JOURNAL of FORENSIC ODONTO-STOMATOLOGY
VOLUME 30 Number 1 July 2012
SECTION TOOLS AND TECHNIQUES

The Tooth for Molecular Analysis and Identification : a Forensic Approach

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The authors declare that they have no conflict of interest.

ABSTRACT

The aim of this study is to optimize laboratory preparation of teeth for DNA identification. By sectioning the tooth topographically into two different radicular portions, it was analyzed whether these portions of mineralized tissue differ in the quantity and quality of DNA they contain.

25 teeth were subject to different experimental conditions and total DNA was quantified for each individual tooth's radicular portion: apical and remaining root, according to a 2003 study by Gaytemenn and Sweet.

We verified, with statistically significant figures, that the apical portion of the tooth is that which contains the greatest quantity of DNA. Different analytical procedures were studied for various polymorphic markers to evaluate the quality of the DNA.

We concluded that the tooth is topographically distinct in both DNA quantity and quality. The tooth's apical portion is the preferential choice in sample preparation of dental mineralized tissue for molecular analysis and identification.

KEYWORDS**: *tooth, apical root, identification, genetic profile***

INTRODUCTION

The dentition is an organ, made up of different types of tissues which are structurally disposed for a specific function [1,2]. Enamel, the protective tissue is acellular, avascular and non-enervated and therefore unimportant for genetic analysis. Cement is responsible for tooth anchoring and suffers constant remodeling. Cement is composed of cement cells located in lacunae and can characteristically invaginate the interior of apical canaliculus and canaliculi[3]. Dentin, [2,3] characterized by its histological organization can be designated secondary or tertiary (which comprises sclerotic dentin).

The choice of a tooth as a sample for genetic analysis occurs only in situations of extreme degradation when other biological tissues are not considered suitable for sampling [4,5,6,7,8,9,10]. In practice, dental pulp, due to being highly vascularized connective tissue, was the first to be studied. However, for that same reason it is also the first part of the tooth to be degraded, therefore in this study mineralized dental tissues are being studied.

Through molecular analysis of the tooth, specifically mineralized tissues, we aimed to establish individual identification and genetic profile typing using DNA analysis.

Our specific objective was to study the apical radicular portion and the remaining radicular portion to analyze whether these portions differed in DNA quantity and quality.

MATERIALS AND METHODS

25 teeth belonging to individuals between the age of 18 and 87 were studied after informed consent was obtained. Equal proportions of intact teeth and those with caries or restorations were present. The teeth were exposed to different experimental conditions, some were buried in different mediums: pH=6 (n=7); pH=5 (n=3); pH=7 (n=5); buried in sand(n=7) for a period of 7 months while others subject to various atmospheric conditions (Iberian Peninsula) for two years (n=3). The material used for sampling was in different stages of genetic alteration after cell death [6,11,12].

Each tooth was chemically cleaned, with hypochlorite, and mechanically, with a rotary device, it was then pulpectomized (fig.1), and only the root portion kept. The root portion was then divided further into two portions, the apex and the remaining portion, in conformity to the topographic drawings of Gaytmenn and Sweet's 2003 study [13] (fig.2).

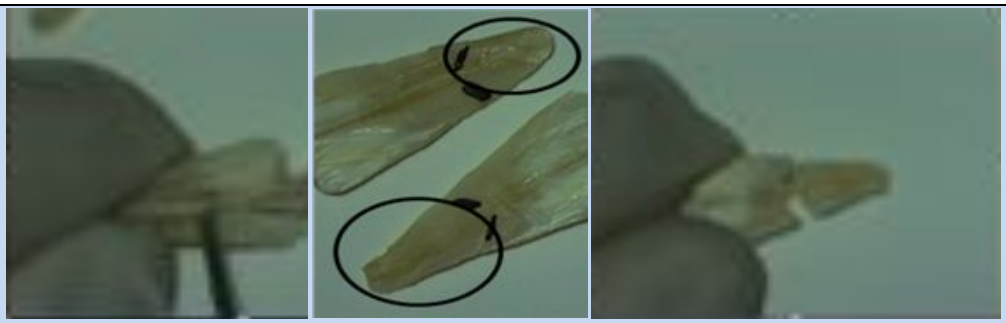

Figure 1: Sequence of the used tooth, cuts by Gaytmenn and Sweet

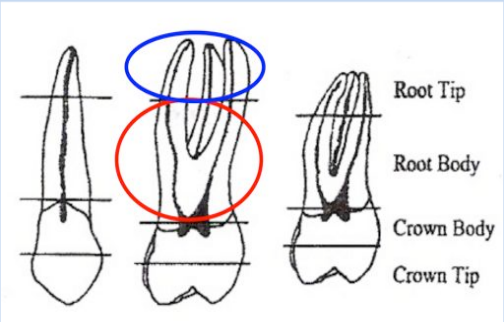

Figure 2: The Gaytmenn and Sweet guidelines

DNA was extracted using the ArchivePure commercial™ kit (5Prime®). Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit (Applied Biosystems®).

Total DNA quantification value was associated with the study viability of the polymorphisms which are most relevant for identification, namely autosomal STRs [14,15], with the aid of AmpFℓSTR® Identifiler™ and AmpFℓSTR® Minifiler™ commercial kits, both by Applied Biosystems.

Mitochondrial DNA analysis was performed on samples over which validation of the 7 markers or any autosomal marker was not viable [16,17,18, 19], and in which it was not possible to obtain an identifying autosomal profile.

The results of the quantitative study of DNA, performed on the apical and remaining tooth radicular portion, were analyzed from 25 paired samples. Quantification results were then statistically analyzed with SPSS (Statistical Package Social Science) software. Such analysis began by studying normality of the variable quantification through the Kolmogorov-Smirnov test. The non-parametric inferential median test was applied for the absence of normality within the variable quantification.

RESULTS

The results for the parameter total DNA quantification (ng/µL) obtained for each of the portions analyzed, the conservation medium and characterization of the analyzed polymorphisms are shown in Figure 3 and Table 1.

DISCUSSION AND CONCLUSIONS

Various types of teeth were sampled for this study: intact, carious, endodontically treated and restored. Teeth without caries were not included as referred to in the studies by Schwartz and colleagues (1991) [20] Chen, Sun e Wu (1994) [21] ou Alvarez-Garcia (1995) [7], López (1996) [22] and Utsuno e Minaguchi (2004) [23]. Over the years, the pulp has been considered the ideal source of DNA. Pulp degradation in extreme forensic situations, endodontic clinical procedures and difficulty anatomically in removing pulp tissue, makes, pulp tissue unsuitable for sampling. In this study the teeth were cleaned and pulpectomized and the mineralized tissues of two root portions were analysed.

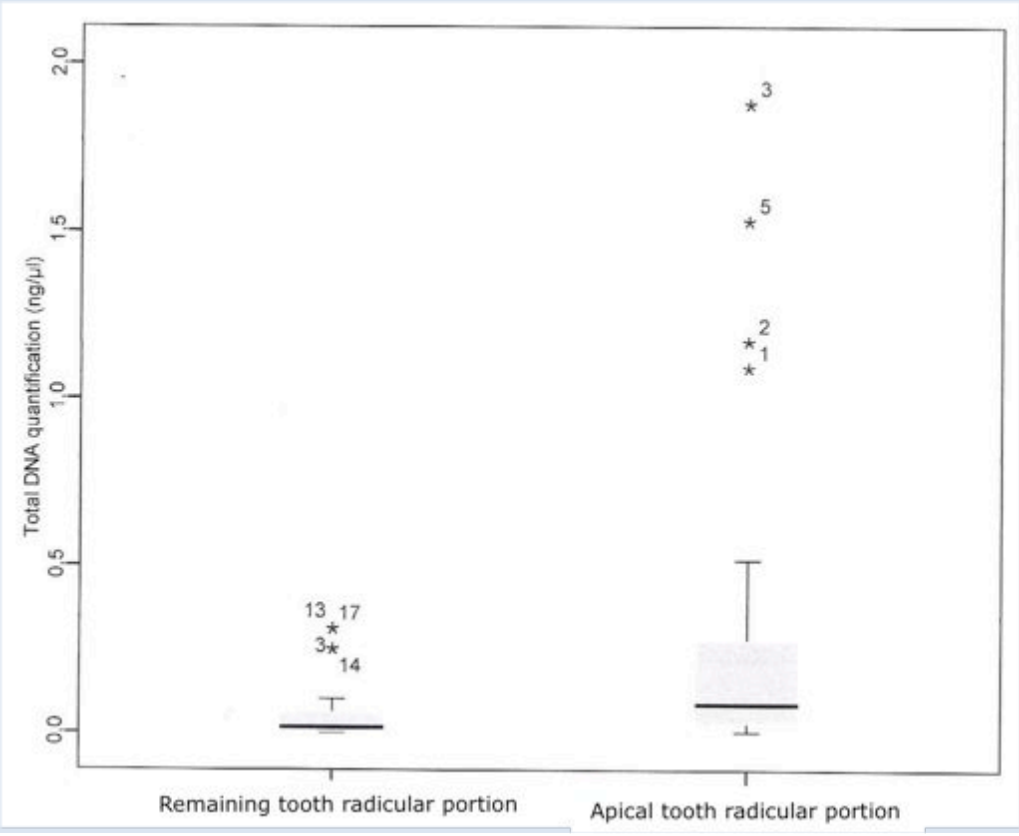

Figure 3: Quantification results of the apical radicular portion and the remaining radicular portion, statistically analyzed with SPSS.

There are several techniques for tooth preparation before DNA extraction [7, 20, 21, 22, 23], the most common is the crushing or grinding, which implies sample destruction. In this paper the tooth was preserved for morphological study, crown and restorations description. The main purpose of this research is to reduce the damage of archaeological specimens, maintain the crown morphology and validate access through the pulp, when this can yield potential samples [25,26].
The apical portion of the tooth was compared to the remaining radicular portion, and a topographic molecular analysis was made considering the two different portions of the root: the apical portion and remaining root.

The values obtained in the quantification of total DNA were higher for the apical portion than for the remaining root (table 1)the same applies to the number of genetic profiles that were obtained in each radicular portion. The type of the tooth did not affect the results obtained. Due to the tooth's anatomy, the pulp chamber communicates with the surrounding environment through the tooth's apical foramen located near the apex, and through which mineralized tissues of the erupted tooth maintain their activity. Elements resulting from cell death are disseminated through the apex: potential elements of DNA degradation and contamination.The pulp that fills the dentinal canalicules is degraded after death in a centripetal manner. Recent studies have focused on mineralized tissuesas an alternative source of DNA. One such study, published in 2003 by Gaytemenn and colleagues, shows that the

Table1: Characterization of samples according to conservation medium, total DNA identification and analyzed polymorphisms in 25 paired samples

SAMPLES	*MEDIUMS*	*RADICULAR TOOTH*	TOTAL DNA QUANTIFICATION (ng/ L)	*STR Profile*	*MITOCHONDRIAL*
1	2YEARS	REMAINING	0,006	*	ANALYZED
		APICAL	1,09	15 POLYMORPHISMS	**
2		REMAINING	0,1	*	ANALYZED
		APICAL	1,17	15 POLYMORPHISMS	**
3		REMAINING	0,311	15 POLYMORPHISMS	**
		APICAL	1,88	15 POLYMORPHISMS	**
4	pH6	REMAINING	0,019	*	ANALYZED
		APICAL	0,022	*	ANALYZED
5		REMAINING	0,003	*	ANALYZED
		APICAL	1,53	15 POLYMORPHISMS	**
6		REMAINING	0,0000	*	ANALYZED
		APICAL	0,03534	*	ANALYZED
7		REMAINING	0,024	*	ANALYZED
		APICAL	0,2396	14 POLYMORPHISMS	**
8		REMAINING	0,0605	*	ANALYZED
		APICAL	0,5172	15 POLYMORPHISMS	**
9		REMAINING	0,0061	*	ANALYZED
		APICAL	0,1027	*	ANALYZED
10		REMAINING	0,017	*	ANALISADO
		APICAL	0,113	*	ANALYZED
11	pH5	REMAINING	0,0158	*	ANALYZED
		APICAL	0,157	*	ANALYZED
12		REMAINING	0,033	*	ANALYZED
		APICAL	0,0846	*	ANALYZED
13		REMAINING	0,031	*	ANALYZED
		APICAL	0,033	*	ANALYZED
14	pH7	REMAINING	0,0250	*	ANALYZED
		APICAL	0,2955	12 POLYMORPHISMS	**
15		REMAINING	0,002	*	ANALYZED
		APICAL	0,0502	*	ANALYZED
16		REMAINING	0,0045	*	ANALYZED
		APICAL	0,047	*	ANALYZED
17		REMAINING	0,2491	15 POLYMORPHISMS	**
		APICAL	0,2756	15 POLYMORPHISMS	**
18		REMAINING	0,0012	*	ANALYZED
		APICAL	0,004	*	ANALYZED
19	SAND	REMAINING	0,0096	*	ANALYZED
		APICAL	0,0158	*	ANALYZED
20		REMAINING	0,0077	*	ANALYZED
		APICAL	0,031	*	ANALYZED
21		REMAINING	0,0605	*	ANALYZED
		APICAL	0,1084	*	ANALYZED
22		REMAINING	0,007	*	ANALYZED
		APICAL	0,004	*	ANALYZED
23		REMAINING	0,001	*	ANALYZED
		APICAL	0,039	*	ANALYZED
24		REMAINING	Indeterminated	*	ANALYZED
		APICAL	0,0069	*	ANALYZED
25		REMAINING	Indeterminated	*	ANALYZED
		APICAL	0,0020	*	ANALYZED

* Validated less than 7 markers, ** Not studied.

mid portion of the root is the ideal mineralized tissue for genetic analysis. Our results contradict their findings (Table 1 and Fig 1).

Our results can be explained based on the fact that apical canicular obliteration through the formation of tertiary dentin can preserve some of the pulpar contents in the topographic region, resembling a "mosquito in amber"; on the other hand cementogenesis in the apical portion can occur by invagination into the canaliculi in a rapid and disorganized fashion enabling the trapping of cementocites in its lacunae (fig. 4). In our study it was the apical portion that through its cellular content presented Ageing processes increase the quantity of collected DNA, which can be explained by the increased numbers of cell repairs and regenerative processes with increased sequestration of cells in the mineralized matrix (fig. 4).

Forensic laboratory practice shows that success in DNA typing depends on the conditions under which bodies are maintained and their capacity to degrade [6,11,12,24]. In order to promote extreme degradation of the pulp we have mimicked extreme forensic conditions as in prior studies by Pfeiffer and colleagues (1999), Burger and colleagues (1999) and

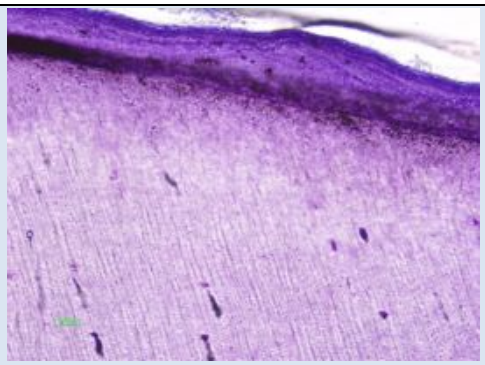

Figure 4: Microscopic photograph of cementogenesis in the apical root portion.

in studies by Alonso and colleagues (2001). To evaluate DNA quality, different polymorphisms were studied: 15 STRs and Amelogenin, the full profile for identification according to the European Standard .

We have verified that sand and pH=5 were the mediums that produced the poorest results in DNA analysis: less than seven nuclear markers were validated, only permitting the characterization of maternal lineage through the mitochondrial polymorphisms chosen for this study.

We verified that DNA quantity and quality are correlated [27]. For samples under 0,179ng/µL (tab. 1) mitochondrial DNA was used to obtain an ID. For the other samples we were capable of detecting more than seven autosomal markers, which are the preferred markers for DNA identification [16,17]. In samples 1,2,5,7,8 and 14, (Table 1) autosomal polymorphic validation for an ID (15 STRs) was obtained in the apical radicular portion while this was not possible in the remaining radicular portion. To increase the chances of compiling a complete profile from the samples (table1), we amplified with the more sensitive next generation kits, such as AmpFℓSTR® NGM™ PCR amplification kit (Applied Biosystems (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, D1S1656, D12S391, D10S1248, D2S441 and the gender determination *locus* amelogenin) and the PowerPlex® ESI 17 System (Promega) (D22S1045, D2S1338, D19S433, D3S1358, Amelogenin, D2S441, D10S1248, D1S1656, D18S51,D16S539, D12S391, D21S11, vWA, TH01, SE33, FGA AND D8S1179)[28,29].

We believe that these preliminary results warrant a further study with a larger sample size. .

This study emphasizes the value of the genetic analysis of mineralized tooth tissues as an alternative to pulp, especially in extreme forensic conditions.

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