roots is produced by DNA-containing cementoblasts which are concentrated in the lower third of the apex and in the area of the bifurcatio of teeth with more than one root (Smith et al. 1993). Also, granulation tissue formed at a site of infection surrounding the roots and remaining fixed to the root after tooth extraction, harbours numerous cells. Furthermore, mitochondrial DNA (mtDNA) which is maternally inherited (Giles et al. 1980) seems to be especially preserved in teeth because the mitochondria of the odontoblastic processes within the dentinal tubules subsequently become occluded with calcium phosphate crystals and thus remain protected even for long time intervals after death (Mörnstad et al. 1999).

In the following we report a DNA recovery method and the results obtained from a tooth with historical implications.

Materials and methods

Materials

A lower molar of the German Emperor Wilhelm II (1859–1941), extracted at the beginning of the twentieth century, was handed over by the museum Huis Doorn, in The Netherlands (Fig. 1). It had to be guaranteed that no visible destruction would occur due to manipulation necessary for the recovery of DNA. The tooth had two golden inlays, extensive carious defects and mummified granulation tissue fixed between the roots. Due to extensive dental manipulation the pulp chamber was largely destroyed and could not be used for DNA recovery.

Sample preparation

Hard metal drills operating with a low rotating micro-motor drive (10,000 rpm) were used for the sample preparation. In order to minimise further loss of material, a special powder collecting device was constructed consisting of a high-grade steel cylinder with a collecting hopper at the upper end and a vacuum at the lower end (Fig. 2). A clean micro-centrifuge filter tube was fixed in the cylinder and the vacuum was connected. The powder produced by

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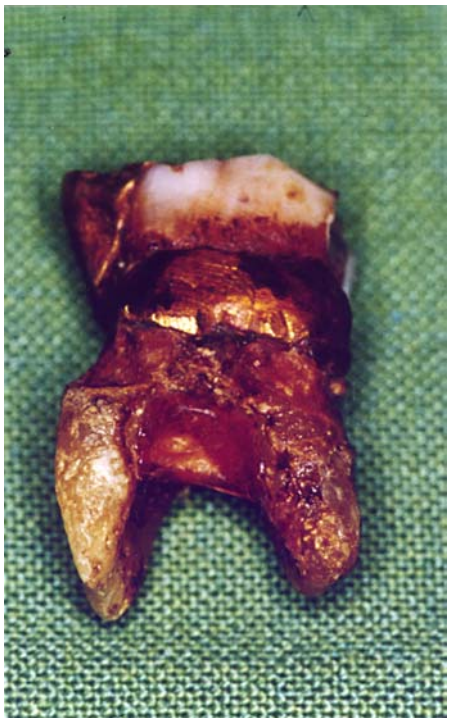

Fig. 1 The Kaiser's tooth?

drilling followed the vacuum gradient and thus was directly transferred into the clean micro-centrifuge filter tube.

To avoid contamination the tooth was rinsed in ethanol (70%) followed by sterile distilled H_2O. After air drying a thin layer from the outer surface of the roots was removed by drilling. Then, two separate samples were prepared using the special powder collecting device:

1. Mummified soft tissue between the roots
2. Cement from the outer layer of the root tips (Fig. 3).

From the putative daughter of Wilhelm II, two molars were obtained after exhumation. DNA was extracted from these teeth after destruction by drilling.

DNA extraction, quantification and PCR amplification

DNA extraction from the mummified soft tissue was performed with Chelex 100 (Walsh et al. 1991). DNA was extracted from the hard dental tissues using the phenol-chloroform method as described previously (Pfeiffer et al. 1998) and the DNA was quantified using the slot-blot method (Waye et al. 1989). The amplification of the 106 and 112 bp fragments of the X-Y homologous gene amelogenin was performed according to Mannucci et al. (1994). Furthermore, DNA was amplified for the five STR systems FGA (Rolf et al. 1998), VWA (Möller et al. 1994), TH01 (Edwards et al. 1992), D12S391 (Lareu et al. 1996) and ACTBP2 (Rolf et al. 1997).

MtDNA amplification and sequencing

The amplification and sequencing of two overlapping short fragments of HV1 was performed as previously described (Pfeiffer et al. 2001) using the primer sets F15971/R16251 and F16144/16410 (Holland et al. 1995).

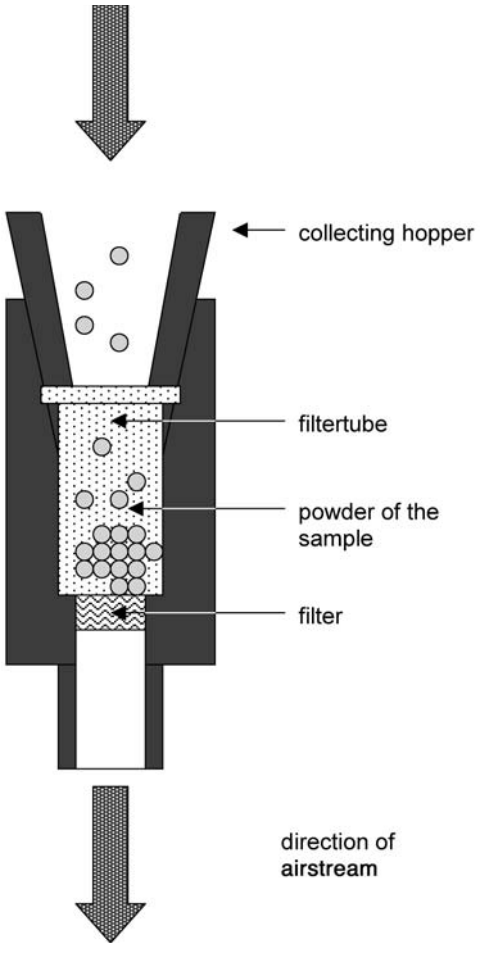

Fig. 2 Material collection device

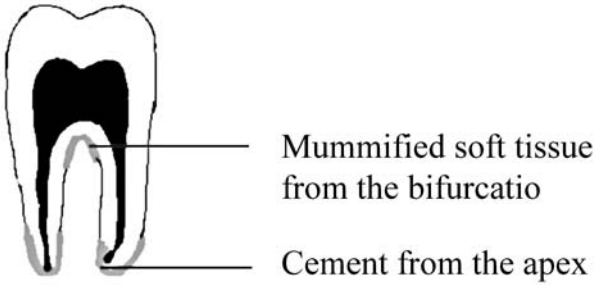

Fig. 3 Areas of sample preparation

Results and discussion

Approximately 50 µl powder could be collected in sterile micro-centrifuge tubes without causing visible damage to the historic tooth.

With ancient DNA the possibility of contamination is very high. Therefore, the two samples obtained from different tissues and different sites of the historic tooth were treated separately. Furthermore, using the powder collecting device the material followed the vacuum into a clean tube without being exposed to contamination during handling.

Table 1 DNA concentrations and results of PCR amplification (*nr* no result; *ni* not investigated)

Sample	DNA concentration ng/µl	ACTBP2	FGA	VWA	TH01	D12S391	Amelogenin
Mummified soft tissue – historic tooth	6.6	18, 26.2	21, 23	15, 16	8, 9	15, 19	XY
Cement – historic tooth	0.42	nr	21, 23	ni	ni	nr	nr
Tooth 1 – putative daughter	0.31	22.2, 29.2	23, 24	14, 19	9.3, 9.3	ni	ni
Tooth 2 – putative daughter	0.31	22.2, 29.2	23, 24	ni	ni	23, 23	ni

Table 2 Consensus mtDNA sequence obtained from the historical tooth and from maternal relatives of Wilhelm II

Position	16111	16357
Reference sequence (Anderson et al. 1981)	C	T
Mummified soft tissue from historic tooth	T	C
Tsarina Alexandra (Gill et al. 1994)	T	C

DNA could be extracted and PCR amplified from both samples of the two individuals. In four STR systems the donor of the historic tooth was excluded as being the father of the woman who died in 1952. The STR results obtained from all extractions were unambiguous, i.e. clear 1- or 2-peak patterns with sufficient intensities and no indication of contamination, reproducible in different extractions were obtained (Table 1). From the extraction of the cement of the historic tooth positive results could be obtained for FGA only. The authentity of the historic tooth was confirmed by mtDNA analysis of the extraction rest from the mummified tissue (Table 2). The mtDNA haplotype determined by sequencing of both directions of the hypervariable region I (16111T; 16357C) was unique in the database "mtradius" of Röhl et al. (2001) among 13,636 HV1 mtDNA sequences. The same sequence had been published before for Tsarina Alexandra (Gill et al. 1994) and Tsarina Alexandra and Wilhelm II had the same maternal ancestry. Also because of the rarity of the mtDNA sequence we strongly believe that this sufficiently proves the authenticity of the tooth. This result is furthermore strong indication that no contamination had occurred with this tooth.

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