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Research article

Genetic identification in endodontic treated tooth root

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Abstract

The tooth is an important sample in adverse forensic conditions. Its morphology and histology contributes for the preservation of the cellular components.

The endodontic root filling is a methodology used for preservation of tooth, in life, supporting a fixed rehabilitation. During endodontic procedure the pulp tissue and the inner layer of dentine are removed (the major nuclear DNA) and replaced for endodontic filling usually thermoplastic and cement. In the dentinal tubules, the odontoblats prolongations are preserved (mitochondrial DNA).

In this paper were studied endodontic tooth and the respective donor blood on compresses as reference samples. The samples were prepared. Teeth DNA extraction was made with a commercial kit, Purigene® DNA Purification System (PE Gentra), and quantified with an ABI Prism® 7000; amplification was performed with Identifiler™ PCR Amplification Kit (Applied Biosystems). In samples without STR profile was sequenced HVRI and HVRII. The samples were genotyped using an ABI Prism® 310 and 3130 Analysers.

Results showed that with endodontic treated teeth were obtained STR profiles. Teeth are extraordinary samples in identification caseworks.

This study showed that even in roots with endodontic treatment were obtained full STR profiles, giving the possibility of individual genetic identification.

Keywords: Identification; STR profile; Endodontic tooth root

1. Introduction

Forensic adverse conditions are usually associated with a high level of putrefaction and too degraded samples to perform genetics analyses. This is particularly true in mass disasters. The tooth is an important sample in different environments [1].

On the same source, usually you can find teeth with different quality: healthy and intact, with carious, with coronary and with endodontic root fillings (treatment to support a complex restoration or a fixed prosthetic rehabilitation often used). In same cases, there is only as biology sample an endodontic treated tooth.

During an endodontic procedure the pulp tissue and the inner layer of dentine are removed (the major contribute of nuclear DNA [1,2]) and replaced for endodontic filling usually thermoplastic and cement. Into the dentinal tubules, the odontoblats prolongations are preserved from the aggressions (mitochondrial DNA) [2].

In this study were analyzed endodontic teeth root in order to aid the identification process.

2. Material and methods

In this study were analyzed 10 endodontic teeth and the respective donor blood on compresses as reference samples. Teeth were placed 1 min in sodium hypochlorite commercial bleach, and washed in sterile distilled water. Using a diamond cutting disc the samples were fragmented by longitudinal section. The endodontic filling was removed using a fine spoon excavator and the root channels were cleaned. The remaining hard tissues were isolated and reduced to powder in cryogenic mill [2–4].

Teeth DNA extraction was performed using the commercial kit Puregene® DNA Purification System (Gentra Systems) with some modifications: 0.06 g of dentine powder and bath at 55 °C

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Table 1
Results obtained with the samples

Samples	Quantification (ng/μl)	Identifiler	mtDNA
1	3.769	Full profile	*
2	8.182	Full profile	*
3	39.423	Full profile	*
4	3.042	Full profile	*
5	0.023	No profile	HVRI HVRII
6	0.008	No profile	HVRI HVRII
7	0.782	Full profile	*
8	0.385	Full profile	*
9	0.491	Full profile	*
10	0.355	Full profile	*

Note: *For the subject of this paper mtDNA analyses was not necessary.

overnight to better dissolution of proteins. DNA from compresses was extracted by the Chelex™ 100 method [5].

DNA quantification was made with the Quantifiler™ Human DNA Quantification kit at the ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

Amplification of autosomic STRs (AmpFlSTR®Identifiler™PCR Amplification Kit—Applied Biosystems) was made according to kit instructions. DNA quantity was adjustable to 3 ng/μl DNA in samples with high quantification values. PCR products were analyzed using an ABI Prism™ 310 Genetic Analyser (Applied Biosystems).

In samples without STR profile Hypervariable Regions (HVRI: L15997-H16395; HVRII: L047-H408) were amplified with primers and amplification conditions according to Wilson et al. [6], using Taq Gold. Amplified fragments were purified by MicroSpin Sephadex HRS 300.

Sequencing reactions were performed in forward direction, using the ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), with the same primers used in amplification. Sequenced product was purified by $MgCl_2$/ethanol precipitation. Detection of sequencing fragments was carried out using an ABI Prism 3130 and analyzed in an ABI Prism 3130 Avant Automatic Sequencer.

3. Results

In eight of the samples it was obtained full STR profiles. In the others additional information was achieved in mitochondrial sequences (Table 1).

All extraction of references samples had good and correspondent STR profile and mitochondrial DNA.

4. Discussion and conclusions

Initially it was thought that the genetic analysis of the tooth could only be made from the pulp tissue [1,7]. Recently the analysis from its hard tissues has became more relevant [2–4]. The first choice is always for the intact and healthy tooth, however this option is not always available [2].

It was only expect a good mtDNA profile with endodontic treated tooth as a sample.

However, during endodontic procedure strong hydraulic forces are created within the dentinal tubules with odontoblast displacement [8]. The cell bodies of odontoblasts are displaced upward into the dentinal tubules.

Results (Table 1) showed that in teeth root with endodontic filling were obtained STR profiles. It is extraordinary samples in identification caseworks.

In conclusion tooth is a valuable source of DNA allowing genetic identification. Particularly even endodontic treated tooth should be collected when an intact tooth is not available.

Conflict of interest

None.

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