TECHNICAL NOTE

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Recovery of DNA from Human Teeth by Cryogenic Grinding

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ABSTRACT: DNA has been previously recovered from human teeth for RFLP and PCR-based forensic analysis. In some cases, the maximum amount of undisturbed tooth structure is required for ulterior forensic analysis. But, in most cases, following comprehensive documentation, it is possible to section the tooth longitudinally or horizontally, or crush it to access the DNA-rich core. This technical report describes an alternative method to recover DNA from whole extracted human molar teeth. A 6700 freezer mill was used to pulverize 20 teeth under frozen preparation in liquid nitrogen and sterile conditions. The mean yield of DNA was 30.9 μg (18.4 μg DNA per gm tooth powder). The resulting fine powder was subjected to organic extraction and subsequently quantified using slot blot hybridization. Aliquots were successfully amplified at three short tandem repeat polymorphic loci. The technique is simple and relatively rapid. Isolation of the samples during pulverization minimizes the risk of contamination.

KEYWORDS: forensic science, forensic dentistry, DNA evidence, teeth, cryogenic grinding, liquid nitrogen

The teeth are the hardest substances in the human body (1). They are known to survive most postmortem circumstances, including but not limited to such events as decomposition, immersion in water, burial, and fires reaching temperatures of 1100°C (2–5). Recently, investigators have focused attention on human teeth as potential sources of forensically significant DNA evidence. Studies have been completed on the efficacy of extracting DNA from teeth exposed to a variety of environmental insults (6).

The dental pulp is a loose connective tissue which occupies the central region within the tooth. It is continuous with the connective tissue of the periodontal ligament through the apex of the root. The components of the pulp are common to all loose connective tissue, comprising cells, fibers, ground substance, blood vessels and nerves (7). The relative size of the dental pulp is approximately equivalent to the relative size of individual classes of teeth. Therefore, molars theoretically are better sources of pulpal DNA than incisors.

Several methods to access the DNA of human teeth have been proposed. The neurovascular tissue that is contained in the pulp cavity is thought to be the best source of DNA (8). Vertical sections through the long axis of the tooth result in exposure of the entire pulp system (5). Horizontal sections through the interface between crown and root allow the crown of the tooth to be retained for ulterior investigations, such as comparison of dental fillings to antemortem dental records (9). Crushing the teeth using a mortar and pestle or a hammer and metal plates has also been attempted (9).

This paper reports an alternative method to maximize the amount of DNA recovered. A freezer mill was used to cryogenically grind recently extracted whole teeth and extracted endodontically treated (root canal filled) teeth. The yield of DNA obtained was calculated as a function of tooth weight. The DNA samples were purified, amplified at several STR loci, and visualized using capillary electrophoresis.

Material and Methods

Twenty human molars (16 upper and 4 lower) were obtained from an oral and maxillofacial surgeon. Additionally, one molar, which had been endodontically treated and restored with a retentive post and full gold crown, was obtained from an endodontist. Adherent materials, such as epithelium, calculus, and blood, were removed from the outer surface of each tooth with an excavator. The teeth were individually placed in bleach (5.25% sodium hypochlorite) for 20 min, rinsed with sterile water, and then soaked in sterile water for 20 min. Each sample was then rinsed with ethanol (95%) and allowed to air dry at room temperature (RT) under a 256 nm ultraviolet (UV) light source (Philips TUV 30 watt, Microzone Corp., Nepean, Ontario) for 20 min (rotated after 10 min). The pre-treatment weight of each tooth was recorded (see Table 1).

A 6700 freezer mill (SPEX Sample Preparation, Metuchen, NJ) was used to cryogenically grind each tooth. The tooth was placed in a sterile grinding vial with a stainless steel impactor. Stainless steel end plugs are positioned at each end of the cylinder. This assembly was placed in the electromagnetic chamber which was precooled for 10 min in liquid nitrogen. During operation, the grinding vial was immersed in liquid nitrogen.

An alternating magnetic field shuttles the steel impactor against the ends of the vial, powdering the frozen tooth sample. The protocol developed by the authors uses a two-stage pulverization process. The first stage employs a stroke rate of 150 strokes per min for 5 min followed by a second stage employing a rate of 330 strokes per min for 5 min. The tooth powder produced by grinding

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was scraped from the vial and weighed (see Fig. 1). The percentage yield of powder was calculated (see Table 1).

The grinding vials and metal components were cleaned between samples by subjecting them to aquasonic cleaning at 80°C for 30 min with Extran 300 (cat. no. VW2305-4, VWR Canlab, Mississauga, Ontario). Each component was rinsed with sterile water and ethanol (95%) and then exposed to UV light for 30 min.

One mL of extraction buffer [Tris-HCl 10 mM, EDTA 10 mM, NaCl 50 mM, SDS 2% (w/v) (pH 8.0)] and 15 μL of proteinase K (10 mg/mL) were added to approximately 0.5 gm of tooth powder in a sterile 1.5 mL eppendorf tube. This was left at 56°C overnight. The sample was centrifuged at 10 000 $\times g$ for 5 min, and the supernatant subjected to organic extraction using buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1) (10) in a phase lock gel tube (5′-3′ Inc., Boulder, CO) and microconcentrated in AMICON-100 tubes (Millipore Canada, Toronto, ON) (11). Subsequently, each sample was quantitated by slot blot hybridization using a human-specific D17Z1 probe (12) and amplified using the AmpFℓSTR Blue triplex system (PE Applied Biosystems, Foster City, CA) at the following loci: D3S1358, HUMvWA and FGA.

TABLE 1—*Yield of tooth powder after grinding, DNA yield following slot-blot quantitation and DNA yield as a function of tooth weight (FDI = Fédération Dentaire Internationale).*

Sample No.	Tooth No. (FDI)	Weight, (gm)	DNA Yield, (μg)	Yield/Weight, (μg/gm)
1	18	2.29	13.5	6.1
2	28	1.30	86.1	66.7
3	48	2.09	5.6	2.8
4	28	1.03	9.9	10.1
5	28	1.90	90.9	48.9
6	38	2.10	61.9	30.3
7	28	1.68	97.5	59.5
8	18	1.40	29.1	21.6
9	18	1.72	59.1	35.0
10	18	1.83	12.8	7.1
11	18	0.98	3.9	4.2
12	38	1.24	1.9	1.6
13	48	1.57	0.5	0.3
14	18	1.99	47.9	25.2
15	28	1.85	2.0	1.1
16	18	1.89	0.9	0.5
17	28	2.08	4.1	2.0
18	18	2.34	5.5	2.5
19	28	2.18	51.1	23.4
20	18	1.87	34.0	18.4
Mean		1.77	30.9	18.4

Results

The mean weight of tooth powder recovered from each pulverized whole molar was 1.77 gm, which represents an average yield of 97%. The average yield of DNA from each molar was 30.9 μg (see Table 1). The average yield of genomic DNA from tooth powder was 18.4 μg DNA/gm. As Table 1 illustrates, the yield of DNA from human teeth is highly variable. The amount of DNA ranged from a minimum of 0.5 μg to a maximum of 97.5 μg. The reason for this variability is currently unknown. Similar results have been previously reported (9).

The DNA was successfully amplified and typed at three forensically significant genetic loci using standard PCR protocols (AmpliTaq Gold enzyme, hot start at 95°C for 11 min, t_1 = 94°C for 1 min, t_2 = 59°C for 1 min, t_3 = 72°C for 1 min, 28 cycles followed by hold at 60°C for 30 min) and capillary electrophoresis (Model 310, Applied Biosystems, Foster City, CA, 50 μm × 47 cm capillary, POP-4 polymer).

Discussion

Prior to cryogenic grinding, each tooth was debrided and decontaminated using bleach, sterile water, ethanol and UV crosslinking. In this study, each tooth was intact and fully developed. No defects in the tooth's crown or root were identified by visual examination. The authors advise against the use of bleach and ethanol on teeth that have visible fractures or do not have fully-developed roots since these substances can potentially enter the pulp system. It appears that they did not access the neurovascular system of an intact tooth under the conditions used in this study.

If a tooth is fractured or incompletely developed, or a pathway to the pulp system exists, it is recommended that DNA decontamination be completed by thorough cleaning and debriding of the

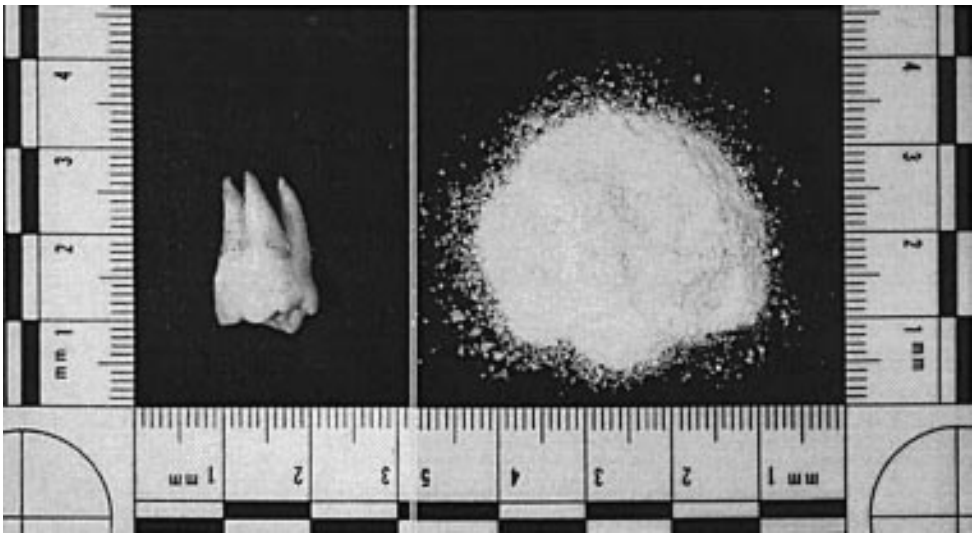

FIG. 1—*Upper second permanent molar tooth shown before and after cryogenic grinding.*

outer aspects of the tooth along with crosslinking using ultraviolet light.

An average of 30.9 μg of DNA was extracted from human molars after cryogenic grinding. Previous authors have reported similar results to those found in this study. Smith et al. in 1993 obtained an average yield of 4.0 μg of DNA by sectioning teeth and 18.1 μg of DNA by crushing teeth (9). Schwartz et al. in 1991 reported a range of 15 to 20 μg of total DNA (6) and, in 1992, Pötsch et al. reported a range of 6 to 50 μg of total DNA (13).

Although cryogenic grinding does not result in a marked increase in DNA yield compared with previous studies, the ability to control contamination of other samples and the laboratory environment through sample isolation is superior. Pulverization of forensic samples in a closed system under frozen preparation in liquid nitrogen and sterile conditions is a distinct advantage over other methods. Additionally, the individual components of the apparatus can be easily cleaned and sterilized between samples, minimizing potential cross-contamination.

Cryogenic grinding has been used as a preparation tool for vital samples, such as cartilage (14) and bone (15–17). The SPEX 6700 freezer mill was employed to extract DNA from skeletal remains of the Romanov family (18). Samples of medieval teeth and bones have been crushed using a mortar and pestle and subsequently ground using a mineralogy mill (19).

The yield of DNA from tooth powder is relatively high and of sufficient quality and quantity for PCR-based analysis. Others concluded that crushing teeth results in higher DNA yields than sectioning but also produces more shearing of the DNA (9). The high yield found in this study is most likely due to the presence of large amounts of pulp tissue in recently extracted whole molar teeth. The degradation previously reported as a result of DNA shearing is inconsequential due to the sensitivity of PCR technology.

Teeth exhibiting carious lesions and amalgam dental fillings were included as samples along with virgin, unrestored teeth. All teeth were manipulated in the same manner. The presence of dental caries (bacterial etiology) and foreign materials, such as silver amalgam, did not affect DNA recovery and amplification. The yield of DNA for these teeth was the same as for noncarious, unrestored teeth.

An endodontically treated molar was also pulverized (data not shown). The quantity of DNA recovered was approximately 100-fold less than the average quantity from the other teeth sampled in this study. However, the amount recovered is adequate for PCR-based analysis. This is significant since, in the case of endodontically treated teeth, the pulp tissue has been removed prior to placement of root canal filling materials. Therefore, sectioning the tooth to access the dental pulp is not warranted. Theoretically, genomic DNA is embedded in the hard tissues of the tooth. Cryogenic grinding may be the most effective method to extract this DNA.

Cryogenic grinding of vital samples using a freezer mill is a relatively simple and very effective method to recover forensically significant amounts of DNA from human molar teeth. The average yield of 30.9 μg DNA per molar is sufficient to provide target DNA for more than 30 000 PCR reactions. Through future studies using this cryogenic grinding protocol on additional samples, such as other human teeth, bone and various animal hard tissues, the effects of several potential postmortem factors will be evaluated. Results of other studies to assess the DNA yield from cellular material trapped in the inorganic matrix of teeth compared with pulpal tissues and the effects of environmental insults, such as soil, salt water and incineration, will be reported at a later date.

The results of this study on freshly extracted human teeth represent a best-case scenario. Teeth from old or skeletal remains have been found to yield less DNA. This study was undertaken primarily to develop and refine a protocol of decontamination and extraction which could be reasonably applied to forensic casework where the levels of endogenous DNA warrant such measures.

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