Noninvasive method for obtaining RNA from buccal mucosa epithelial cells for gene expression profiling

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Swabs and scrapings from the buccal mucosa in the mouth have been used to obtain DNA from epithelial cells for genetic studies (1,2). RNA has been obtained from resected tissues and from biopsy samples of mouth epithelium in various disease states to measure gene expression (3,4). However, RNA has not been extracted from scrapings of buccal mucosa because ribonucleases that are present in saliva rapidly degrade epithelial cell RNA (5) during collection.

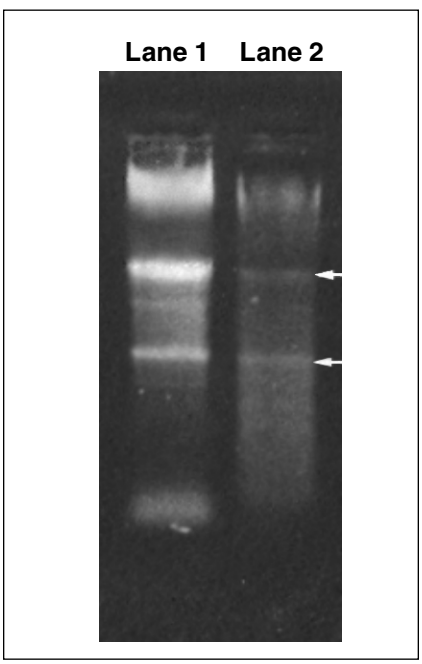


Figure 1. RNA denaturing gel (1% agarose). (Lane 1) RNA extracted from epithelial cell lines and (lane 2) buccal mucosa scrapings. Bands for (upper arrow) 28S rRNA and (lower arrow) 18S rRNA are shown. This is one of the highest quality, rather than a representative gel. Most scrapings produced too little RNA for a gel or displayed evidence of some RNA degradation. This partial degradation did not hinder the measurement of RNA by real-time PCR or mass spectrometry.

To collect intact RNA from buccal mucosal epithelium for studies of the biologic effect of smoking on the airway epithelium, we developed a relatively noninvasive method for obtaining small amounts of RNA from the mouth. We measured the expression of selected genes in individual subjects using real-time PCR and used a recently described mass spectrometry method that requires only nanogram amounts of total RNA for analysis and lends itself to high-throughput analysis of hundreds of genes (6).

Initially, we used a micropipet tip cut lengthwise to collect epithelial cells from the buccal mucosa in a relatively noninvasive fashion. We subsequently designed a standardized plastic tool that is concave with serrated edges. It is 5/16 inches wide and 1 6/16 inches long with a 3 inch handle that can be broken off when the scraping tool with the collected cells is inserted into a 2-mL microfuge tube containing 1 mL RNA*later*™ solution (Qiagen, Valencia, CA, USA). The tool has two features that allow the collection of a significant amount of good-quality RNA from the buccal mucosa: a finely serrated edge that can scrape off several layers of epithelial cells and a concave surface that collects the cells. Using gentle pressure, the serrated edge was scraped (10 times) against the buccal mucosa on the inside of the cheek, and the cells collected were immediately immersed in 1 cm^3 RNA*later* solution. After storage at 4°C for up to 24 h, total RNA from buccal epithelial cells was isolated from the cell pellet using TRIZOL® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The integrity of the RNA was confirmed in select cases on a RNA denaturing gel (Figure 1). Epithelial cell content was quantified by cytocentrifugation at 700× *g* (Cytospin®; ThermoShandon, Pittsburgh, PA, USA) of the cell pellet and staining with a cytokeratin antibody (Signet, Dedham, MA, USA) (Figure 2). Using this protocol, we were able to obtain 300–1500 ng RNA from each subject [the mean (± SD) = 983 ± 667 ng].

The procedure was well tolerated by all subjects recruited into this study, and none of the subjects experienced bleeding or pain during or after the scrapings. We have tried several other instruments including an endoscopic cytobrush (CELEBRITY™ Endoscopy Cytology Brush; Boston Scientific, Boston, MA, USA), a Costar® 3010 Cell Scraper (Corning, Corning, NY, USA), a pap smear kit, and a tongue depressor and have not been able to obtain significant quantities of intact RNA using the above protocol. In addition, we have found that the storage of the epithelial cells in RNA*later* significantly improves the preservation of the RNA integrity compared to placing the cells directly into TRIZOL. We have found that cells can also be preserved in RNA*later* at room temperature for

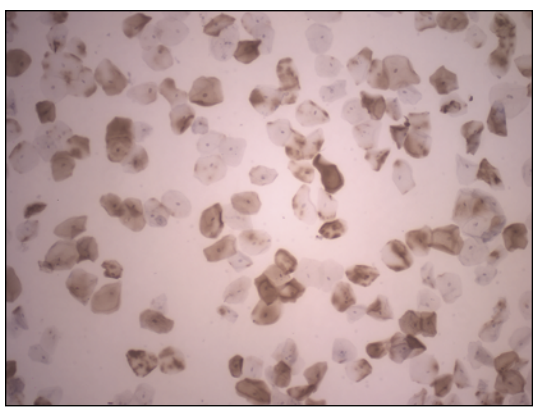
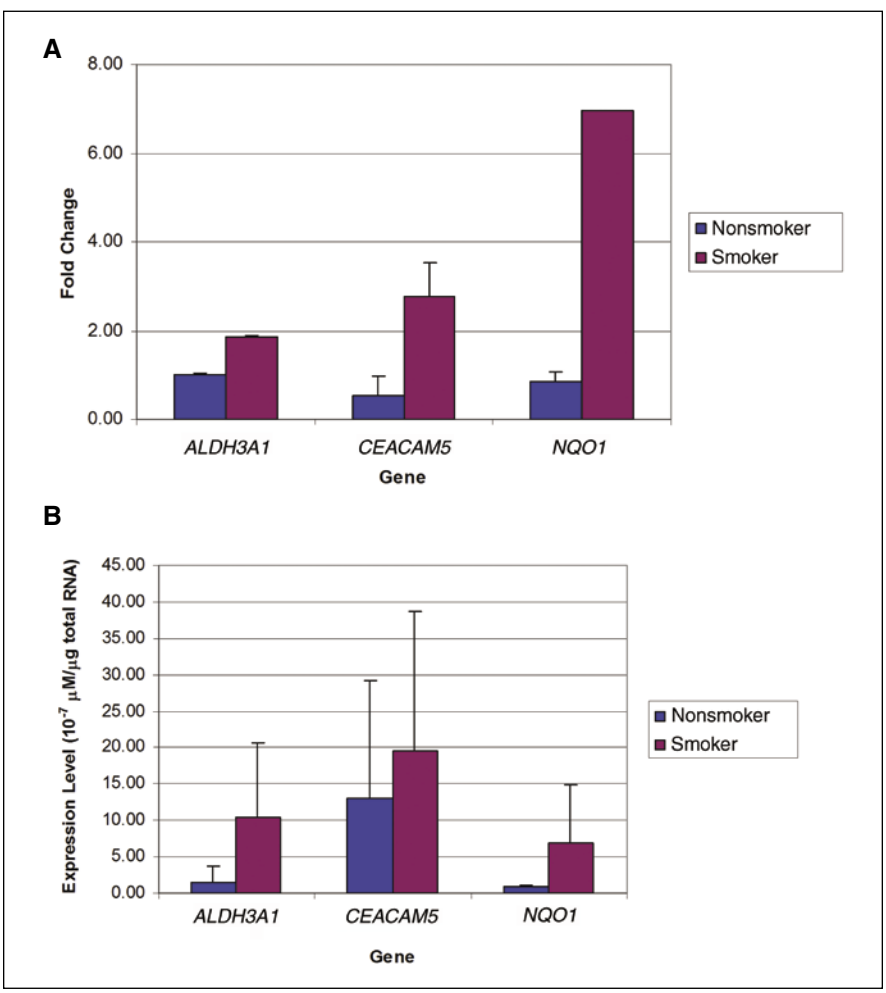


Figure 2. Immunocytochemical stain for the pancytokeratin protein in buccal mucosa cells obtained using our protocol. All cells have epithelial morphology and stain positive (brown) for the antibody to various degrees.

Table 1. Forward and Reverse Primers for Three Genes Measured by Real-Time RT-PCR and MALDI-TOF Mass Spectrometry

A. Primers for Real-Time RT-PCR		
ALDH3A1 Forward	5′-ATGGGATCCTACCATGGCAAG-3′	
CEACAM5 Forward	5′-GTCTTG TTTCCCAGATTTCAGGAA-3′	
NQO1 Forward	5′-TGGGAGACAGCCTCTTACTTGC-3′	
ALDH3A1 Reverse	5′-GCGGCGGTGAGAGAAAGTCT-3′	
CEACAM5 Reverse	5′-AGAGTGGATAGCTTAAAAGAAAAAAAGTTTC-3′	
NQO1 Reverse	5′-CAGCTCGGTCCAATCCCTTC-3′	
B. Primers for Competitive PCR and MALDI-TOF Mass Spectrometry		
PCR Primers	ALDH3A1 Forward	5′-ACGTTGGATGCACTGAAAGAGTTCTACGGG-3′
	CEACAM5 Forward	5′-ACGTTGGATGATGTGAAACCCAGAACCCAG-3′
	NQO1 Forward	5′-ACGTTGGATGCCACAGAAATGCAGAATGCC-3′
	ALDH3A1 Reverse	5′-ACGTTGGATGCGGGCACTAATGATTCTTCC-3′
	CEACAM5 Reverse	5′-ACGTTGGATGTCCGGGCCATAGAGGACATT-3′
	NQO1 Reverse	5′-ACGTTGGATGTGTACTCTCTGCAAGGGATC-3′
Extension Primers	ALDH3A1-E	5′-GGGAAGATGCTAAGAAATC-3′
	CEACAM5-E	5′-CAGGCGCAGTGATTCAGT-3′
	NQO1-E	5′-GAATGCCACTCTGAATT-3′

RT-PCR, reverse transcription PCR; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; *ALDH3A1*, aldehyde dehydrogenase family 3 member A1; *CEACAM5*, carcinoembryonic antigen-related cell adhesion molecule 5; *NQO1*, NADPH dehydrogenase quinone 1; E, extension.



up to 24 h prior to RNA isolation.

To assess the biological integrity of the RNA collected from the buccal mucosal cells, we measured the expression of a select number of detoxification-related genes that might be expected to be altered by exposure to cigarette smoke (7) as well as a gene involved in cell adhesion. Using the protocol described above, buccal mucosa RNA

Figure 3. Expression level for select buccal mucosa epithelial cell genes in smokers and nonsmokers. (A) The measurement of buccal mucosa epithelial gene expression by real-time reverse transcription PCR (RT-PCR). The mean (± SD) expression fold changes for three nonsmokers and two current smokers for each gene are shown (only one smoker sample was measured for *NQO1*). Fold change refers to the ratio of the mean expression level of a gene in a group of samples as compared to one of the nonsmoker samples. All real-time PCR experiments were carried out in duplicate for each sample. (B) The measurement of buccal mucosa epithelial gene expression by competitive PCR and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Expression levels were normalized to total RNA concentration (10^{-7} µM/µg total RNA). The mean (± SD) expression level for 7 nonsmokers and 10 smokers for each gene are shown. There was a significant ($P < .05$) increase in gene expression for *ALDH3A1* and *NQO1* in smokers. *ALDH3A1*, aldehyde dehydrogenase family 3 member A1; *CEACAM5*, carcinoembryonic antigen-related cell adhesion molecule 5; *NQO1*, NADPH dehydrogenase quinone 1.

was collected from 12 nonsmokers and 14 current smokers. The study was approved by the Boston University Medical Center Institutional Review Board (Boston, MA, USA).

Real-time reverse transcription PCR (RT-PCR) (8) was used to measure the expression of NADPH dehydrogenase quinone 1 (*NQO1*), aldehyde dehydrogenase family 3 member A1 (*ALDH3A1*), and carcinoembryonic antigen-related cell adhesion molecule 5 (*CEACAM5*) from samples obtained from three nonsmokers and two smokers (Figure 3A and Table 1A). The mean expression of *NQO1*, *ALDH3A1*, and *CEACAM5* were increased 7-, 2- and 3-fold, respectively, in patients exposed to tobacco smoke. Using competitive PCR and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (6), we measured the expression of *ALDH3A1*, *NQO1*, and *CEACAM5* in 7 nonsmokers and 10 smokers (Figure 3B and Table 1B). The expression of all three genes was up-regulated in smokers compared to nonsmokers, with statistically significant changes for *ALDH3A1* and *NQO1*.

These studies represent the first successful approach to obtaining RNA from buccal mucosal cells in a noninvasive fashion for measuring gene expression. The method may be useful for understanding molecular mechanisms of a variety of diseases that involve the mouth, in assessing the response to and damage caused by inhaled pollutants such as cigarette smoke, the diagnosis and biologic impact of inhaled infectious agents, and for developing simple early diagnostic biomarkers of airway and lung cancer that might be applied to screen at-risk populations. The mass spectrometry system allows high-throughput analysis of large numbers of genes (100–200) in short periods of time and could be adapted to mass screening of large numbers of samples.

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