ISSNe 2178-1990 10.7308/aodontol/2017.53.e17

Assessment of the quantity and purity of DNA obtained from buccal cells under different storage methods

Avaliação da quantidade e pureza do DNA obtido por meio de raspagem de células da mucosa buccal sob diferentes condições de armazenamento

Tayla Cavallari[1], Letícia Yumi Arima[1], Simone Tetu Moysés[2], Samuel Jorge Moysés[2], Renata Iani Werneck[2]

ABSTRACT

Aim: This study aimed to verify the quantity and purity of DNA obtained from buccal cells under different storage conditions. **Methods:** Thirty students, between 18 and 23 years of age participated in the study. Three samples of genetic material were collected from each student (samples A, B, and C) through a mouth rinse with 5 mL of 3% glucose. The first phase of DNA extraction from sample A was carried out on the same day of sample collection, whereas samples B and C were stored in a refrigerator and a freezer, respectively, for 1 month before the first extraction phase. DNA extraction was performed with 10 M ammonium acetate and 1 mM EDTA. Sample purity was assessed by spectrophotometry. Statistical analyses were performed through descriptive analysis and analysis of variance ANOVA using the SPSS software, version 21.0. **Results:** the samples presented no statistically significant differences between the DNA quantity ($p = 0.37$) or quality ($p = 0.16$). **Conclusion:** the quantity and purity of DNA extraction from the three samples were satisfactory, and no differences in storage conditions were found.

Uniterms: DNA. Mouth. Mucosa.

Data de recebimento: 06/11/2017
Data de aceitação: 22/02/2018

INTRODUCTION

The study of human genetics, combined with other health areas, has been growing considerably and has led to the development of new methods of DNA collection and extraction. DNA extraction from the blood provides an excellent DNA source; however, this is an invasive process that demands high laboratory costs[1]. Therefore, various DNA kit extractions and chemical protocols using buccal cells have been developed[2]. Buccal epithelial cells are a good source of genetic material, which explains how many protocols can perform in a faster, simpler, and more cost-effective manner. Furthermore, these procedures allow several samples to be processed at the same time[3]. in addition to being painless and noninvasive. The efficacy of buccal sampling has proven to be the same as blood sampling[1].

The procedure for obtaining DNA from saliva may vary according to the materials, protocol sequence, and storage conditions[4-6]. Large-scale studies have used different types of collection methods to assess DNA yields, with the aim of optimizing procedures in large research projects[1,7]. The diversity of collection methods may lead to some discrepancies between the compared groups; however, in many studies using saliva, the DNA purity and quantity have proven to be

[1] Mestre, Departamento de Pós-Graduação em Saúde Coletiva, Pontifícia Universidade Católica do Paraná (PUC/PR), Curitiba, Paraná, Brasil.
[2] Doutor (a), Departamento de Pós-Graduação em Saúde Coletiva, Pontifícia Universidade Católica do Paraná (PUC/PR), Curitiba, Paraná, Brasil.
Autor para correspondência: Leticia Yumi Arima. Departamento de Pós-Graduação em Saúde Coletiva. R. Imac. Conceição, 1155 - Prado Velho, Curitiba, PR, Brasil. CEP: 80215-901. Telefone: +55 41 3271 1555.
Contatos: taylacavallari@gmail.com; le_arima@hotmail.com; simone.moyses@pucpr.br; s.moyses@pucpr.br; renata.iani@pucpr.br

10.7308/aodontol/2017.53.e17

acceptable[4,8-12]. This occurs even when a specific step of the protocol to increase the DNA yield is altered[13] or when simple ancillary materials, such as tooth brushes, are used to collect buccal cells[14].

The assessment of methods to improve DNA extraction for genetic studies also aims to find simpler collection and storage procedures that simplify the process. Increasing practicability allows researchers and clinicians to conduct their studies even under limited conditions of genetic material storage or inexpensive materials. More recently, progressively more genetic studies have been conducted. According to the standard DNA extraction protocol, the first phase of the extraction process must be performed immediately after data collection. Unfortunately, the researchers and clinicians often fail to dispose of the equipment immediately after collection. Since the DNA consists of a resistant molecule, the hypothesis of this research was to test if different methods of sample storage (in a refrigerator or a freezer) after collection can be carried out as well. This study sought to verify the quantity and purity of DNA obtained from samples stored for one month and under different temperature conditions. This study also tested whether or not there is a difference in the DNA yield when the sample is subjected to the first step of DNA extraction shortly after collection.

MATERIAL AND METHODS

SAMPLE COLLECTION

This study was approved by the Ethics and Research Committee from the Pontific Catholic University of Parana (PUC-PR), logged under protocol number 69491, and was supported by the Brazilian National Council for Scientific and Technological Development (CNPq). Saliva samples were collected from 30 students, between 18 and 23 years of age, at the PUC-PR in Curitiba, Brazil. The students who consumed tobacco or alcohol; who used certain medications, such as antidepressant drugs (or others that could decrease the salivary flow); and who presented systemic diseases (which could affect the morphology and function of the buccal mucosa cells) were excluded. Samples were collected between September and December 2016.

The following auxiliary materials were used for collection: 5 mL of a 3% glucose solution, a wooden spatula, 15-mL Falcon tubes, and sterilized disposable straws.

All samples were collected in the afternoon. The students were instructed to vigorously rinse their oral cavities with the glucose solution for 1 minute. Subsequently, they were asked to transfer the glucose solution into a Falcon tube using a plastic straw. The malar mucosa was then scraped with a wooden spatula, which was stirred into the solution. Three samples were collected from each student (A, B and C) on the same day. For sample A, the first phase of DNA extraction (i.e. centrifugation and addition of extraction buffer) was performed on the same day of collection. The samples were sent to the genetics laboratory at PUC-PR and centrifuged at 2000 rpm for 10 minutes. Centrifugation was performed again if there were cells in the suspension. Cells and debris formed a pellet at the bottom of the tube. The supernatant (saliva and glucose) was separated, and 1300 µL of extraction buffer (10 mM TRIS, 4 mM EDTA, 0.5% SDS; pH, 7.76) was added to each tube. Finally, the samples were stored at −20°C[15].

The collection sequence was the same for samples B and C. However, after the mouth had been rinsed and the oral mucosa scraping had been collected, sample B was stored for 1 month in a refrigerator at 5°C, while sample C was stored for 1 month in the freezer at -20°C. After this period, the samples were thawed and prepared for DNA extraction in the same manner described for sample A.

DNA EXTRACTION

DNA extraction was performed for all samples according to the protocol described by Trevillato & Line, 2000[15]. The samples were thawed and incubated overnight with 10 µL of proteinase K. DNA was extracted the following day with 10M ammonium acetate and 1 mM EDTA. All of the DNA extractions were carried out within one week by an experienced researcher in laboratory procedures.

DNA QUANTIFICATION AND PURITY ANALYSIS

DNA concentration and purity were determined by spectrophotometry (NanoDrop™). In the spectrophotometric method used in this study, the DNA and proteins selectively absorb ultraviolet light at wavelengths of 260 nm and 280 nm, respectively. Therefore, the analysis of the solution at these two wavelengths allowed for the quantification of both DNA and proteins present in the sample. The ratio of 260/280 mM/µL indicated the degree of purity in each sample. A260/280 values of greater than 1.8 are appropriate for analysis. Lower A260/280 values may indicate protein contamination. The samples were quantified 1 month after extraction.

STATISTICAL ANALYSIS

After collecting the necessary information and DNA analysis, data were tabulated using the Statistical Package for the Social Sciences software, version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive analyses of data with frequency distribution were used to indicate the sample profile. To compare the purity of the samples, it was used the parametric ANOVA test.

10.7308/aodontol/2017.53.e17

RESULTS

This study analyzed three different storage methods of oral mucosa scraping for DNA extraction. The sample included 30 Dental School students (from 18 to 23 years of age), on which 15 were males and 15 were females. The average age of the participants was 22 years old. No statistically significant differences were observed regarding the DNA quantity (p = 0.37) and quality (p = 0.16) (Table 1).

Table 1 - Assessment of DNA quantity and quality

Samples	Storage	DNA concentration (ng/µL)(mean and standard deviation)	Ratio 260 nm/280 nm (mean and standard deviation)
A	1 month at -20°C after first phase of extraction	373.47 (±434.04)	1.957 (±0.147)
B	1 month at 5°C	200.26 (±243.90)	2.035 (±0.113)
C	1 month at -20°C	185.9 (±338.26)	2.049 (±0.264)

DISCUSSION

All methods were effective to measure the quantity and purity of DNA, regardless of the storage method used. Method A resulted in a higher quantity of DNA.

In the literature, the results obtained for DNA extraction from blood cells are similar to those for DNA extraction from buccal cells. The latter have been proposed as a promising alternative source of genomic DNA for molecular studies, primarily because their collection requires a painless and noninvasive method with no risk of disease transmission[16]. Although the scientific community has embraced the practicality of obtaining DNA from saliva, different procedures have been proposed for saliva collection, storage, and processing for DNA extraction[3,12]. Moreover, buccal epithelium samples have an advantage over blood samples, since the latter contains PCR inhibitors that can consequently be affected by the presence of DNA after extraction, which leads to problems in amplification[17]. It is therefore necessary to compare different protocols to determine the DNA yield and integrity, the costs of sample collection, and laboratory processing.

The protocol used to prepare sample A was in accordance with the extraction procedures proposed by Trevilatto & Line[15]. The authors recommend sending the samples to the laboratory immediately after collection for the first phase of extraction, followed by storage at −20°C. However, some studies have demonstrated that when samples are frozen before the first phase of extraction, a higher quantity of DNA can be obtained, given that some salivary enzymes that degrade DNA are inactivated by immediate freezing[18]. In the present study, sample C was immediately frozen after collection. Nevertheless, all conditions yielded DNA concentration and purity suitable for molecular studies. One study also reported that the storage of saliva at room temperature for up to 30 days before extraction allows for the collection of field specimens and an increase in the sample amount[19]. The DNA consists of a stable molecule; thus, regardless of the storage process, the DNA purity and quantity were similar in the tested methods[9].

All samples from each individual were collected on the same day in this study. This was a limitation and probably the reason why sample A produced a higher quantity of DNA. However, there were no significant differences among the three samples with regard to DNA quantity.

CONCLUSION

All methods tested in this study produced a satisfactory quantity and purity of DNA, indicating that the collection of saliva in a medical office and the freezing of the sample for subsequent DNA analysis is feasible and does not alter the purity of extracted DNA.

RESUMO

Objetivo: O objetivo desta pesquisa foi avaliar a quantidade e pureza do DNA obtido por células bucais utilizando diferentes meios de armazenagem. **Métodos:** trinta estudantes do curso de Odontologia entre 18 e 23 anos participaram desta pesquisa. O material genético foi coletado 3 vezes de cada indivíduo (amostras A B e C) por meio de bochechos com 5 ml de glicose 3%. Para a amostra A, foi realizada a primeira fase da extração do DNA no dia da coleta, já

as amostras B e C, ficaram armazenadas em geladeira e freezer, respectivamente, por um mês antes da primeira extração. A extração do DNA foi realizada com acetato de amônio 10M e EDTA 1mM. Avaliou-se a pureza das amostras por espectrofotometria. **Resultados**: as amostras não apresentaram diferenças estatisticamente significativas entre a quantidade (p = 0,37) ou pureza (p = 0,16) do DNA. **Conclusão**: a quantidade e a pureza do DNA das três amostras foram satisfatórias e não houve diferenças nas condições de armazenamento.

Descritores: DNA. Cavidade oral. Mucosa.

REFERENCES

1. Juárez-Cedillo T, Sánchez-García S, Mould-Quevedo JF, García-Peña C, Gallo JJ, Wagner FA, et al. Cost-effective analysis of genotyping using oral cells in the geriatric population. Genet Mol. 2010; 9:1886-95.
2. Saab YB, Kabbara W, Chbib C, Gard P.R. Buccal cell DNA extraction: yield, purity, and cost: a comparison of two methods. Genet Test. 2007 Winter;11(4):413-6.
3. Aidar M., Line S.R. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007;18:48-52.
4. Nedel F, André Dde A, Oliveira IO, Tarquinio SB, Demarco FF. Buccal cells submitted to three different storage conditions before DNA extraction. J Appl Oral Sci. 2009;17:113-5.
5. Hearn RP, Arblaster KE. DNA extraction techniques for use in education. Biochem Mol Biol Educ. 2010;38:161-6.
6. Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, Amorim LM. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. J Appl Oral Sci. 2012; 20:467-71.
7. Koni AC, Scott RA, Wang G, Bailey ME, Peplies J, Bammann K, et al. DNA yield and quality of saliva samples and suitability for large-scale epidemiological studies in children. Int J Obes (Lond). 2011 Apr;35 Suppl 1:S113-8.
8. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol Biomarkers Prev. 1998 Aug;7(8):719-24.
9. Mattana C, Fernandes MM, Tinoco R, Oliveira R, Mattana R, Rodrigues B. Forensic importance of DNA and the dentist participation. Braz J Forensic Sci. 2012;2:65-82.
10. Ng DP, Koh D, Choo SG, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA from saliva. Clin Chim Acta. 2004 May;343(1-2):191-4
11. Rogers NL, Cole SA, Lan HC, Crossa A, Demerath EW. New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. Am J Hum Biol. 2007 May-Jun;19(3):319-26.
12. Waltrick-Zambuzzi M, Santos TV, Freire HR, Macedo E, Granjeiro J, Kuchler E. Avaliação do rendimento, pureza e integridade do DNA genômico em diferentes protocolos de coleta de células bucais. Int J Dent. 2012;11:12-8.
13. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PLoS One. 2014;9:1-18.
14. Imamura Y, Usui S, Oomori Y, Fujigaki Y, Ota N, Wang PL. Recovery of genomic DNA from lingual mucosal cells in sufficient quantity and quality. Am J Dent. 2007;20:241-4.
15. Trevilatto PC, Line SR. Use of buccal epithelial cells for PCR amplification of large DNA fragments. J Forensic Odontostomatol. 2000 Jun;18(1):6-9.
16. Vries HG, Collèe JM, Van Veldhuizen MH, Achterhof L, Sibinga CTS, Scheffer H, et al. Validation of the determination of deltaF508 mutations of the cystic fibrosis gene in over 11 000 mouthwashes. Hum Genet. 1996 Mar;97(3):334-6.
17. Barea J, Pardini M, Gushiken T. Extração de DNA de materiais de arquivo e fontes escassas: revisão e apontamentos sobre as possíveis aplicações. NewsLab. 2004;63:96-114.
18. Scorsato A, Telles J. Factors that affect the quality of DNA extracted from biological samples stored in paraffin blocks. J Bras Patol Med Lab. 2011;47:541-8.
19. Aidar M. Extração do DNA genômico a partir de células epiteliais bucais utilizando acetato de amônio. Piracicaba. Dissertação [Mestrado]. - Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas; 2006.