EDITORIAL

Oral Cavity–A Resilient Source for DNA Sampling

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Dear Editor,

DNA is a double-stranded structure present inside the nucleus that carries all the inherited genomic information. Dental tissues are excellent sources of DNA as they show resistance to chemical and physical conditions.[1] Dental enamel can withstand adverse conditions like humidity, microbial action, and high temperatures, thus preserving the enclosed DNA.[2] DNA can be obtained from odontoblastic processes of dentin, cellular cementum, neurovascular tissues of the pulp, radicular canals, periodontal ligament, and alveolar bone.[3] A high concentration of DNA is found in the root body, then in the root tip, coronal body, and coronal tip. The pulp is rich in vascularity, which makes it a rich source of DNA. Cementocytes from cellular cementum are one of the DNA sources that are present within the extracellular matrix.[4] In addition, the odontoblastic cell processes are rich in mitochondrial DNA (mtDNA).

Overall teeth are a good source of high molecular weight mitochondrial DNA, which aids in human identification from the remains.[5] Demineralized root tips are also a viable source of nuclear DNA because cementum shows better resistance to decay than pulp and dentine, so the preservation of nuclear DNA is better.[6] *AMELX* and *AMELY* are amelogenin genes, used in identifying gender. Pulpal DNA aids in gender identification through gel electrophoresis-mediated assessment of X and Y loci peaks.[5] Extraction of DNA can be done by crushing or grinding the tooth, and the pulp can be extirpated by tooth irrigation and sectioning horizontally and nitrogen liquid cryogenic pulverization.[5] The disadvantages of crushing or grinding are that additional assessment of tooth morphology or histological examination is not possible. Thus, before grinding, it is necessary to document the tooth morphology, which includes taking photographs from various angles and taking radiographs.[7] Although phenol can be used to extract DNA, the preferred methods for extracting DNA from human teeth are chelex, silica, and magnetic bead systems.[8]

Dental pulp can be extracted using standard cavity preparation techniques. It is simple, low-cost, and preserves tooth integrity.[9] Pulp tissue obtained is kept in Eppendorf vials. Adding 1 mL sterile deionized water, followed by centrifugation thrice in 5 minutes. EDTA and Tris-HCl are added to the buffer and it is incubated for 18 hours with proteinase K. It is then followed by a series of centrifugation and purification stages. A 1% agarose gel is added to a TBE (Tris-HCL, boric acid, and EDTA) buffer and gel electrophoresis is performed. If the tooth is without pulp, as in endodontically treated teeth, it can harbor microorganisms. Natural human DNA can enter the sample. As an alternative, cementum and dentin are utilized to extract DNA. Nonhuman DNA is found less in calcified structures, except carious lesions. Pure DNA can be recovered easily from the organic matrix of calcification. Dentin and dentin–cementum powder can be obtained by grinding the root. The powder is dissolved and stored in vials. DNA extraction from pulpal tissue is done similarly. The ultrasonication of teeth is an alternative to crushing, sectioning, or destructing the tooth, for obtaining genetic material to perform DNA analysis. Unfortunately, this method yields an insufficient quantity of DNA. However, it can be used in cases where other methods are not feasible.[7] Genetic analyses can also be done on endodontically treated teeth. Preferably, the tooth has to be intact and healthy. Odontoblastic displacement into the dentinal tubules occurs during the endodontic procedure with strong hydraulic forces that are created inside of the dentinal tubules. Mitochondrial DNA can be extracted and STR profiles can be obtained which can be used in identification cases.[10]

Saliva contains a lot of DNA because it contains desquamated epithelial cells that are being flushed away. Whole saliva can be collected in stimulated or resting conditions. Diluted saliva is collected by rinsing the mouth with mouthwash then spitting it into the test tube. The collected samples have to be transported to the lab with normal or refrigerating transport conditions. The FTA cards (Flinders Technology Associates, manufactured by Whatman Co, GE Healthcare, UK) are chemically processed filter papers that are prepared to collect and store biologic samples at room temperature for molecular analyses. DNA is well protected by the

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cards and prevents oxidation or UV damage, microbial infection by impregnating with denaturants. Saliva can be dabbed on the FTA cards. Studies have shown that DNA obtained from buccal swab samples is immobilized on FTA cards and can be stable for a long period under room temperature, so any delay in transporting the sample to the lab would not interfere with the integrity of the analysis. RT-PCR is better than fluorometry and spectrophotometry which has failed to differentiate between bacterial DNA and human DNA.[7]

The swab is often used to collect DNA from the oral cavity. A cotton tip is swabbed inside the mouth. Dacron-coated or foam-coated tips and tongue depressors are used to collect the exfoliated mucosal cells. Usually, the mouth is cleaned using water and mouthwash. A firm and continuous scraping are performed to collect the samples. Living cells are collected by a cytobrush. It is placed gently and pressed firmly on the site, removed after rotating it once. The suprabasal layer and basal cells have high nuclear content which can be extracted. Cytobrushes and swabbing are of greater advantage for young children who are not cooperative during saliva sampling. Nonhuman DNA is seen in samples, but the quantity is very less in comparison with salivary samples. Burger et al. used a buccal DNA collector to collect the buccal cells, with the help of filter paper attaching to a plastic handle. The DNA yield increases with the number of swipes.[7] To conclude, the oral cavity is a valuable source of DNA and in specific scenarios including mass disasters, advanced decomposition, they potentially could be the sole DNA source.

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