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Different Dental Tissues as Source of DNA for Human Identification in Forensic Cases

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Aim. To evaluate different dental tissues (pulp, dentin, and cement) as sources of DNA for forensic analysis.

Methods. A total of 20 teeth were obtained from unidentified bodies buried at the Central Cemetary in Bogotá in 1995 and exhumed in 2000. The tissues from three teeth obtained after surgery were used as controls. The pulp cavity was exposed after cutting each tooth with a high-speed handpiece. The pulp was removed and processed separately from the dentin and cement. The dentin and cement were obtained by drilling with a high-speed handpiece and placed into separate tubes. DNA was extracted from mineralized tissues after a short decalcification step with EDTA. The DNA was quantified by dot-blot hybridization with D17Z1 probe, a primate- and human-specific alpha satellite DNA for the D17Z1 locus. Polymerase chain reaction was carried out for the hypervariable control region between nucleotides 29 and 408 bp (HV2 region) of the mitochondrial DNA, followed by gel electrophoresis to evaluate the amount and efficiency of the amplification.

Results. The pulp yielded the strongest amplification signals. The signals for dentin and cement were very similar because of the presence of cells of the periodontal ligament in the cement. The results of the amplification of the HV2 region of the mitochondrial DNA showed that dentin and cement acted as protective factors for the cells and allowed the conservation of the DNA.

Conclusion. Cementoblasts and odontoblasts located within the cement and dentin are surrounded by the mineral matrix of the dental structure and thus protected from any environmental degradation forces, which makes them suitable sources for the DNA analysis.

Key words: *dental pulp; dentin; DNA, mitochondrial; DNA probes; forensic dentistry; nucleic acid hybridization; polymerase chain reaction; tooth*

The identification of human remains can be carried out by means of anthropological methods (1), dental structure analysis (2), and DNA-based analytical methods (3). DNA analysis remains the only method of identification in cases with very small, fragmented, or mixed forensic material.

The teeth differ in form and size but have similar histological structure. Dentin, the connective mineral tissue, forms the major structural axis of the tooth. Dentin on the crown of the tooth is covered by enamel, a strong tissue of ectodermic origin, whereas the radicular dentin is covered by cement, another type of calcified connective tissue. Dental pulp, formed of lax connective tissues, fills the pulp chamber – the cavity at the core of a tooth surrounded by dentin. The pulp and dentin tissues form a structural, embryological, and functional unit (4). The dentin contains a network of tubules – between 20,000 and 45,000 per mm^2 – which contain odontoblastic processes rich in mitochondria (5).

Analysis of DNA from human remains can be carried out from genetic material obtained from bones or teeth. The teeth are the hardest tissue in the human body because of the dental enamel, which makes them resistant to adverse conditions degrading the DNA, such as humidity, high temperature, and the action of fungus and bacteria. The pulp and dentin can be used as a source of DNA (5), whereas dental cement as a source of DNA has not been tested yet. Although the soft pulp tissue is protected by hard tissues (e.g., dentin), it is affected by putrefaction until its total decomposition. When teeth are extracted and stored at environmental temperature, a rapid dehydration of the pulp tissue occurs (6). Also, when teeth are loose inside the dental alveoli, humidity and bacterial action destroy the pulp (7). In other instances, when no humidity is present, dry atmosphere makes ideal conditions for the mummification of soft tissues (7). The cellular cement is characterized by the presence of numerous lacunae occupied by cementocytes, which are less exposed to external damages, especially to chemical or bacterial degradation (8).

The aim of our study was to determine if and in what degree different dental tissues – pulp, dentin, or

cement – could serve as a source of DNA for human identification in forensic casework.

Material and Methods

Twenty teeth – 10 canines, 1 incisive, 4 premolars, and 5 molars – were obtained from bodies of unidentified people buried in 1995 at the Central Cemetery of Bogotá and exhumed in July 2000. The estimated age of the unidentified persons ranged between 25 and 55 years (median, 35 years). The teeth were washed with distilled water and the external surface was cleaned with curettes to remove other material. Each dental structure was washed 5 minutes in commercial bleach, 5 minutes in 100% ethanol, once again in distilled water, and then dried at room temperature. A high-speed handpiece with a 700 SS White® bur (SS White Burs Inc., Lakewood, NJ, USA) cooled by sterile water was used to make a cut along the longitudinal axis of the tooth and access the different dental tissues (pulp, dentin, and cement). The pulp, present in 5 samples, was recovered with a sterile curette and placed into a 1.5-mL tube (QSP, Porex Bio Products Inc., Petaluma, CA, USA). The dentin from the inside surface of the tooth was drilled with the burr and isolated directly into a 1.5-mL tube. The cement was obtained from the apical root by using a Zecrya® bur (SS White Burs Inc.) cooled with sterile water. The excess water was removed after centrifugation.

Dental tissues of three molars, each obtained from 20-year-old patients after oral surgery, were used as controls and processed under the identical conditions as the teeth of exhumed bodies.

DNA Extraction

A total of 45 samples were analyzed (5 pulps, 20 dentin, and 20 cement samples). The dentin and cement samples were subjected to two decalcification washes with 0.5 mol/L ethylenediaminetetraacetic acid (EDTA), pH 8.0, for 5 minutes each. The tissues (pulp, dentin, and cement) were then lysed in a buffer containing 10 mmol/L Tris-HCl, 50 mmol/L NaCl, 2% (w/v) sodium dodecylsulfate (SDS) (pH 8.0), and 15 μL of proteinase K (10 mg/mL) at 56 °C, and rotated for 16 hours. Each sample was extracted three times with phenol/chloroform/isoamyl alcohol (24:25:1). The upper aqueous layer was transferred to a 1.5-mL tube and extracted once with chloroform/isoamyl alcohol (24:1). The upper aqueous layer was washed with distilled water three times in a Centricon-100 concentrator (Amicon, Millipore, Toronto, Canada) and concentrated with 1X Tris-EDTA, pH 8.0, to a final volume of 100 μL. The samples were stored at -20 °C until further analysis.

DNA Quantitation

DNA samples were quantified in a 0.6% agarose gel by using standards of known concentration. In addition, samples were quantified by dot-blot hybridization with the D17Z1 alpha-satellite probe by using Quantiblot Kit (Applied Biosystems, Roche®, Branchburg, NJ, USA), according to manufacturer's recommendations.

HV2 Mitochondrial DNA Amplification

The hypervariable region 2 (HV2) of mitochondrial DNA (mtDNA) from five dental pulps, nine dentin, and eight cement samples was amplified in a PTC100VG thermal cycler (MJ Research Inc., Watertown, MA, USA) with L29 and H408 primers (9). The amplified product was visualized by gel electrophoresis in a 3% agarose gel (2% Nusieve®, 1% SeaKem®; Biocompare Inc., Burlingame, CA, USA), stained with ethidium bromide and scanned in an FMBIO II fluorescent scanner (Hitachi Corporation, Alameda, CA, USA). ΦX 174/Hae III marker (Promega Corporation, Madison, WI, USA) was used as a reference.

Results

Out of 20 teeth, 45 samples were obtained: 5 of pulp, 20 of dentin, and 20 of cement (Table 1). The mean ± SD weight of the teeth was 1.56 ± 0.45 g. Pulp tissue was found in only five of the 20 dental structures analyzed. To verify the quality and quantity of DNA, the samples as well as controls were analyzed by gel electrophoresis. However, DNA was found only in the three controls (fresh pulp, dentine, and cement; data not shown). No signals were detected for any of the forensic samples analyzed. Even when dot blot hybridization was used, the investigated samples gave no signals, ie, they were below the standard detection limit. Once again, signals were detected for the control samples.

Table 1. Samples obtained from 20 teeth of the bodies of unidentified persons buried in 1995 at the Central Cemetary in Bogota and exhumed in 2000

Protocol	FDI* No. of tooth	weight (g)	Sample tissue		
			pulp	dentin	cement
6580	13	1.3	+	+	+
3201	35	1.3	+	+	+
513	36	2.4	–	+	+
5795	38	2.2	–	+	+
5537	13	1.3	+	+	+
6580	11	1.1	–	+	+
1453	25	1.1	–	+	+
124	23	1.6	–	+	+
6503	23	1.3	–	+	+
ScSJo 32	23	1.3	+	+	+
4327	13	2.3	–	+	+
4986	13	1.2	–	+	+
4568	23	1.9	–	+	+
3201	34	1.1	+	+	+
4439	46	2.0	–	+	+
513	27	2.1	–	+	+
4915	23	1.4	–	+	+
3948	16	2.0	–	+	+
3917	13	1.2	–	+	+
ScSJ 45	25	1.1	–	+	+
Mean ± SD		1.56 ± 0.45			

*FDI – International Dental Federation.

Out of 45 samples, 22 samples of the pulp, dentin, and cement were randomly selected and amplified for the HV2 mtDNA region. Positive amplification signals were obtained in all samples but one dentin sample. Negative controls gave no amplification signal (Fig. 1).

Discussion

We focused on the teeth obtained from exhumed bodies, which had been buried for 5 years, to assess whether and in what degree the teeth material – pulp, dentin, and cement – can be used as a DNA source. Previous studies evaluated the use of dental structures as sources of DNA for identification purposes (8,10-12). However, most teeth used in those studies were obtained after surgical removal and some were subjected to different environmental factors to imitate the actual decomposition in a real forensic case.

Different methods are used to gain access to dental tissues for DNA isolation, such as conventional endodontic access, horizontal section (a cut through the neck of the tooth), and vertical section (a cut along the longitudinal axis of the tooth) (11). We opted for the vertical section, using a high-speed handpiece cooled with distilled water, because it allowed us to recover the pulp tissue and effectively separate the dentin and cement tissues.

For mineralized tissues (dentin and cement), we used a brief decalcification step with EDTA to gain access to cellular structures within the mineralized tis-

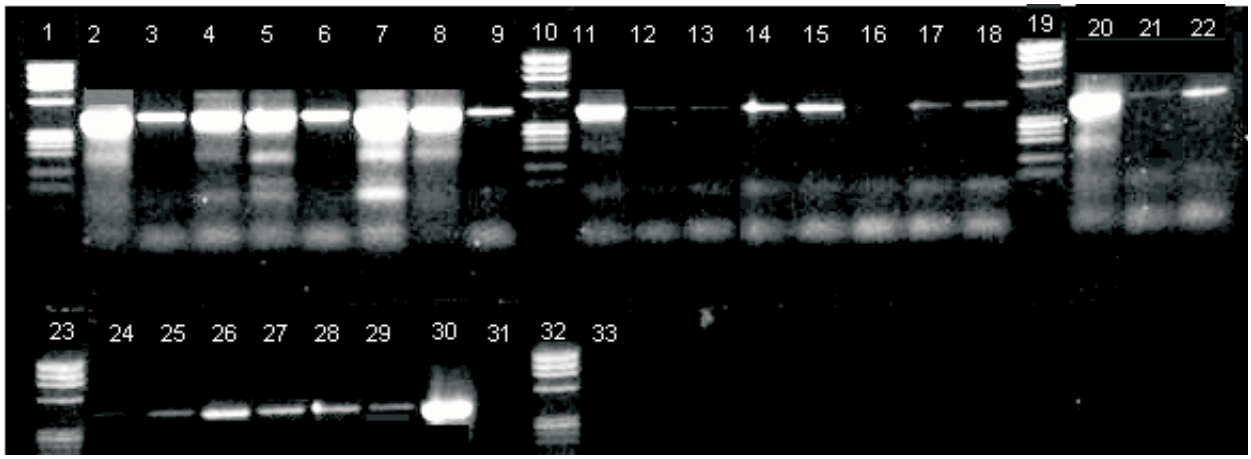

Figure 1. Polymerase chain reaction amplification of the hipervariable region II of the mtDNA. Lanes 1, 10, 19, 23, and 32 – ΦX 174/Hae III marker; lane 2 – positive control of the pulp tissue (material isolated from sugical samples); lanes 3-7 – pulp tissue (exhumed samples 3, 4, 5, 6, and 7); lane 8 – positive control for dentin (material isolated from surgical samples); lanes 9 and 11-18 – dentin tissue (exhumed samples 10, 11, 12, 13, 14, 16, 17, 18, and 19); lane 20 – positive control for the cement (surgical samples); lanes 21, 22, and 24-29 – cement tissue (exhumed samples 33, 43, 47, 36, 37, 38, 39, and 40); lane 30 – positive control of the genomic DNA; lane 31 – isolation control (water and reactives used for the extraction of DNA); and lane 33 – negative control (no DNA).

sues. In a previous study (13), a four-day decalcification step was used and followed by a significant loss of DNA for analysis. In another study (14), an 8-minute decalcification step was used without any significant loss of DNA. Our results showed that DNA could be obtained from forensic teeth with such a brief decalcification step.

In general, DNA quantitation with the Quantiblot kit gave no signals for most forensic samples we tested. These results suggested that the amount of DNA in the teeth was below the limits of detection for this system, indicating the need to develop methods that could accurately determine the amount of DNA in teeth used in forensic casework. In this regard, real-time PCR has been recently used to quantitate samples with low amounts of DNA, both nuclear and mtDNA, from human bones which had been found negative with slot-blot hybridization techniques (15). This technique provides a linear relationship between the amount of DNA and the number of cycles required to obtain signals (16). In addition, liquid hybridization increases the specificity of the system when compared with hybridization techniques that require fixation of the DNA to a membrane (15).

Due to the absence of DNA signal with the Quantiblot kit, we tried to determine if mtDNA was present in 22 of the 45 tissue samples. The results of the amplification of the region HVII of the mtDNA were as expected: the pulp samples gave the strongest amplification signal. As for the dentin and cement, some samples gave stronger signals from the dentin, whereas others gave stronger signal from the cement. The dentin signals were expected due to the odontoblastic processes present in this tissue. The signals obtained for the cement samples could be the result of the presence of cells from the periodontal ligament anchored inside the root cement. The results obtained in this study gave evidence that the tooth and tissues composing it can be used as a source of DNA, in spite of having been subjected to putrefaction, and that dental tissues, especially dentin and cement, contain enough DNA to allow the amplification of the mtDNA, which can be used in the human identification.

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