TECHNICAL NOTE

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A Systematic Approach to the Sampling of Dental DNA

REFERENCE: Smith, B. C., Fisher, D. L., Weedn, V. W., Warnock, G. R., and Holland, M. M., **"A Systematic Approach to the Sampling of Dental DNA,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 5, September 1993, pp. 1194–1209.

ABSTRACT: As investigations into the forensic aspects of DNA analysis continue, the human tooth will play a dual role in identification. Dentin and enamel provide a protective enclosure for genomic and mitochondrial DNA as well as providing the basis for radiographic, biochemical, and ultrastructural forensic studies. The purpose of this investigation is to establish technical guidelines, based on histology and experimental evidence, for the management and sampling of dental DNA. The anatomic location of dental DNA is discussed with emphasis on the conservation of tooth structure during sampling. Ten pairs of maxillary right and left third molars were sampled for DNA following storage for 18 weeks at ambient temperature and humidity. Right third molars were crushed, whereas the left third molars were sectioned conservatively prior to sampling the DNA. The quantity and quality of human DNA obtained from each tooth was compared, as well as the radiographic appearance of remaining hard tissue and the overall simplicity of each approach. DNA typing was performed, both sequence and length based analyses, comparing teeth from the same individual and teeth from different donors. The results of this study suggest that the odontologist will maximize the dental DNA yield by crushing the entire specimen but that substantial yields of human DNA can be obtained by using a conservative technique that preserves the tooth structure. In addition, the method of sampling does not affect the ability to perform DNA typing analyses.

KEYWORDS: odontology, DNA typing, ACTBP2 (SE33), HLA-DQalpha, dental identification, teeth

Technical advances in molecular biology have propelled the analysis of DNA into routine usage in crime laboratories. This has prompted the investigation of various human tissues as potential sources of genetic evidentiary material. Recently, teeth have been the subject of DNA studies, since the dental hard tissue physically encloses the pulp,

Received for publication 30 June 1992; revised manuscript received 20 Jan. 1993; accepted for publication 23 March 1993.

This paper was presented at the Annual Meeting of the Academy of Forensic Sciences, Odontology Section, February 1992.

The opinions and assertions expressed herein are solely those of the authors and are not to be construed as official or as the views of the US Army, US Navy or Department of Defense.

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offering an anatomic configuration of great durability [*1,2*]. Preliminary work suggests that dental DNA of sufficient quantity for forensic analysis may be recovered following a broad range of environmental insults [*3–5*]. Until now, a systematic approach has not been established for sampling dental DNA and teeth have been "cracked," "broken," or "crushed" in the process of removing the pulp. Retrieving DNA has been the primary issue without considering the importance of conserving the dental hard tissue for its multi-faceted value as forensic evidence. Enamel and dentin are the basis for radiographic comparison, aspartic acid racemization [*6–10*], incremental line studies [*11–15*] and scanning electron microscope examination with energy dispersive X-ray analysis (SEM/EDX) [*16,17*]. Therefore, a technique is required that conserves tooth structure for possible use in other postmortem studies without significantly compromising the DNA yield. Such a technique should consider the complexity of dental histology, the specific requirements of DNA analysis, and the preservation of sufficient hard tissue necessary for other forensic tests. The purpose of this study is to establish technical guidelines, based on dental anatomy and experimental evidence, for the management and sampling of dental DNA for forensic case work.

Relevance of Dental Micro-Anatomy

A diploid complement of genomic DNA is present in all nucleated somatic cells. In addition, most human cells contain hundreds of mitochondria in their cytoplasm and each mitochondrion has multiple mitochondrial DNA (mtDNA) molecules [*18,19*]. DNA is abundant throughout the human body and there are distinct anatomic locations within the tooth where DNA is found (Fig. 1).

On the basis of gross volume, the *coronal pulp chamber* and *radicular canals* are obvious targets for DNA sampling. The average volume of pulp tissue for all adult teeth approximates 0.02 cubic centimeters (cc) with third molar pulp volumes ranging from 0.023 cc for maxillary teeth to 0.031 cc for mandibular molars [*20*]. The pulp chamber size normally decreases with age and irritation, as secondary dentin deposition occurs on all surfaces. The root canal diameter undergoes a similar constriction that may be accentuated with apical periodontal disease [*21*]. The soft tissue within the coronal and radicular pulp chambers consists of odontoblasts, fibroblasts, endothelial cells, peripheral nerve cells, undifferentiated mesenchymal cells and the nucleated components of blood. The cellularity of pulp tissue decreases with age as the fibrous intercellular elements increase.

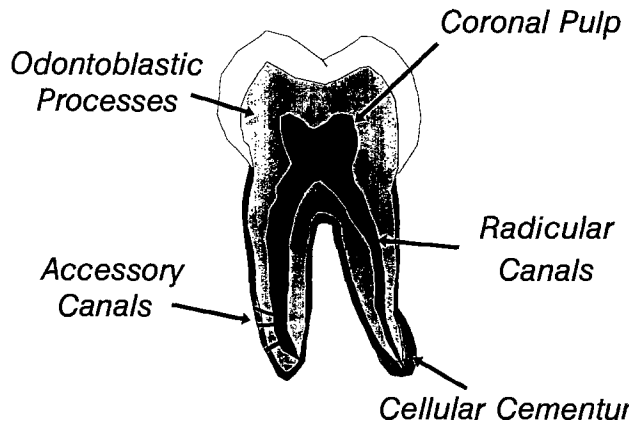

FIG. 1—*Sites within the tooth where the deoxyribonucleic acid molecule can be found. Note that enamel does not contain DNA.*

The *odontoblastic processes* that extend into the dentinal tubules contain numerous mitochondria. These organelles are seen routinely during odontogenesis, but their number progressively decreases with tooth maturity [*20,22*].

Accessory canals are more common in the apical half of the root, but may occur wherever the developing root encounters a blood vessel. In a similar manner, the apical foramen may have eccentric or multiple canals [*21*]. DNA located in these canals will not be sampled during a simple pulpectomy but require aggressive extirpation or apicoectomy.

Cellular cementum is so named because it contains nucleated cells with numerous branching canaliculi. The cementocytes are found in lacunae, similar to the osteocytes of bone, with processes extending toward the periodontal surface. Although layers of cellular and acellular cementum may alternate, cellular cementum usually appears on the outer surface and most commonly in the apical half of the root. The overall thickness of cementum may reach 100 to 150 μm near the root apex, but increasing numbers of empty lacunae at 60 μm suggest that viability of cementocytes in the deeper layers is unlikely [*20*]. Because nutrition to cementocytes is provided by the periodontium, not the pulp, it is possible to find viable cementocytes after the tooth has undergone endodontic therapy. Theoretically, sampling the root apices of a non-vital tooth could result in the harvest of DNA if cellular cementum is present.

Adherent bone, periodontal fibers, and blood may be present in addition to the normal tooth structure. Although these potential sources of DNA should be considered in a case-workup, they are subject to more degradation and non-human DNA contamination by virtue of the external location. The contribution of external sources to the total quantity of dental DNA was not targeted in this study.

Approaches to Sampling Dental Source DNA

Based on the micro-anatomy of the human tooth and the location of cells that harbor potentially useful DNA, four techniques were considered for study. The advantages and disadvantages of each follows.

Crush Entire Tooth

Grossly, this technique would appear to be the most thorough in accessing dental DNA. The disadvantage however, is that the tooth and any restorative features are lost to further radiographic, biochemical or morphologic studies. In addition, this approach ignores the specific locations of dental DNA and may increase the likelihood of contamination by bacterial DNA endonucleases and potential polymerase chain reaction (PCR) inhibitors found on the exposed surfaces of the tooth. Studies have shown that skeletal remains that have not been cleaned and sanded prior to DNA extraction will result in subsequent inhibition of the PCR [*23*].

Conventional Endodontic Access

The removal of the pulp chamber contents by a conventional endodontic procedure is a technique with well established clinical parameters. The technical difficulty of this approach depends on the pulp chamber morphology and size of the access opening. The greatest disadvantages are the disruption of the occlusal surface and restorations, as well as assuring that all portions of the chamber are completely debrided.

Vertical Split

A section along the vertical axis of the tooth allows convenient access to the entire length of the pulp chamber. Unfortunately, the eccentric nature of tooth roots in the

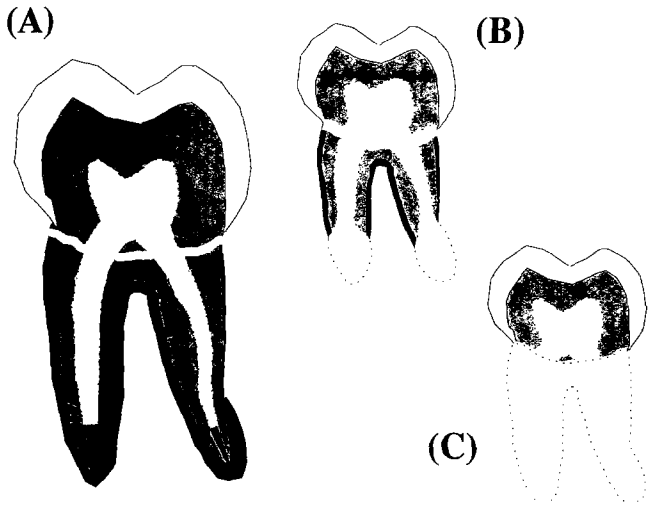

FIG. 2—(A) *Horizontal section of tooth with partial extirpation of coronal and radicular pulp.* (B) *Horizontal section of tooth with aggressive extirpation and apicoectomy.* (C) *Horizontal section of tooth with aggressive pulpectomy and crushing of radicular half of tooth.*

vertical plane make it nearly impossible to achieve longitudinal access to the entire pulp chamber in most multirooted teeth. Additionally, a vertical split is likely to violate coronal restorations as previously discussed.

Horizontal Section

A horizontal section through the cervical root, subjacent to the cementoenamel junction (CEJ), avoids most restorations and provides the operator access to both the radicular and coronal pulp chambers. This unhindered approach to the root canals is an improvement over the conventional endodontic access and can be achieved with minimal changes in the tooth morphology. A similar procedure has been described by Duffy et al. in their work on dental pulp sex chromatin studies [*24*]. This approach permits rotary instrumentation of the inner dentin and independent sampling of the cementum and accessory canals without altering the coronal enamel (Fig. 2*A*).

Maximum DNA yield and conservation of coronal morphology were regarded as the two most important criteria in developing a standard procedure for sampling dental DNA. Based on these criteria, the *crushing* technique was compared with that of the *horizontal section*.

Methods and Materials

Ten pairs of teeth (A to J) were selected for the study. Each pair of teeth consisted of a right and left maxillary third molar extracted from the same patient at the same appointment. Patients' ages ranged from 17 to 23 years with the exceptions of samples A and B, which were obtained from 40 and 72 year old patients, respectively. This difference is noted because of the tendency for pulp volume to be reduced with age [*25*].

On gross examination, the root apices of all samples were closed and the teeth were without caries or restorations. Soft tissue remnants were removed and each tooth was rinsed in sterile water to dislodge loose debris and blood. Each pair of teeth was air dried and placed in a sterile plastic bottle with a loose fitting cap. All containers were labeled with the patient's age and gender. The samples were left undisturbed for 18 weeks at ambient room temperature and humidity.

All maxillary right third molars were crushed by placing them inside a sterile plastic bag between two steel plates. The tooth was struck repeatedly with a mallet until resulting fragments were 0.4 cm or less in diameter. The plates reduced perforation of the bag and subsequent loss of sample. The fragmented tooth was shaken from the bag into a 15 mL polypropylene tube. New sterile bags and operator's latex gloves used for each sample. The plates were washed in 95% ethanol, rinsed in distilled sterile water and wiped dry each time. The teeth were weighed to the nearest milligram before and after crushing.

All maxillary left third molars were scored horizontally on the lingual surface immediately below the CEJ with a small triangular file. The teeth were placed in a sterile plastic bag, buccal surface down, on a hard rubber surface and stabilized with rubber tipped clamps. A surgical chisel was placed in the lingual notch and struck sharply with a steel mallet. Visible pulp tissue was removed with a 38/39 spoon excavator. All canals were debrided with small broaches, followed by instrumentation with endodontic files up to size #30. Dust and tissue removed from each tooth were placed in a 15 mL polypropylene tube. New sterile bags and operator's latex gloves were used for each sample.

The samples were submitted to a conventional organic DNA extraction method including an overnight incubation at 56°C in extraction buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 2% sodium dodecyl sulfate, and 10 mM EDTA), and three chloroform/phenol/isoamyl alcohol protein extractions. Each sample was spun at 2500 rpm in a Beckman GPR Centrifuge using a Centricon 30 microconcentrator to further purify the DNA and to reduce the volume. The final volume of each sample approximated 0.4 mL. The DNA was ethanol precipitated (0.3 M sodium acetate and two volumes of 100% ethanol), washed in 70% ethanol, dried in a vacuum microcentrifuge, and resuspended in 400 uL of TE (10 mM Tris, pH 7.6, 1 mM EDTA).

One microliter samples from each specimen were electrophoresed on a Bio-Rad Wide Mini Sub™ Cell in a 1% agarose gel with 1 μg/mL ethidium bromide. The bacterial phage Lambda 123 base pair ladder and quantification standards were run on the gel for comparison purposes. The results were evaluated under ultraviolet (UV) light for relative quantity of DNA and degree of degradation.

Samples (10 μL) were submitted from each specimen in a 10:1 dilution with dH_2O for UV absorption at wavelengths of 260 nm, 270 nm, and 280 nm. Purity of samples were determined by evaluating the A_{260}/A_{270} and A_{260}/A_{280} ratios. Results approximating 1.2 and 1.8, respectively, were considered significant for the effective removal of phenol and protein [*26*]. The total DNA recovered from each specimen (the values used in Table 1) was estimated using the Warburg-Christian equation

$$[(-36 \times A_{280}) + (62.9 \times A_{260}) = \text{Nucleic Acid } (\mu g/mL)] \quad [27].$$

Since bacterial DNA may account for some of the total DNA recovered, the percentage of human DNA was determined for each specimen. A Gibco BRL slot blot apparatus was used with a human-specific oligonucleotide probe for an alpha satellite on the short arm of chromosome 17 (p17) (Lumi-Phos 530 from Gibco BRL). The probe was tagged with a chemiluminescent label activated by the addition of alkaline phosphatase [*28*]. Samples of 40 nanograms (ng) of total DNA (determined by UV spectrophotometer analysis) were submitted from each specimen and blotted onto Pall Biodyne A 0.45 micron membrane (Pall Trincor Corporation) along with 0.2 to 40.0 ng of known K562 human DNA standards. Following activation with alkaline phosphatase, the membranes were exposed to Kodak XAR film for approximately 3 hours.

PCR (polymerase chain reaction) based DNA typing methods were used to analyze the DNA extracts. The HLA DQ A1 Amplitype kit developed by Cetus, is a PCR based

TABLE 1—*Total DNA recovered from each specimen compared to that portion determined to be human DNA by oligonucleotide probe specific for human p17.*

Tooth	Wt (gm)	Total DNA (μg)	Human DNA (μg)	% Human
A (c)	2.00	70.0	2.66	3.8
A (s)	*2.08*	*11.2*	*0.67*	*6.0*
B (c)	1.75	12.6	2.14	17.0
B (s)	*2.37*	*48.2*	*0.82*	*1.7*
C (c)	1.65	117.6	31.75	27.0
C (s)	*1.80*	*31.4*	*5.97*	*19.0*
D (c)	2.09	160.6	38.54	24.0
D (s)	*2.18*	*33.0*	*6.27*	*19.0*
E (c)	1.29	104.8	20.96	20.0
E (s)	*1.25*	*31.0*	*2.79*	*9.0*
F (c)	1.70	173.6	39.06	22.5
F (s)	*1.58*	*28.4*	*6.04*	*21.3*
G (c)	1.87	98.4	2.46	2.5
G (s)	*1.75*	*39.6*	*2.48*	*6.3*
H (c)	2.09	138.8	2.78	2.0
H (s)	*1.98*	*41.0*	*4.10*	*10.0*
I (c)	1.24	128.8	32.20	25.0
I (s)	*1.17*	*26.0*	*1.95*	*7.5*
J (c)	0.99	100.6	8.80	8.8
J (s)	*1.06*	*88.8*	*8.90*	*10.0*
Mean (c)		110.6	18.1	15.3
Mean (s)		*37.9*	*4.0*	*11.0*

NOTE: (c — crushed teeth, s — sectioned teeth).

analysis currently being used in crime laboratories [*29,30*]. The second exon of the DQ-alpha region, located within the HLA locus, contains six common and two uncommon sequence polymorphisms. The six common alleles result in twenty-one possible genotypes with a power of discrimination of approximately 0.93. DNA extracts from two individuals (E and I) were analyzed, comparing the conservative and destructive methods of sampling, as well as comparisons of DQ-alpha genotypes in order to discriminate between individuals. HLA DQ-alpha genotypes were determined using the procedure provided in the Cetus kit.

Current research is directed towards development and validation of AmpFLPs (amplified fragment length polymorphisms), which are expected to be the second generation method of forensic DNA analysis. The AmpFLP technique is similar to restriction fragment length polymorphism (RFLP) analysis with three important exceptions. First, AmpFLP alleles are generated using the PCR, allowing for the analysis of subnanogram levels of DNA. Second, transfer of DNA to a membrane following electrophoresis is not necessary, and detection of the target DNA bands can be achieved using non-isotopic methods; silver staining [*31*] and fluorescence (Applied Biosystems, GeneScanner 362 Fluorescent Fragment Analyzer). Finally, AmpFLP alleles (100–1500 base pairs) are significantly smaller than RFLP fragments (some greater than 10 000 base pairs), making AmpFLP analysis less sensitive to DNA degradation.

Microsatellites, recently designated short tandem repeats (STRs), are AmpFLP loci that contain small repeat units (2 to 5 base pairs). A tetranucleotide repeat (AAAG) at the ACTBP2 locus (common name SE33), in the 5′-region of a human beta-actin related pseudogene, is highly informative with a heterozygosity value of 95%, and at least 21 observed alleles [*32–35*]. The 21 alleles result in at least 231 different genotypes, although all possible genotypes have not been observed. DNA from two individuals (B and E) was typed using SE33 analysis. Comparisons of the conservative and destructive methods

of pulp extraction were made, as well as testing the ability to type DNA extracted from molar root tips. The PCR conditions were as follows; 50 μL reaction containing 1X polymerase buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/mL gelatine) (Boehringer Mannheim Biochemicals), 0.2 mM dATP, dCTP, dGTP, dTTP, 0.2 μM primers (5′-AAT CTG GGC GAC AAG AGT GA and 5′-ACA TCT CCC CTA CCG CTA TA) [*33*], 2.5 units of Taq polymerase (Cetus) and 20 ng of DNA. The thermal cycling parameters were; 94°C for 10 s, 66°C for 10 s, 72°C for 5 s for 10 cycles, and 94°C for 10 s, 64°C for 10 s, 72°C for 5 s for 20 cycles.

Analysis of PCR product for SE33 was performed using horizontal, discontinuous polyacrylamide gel electrophoresis [*31*]. The gel contained 7% acrylamide and 3.3% piperazine diacrylamide (w/w), 60 mM formate (leading ion), and 37.5 Mm Tris-HCl, pH 9.0. Agarose plugs (2%, w/v) provided the trailing ion, 280 mM borate, with 1.04 M Tris, pH 9.0, and 0.00005% bromophenol blue. Sample applicator tabs (5 × 5 mm fiberglass tabs from Pharmacia LKB Biotechnology, Piscataway, NJ) were placed on the gel, 1 cm from the cathodal agarose plug. PCR product (5 μL) was loaded directly onto the tabs, and the electrophoresis was carried out on an EC-Apparatus Standard Electrophoresis Unit at 600 volts, 15 watts, and 20 mAmps of constant current (at 15°C). Electrophoresis was terminated when the bromophenol blue reached the anodal agarose plug (17 cm, and approximately 2.5 h). The gel was subsequently silver stained as previously described [*31*].

SE33 analysis was performed on the same two individuals (B and E) using the fluorescence based chemistry provided by the ABI 362 Fragment Analyzer (Gene Scanner). Approximately 20 ngs of DNA extracts B and E (B#1, B#16, E#1, E#16, and E_{Root}) were amplified in a 25 μL reaction containing 1X Taq DNA polymerase buffer (same as above, Boehringer Mannheim Biochemicals), 200 μM of each dNTP, 1.25 units of Taq DNA polymerase (Cetus), and 5 pmols of each SE33 primer (described above). The TTTC-strand primer was labeled at the 5′-end with the fluorodye FAM (Applied Biosystems, Inc.). Samples were amplified on the Perkin Elmer 9600 Thermal Cycler as previously described.

Following amplification, 1 μL of each PCR product was coelectrophoresed with 4 fmols of ROX labeled internal molecular weight standard GS-2500 (lambda phage DNA cut with *Pst* I) on a 4 mm 6% denaturing polyacrylamide gel. Samples were run into the gel for 1 h at 900 volts. The gel was then placed on the Gene Scanner and electrophoresis was carried out at 900 volts, with an 18 cm well to read distance, for an additional 4 h. The fluorescently labeled fragments were detected by a laser, and the size (base pairs, bp) of the samples were determined automatically based on the internal standards using Local Southern Analysis. To confirm the size calls made by the Gene Scanner software, TAMARA labeled SE33 allelic ladder was run in a separate lane with the GS-2500 molecular weight standards. The alleles comprising the ladder were sized using the internal lane standards as described above.

When nuclear DNA cannot be analyzed due to severe degradation and/or insufficient nuclear DNA available for typing, DNA sequence analysis of mitochondrial DNA (mtDNA) can be useful for identification purposes. The mtDNA genome is 16 569 base pairs (bp) in length and circular, consisting of conserved coding regions and two hypervariable segments within a defined control region [*36*]. The mtDNA genome evolves 5 to 10 times faster than nuclear DNA, is passed through the maternal lineage, and there are 100 to 10 000 copies of the mtDNA genome in each human cell [*37,38*]. DNA sequence analysis of the control region has been used for identification purposes of DNA extracted from both skeletal remains [*39*] and dental remains [*40*]. The control region of all molar pulp DNA extracts was amplified, and two of the samples (B and E) were subjected to DNA sequence analysis.

The PCR conditions for amplification of the mtDNA control region were the same as stated above for SE33 with two exceptions. The primers used for amplification were F15997 (5'-CAC CAT TAG CAC CCA AAG CT) and R580 (5'-TTG AGG AGG TAA GCT ACA TA) [*41*], and the thermal cycler parameters were 95°C for 20 s, 52°C for 10 s, and 72°C for 10 s for 30 cycles with 20 ng of total DNA. The PCR products were analyzed on an agarose gel and compared to known size standards.

DNA sequence analysis of the mtDNA control region was carried out using the ABI Taq DyeDeoxy Terminator Cycle Sequencing Kit. One microliter of purified PCR product (Amicon, Inc. Centricon 30 or 100 column dialysis purification) was subjected to cycle sequencing according to the protocol provided in the kit. The sequencing reactions were purified using G-50 Quick Spin Columns (Boehringer Mannheim Biochemicals) according to the protocol provided in the ABI cycle sequencing kit. Sequencing reaction products were run on an 8% denaturing polyacrylamide gel on the ABI 373A DNA Sequencer according to the protocol provided by ABI.

Results

Crushing of the maxillary right third molars was complicated by an occasional perforation in the plastic bag and accounts for the small amount of sample loss. Based on weight before and after crushing, sample loss ranged from 1% to 14% per tooth with an average of 0.08 g or 5% for all samples (data not shown).

For seven specimens, splitting of the maxillary left third molars resulted in coronal and radicular sections as predicted. However, the three remaining teeth broke into multiple fragments; sample *C* (four pieces), *H* (seven pieces), and *K* (three pieces). All sectioned teeth could be reassembled with negligible changes in their external and radiographic morphology. The most frequently noted postprocedural alterations were hairline fractures, pulpal enlargement on radiographs, and loss or fracture of root apices (Fig. 3).

A 1% agarose yield gel showed high molecular weight (HMW) DNA present in all 20 samples. With the exception of specimen B, the crushed teeth appeared to provide the larger quantity of total DNA but with greater degradation (Fig. 4).

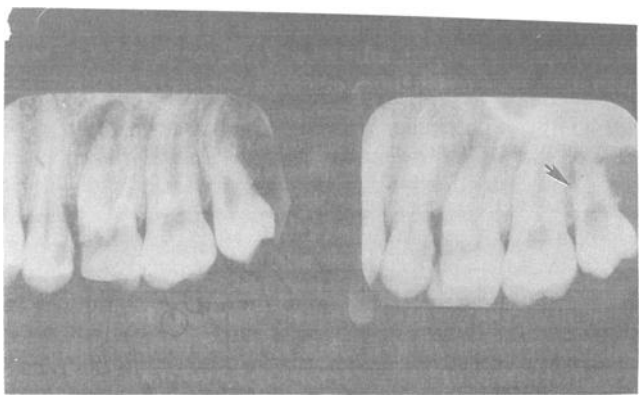

FIG. 3—*Radiographic comparison of maxillary left third molar before (left) and after procedure (right). The horizontal section technique with aggressive extirpation was used, as illustrated in Fig. 2B. The arrow indicates post-procedural loss of root apices and enlargement of pulp chamber. The tooth's radiographic image is otherwise unchanged.*

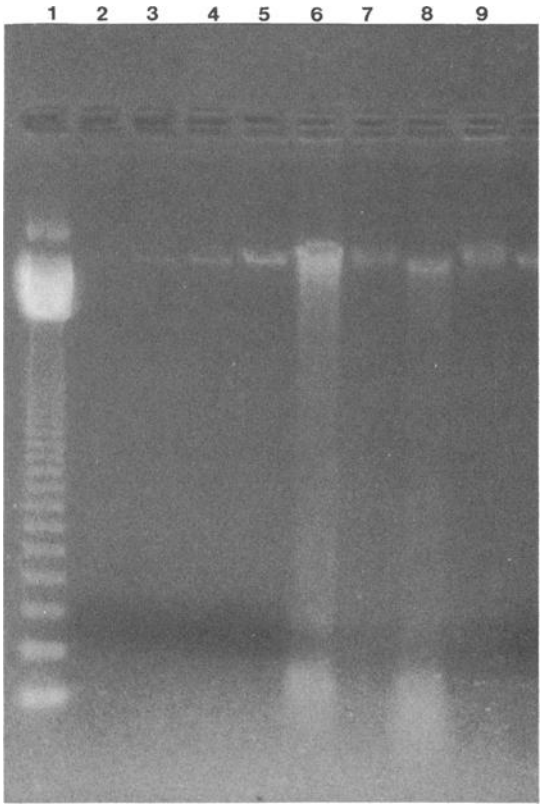

FIG. 4—*Agarose gel (1%) comparing DNA obtained from crushed teeth with that from sectioned teeth. Samples D and I shown. Lane 1 is the bacterial phage Lambda 123 base pair ladder. Lanes 2 through 5 are HMW quantification standards ranging from 7.5 ng to 60 ng. Lanes 6 and 8 are crushed teeth D and I, respectively. Lanes 7 and 9 are sectioned teeth from teeth D and I.*

The ultraviolet spectrophotometer results showed A_{260}/A_{280} ratios ranging from 1.88 to 2.17 with an average of 2.03, indicating the successful removal of protein from the sample. The A_{260}/A_{270} ratio ranged from 1.23 to 1.30 with an average of 1.27. This result indicated the absence of contaminating phenol. Table 1 shows the total DNA content of each specimen followed by the amount of human DNA. The third column shows the percentage of total DNA for each specimen that was positive for the human specific oligonucleotide probe.

Some samples (C, D, E, F, I) showed that significantly less human DNA was obtained from the sectioned teeth. This observation prompted further investigation of the remaining hard tissue. The horizontally sectioned teeth were re-instrumented with a medium peeso reamer, followed by a #4 round bur on a slow-speed rotary handpiece (Fig. 2B). The dentin powder generated by this procedure was saved as a separate specimen. Sterile water used to flush the pulp chamber was saved in a separate tube as a second specimen. Finally, the entire radicular half of the sectioned teeth were crushed and submitted as a third sample (Fig. 2C). The DNA in two of these samples, specimens D and I, was evaluated as previously described. The results are shown in Table 2.

The irrigation fluid did not contain detectable levels of nucleic acid, whereas the roots and powdered dentin both provided notable quantities of HMW DNA. The DNA ob-

TABLE 2—*Human DNA recovered from sectioned teeth by irrigation with sterile water, rotary instrumentation of dentin, and complete crushing of the root.*

Tooth	DNA in micrograms		
	Irrigant	Dentin	Roots
D	0	0.1	0.92
I	0	0.54	2.31

tained from the roots was greater in both total amount and degree of degradation (Fig. 5).

DNA typing results from representative DNA extracts confirmed the expectation that DNA profiles were identical for teeth originating from the same individual. In addition, the profiles for each individual analyzed were different from one another. HLA DQ-alpha typing (HLA DQ A1 Amplitype kit from Cetus; see Methods and Materials for details) of DNA extracted from individuals E and I resulted in two different genotypes. Individual E was a 3,4 and individual I was a 1.3,4. In addition, DNA extracted from

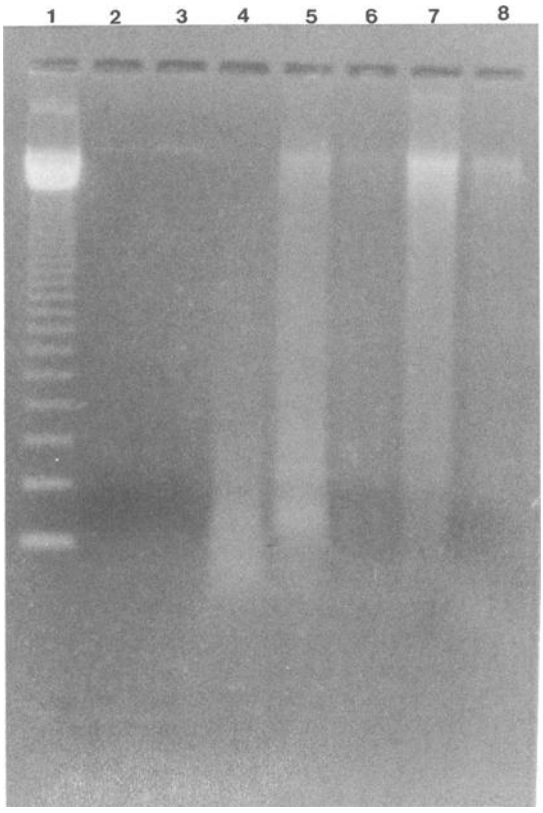

FIG. 5—*Agarose gel (1%) comparing DNA obtained from irrigation, rotary instrumentation, and crushing the roots of previously sectioned teeth. Lane 1 is the bacterial phase Lambda 123 base pair ladder. Lanes 2 and 3 are HMW quantification standards of 7.5 and 15 ng. Lanes 5 and 7 are crushed roots from samples D and I, respectively. Lanes 6 and 8 are dentin removed from the outer pulp chambers of teeth D and I.*

both molar #1 and #16 (conservative and destructive sampling, respectively), as well as DNA extracted from the root tip of molar #1, gave the identical DQ-alpha genotype.

AmpFlp analysis of the SE33 locus (see Methods and Materials for details) was performed for DNA extracted from individuals B and E. PCR amplified fragments were run on a discontinuous polyacylamide gel directly next to an allelic ladder. The alleles in the ladder have been arbitrarily designated even numbers from 2 to 20 [*34,35*]. Fragments which fall on an allele in the ladder are given even numbered allele designations, and alleles which fall between bands in the ladder are designated odd numbered alleles. Based on comparison of the amplified fragments to the allele specific ladder, the genotype of individual B was 9,17, and individual E was 5,13 (Fig. 6).

The ABI Gene Scanner was used as an alternative method of characterizing the SE33 alleles amplified from extracts B and E. Co-electrophoersis of the alleles with GS-2500 internal molecular weight markers resulted in sizing of the alleles in bases. The allele sizes for individual B were 266 and 305 bases, and for individual E were 254 and 284 bases (Fig. 7A). In addition, the sizes observed for the same individual, but from molar #1, #16, or root DNA, differed by no more than a single nucleotide. The alleles from individual E ranged from 253.92 and 284.65 bases, to 253.37 and 284.28 bases, and finally to 253.56 and 284.28 bases for molar #1, #16, and root DNA, respectively. In all cases, the allele sizes fell within the 253 and 284 base range. The greatest difference for any allele size was 0.55 bases. These results illustrate the precision of the Gene Scanner analysis.

The amplified SE33 alleles were also compared to an allele-specific ladder (nine of the ten original alleles from the manual analysis; alleles 2-18) using the Gene Scanner. The genotype of each individual remained the same (Figs. 6 and 7B). In addition, the actual size of the alleles in the ladder was determined. The allele sizes (241, 246, 258, 262, 270, 281, 289, 297, 309 bases), were consistent with the published values using conventional sequencing polyacrylamide gel methods [*33*].

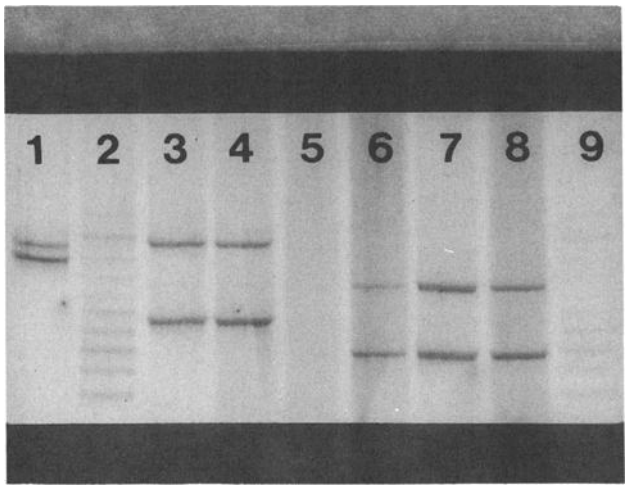

FIG. 6—*Discontinuous polyacrylamide gel electrophoresis of SE33 alleles from individuals B and E. Lanes 2 and 9 are the allele specific ladder containing ten alleles. The bottom allele in the ladder is designated allele 2 and proceeds by even numbers to the top DNA band, allele 20. All alleles which fall between bands in the ladder are odd numbered alleles. Lane 1 is the K562 human control cell line (genotype 16,17). Lanes 3, 4, and 5 are molar #1, molar #16, and root tip of molar #1, respectively, from individual B. Lanes 6, 7, and 8 are the same samples from individual E.*

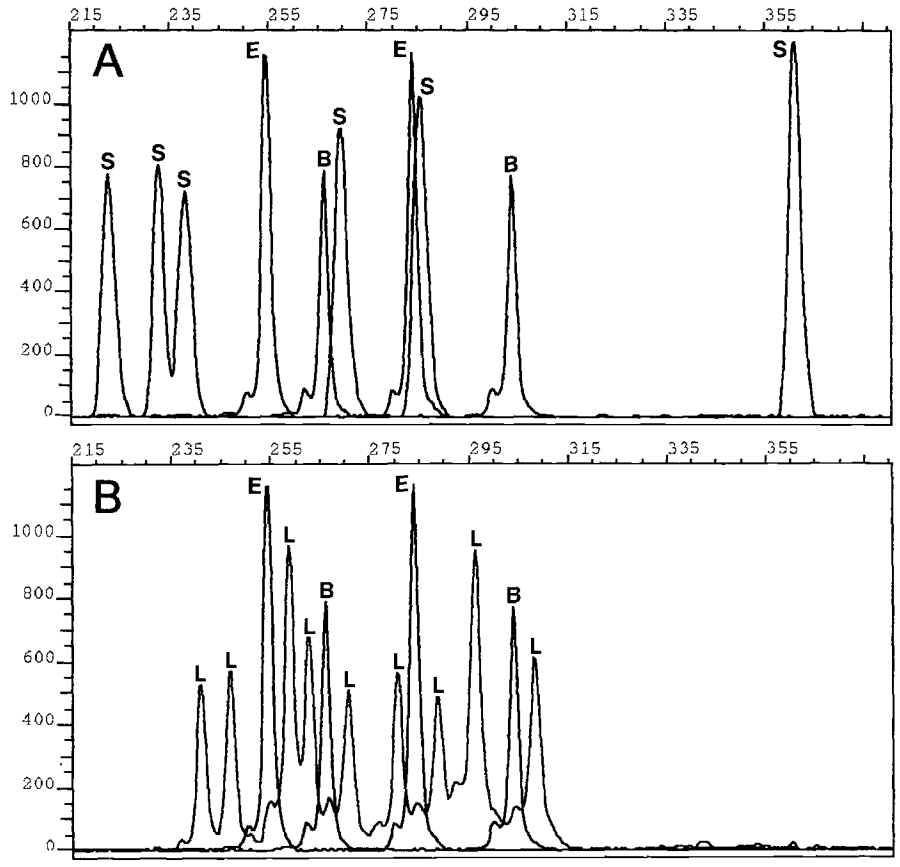

FIG. 7—*Gene Scanner analysis of SE33 alleles from individuals B and E. The Y axis is fluorescence peak intensity and the X axis is DNA base pairs. Panel A: Peaks labeled "S" represent GS-2500 molecular weight standard. Peaks from individuals B and E are labeled "B" and "E" respectively. Note that the peak intensity of each allele is uniform for teeth of the same donor. Panel B: Peaks designated "L" represent the allele specific ladder and individuals B and E are labeled as in Panel A. Note that the relative peak positions are similar to the results obtained using discontinuous polyacrylamide gel electrophoresis techniques (Fig. 6).*

The mtDNA control region was amplified for all molar pulp extracts. The result was a DNA band of approximately 1100 bp which co-migrated with the known control band (data not shown). The PCR product from individuals B and E was subjected to DNA sequence analysis. A total of 330 base pairs of sequence was generated for each individual. The DNA sequence of molars #1 and #16 was identical, and when the sequence from individuals B and E were compared, the sequences were different. The use of DNA sequence analysis to identify the origin of dental source DNA will be addressed in a future publication.

Discussion

Forensic dental identification is at a technologic crossroads. The incidence of dental restorations, the mainstay of radiographic dental identification, have declined, whereas molecular biology laboratory procedures are rapidly increasing in efficiency and availa-

bility. The extent to which teeth may suffer environmental changes and still provide useful DNA typing data has not been clarified. While these new methodologies are being refined, conventional techniques should not be abandoned. The odontologist's role will be to lead the forensic team in the correct management of the dental evidence for each case, regardless of the method of analysis. In the present study, we have proposed a systematic approach to the sampling of dental DNA. With mico-anatomy as a guide, a conservative method of obtaining DNA has been presented that preserves coronal enamel, dentin, and restorations. In this manner, DNA analysis of dental evidence need not be a destructive alternative.

Crushing the teeth was technically simple, requiring only a few minutes for each tooth. The approximate 5% loss of total tooth weight was not worrisome in light of the microgram quantities of DNA obtained. Although degradation was more obvious among the crushed tooth samples, a bright band was obvious in the HMW region of the gel for most crushed samples. By comparison, the horizontal sectioning and extirpation of left third molar teeth required 15 to 30 min for each tooth. The debridment of the pulp chamber was a challenging procedure, even for a dentist familiar with the anatomy and instrumentation, and the dust created by the process was difficult to capture efficiently. The results showed less total human DNA from the sectioned teeth than the crushed teeth in seven of the ten pairs tested. However, less degradation was noted on the yield gel for the DNA obtained through conservative means. Three of the sectioned teeth (G, H, J) actually provided similar or greater quantities of human DNA than their crushed counterpart.

The finding of additional DNA in the dentin powder and in previously instrumented roots highlights the relevance of dental anatomy. Not all dental DNA is likely to be obtained through simple removal of the pulp alone. Dentin from the pulp chamber walls, roots and cementum should be considered as potential additional sources for DNA.

DNA typing methodologies were performed on selected samples obtained through both the conservative and destructive approaches. Although RFLP (restriction fragment length polymorphism) typing of the specimens was not conducted, the quality and quantity of all samples was consistent with the ability to perform RFLP analysis. PCR-based (polymerase chain reaction) methods, HLA DQ-alpha [*42–45*] and SE33 [*32–34*], were successfully conducted. Both the DQ-alpha and SE33 data alone discriminated between the individuals tested.

SE33 analysis was carried out using two separate methods of resolving and detecting the PCR amplified alleles. Identical typing results were observed using either discontinuous polyacrylamide gel electrophoresis and silver staining, or denaturing gel electrophoresis and fluorescence detection on the ABI Gene Scanner. The individual alleles were typed on the Gene Scanner using either an allele-specific ladder or an internal molecular weight standard. Typing the individual samples using an allele-specific ladder resulted in identical genotypes. In addition, the allele sizes were precisely determined in nucleotide bases using an internal molecular weight standard. In the future, internal molecular weight standards may replace the need for allele-specific ladders.

When DNA is highly degraded, or in limited quantities, mitochondrial DNA (mtDNA) can be used for identification of human remains and evidentiary material [*39,40*]. Forensic analysis of mtDNA is expected to become an increasingly common approach in typing degraded specimens. Mitochondrial DNA constitutes a fraction of 1% of the total DNA in a cell. More importantly, however, there are hundreds to thousands of copies of mtDNA for every copy of nuclear sequence, improving the chances of DNA recovery for analysis. In addition, the maternal pattern of inheritance increases the availability of matching sequences in that siblings and even distant maternal cousins, carry identical mtDNA sequence. As the library of mitochondrial DNA sequences grows and the population statistics are determined, mtDNA will be expected to play a major role in dental DNA analysis. The DNA sequence analysis of selected samples from our study demonstrate the suitability of DNA obtained by either technique for this typing method.

The practical implication of our results is that the odontologist has a range of options in his approach to sampling dental DNA. Some cases may necessitate conservation of dental hard tissue at the request of a family member, a medical examiner or museum curator. In situations with minimal dental material, preservation of tooth structure may simply be prudent. These cases can be managed with tooth sectioning and pulpal extirpation. The halves can be reapposed and cemented with minimal changes in the radiographic, biochemical, or gross appearance of the enamel and dentin. Based on our findings, the total DNA yield from this procedure is sufficient for most DNA typing procedures.

If more aggressive sampling is necessary but the conservation of dental structure is vital, the radicular one-half of the tooth may be crushed and processed coincidentally with the pulp. The present study demonstrates that additional quantities of DNA may be obtained from the crushed roots and from dentin removed by a rotary instrument. In this manner the chances for DNA yield are increased and coronal enamel, dentin and restorations are still preserved. If pulp tissue is not visible on sectioning or, if conservation of the dental hard tissue is not a concern, the odontologist will maximize DNA yield by crushing the entire tooth. A greater likelihood of DNA degradation and non-human contamination should be anticipated when using this approach.

Teeth are unique forensic specimens. They harbor a complexity of biologic data that appropriately analyzed can contribute significantly to an investigation. The odontologist should be consulted on the management of all dental evidence including the sampling of teeth for DNA. A systematic approach based on dental anatomy, tailored for the limitations of the specific case and needs of the intended laboratory procedure, will produce the best results with the least alteration to the evidence.

Acknowledgments

The authors wish to express their appreciation to Paul Sledzik of the Army Medical Museum for the opportunity to study the American Civil War collection and to Demris Lee and Rhonda Roby of the Armed Forces DNA Identification Laboratory for their technical support. We are further indebted to Debbie Cooper of the Walter Army Medical Center Dental Activity for her assistance in specimen selection.

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