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Original article

DNA amplification using phi29 DNA polymerase validates gene polymorphism analysis from buccal mucosa samples

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Abstract

Venous blood is currently the most common source of DNA for gene polymorphism screening; however, blood sampling is invasive and difficult to perform in general dental treatment. Buccal mucosa samples provide an alternative source of DNA, but it is frequently difficult to effectively amplify the DNA owing to the small amounts of sample material obtained. This study was performed to establish a method for performing total genomic DNA amplification from buccal mucosa samples using phi29 DNA polymerase. Total genomic DNA was isolated from buccal mucosa samples obtained from healthy subjects and was amplified using phi29 DNA polymerase. To determine the suitability of the extracted DNA for genotyping, polymerase chain reaction and restriction fragment length polymorphism analyses were performed for the IL-1 gene polymorphism. Genotyping of the IL-1 polymorphism was successful using the amplified DNA from a buccal mucosa, but genotyping was unsuccessful using the unamplified control because of low DNA purity. The method of extracting DNA from a buccal mucosa is painless, simple, minimally invasive, and rapid. Genomic DNA from a buccal mucosa can be amplified by phi29 DNA polymerase in sufficient quantity and quality to conduct gene polymorphism analyses.

Keywords: Gene polymorphism; Buccal mucosa; DNA amplification; Peri-implantitis

1. Introduction

Since the determination of the human genome sequence, the relationships between various diseases and gene polymorphisms have been proposed [1]. Over the last several years, a number of studies have suggested the relationships between severe chronic periodontitis and gene polymorphisms. The IL-1 cluster of genes, particularly the composite genotype (allele 2, at IL-1α +4845 and IL-1β +3954) described by Kornman et al. [2] is the most extensively studied in relation to distribution frequencies in populations of different ethnic origins. Polymorphisms in genes encoding other important immunological molecules such as TNF-α are also associated with periodontitis. As for peri-implantitis, it has been reported that IL-1 genotype-positive heavy smokers have a significantly higher risk for peri-implant bone loss after an average of 5–6 years of function, compared with IL-1 genotype-negative smokers [3,4]. It is increasingly important to examine the relationship of gene polymorphisms to peri-implantitis and periodontal diseases. To perform such a examination, collaborative study among multiple facilities is necessary to obtain a large number of human samples.

Blood sample is a most favorable source to obtain large amount of DNA and is frequently used for epidemiological study. Nevertheless, this method is invasive for subjects and is expensive for large-scale studies. In contrast to blood, buccal cells offer a minimally invasive and more easily collected source of DNA [5–13]. As for buccal mucosa sampling, although successful genetic analyses have been reported, amount of DNA obtained still remains problematic [10]. Particularly for multicenter study, variable sampling and damage of sample during transport from one facility to another may threaten successful analysis. The use of whole-genome amplification (WGA) is vital for success for large-scale studies. Currently available WGA technologies include isothermal multiple displacement amplification (MDA) procedures based

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on phi29 DNA polymerase [14,15] and several PCR-based technologies, such as degenerate oligonucleotide primed PCR (DOP-PCR) [16], Primer extension PCR (PEP) [17], and fragmentation ligation PCR (FLP) [18]. Comparative studies suggest that MDA-based WGA procedures produce amplified DNA that is more suitable for a wide range of genetic analysis than DNA from PCR-based WGA methods. The MDA procedures are also very simple to perform, requiring only the addition of reagent (primers, polymerase, and nucleotides) in a single, isothermal step to produce microgram quantities of representative DNA [14,15,19,20].

The present study was performed to determine whether genomic DNA can be amplified quickly and conveniently from buccal mucosa samples, in sufficient quantity and quality to allow gene polymorphism analysis.

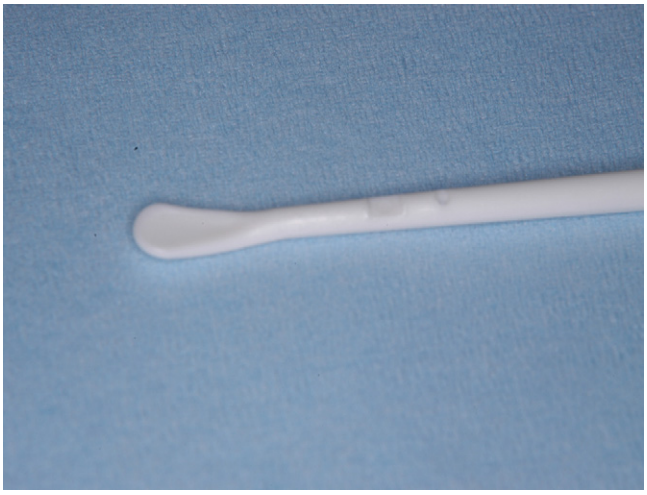

Fig. 1. PP Spatula (AS ONE CORPORATION)

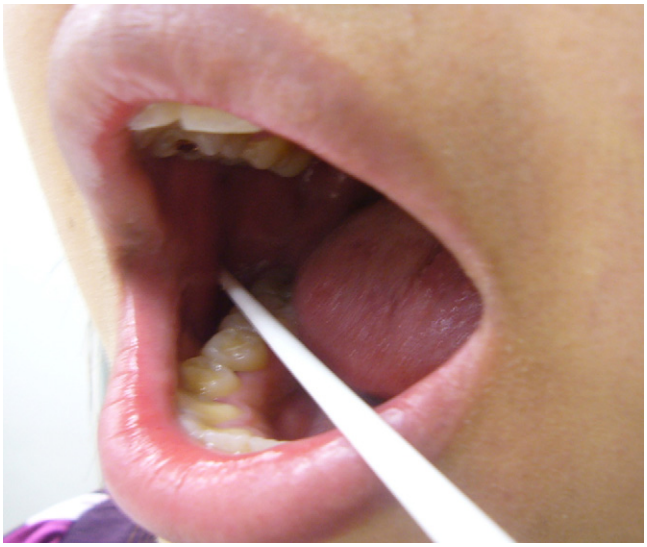

Fig. 2. Buccal mucosa cells were harvested using 5 strokes with an applicator.

2. Methods

2.1. Sample collection

2.1.1. Extraction of DNA

DNA was isolated from buccal mucosa samples from five healthy volunteers, all of whom provided appropriate informed consent. The Ethics Committee of Kyushu Dental College approved the study protocol. Buccal mucosa cells were harvested from inside the cheek using five strokes with an applicator (PP Spatula; As One Corp., Osaka, Japan) (Figs. 1 and 2). Buccal cells were washed with PBS and collected by centrifugation. The cell pellet was suspended in an adequate amount of cell lysis solution (400 mM KOH, 10 mM EDTA, 100 mM DTT) and incubated on ice for 10 min. Neutralization buffer (400 mM HCl, 600 mM Tris–HCl) was added to the cell lysate, and the sample was mixed and stored on ice. As a control sample, DNA was purified using 1.5 M sodium acetate/250 mM EDTA and precipitated with ethanol.

2.2. Yield and purity determination

Total DNA yield was measured by UV absorbance at 260 nm. DNA purity was assessed using the ratio of A_{260}:A_{280}.

2.3. DNA amplification

We used strand displacement amplification with phi29 DNA polymerase (Genomiphi V2 DNA Amplification Kit®; GE Healthcare, Waukesha, WI, USA) to increase the amount of DNA available. With this method, a limited amount of genomic DNA is combined with sample buffer containing random hexamers and appropriate reagents to produce copies of the high-molecular-weight fragments in the source DNA.

For each amplification reaction, we combined 9 μl of reaction buffer containing deoxynucleotide triphosphates and 9 μl of sample buffer containing primers with 1 μl of enzyme mix (phi29 DNA polymerase) on ice, and added the entire 1 μl volume of neutralized cell lysate with gentle mixing. The tube was then incubated at 30 °C for 2 h. The samples were heated to 65 °C for 10 min to inactivate the enzyme and then stored at 4 °C.

2.4. PCR analysis

For PCR, the reaction mixture contained 20 μM each primer, 10× PCR buffer, 200 μM dNTPs, 2.5 mM $MgCl_2$, and 2.5 U of Taq DNA polymerase in a total volume of 50 μl. The primers used were: forward, 5′-CTC AGG TGT CCT CGA AGA AAT CAA-3′; and reverse, 5′-GCT TTT TTG CTG TGA GTC CCG-3′. PCR was carried out at 95 °C for 2 min, followed by 40 cycles of 1 min each at 95 °C, 67.5 °C, and 74 °C, with a final extension at 72 °C for 8 min.

The resulting products were resolved in 3% agarose gels, stained with ethidium bromide, and visualized under ultraviolet (UV) light. Samples that were positive for the genotype had a 194-bp band in the case of IL-1β +3954.