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Original Article

Design of a protocol for obtaining genomic DNA from saliva using mouthwash: Samples taken from patients with periodontal disease

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ABSTRACT

Background: Obtaining high quality genomic DNA safely and economically is vital for diverse studies of large populations aimed at evaluating the role of genetic factors in susceptibility to disease.

Aim: This study was to test a protocol for the extraction of high quality genomic DNA from saliva samples obtained with mouthwash and taken from patients with periodontal disease.

Methods: Saliva samples were taken from 60 patients and then stored at room temperature. DNA extraction was carried out at distinct post-sampling times (10, 20 and 30 days). Evaluation of genomic DNA was performed with spectrophotometry, electrophoresis, and PCR genotyping and sequencing.

Results: The greatest concentration of DNA obtained was 352 μg at 10 days post-sampling, followed by 121.025 μg and 19.59 μg at 20 and 30 days, respectively. When determining the purity of DNA with the spectrophotometric ratio of 260/230, the relations of 1.20, 1.40 and 0.781 were obtained for 10, 20 and 30 days, respectively. In all samples, it was possible to

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amplify the product of 485 bp and the sequence of the amplicons showed 95% similarity to the reference sequence.

Conclusion: The present protocol represents an easy, safe and economical technique for obtaining high quality genomic DNA.

1. Introduction

It is of vital importance to carry out genetic analyses of the most prevalent diseases in large populations. This need has fueled research into techniques for obtaining high quality genomic DNA in an easy, safe and economical manner. Although blood samples provide an excellent means of obtaining DNA of sufficient quantity and quality for such genetic analyses, this procedure is invasive and unpleasant for many people, making it difficult for studies of large populations. Consequently, there is a search for alternatives.

An easier and less invasive way to obtain DNA is with saliva samples. Saliva provides a great number of nucleated cells, such as epithelial cells, leukocytes, and Langerhans cells. Additionally, it contains bacteria, virus, fungus, salts, and food residues.[1,2] The presence of bacteria represents a disadvantage , because these microorganisms can degrade human DNA. Moreover, if the sample is maintained at room temperature, these organisms can easily grow and proliferate.[3]

Different protocols have been developed for obtaining DNA from saliva. The commonly used methods employ polyethylene swabs or brushes, treated Guthrie-type cards, rinses with saline solution or 3% sucrose.[4–13] However, these techniques also have their disadvantages since the sample must be frozen or processed immediately so as to assure the quantity and quality of DNA. This requirement represents a difficulty for their use in studies that require transportation of the sample and its maintenance at room temperature. Additionally, Rogers et al. documented that the use of cotton swabs and polyethylene brushes results in the worst quantity and quality of DNA.[14]

Garcia-Closas et al. documented in an epidemiological study of cancer that with this technique the average percentage of bacteria is 50.5% than that found in saliva samples obtained without mouthwash.[15] Feigelson et al. reported that only 40% percent of the bacteria remained in saliva samples after using mouthwash with alcohol, and that the quantity and quality of DNA was stable during one week at room temperature.[16] Lum and Le Marchand proposed and documented the utilization of a mouthwash with alcohol (Listerine®, Johnson & Johnson) for obtaining high quality genomic DNA from saliva samples, demonstrating diminished bacterial growth at a temperature of 37 °C for one week. This technique can be used for self-collection of samples sent by mail.[17,18] The drawback of using this technique for extracting DNA is that it employs phenol–chloroform, which is a known toxic, mutagenic and carcinogenic agent.[19] There are kits on the market for the extraction of DNA that are non-toxic and easy to use, but the cost represents a problem for studies involving large populations. Therefore, there is the need to search for an easy, safe and economical manner to obtain high quality genomic DNA.

The aim of the present study was to evaluate a technique for the extraction of high quality genomic DNA that fulfills these requirements. For this purpose, we designed a model for obtaining saliva samples with a mouthwash, and tested it under the difficult buccal conditions of patients with severe chronic periodontitis that were not under treatment. This infection is a complex and multifactorial disease that results from the interaction of the host defense mechanisms with the plaque microorganisms. This interaction leads to the primary clinical features of periodontitis, which are gingival inflammation, attachment loss periodontal pocketing and alveolar bone loss.[20–22] The oral conditions of these patients give us an ideal scenario for assessing our protocol.

2. Materials and methods

This study was registered on https://clinicaltrials.gov/ as NCT02523326.

2.1. Patients

The study was conducted with 60 patients diagnosed with severe chronic periodontitis in the Periodontic Clinic of the Interdisciplinary Health Sciences Center (Santo Tomás Unit), Instituto Politécnico Nacional. The protocol of this study was approved by the Research and Bioethics Committee of the Escuela Superior de Medicina, Instituto Politécnico Nacional. Informed consent was obtained from all participants included in the study, who signed the appropriate form allowing for the collection of saliva samples and the extraction of genomic DNA. Ethical norms for research on human beings, established by the Declaration of Helsinki (1975; updated most recently in 2008) were strictly followed. Each patient was instructed how to prepare for saliva sampling, which involved brushing their teeth at least 2 h previously and abstaining from eating or drinking anything.

2.2. Taking the sample

In a tube of 50 ml was placed 10 ml mouthwash (Listerine Cool Mint, *Johnson & Johnson*, 21.6% alcohol). Patients were instructed to rinse their mouth vigorously with this quantity of mouthwash during 30 s, then spit it into the tube. Once collected, the samples were stored at room temperature (20–24 °C). The 60 samples were divided into 3 groups ($n = 20$), and the extraction of DNA was performed at distinct times: group A at 10 days, group B at 20 days and group C at 30 days.