

Comet Assay-A Diagnostic Tool to Assess DNA Damage in Buccal Epithelial Cells for Early Detection of Oral Cancer

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Abstract

Review Article

Introduction: The purpose of this article is to provide an overview on the application of single-cell gel (comet) assay as a promising tool for the detection of DNA damage induced by the use of tobacco, smokeless tobacco products and alcoholic beverages to assess the development of oral cancer risk. Comet Assay is a versatile method which is utilized to detect genotoxicity and cytotoxicity at the cellular level and can be utilized in regular practice as a pre-diagnostic tool in the prevention of oral carcinogenesis. **Discussion:** Buccal epithelial cells prove to be a potential replacement for lymphocytes collected from blood as the least invasive method for collection of cells for the early detection and prevention of oral cancer by the assessment of DNA damage.

Keywords: Comet assay, Buccal Epithelial Cells, DNA damage, Oral Cancer.

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INTRODUCTION

The purpose of this article is to provide an overview on the application of single-cell gel (comet) assay as a promising tool for the detection of DNA damage induced by the use of tobacco, smokeless tobacco products and alcoholic beverages to assess the development of oral cancer risk. The single-cell gel electrophoresis (Comet Assay) is a microgel electrophoresis technique which can be utilized for the detection of single strand or double DNA strand breaks. The alkaline version of Comet Assay is the most commonly used version to detect DNA single-strand breaks, DNA double-strand breaks, alkali-labile sites, and single-strand breaks containing incomplete excision repair sites which leads to increased DNA migration. Comet Assay is a versatile method which is utilized to detect genotoxicity and cytotoxicity at the cellular level and can be utilized in regular practice as a pre-diagnostic tool in the prevention of oral carcinogenesis. Buccal epithelial cells are the most common sites of malignant transformation and thus they can be a potential replacement for lymphocytes which are withdrawn from blood as cells taken from buccal mucosa causes minimal trauma and least invasion. The work of Rojas et al. described the use of buccal cells in the comet assay [1, 2]. Buccal cells are collected by a

gentle scraping of the lining of the cheek of the mouth and are most commonly affected by harmful carcinogens like tobacco chewing or the consumption of Smokeless Tobacco products like Khaini or Paan Masala [3]. This makes a buccal cell comet assay model an effective and potentially useful tool for investigating in vitro and in vivo effects on DNA damage in the users of tobacco or smokeless tobacco products.

Single cell gel electrophoresis (SCGE) or the Comet Assay is a versatile, sensitive yet simple and economical technique used to measure DNA damage in various cells obtained from buccal mucosa or nasal mucosa or from brain cells. The comet assay helps to measure the single/ double-strand DNA breaks, alkali labile sites (apurinic/ apyrimidinic sites), DNA cross-links, base/ base-pair damages [4]. In 1988, Singh and his co-workers modified the technique using alkaline conditions which substantially increased its specificity and reproducibility [5]. Since then SCGE has gained huge popularity and evolved as a standard technique for evaluation of DNA damage/repair [6]. The most widely used method for assessment of DNA damage is the alkaline comet assay; hence this article describes the application of alkaline comet assay using buccal epithelial cells.

PROTOCOL FOR COMET ASSAY

Buccal epithelial cells were collected from subjects by using a soft bristle tooth brush gently from the oral mucosa of the cheeks. The brush was then swirled into a tube containing cold phosphate buffered saline (PBS) and centrifuged at 2000 rpm for 10 min. The supernatant was removed and 300 μ l of trypsin solution (0.25% trypsin, 1 mM EDTA in PBS) was added to the buccal cells and incubated for 30 min at 37 °C. The cells were centrifuged and the supernatant was discarded. The cells were then washed thrice by centrifugation at 2000 rpm for 10 min in cold PBS. About 40 μ l of cell suspension and 60 μ l of 0.5% low melting agarose (LMA) were mixed and placed on frosted slides previously coated with 1% normal melting agarose. To the solidified agarose, a third layer of 1% low melting agarose was applied and the slides were dipped in freshly prepared cold lysing solution (2.5 M NaCl; 100 mM EDTA; 10 mM Trizma base; 1% Triton X; 10% DMSO) for 24 h [7]. Then the slides were subjected to electrophoresis (300 mM NaOH/1 mM EDTA) (pH >13), followed by neutralization (0.4 M Tris-HCl) and stained with ethidium bromide (20 μ g/ml) [11]. Three slides were prepared per individual and a total of 50 randomly captured comets per slide, under a fluorescence microscope were analysed for scoring comet tail length by using comet score 1.5 software (TriTek corporation).

DISCUSSION

Buccal epithelial proves to be a potential replacement for lymphocytes collected from blood as the least invasive method for collection of cells for the early detection and prevention of oral cancer by the assessment of DNA damage [12]. Alkaline comet assay (pH 9.1) is more suitable for buccal epithelial cells, as they are more prone to DNA strand breaks [13, 14]. The buccal cell comet assay model is of potential value as it can be applied for the study of genotoxicity and cytotoxicity at cellular level in humans and can be used in the users of tobacco smoking or chewing [15] or smokeless tobacco users and alcohol users which lead to the highly potential risk for the development of oral cancer.

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