REVIEW ARTICLE

Cryogenic Grinding: An Insight into The New Era

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ABSTRACT

As we enter a new millennium, society is faced with fresh challenges in every conceivable area. Through the speciality of forensic odontology, dentistry plays a small but significant role in identification of individuals. Cryogenic Grinding is the act of cooling or chilling a material and then reducing it to a small particle size. DNA is extracted from human teeth with the help of cryogenic grinding for PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) based forensic analysis. This technique provides with a high DNA yield of 30.9 micrograms DNA per tooth which is sufficient to provide the target DNA for more than 30,000 PCR from these samples which are too flexible or sensitive to be ground at ambient temperatures. This simple and rapid technique can be used to extract the DNA which is embedded in the hard tissues of the teeth. RNA can also be extracted by this method. This technique is advantageous over other techniques because it provides isolation of the samples in a closed system and in sterile conditions and also prevents cross contamination because the components of the apparatus can be easily cleaned and sterilized between samples. This versatile technique has evolved through many stages of using mortar and pestle to the recent homogenizer system and hence an important tool for further research in the field of forensic odontology.

KEYWORDS: Cryogenic, Forensic, Forensic Dentistry, Forensic Odontology

INTRODUCTION

Cryogenic grinding, also known as freezer milling, freezer grinding, and cryomilling, is the act of cooling or chilling a material and reducing it into smaller particles. The interest in using dental tissues as DNA as a source of individual identification falls within the particular character of resistance of this organ towards physical or chemical exterior aggressive agents.[1] When the routine dental identification methods fail, this biological material can provide the necessary link to prove identity. With the advent of the polymerase chain reaction (PCR), a technique that allows amplification of DNA at pre-selected specific sites, this source of evidence is becoming increasing popular with investigators.

Comparison of DNA from the teeth of an unidentified individual can be made to a known ante mortem sample like stored blood, hairbrush, clothing, cervical smear and etc or to a parent or sibling.[2] Generally the most DNA-rich site will be the dental pulp which is enclosed by coronal pulp chamber, root canals

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and accessory canals.[3,4] Some DNA maybe recovered from dentin or cementum but none should be expected within the enamel. On the basis of gross volume, the coronal pulp chamber and radicular canals are the targets for DNA sampling including odontoblastic processes, accessory canals and cellular cementum.[4] Pulp tissue is most commonly used because it is usually abundant and has least chance of contamination by nonhuman DNA. Teeth as usually cleaned and stored in normal saline and may or may not be refrigerated, depending on time and facilities.[5]

METHODS FOR DNA ISOLATION

The various methods used for isolation of DNA are:

- Cryogenic grinding
- Conventional endodontic access
- Vertical splitting
- Horizontal Section
- Crushing

Cryogenic grinding: Sweet and Hildebrand have pioneered the use of this technique which involved the pulverization of a cleaned tooth in a freezer mill which was later cooled by liquid nitrogen. The workers reported that their method greatly minimizes risk of contamination and takes as little as two minutes. The powder was solubilized in proteinase K buffer solution and incubated overnight. DNA was quantified using Slot blot hybridization in which nucleic acids are blotted using rectangular slots. The yield of DNA per gram of tooth powder was found to be around 18. 4 µg.[6]

Cryogenic Grinding is used to extract DNA from calcified tissues such as teeth. A ferromagnetic plunger is used in freezer mills to produce oscillations which generate alternating electric current. Liquid nitrogen is used to cool the sample which results in making it extremely brittle but the advantage is that it also protects the DNA from heat degradation. The tooth is reduced to a powder to increase surface area and expose trapped cells to biochemical agents that release DNA into solution. Even root filled teeth supply sufficient biological material for PCR analysis. [2,6]

Conventional endodontic access: If the tooth is intact and has been removed from alveolus recently, a conventional endodontic access along with instrumentation can be done.[7] It is difficult to obtain enough DNA through conventional endodontic access. In addition, the occlusal morphology and restorations are damaged. [8]

Vertical splitting: A lot of pulp tissue can be obtained with the vertical splitting method, although the restoration and tooth are also damaged. However, this technique is practically impossible due to various forces of the root.[9] Splitting is done with carborundum discs, with protection gear such as heavy duty gloves, preferably under a hood, as aerosol is harmful and may contact the skin or eyes or can be inhaled. The tooth is held securely or maybe mounted in dental stone. The carborundum discs are used to split the tooth from the incisal edge along with frequent washing using distilled water. When the pulp cavity is reached, the tooth is split by a chisel to avoid damage to pulp tissue. The pulp is then excavated and transferred to several vials. The sliced tooth is used for histological evaluation.[7,9]

Horizontal section: A fourth possible approach is the horizontal section, in which the tooth is split at the enamel-dentine border. The access of the pulpal room and the roots is sufficient and also the crown remains intact.[9] Either a chisel or rotary disk maybe used to section the tooth. Potential risks are that a chisel is likely to

fragment a severely dried specimen and the rotary instrument may create heat that could theoretically damage the DNA.[7,9]

The recently developed method of cryogenic grinding is easier and very effective. It also allows obtaining DNA from endodontic treated teeth because of the presence of DNA in hard tissues. Although, cryogenic grinding does not result in a marked increase in DNA yield compared with previous studies, the ability to control contamination of other samples and the laboratory environment through sample isolation is superior. Pulverisation of forensic samples in a closed system under frozen preparation in liquid nitrogen and sterile conditions is a distinct advantage over other methods. Also, the individual components can be easily cleaned and sterilized between samples which reduce cross contamination.

Cryogenic grinding has been used as a preparation tool for vital samples, such as cartilage and bone. The SPEX 6700 freezer mill was employed to extract DNA from skeletal remains of Romanov family. Samples of medieval teeth and bones have been crushed using a mortar and pestle and subsequently ground using a mineralogy mill. The yield of DNA from tooth powder is relatively high and of sufficient quality and quantity for DNA analysis. Theoretically genomic DNA is embedded in the hard tissues of the tooth. Cryogenic grinding maybe the most effective method to extract this DNA.[6,9]

CONCLUSION

Cryogenic grinding of vital samples using a freezer mill is a relatively simple and very effective method to recover forensically significant amounts of DNA from human molar teeth. The average yield of 30.9 µg of DNA per molar is sufficient to provide target DNA for more than 30,000 PCR reactions.

PCR based analysis produces a DNA profile that can be compared with known ante mortem samples or paternal DNA. The technique has allowed criminal investigators to link victims to crime scenes once the body has been removed and incinerated. In cases of mass disaster and other calamities, dental remains are helpful in establishing identity. Whenever dental professionals are required to provide such samples, knowledge of storage and sampling for the optimization of DNA analysis procedure can greatly enhance efficiency of the investigation.

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