

## ORIGINAL RESEARCH

# Extraction of DNA From Odontoblastic Process of Permanent Tooth Dentin- A Pilot Study

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## ABSTRACT

**Introduction:** Pulp was earlier thought to be the major source of DNA in the tooth; but the extraction of DNA from the dentin is also on the rise where the odontoblastic processes are rich in mitochondria which in turn contain mtDNA. The present study was done to extract the DNA from odontoblastic process of permanent tooth dentin and to analyze the role of DNA in forensic dentistry.

**Material and Method:** In this study, Permanent maxillary and mandibular premolar teeth which were extracted for orthodontic reasons were taken and were subject to chemical process to extract the dentin. The extracted dentin was used for PCR analysis to study the DNA.

**Result:** Most of the samples inclusive of DNA from both coronal and root dentin samples showed both higher molecular weight band and smear. The concentration of DNA varied among samples. The mean of total DNA concentration in Radicular dentin samples was higher than that of coronal dentin samples.

**Conclusion:** Dentin is a source of DNA. However radicular dentin is a better source of DNA extraction compared to the coronal dentin.

**Keywords:** DNA, PCR, Teeth, Dentin

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## INTRODUCTION

Forensic odontology is the branch of science that has evolved from simple methods of age estimation and bite-mark analysis to a new era of genetic and serological investigations in identifying an individual.<sup>1</sup> DNA is a molecule that builds genes and this gene carries all genomic information that determines the inherited characteristics. DNA, found in both nucleus and mitochondria is well-suited for biological information storage.<sup>2</sup> It is resistant to cleavage and its double-stranded structure provides the molecule with a built-in duplicate of the encoded information.

Though DNA is found in both the nucleus and mitochondria, only the mitochondria increases the chance of DNA to be preserved for a longer period, where it could be used as a means of identification and also in age estimation of an individual.<sup>1</sup> Pulp was earlier thought to be the major source of DNA in the tooth; but the extraction of DNA from the dentinal tubules is also on the rise where the odontoblastic processes are rich in mitochondria which in turn contain mtDNA.<sup>2</sup>

Forensic science has evolved to analyze the genetic content (DNA molecules) from odontoblasts of dried tooth material. Nuclear DNA can be obtained from the pulp whereas the mitochondrial DNA (mt DNA) is abundantly available from dentin. mtDNA has a higher chance of being detected from odontoblasts relative to nuclear DNA because of its well protected nature.

A tooth is made up of 3 major tissues; enamel, dentin and dental pulp. Dental pulp cavity contains blood capillaries whereas the dentin contains a network of tubules where the odontoblastic processes rich in mitochondria occur. Since

dentin and pulp is covered with enamel; the outer layer as well as the hardest part of tooth, both are resistant to incompatible conditions for DNA. Thus, in extreme conditions such as exposure to the bacterial infection, dentin remains as a better source of DNA.<sup>2</sup>

This study focuses on extraction of DNA from odontoblastic process of permanent tooth dentin. The study was aimed at deciding to use this technique, we aimed at extracting the DNA from the permanent tooth dentin and to compare the efficacy of the coronal and the radicular dentin as a source of DNA.

## MATERIALS AND METHODS

### PREPARATION OF TOOTH SAMPLES

Maxillary and Mandibular Premolars extracted for orthodontic treatment was used as study samples. 12 of such samples were collected.

### METHODOLOGY FOR DNA EXTRACTION

First the crown and root portion of the tooth was separated. Pulp extirpation was done. The dentin powder was obtained using micromotor with carborundum disc and straight fissure bur from coronal and radicular portion. This powder was added to 1.5ml microcentrifuge tube, 650µl of buffer solution added and mixed vigorously and incubated at 60°C for 1 hr. After incubation, centrifugation of the tubes was done at the rate of 13,000rpm for 3 minutes. Transfer of 350µl of the clear supernatant to another 1.5 ml tube was done. 350µl of 100% Ethanol added for binding and the tube was inverted few times to mix properly. 600µl of lysate loaded on to the SMS column with collection tube and centrifugation of the tubes was done at 10,000 rpm for 30 seconds. The flow through discarded and the SMS column placed back in the collection tube. 600µl of 70% ethanol was added to the SMS column and centrifuged at 10,000 x g for 15 seconds. The flow through was discarded and the SMS column placed into the new collection tube and centrifuged at 2 minutes at the rate of 13,000 rpm to remove the residual ethanol. The SMS column cup was transferred to a new 1.5ml tube. 30-50µl of buffer SE was added on the membrane and

incubated for 1 minute. The tubes was centrifugated at 13,000 rpm for 1 minute. DNA was eluted from the SMS column and collected in the 1.5ml tube. The column was discarded. Validity of DNA was checked using agarose gel electrophoresis processes for 30 minutes. DNA amplification was done using PCR. The PCR amplification product was run again in agarose gel electrophoresis.

## RESULTS

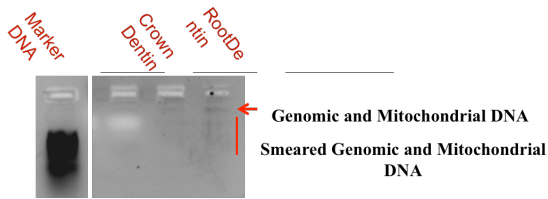
The dentin powder collected from all the samples was processed simultaneously. The samples was first weighed, which ranged between 120mg to 160mg. 100mg of dentin powder from each of the above samples was transferred to fresh DNase/RNase free 1.5ml microfuge tubes and was processed to extract by addition of dentin lysis buffer as described in the methods section. Following extraction of DNA, the quality of the sample was analyzed by agarose gel electrophoresis. This was performed by loading a 10 µl aliquot of DNA from each of the sample into the sample wells of 0.8% agarose gel, which was then subjected to electrophoresis in a 0.5X TBE buffer. Most of the samples inclusive of DNA from both coronal and root dentin samples showed both higher molecular weight band and smear. The smear was expected as dentin powder was prepared from dried tooth, which hence may be expected to contain degraded DNA as DNA often degrades rapidly in vitro.

The concentrations of DNA in the samples was measured in a fluorometric DNA spectrophotometer to precisely quantify the total DNA concentration. The concentration of DNA varied among samples. The mean of total DNA concentration in Radicular dentin samples was higher than that of coronal dentin samples.

## DISCUSSION

Forensic dentistry is a role in human identification in mass disasters with little material remains to perform visual identification. Conventionally, genomic DNA is obtained from pulp for identification of an individual; but recent advances have shown that even mitochondrial DNA can also be used. Odontoblastic process of

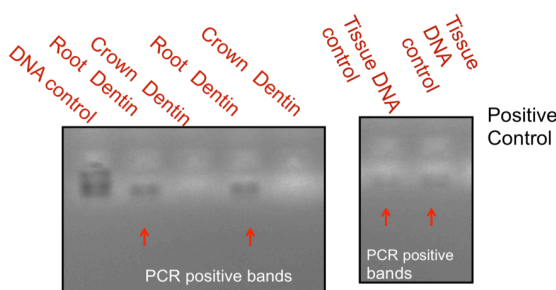
### DNA extraction from DENTIN



- ✓ Genomic and Mitochondrial DNA was extractable from both Crown Dentin and Root Dentin.
- ✓ The DNA extraction yielded optimal amount of DNA - to perform PCR amplification

**Figure-1:** DNA extraction from dentin.

### Gel Electrophoresis After PCR Amplification of DENTIN DNA



clear amplification of the DNA extracted from root dentin.

**Figure-2:** Gel electrophoresis after PCR amplification of dentin DNA.

dentin provides a good source of DNA.

In the present study, we have analyzed a total of twelve permanent tooth to test their ability to yield workable quantity of DNA. Dentin powders obtained from permanent premolars that were extracted during routine orthodontic treatment were processed to extract DNA as explained in the results section. The collected teeth were stored at room temperature for period of one to four months before being processed for DNA extraction, in an attempt to simulate forensic environment. There are different methods available to sample the dentin for DNA analysis which includes the splitting, crushing, scrapping and filing of the teeth. But all these methods encountered in technique failure and destructive tooth sample.<sup>7</sup> Therefore modified Pfeiffer method is used to extract DNA among study. Gel analysis of the extracted DNA showed high molecular weight chromosomal DNA band along with DNA smear in almost all the samples.<sup>2</sup> While

DNA smear from forensic samples is expected, the observation of high molecular weight DNA, though in varied quantity, in the samples suggests that the DNA might have come from the odontoblasts or necrotic pulp tissue. Since all samples were stored at dry room temperature condition, sample contamination by either bacteria or fungi is not likely to have contributed to the high molecular weight DNA.<sup>10</sup> This issue has to be addressed in the subsequent studies, by cleaning the pulp chamber with reamers and broach before preparing dentin powder.

The composition of lysis buffer used in the present study differs from those that have been published earlier, but certainly appears to yield comparable quantity of DNA. It is possible that the strength of lysis buffer used in the present study may have more efficiently disrupted odontoblasts than those that have been published earlier, with the resultant extraction of higher molecular weight DNA. This issue, however, has to be verified by comparing the lysis potential of the buffer used in the present study along with those published earlier by lysing identical quantity of same dentin powder, with each of the buffer, simultaneously. Besides the above, several other criteria have to be standardized, that includes: the type of tooth, portion of tooth (crown and root dentin), age of subject, and dentin powder preparation procedure such as use of airtor and frozen tooth to minimize damage to dentinal cells by heat generated. Until such time, it is not be possible to categorically define a particular protocol to be superior to the rest.

Remauldo Mark A Ellis subjected the teeth to extreme temperture during DNA extraction. The study results showed that extraction of mtDNA was better even with higher temperatures compare to genomic DNA. Even if few generations have passed in between, showing the chances of its preservation for a longer period. Therefore all these studies elaborated the advantages of DNA and mtDNA and its utility in forensic dentistry.

The total concentration of DNA obtained from the radicular portion of permanent tooth group was higher relative to coronal portion in most of the samples, although the amount of dentin powder used for DNA extraction in either of the sample were same. Since the protocol followed for lysis was similar for both sample groups, it

may safely be assumed that the radicular dentin yields relative higher quantity of DNA compared to coronal dentin.

## CONCLUSION

In conclusion, we believe that the present study is fairly efficient in extracting amplifiable quantities of DNA, but would add further value when compared for its efficiency with other published protocols. Despite that, there is one clear advantage with the present protocol, which is that, the technique utilized a simple, non-column based protocol and hence may be expected to reduce the sample processing costs significantly.

Within our limitations we could conclude that Dentin is a source of DNA. However Radicular dentin is a better source of DNA extraction compared to the coronal dentin.

Furthermore, DNA collected from dentin will provide high amount of source material which in turn can be useful in forensic dentistry.

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