Original

Evaluation of an innovative oral brush for potential applications using liquid based cytology

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Abstract: The present study was conducted to assess the applicability of liquid-based cytology (LBC) using an innovative oral brush, Orcellex. Fifty healthy volunteers were recruited. From each subject, four samples were collected using “Orcellex” from apparently normal oral mucosal sites. A plastic spatula was also used to obtain an additional sample. Data on the tolerability and acceptability of the Orcellex were collected from the subjects, together with assessments of the adequacy of LBC slide preparations for cellularity, preparation quality, and the types of cells observed. The Orcellex brush was well accepted by the volunteers, who reported relatively little pain. Orcellex brush LBC preparations were of good quality in terms of cell morphology and staining, with a clean background. Only two smears (2/200; 1%) were found to be inadequate due to low cellularity. All of the plastic spatula LBC preparations were inadequate. Representative cells from all layers of the different oral epithelia examined were documented. Oral liquid-based cytology using the Orcellex brush may have considerable potential for early detection of oral cancer and precancer.

Keywords: oral; brush; liquid-based cytology; oral cancer screening; Orcellex.

Introduction

Oral cancer is a global health problem with an increasing incidence and mortality rate. Approximately 600,000 patients are diagnosed with oral cancer worldwide annually (1). There is considerable scope for oral cancer prevention through detection of early epithelial dysplasia and cellular atypia (2). Early detection of oral cancer, particularly squamous cell carcinoma, can reduce the rate of both morbidity and mortality (3). Exfoliative cytology is a safe, well-tolerated and minimally invasive technique for collecting cells from the oral mucosa. It is also a useful tool for collection of DNA (4). The patient acceptability of this procedure is high, making it an attractive option for detection of early oral cancer.

Several studies have reported the feasibility and reliability of oral brush sampling for collecting an adequate number of cells from the oral mucosa for subsequent liquid-based cytology (LBC) (5-8). However, a good, custom-designed brush for oral cavity sampling has been lacking.

The authors (OK, PS) have collaborated with Rovers Medical Devices B.V. to develop a new oral sampler called the “Orcellex brush”. This brush was designed specifically to permit sampling of cells from within intra-epithelial locations, where most dysplastic cells are found.

In this study, we evaluated the ability of the Orcellex to collect an adequate number of cells from the oral mucosa for LBC studies. Our specific objectives were: 1)

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Table 1 Subjects fulfilling the following criteria were eligible for entry into the study

Inclusion criteria	Exclusion criteria
All healthy males or females aged 18 and over	Subjects with a known history of oral cancer
All subjects with apparently normal oral mucosa	Subjects with a known history of oral mucosal lesions
	Subjects with known systemic diseases
	Subjects who were too distressed or upset to be approached

Fig. 1 The Rovers Orcellex brush.

to determine patient tolerability, 2) to assess cell-capture rates including the number of cells, and the types of cells collected, for subsequent LBC tests, 3) to determine the specificity of cell capture through analysis of the cytological structure of normal cells taken from a different location within the oral mucosa.

Materials and Methods

This study was reviewed and approved by the University of Manchester Research Ethics Committee, United Kingdom (Ref: Kujan-05/102).

Study design and health volunteer recruitment

Fifty healthy volunteers comprising dental nurses, and undergraduate and postgraduate dental students, were recruited from the University Dental Hospital of Manchester, United Kingdom. All volunteers were asked to give informed consent. The data were anonymised by allocating a research number for each volunteer. Patients were recruited in accordance with strict inclusion and exclusion criteria (Table 1).

Study procedures

The study consisted of three stages.

Stage 1: screening

Volunteers were assessed for study eligibility. A thorough oral examination was carried out to rule out any pre-existing oral lesions. Subjects meeting the eligibility criteria were accepted into the study and allocated a research identity number.

Stage 2: sampling

Samples were collected from the oral mucosa using the Orcellex brush (Fig. 1) (Rovers Medical Devices B.V., Oss, The Netherlands). Four oral brush biopsy samples were obtained from four different sites in the oral cavity

including the right and left buccal mucosa, the floor of the mouth, the gingiva, and the lateral border of the tongue. A plastic spatula was used to collect an additional LBC sample from the buccal mucosa. A previous optimization study had revealed that twisting the brush ten times was optimal for capturing an adequate specimen. After sample collection, the brush head was transferred directly to alcohol-based preservative (SurePath, BD Diagnostics, Franklin Lakes, NJ, USA), and transported to the laboratory.

Stage 3: lab processing

A standard protocol for preparation of thin-layer LBC using the SurePath system was undertaken for all LBC specimens. A cytological assessment of the quality of the obtained cells and the yield from each sample was carried out.

LBC sample adequacy and cellularity

Cytological assessment of the quality and yield of cells obtained from each sample was performed in collaboration with the Manchester Cytology Centre. For each sample, cellularity, preparation quality, and the types of cells present were recorded, together with data on microbiota, leucocytes/inflammatory cells and artefacts.

The British Society for Clinical Cytology (2000) has reported that specimen inadequacy is often due to poor cellularity, poor fixation (air drying), and/or excessively thick spreading or obscurity (9). In this study, cellularity was evaluated using the 2001 Bethesda guidelines for liquid-based squamous cellularity in cervical smears (10,11). Using a marking pen, a line was drawn across the centre of each preparation. Ten discontinuous fields across the middle diameter of each preparation were evaluated using a $\times 10$ eyepiece and $\times 40$ magnification. Only well-visualized squamous cells were counted. Large groups containing more than five squamous cells

Table 2 Characteristics of the study subjects

Characteristics	Number (%)
Sex	
Male	22 (44)
Female	28 (56)
Smoking habits	
Smoker	7 (14)
Non-smoker	41 (82)
Ex-smoker	2 (4)
Alcohol habits	
Drinker	21 (42)
Non-drinker	29 (58)

Table 3 Discomfort or pain felt by the study subjects

	Discomfort degree			Pain (yes) n (%)
	No discomfort very mild (0-20%) n (%)	Mild-moderate (21-60%) n (%)	Severe-very severe (61-100%) n (%)	
Buccal smears	37 (74)	14 (28)	2 (4)	3 (6)
Lateral border of tongue smears	13 (26)	30 (60)	7 (14)	12 (24)
Gingiva/hard palate smears	11 (22)	25 (50)	14 (28)	19 (38)
Floor of the mouth smears	4 (8)	33 (66)	13 (26)	24 (48)

Table 4 Reported tolerability of oral scraping using the Orcellex brush

Tolerability question	Responded yes (%)
Would you have the same procedure done again?	92
Would you have, in theory, tolerate six samples in a day?	66
Would you have, in theory, tolerate six samples (consecutive days)?	70
Would you have, in theory, tolerate six samples (consecutive weeks)?	78

were counted in a single focal plane. The average cell count from the 10 fields ($\times 40$) was used for comparison of cellularity. An average of at least seven cells per field was required in order to achieve the 5,000 cells/preparation minimum guideline for specimen adequacy.

Statistical analysis

A sample size of 50 subjects was considered to have 90% power to detect a 5% difference in proportion using the investigators' approach at a two-sided significance level of 0.05. Statistical analyses were performed using the SPSS software package (SPSS, Chicago, IL, USA). Comparisons of sample variance between anatomical sites in the oral cavity and other variables (smoking and alcohol consumption) were tested using X^2 or Fisher's exact test. All tests were two-sided, and differences at $P < 0.05$ were considered to be statistically significant.

Results

Characteristics of the study participants

The demographic characteristics of the 50 healthy volunteers included in the study are presented in Table 2. Most of the subjects were non-smokers (41; 82%). However, seven were smokers (14%), and two were ex-smokers (4%).

Subject feedback on clinical smear collection

The subject acceptability of the procedure was evaluated in terms of two domains: pain and discomfort (Table 3). Over two-thirds (37/50; 74%) of the subjects reported either no or only very mild discomfort due to sampling. In terms of specific oral sites, there were variable degrees of discomfort ranging from very mild to severe. Specifically, brushing the floor of the mouth elicited the highest rate of discomfort compared with buccal sampling. Obtaining smears from the mouth floor was associated with more pain than for other oral sites, 24 subjects reporting pain (48%).

Volunteers answered four questions relating to overall tolerability and willingness to undergo repeat sampling. Their answers were based on both actual and hypothetical experience (Table 4). Any adverse events were recorded. Three subjects reported post-sampling traumatic ulcers, particularly in the floor of the mouth. These ulcers healed normally without leaving any scars.

Cytological assessment of Pap slides

Two hundred and fifty Pap smears were valid for cytological evaluation.

Fig. 2 Oral liquid-based cytology preparation from normal oral mucosa (buccal mucosa): a) Overview ($\times 100$), b) Basal and parabasal cells ($\times 400$), c) Spinosal (Intermediate) cells ($\times 400$).

Table 5 Differences in smear composition according to location

Location of the sample	Superficial cells (%)	Spinosal (intermediate) cells (%)
Buccal mucosa	30-40	60-70
Lateral border of tongue	25-30	70-75
Gingiva/hard palate	40-50	50-60
Floor of the mouth	10-20	80-90

Fig. 3 Inadequate oral LBC preparation collected by a plastic spatula ($\times 100$).

Smear adequacy

All LBC preparations showed good (adequate) preparation quality, including cytoplasmic and nuclear staining with a clear background. However, two smears (2/200; 1%) were found to be inadequate; both had low cellularity. The cellularity of the LBC preparations ranged from 35,000-90,000 cells with a mean of 55,500 cells on each slide. In all studied smears, the cells were distributed evenly. The vast majority of the observed cells were single, but a few cell clumps were evident (Fig. 2a). There were no other reasons for “smear inadequacy” such as blood staining, inflammatory exudates, air-drying artefacts and excessive smear thickness. In fact, the presence of mucus, polymorphs and bacteria was so low as to have no impact on specimen adequacy.

Types of cells observed

All specimens were made up of cell populations representative of all layers of the normal epithelium: basal/parabasal, spinosal (intermediate) and superficial cells (Fig. 2b, c). Keratin-related features such as parakeratosis,

single keratoses and keratin pearls were also evident. Also, anucleate cells suggestive of ortho-keratinisation were also seen. We observed differences in cell populations according to the location from which smears were taken (Table 5). LBC preparations from either the buccal mucosa or lateral border of the tongue had more superficial cells (red cells) than those taken from the floor of the mouth. This is a distinctive feature that can be used to differentiate between these preparations. Additionally, preparations from either the gingiva or hard palate had a characteristically high percentage of anucleate cells. Although preparations from the gingiva tended to be less cellular than those from other sites, no significant inter-site differences in the mean average number of cells were evident ($P = 0.627$). All samples that had been collected using the plastic spatula were inadequate due to low cellularity (Fig. 3).

Discussion

As oral exfoliative cytology is a simple non-aggressive technique that is well accepted by patients, it was used for a large number of studies between 1955 and 1975. However, the role of this technique in screening for oral cancer and precancer has never matched the degree of success reported for diagnosis of cervical cancer (12), and in fact has low sensitivity for the former purpose. The high rate of false negativity has been attributed to several factors, including inadequate sampling, a high risk of procedural error, and the need for subjective interpretation of the findings (13,14). However, in the last few years, interest in oral cytology as a diagnostic and prognostic methodology, and also for monitoring patients with oral cancer and potentially malignant disorders, has re-emerged (4,15,16).

In the scientific or commercial field, no cytobrush specifically designed for oral use has emerged, except for the Oral CDx brush, which was distributed as part of a kit. Oral CDx is quite similar to a conventional brush for oral cytology except that the collection device differs, and computer-assisted screening is employed (17,18). The oral CDx system has been shown to be capable of

obtaining transepithelial specimens. Several studies have assessed the accuracy of the Oral CDx and reported high sensitivity and specificity for detection of oral squamous cell carcinoma and precancerous lesions in comparison with scalpel biopsy (16,18-20). However, there has been some controversy about the sensitivity and specificity of this system (17,19,21,22). A modified LBC technique using the Oral CDx brush without computer-assisted analysis to detect oral lesions has been tested (23,24). The results were encouraging, with high specificity, sensitivity and accuracy for detection of oral malignancy/dysplasia in transepithelial cells (23,24). However, none of these previous studies specifically investigated patient acceptability of the technique, or the number and types of cells that were obtained. Moreover, a comparative study of the cytobrush, curette and Oral CDx brush using LBC failed to demonstrate any differences among the cytological preparations of these three instruments. Furthermore, no basal cells were found in any of the samples analysed (24).

A modified oral brush cytology technique using a commercially available nylon baby toothbrush (Johnson & Johnson, Mumbai, India) without LBC preparation has been assessed (25,26), and found to have potential for low-cost screening of oral cancer. However, the degree of cellularity and the types of specimens obtained were not assessed.

Interestingly, we have shown in a previous publication that oral cytology using LBC is a useful tool to obtain adequate samples from the normal oral mucosa. However, a custom-designed oral brush was warranted (8). We previously collected samples using the “Cytobrush Plus GT” (Medscand Medical AB, Stockholm, Sweden) and “CervexBrush” (Rovers) (8). The observed cellularity of the LBC preparations produced by these brushes ranged from 15,000-40,000 cells with a mean of 25,000 in each slide (8). In the current study, the Brush collected representative cells from all oral mucosa layers; basal/parabasal, spinosal (intermediate) and superficial cells. Also, the marked cellularity of the Orcellex LBC preparations ranged from 35,000-90,000 cells with a mean of 55,500 cells in each slide. We believe the unique geometry design of the Orcellex brush (Fig. 1) can be attributed to its success in obtaining representative cells from all layers of the oral epithelium. According to the Rovers manufacturer, the brush was made of a special polyethylene that can give rigidity to help the fine bristles penetrate the epithelium layers and harvest the cells with minimum discomfort. Additionally, the bristles length and orientation were configured to meet the diverse oral anatomical subsites.

Several studies have investigated oral pathological samples obtained using oral brushing and liquid-based cytology (5-7,23-25,27-29) and reported that oral brushing with liquid-based cytology was useful for obtaining samples from oral lesions, giving better results in terms of both sensitivity and specificity, and providing material for further investigation. However, none of those studies used the Orcellex brush as a sample collection tool.

In the present study, the Orcellex yielded adequate samples for both diagnostic and research purposes using LBC. It has well accepted by the volunteers, and considered relatively painless. Therefore, the Orcellex brush appears to be a non-invasive tool for obtaining samples from the oral mucosa. The present oral liquid-based cytology findings suggest that this device can be used with confidence to sample the oral epithelium, showing a capacity to obtain intraepithelial samples representative of all layers of the oral epithelium. If a non-invasive method of DNA collection is required, then the Orcellex brush Kit would be an appropriate choice. Intriguingly, the Orcellex brush yielded an adequate sample for chip-based quantitative assessment of malignancy risk in a cohort of 714 patients with potentially malignant oral lesions (30). Further research is needed to compare the yield obtained using the Orcellex brush with other collection tools and devices.

In conclusion, the Orcellex brush yields adequate oral samples for diagnostic and research applications. Oral brush cytology with liquid-based preparation might have considerable potential for early detection of oral cancer and potentially malignant disorders.

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Conflict of interest

We would like to thank Rovers Medical Devices for their help in designing and prototyping the new oral brush, Orcellex, and for sponsoring the consumables used in the study. Also, we are grateful to BD Diagnostics (SurePath Systems) for providing the vials for the LBC study. We have no potential conflict of interest to declare.

References

1. Shield KD, Ferlay J, Jemal A, Sankaranarayanan R, Chaturvedi AK, Bray F et al. (2017) The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. *CA Cancer J Clin* 67, 51-64.
2. Speight PM, Epstein J, Kujan O, Lingen MW, Nagao T, Ranganathan K et al. (2017) Screening for oral cancer-a

- perspective from the Global Oral Cancer Forum. *Oral Surg Oral Med Oral Pathol Oral Radiol* 123, 680-687.
3. Mulot C, Stucker I, Clavel J, Beaune P, Loriot MA (2005) Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash, and treated card. *J Biomed Biotechnol* 2005, 291-296.
 4. Alsarraf HA, Kujan O, Farah CS (2017) The utility of oral brush cytology in the early detection of oral cancer and oral potentially malignant disorders: a systematic review. *J Oral Pathol Med*, doi: 10.1111/jop.12660.
 5. Kujan O, Desai M, Sargent A, Bailey A, Turner A, Sloan P (2006) Potential applications of oral brush cytology with liquid-based technology: results from a cohort of normal oral mucosa. *Oral Oncol* 42, 810-818.
 6. Navone R, Pentenero M, Gandolfo S (2011) Liquid-based cytology in oral cavity squamous cell cancer. *Curr Opin Otolaryngol Head Neck Surg* 19, 77-81.
 7. Dona MG, Giuliani M, Vocaturo A, Spriano G, Pichi B, Rollo F et al. (2014) Cytology and human papillomavirus testing on cytobushing samples from patients with head and neck squamous cell carcinoma. *Cancer* 120, 3477-3484.
 8. Braz-Silva PH, Santos RT, Schussel JL, Gallottini M (2014) Oral hairy leukoplakia diagnosis by Epstein-Barr virus in situ hybridization in liquid-based cytology. *Cytopathology* 25, 21-26.
 9. Payne N, Chilcott J, McGoogan E (2000) Liquid-based cytology in cervical screening: a rapid and systematic review. *Health Technol Assess* 4, 1-73.
 10. Wright TC Jr, Cox JT, Massad LS, Twigg LB, Wilkinson EJ (2002) 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. *JAMA* 287, 2120-2129.
 11. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M et al. (2002) The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 287, 2114-2119.
 12. Ogden GR, Cowpe JG, Wight AJ (1997) Oral exfoliative cytology: review of methods of assessment. *J Oral Pathol Med* 26, 201-205.
 13. Diniz-Freitas M, Garcia-Garcia A, Crespo-Abelleira A, Martins-Carneiro JL, Gandara-Rey JM (2004) Applications of exfoliative cytology in the diagnosis of oral cancer. *Med Oral* 9, 355-361.
 14. Dabelsteen E, Roed-Petersen B, Smith CJ, Pindborg JJ (1971) The limitations of exfoliative cytology for the detection of epithelial atypia in oral leukoplakias. *Br J Cancer* 25, 21-24.
 15. Acha A, Ruesga MT, Rodriguez MJ, Martinez de Pancorbo MA, Aguirre JM (2005) Applications of the oral scraped (exfoliative) cytology in oral cancer and precancer. *Med Oral Patol Oral Cir Bucal* 10, 95-102.
 16. Sciubba JJ (1999) Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. *J Am Dent Assoc* 130, 1445-1457.
 17. Greenberg MS (2002) The "brush" controversy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 93, 217-218.
 18. Mehrotra R, Mishra S, Singh M, Singh M (2011) The efficacy of oral brush biopsy with computer-assisted analysis in identifying precancerous and cancerous lesions. *Head Neck Oncol* 3, 39.
 19. Svirsky JA, Burns JC, Carpenter WM, Cohen DM, Bhattacharyya I, Fantasia JE et al. (2002) Comparison of computer-assisted brush biopsy results with follow up scalpel biopsy and histology. *Gen Dent* 50, 500-503.
 20. Kosicki DM, Riva C, Pajarola GF, Burkhardt A, Gratz KW (2007) OralCDx brush biopsy--a tool for early diagnosis of oral squamous cell carcinoma. *Schweiz Monatsschr Zahnmed* 117, 222-227.
 21. Rick GM (2003) Oral brush biopsy: the problem of false positives. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 96, 252.
 22. Lingen MW, Kalmar JR, Karrison T, Speight PM (2008) Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol* 44, 10-22.
 23. Delavarian Z, Mohtasham N, Mosannen-Mozafari P, Pakfetrat A, Shakeri MT, Ghafoorian-Maddah R (2010) Evaluation of the diagnostic value of a Modified Liquid-Based Cytology using OralCDx Brush in early detection of oral potentially malignant lesions and oral cancer. *Med Oral Patol Oral Cir Bucal* 15, e671-676.
 24. Reboiras-Lopez MD, Perez-Sayans M, Somoza-Martin JM, Antunez-Lopez JR, Gandara-Vila P, Gayoso-Diz P et al. (2012) Comparison of three sampling instruments, Cytobrush, Curette and OralCDx, for liquid-based cytology of the oral mucosa. *Biotech Histochem* 87, 51-58.
 25. Mehrotra R, Singh MK, Pandya S, Singh M (2008) The use of an oral brush biopsy without computer-assisted analysis in the evaluation of oral lesions: a study of 94 patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 106, 246-253.
 26. Gupta S, Shah JS, Parikh S, Limbdiwala P, Goel S (2014) Clinical correlative study on early detection of oral cancer and precancerous lesions by modified oral brush biopsy and cytology followed by histopathology. *J Cancer Res Ther* 10, 232-238.
 27. Navone R, Burlo P, Pich A, Pentenero M, Broccoletti R, Marsico A et al. (2007) The impact of liquid-based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. *Cytopathology* 18, 356-360.
 28. Navone R, Pentenero M, Rostan I, Burlo P, Marsico A, Broccoletti R et al. (2008) Oral potentially malignant lesions: first-level micro-histological diagnosis from tissue fragments sampled in liquid-based diagnostic cytology. *J Oral Pathol Med* 37, 358-363.
 29. Hayama FH, Motta AC, Silva Ade P, Migliari DA (2005) Liquid-based preparations versus conventional cytology: specimen adequacy and diagnostic agreement in oral lesions. *Med Oral Patol Oral Cir Bucal* 10, 115-122.
 30. Abram TJ, Floriano PN, Christodoulides N, James R, Kerr AR, Thornhill MH et al. (2016) 'Cytology-on-a-chip' based sensors for monitoring of potentially malignant oral lesions. *Oral Oncol* 60, 103-111.