Original Article

Oral epithelial cell harvesting techniques
-A comparative cytohistologic evaluation

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ABSTRACT

Abstract: The concept of trans-epithelial cellular sampling was boosted by the use of cytobrush, which claimed superiority in harvesting epithelial cells over that of the conventionally used wooden and metal spatulae.

Aim: To compare the efficacy of oral epithelial cell harvesting using perio-interdental brush and the conventional wooden spatula.

Objective: To assess the quantity of cells (in area percent), distribution of cells, and proportion of oral epithelial cells exhibiting cytotoxicity in sampling techniques using the Perio-interdental brush and wooden spatula.

Methodology: The study group comprised twelve informed, consenting patients exhibiting clinical white lesions. Two smears were collected from each patient from closely adjacent lesional sites using Perio-interdental brush followed by wooden spatula. One hundred cells were counted in each Papanicolaou-stained smear under ×400 magnification and categorized as basal, intermediate and superficial cells. The number of cells exhibiting evidence of genotoxicity (as evidenced by cytoplasmic granules, micronuclei, binucleation) and atypia were tabulated. Distribution of cells was analyzed by Image J. Statistical analysis was carried out using SPSS software. Student T test was used to assess the efficacy of sampling between the two techniques.

Results and discussion: Yield of cells (in area percentage) and proportion of identification of cytotoxicity (p value= 0.340 and $p < 0.6$ respectively) was greater with Perio-interdental brush compared to wooden spatula. No significant difference was noted in dispersion of cells between the two techniques (p value = 0.751). The high yield of cells per sample and superiority in identifying cellular cytotoxic changes using the Perio-interdental brush as seen in this study may potentially make it a **cost-effective tool in screening programmes for oral cancer,** and the findings may be validated using a larger sample.

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INTRODUCTION

Oral exfoliative cytology, the study of cells obtained from oral epithelium, is a simple, inexpensive, rapid, patient-friendly technique[1]. This is accomplished by scraping the surface of the mucosa (using wooden spatulas, metal spatulas, cotton swabs, plastic spatulas etc.), rinsing the oral cavity, sampling of saliva, and by using a cyto-brush[2]. Various reviews on the reliability of different instruments used for *oral epithelial cell sampling* have shown that a cyto-brush is an efficient instrument to obtain transepithelial cellular samples. However, its relatively higher cost has limited its routine use in exfoliative cytology[2].

An endocervical brush that resembles a small bottlebrush with fine bristles made of nylon at one end (Fig 1) was introduced in the 1980's for the purpose of cervical smears for gynaecological lesions and demonstrated a better cell spreading when compared to smears obtained using a wooden spatula[2]. Since the perio interdental brush, used as an interdental cleaning aid, bears close resemblance to the endocervical brush (Fig 2), the present study used the perio interdental brush to know its efficacy in harvesting epithelial cells from oral cavity.

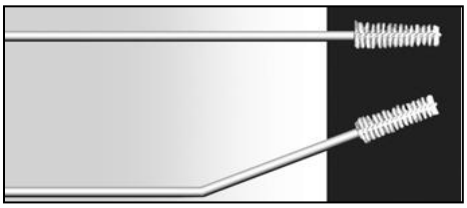


Fig 1. Endocervical brush

Fig 2. Perio interdental brush

The study was initiated upon receiving clearance from Institutional Ethical committee and the informed consent from 12 subjects exhibiting clinical white lesion (Leukoplakia) in the buccal mucosa. Relevant habit history was recorded. Two samples were collected from each patient using perio interdental brush and wooden spatula. The bristled end of the perio interdental brush was rotated over the lesion (10 times in clock-wise direction)[3] and the moistened wooden spatula was scraped over the lesion to harvest epithelial cells. The samples thus obtained were spread on the glass slide, immersed in cytological fixative (1:1 mixture of ether and alcohol) and followed by PAP staining method.

100 cells were counted in each smear under 40 x magnification. Cells were categorised as basal (blue), intermediate (pink) and superficial (orange) respectively (Fig 3). Cytoplasmic granularity was interpreted as evidence of 'Genotoxicity' (Fig 4). Cells exhibiting nuclear changes like micronuclei and binucleation were categorized as 'Atypical' (Fig 5).

Fig.3 Cells of different strata

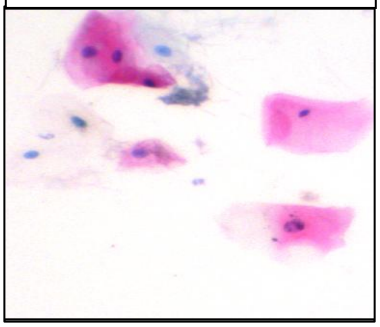


Fig. 4 Cytoplasmic granules

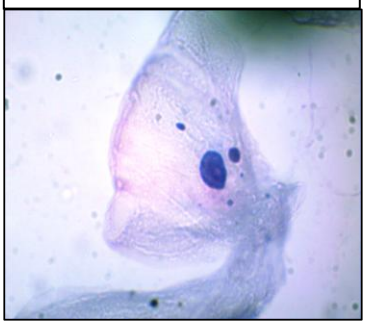
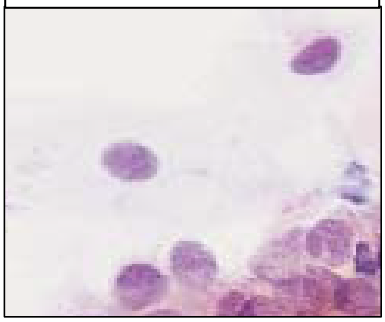


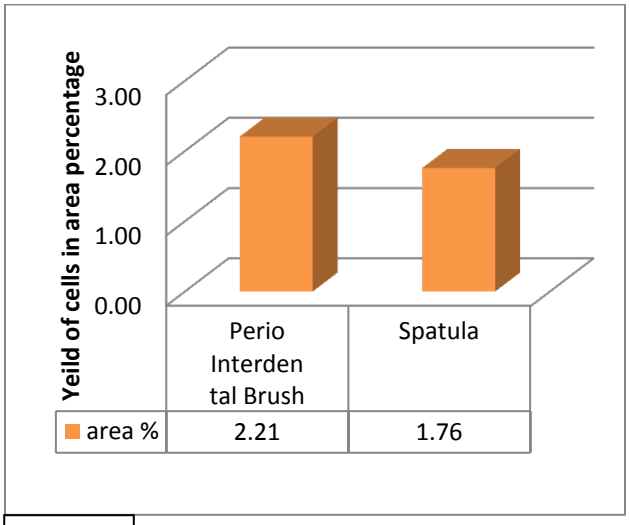
Fig. 5 Cells with Nuclear



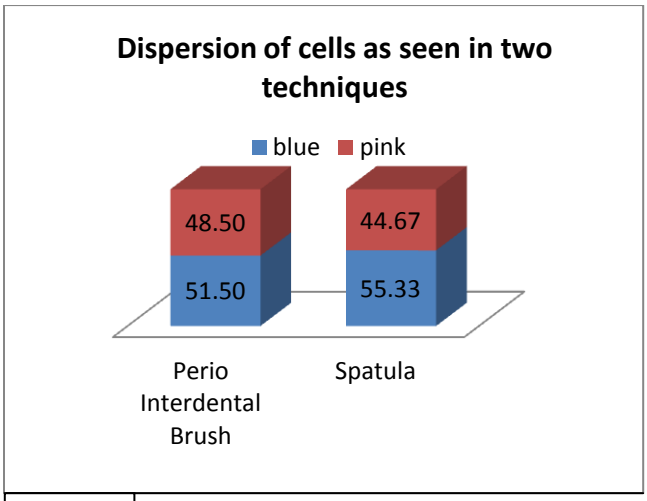
The area of slide showing cells were highlighted semiautomatically using threshold tool. The percentage of area occupied by cells was then measured using Image J software (version 1.46r, NIH USA).The percentage of cells in each stratum, genotoxicity and atypia were compared between the two sampling techniques using student's t test or Mann Whitney U test based on the distribution of the data. Statistical analysis was carried out using SPSS software.

RESULTS

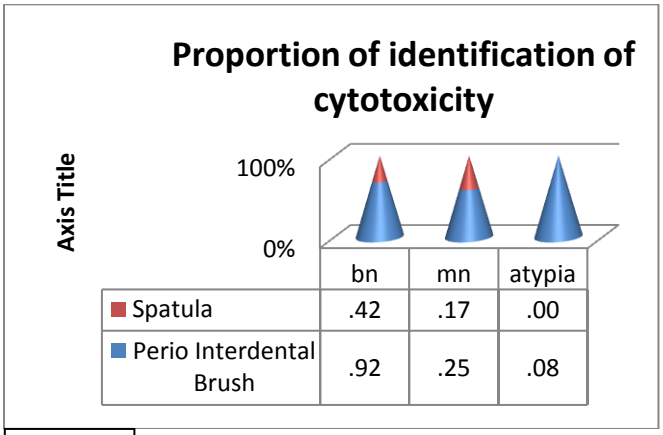
Yield of cells in area percentage was comparatively greater in samples obtained from perio interdental brush than from the wooden spatula (p value = 0.34; Graph 1). No significant difference was noted between dispersion of cells (basal and para basal) between both instruments (p value = 0. 71; Graph 2). Use of perio interdental brush enabled significantly higher identification of cells exhibiting cytotoxic changes (p value = 0.32; Graph 3).



Graph 1



Graph 2



Graph 3

DISCUSSION

Dentists and medical practitioners are generally the first to encounter patients with oral mucosal alterations, and may thus be the critical link in screening for cases of oral cancer. Surgical biopsy followed by histopathology, a gold standard for diagnosis, may not be feasible in all such cases as it is invasive, time consuming, and demands patient cooperation[4,5]. The Brush Biopsy (CDx Laboratories, Suffren, NY) was introduced as a *"potential oral cancer case-finding device"* in 1999[6]. While exfoliative/scrape cytology (or an oral brush biopsy) is not a substitute for a tissue/scalpel biopsy, its convenience can be utilised as a 'first-level' test to identify dysplastic cells or molecular alterations that

would indicate histological control, even in clinically apparently benign oral lesions[7,8.]

Edris AM et al (2011) in a study of involving 50 cases each of normal buccal mucosa and oral lesions (OSCC = 28, Leukoplakia = 8, dysplasia = 3), showed that scrape cytology offered 100% specificity, 87.5% sensitivity and an accuracy of 95%[9]. Similarly, Dolens E et al (2013) in a review emphasized 87.4% sensitivity of scrape cytology[1,10.] Ogden GR, Cowpe JG and Green M in an identical comparative study on oral lesions (1992) found greater cell yield and dispersion with cytobrush than with a wooden spatula, with the former being more efficient in sampling the less accessible sites[11]. Similar findings were reported by Reboiras-Lopez MD et al[5], and Mehrotra R[12] and Queiroz JB et al, who also reported qualitatively better smears in terms of cellularity and homogeneity with cytobrush compared to a metal spatula[13]. Commercially available tooth brushes were also used by Babshet M et al for sampling with reported sensitivity and specificity of 77% and 100% respectively[14]. Furthermore, the brush was also used to diagnose oral infections caused by bacteria, fungus and HSV[5,10,15].

Our study results showed that cell yield and proportion of identification of cytotoxic changes was more in samples obtained by cytobrush than in wooden spatula. The bristles of cytobrush access larger surface area and provide multidirectional cell sampling as compared to uni-directional sampling that occurs with a wooden spatula (p = 0.34).

The dispersion of basal and parabasal cells did not differ much between the two instruments/techniques (p value = 0. 71). This parallels the findings of a comparitive study by Queiroz JB et al (2010), who used a cytobrush and a metal spatula on normal oral mucosa, and found no difference in dispersion of cells from different epithelial strata[13].

Since the cells of the basal layers are more accessible to the cytobrush than the wooden spatula, and due to the fact that cytotoxic changes are first noted in the basal cell compartment[5], the identification of such cells exhibiting cytotoxic changes was enhanced with the perio interdental brush (p = 0.32).

Conclusion:

Being aware of the limitations of time, and factors such as cost-effectivness and patient compliance, the perio-interdental brush is ideally poised to be an effective chair-side tool in screening for oral cancer, monitoring of radiation response in oral mucosa, diagnosis of oral infections like candidiasis, viral infections (HSV), and oral diseases like pemphigus[5]. Further, it can also assist in cytological evaluation by newer techniques like DNA analysis, immunocytochemistry, and molecular analysis, besides liquid-based preparations[1].

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