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# Exploring the potential of interdental brush in oral cytology: a pilot study on sampling efficiency

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## Abstract

**Background** With the renewed interest in brush biopsies, particularly for screening oral potentially malignant disorders and cancers, understanding the efficacy of available brush types in oral cytology is crucial. This study aims to compare the effectiveness of interdental brush sampling with commonly used cytology brushes in oral cytology evaluation.

**Methods** The study design was an observational clinical study. Brush biopsies were collected from the buccal mucosa of 99 healthy volunteers using an Interdental Brush- TePe Original Interdental Brush (TePe, Malmö, Sweden) (Cell sampling-Interdental Brush (CSIB)), Cytobrush Plus GT (Medscand Medical AB, Sweden) (Cell sampling device 1 (CSD1)), and CervexBrush (Rovers, Oss, Netherlands) (Cell sampling device 2 (CSD2)). Samples were evaluated for cellularity, depth of the epithelial layer, and cellular integrity. Results were compared between brush types using the Friedman test, and multiple comparisons were examined using the Dunn test. The significance level was set at  $p < 0.05$ .

**Results** Of the 99 cases, 59.6% were female and 40.4% were male, with a prevalence of smoking and alcohol consumption at 43.4%. The mean age was 24.3 years ( $\pm 4.5$ ). There were no significant differences in cellularity between the brush types ( $p = 0.205$ ). Mild cellularity was observed at rates of 3%, 9.1%, and 9.1% for CSD1, CSIB, and CSD2, respectively. Parabasal/basal layer cells were detected in 1% of samples collected with CSD1 and 4% with CSD2, while CSIB samples contained only superficial/intermediate cells. CSIB produced an inadequate integrity rate of 30.3%, whereas neither CSD2 nor CSD1 yielded samples with inadequate integrity.

**Conclusions** Interdental brushes may have potential as accessible and practical tools for chairside cytological screening. The CSIB obtained high-cellularity samples; however, it demonstrated low scores in cellular integrity. Future oral cytological studies may explore the modifications in interdental brush design to improve its potential in sampling for cell preservation and integrity.

**Keywords** Cyodiagnosis, Oral mucosa, Exfoliative cytology

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## Background

Cancer constitutes a leading global cause of mortality, with particularly dismal survival rates observed among patients afflicted with oral squamous cell carcinoma (OSCC). OSCC stands as the most common malignancy affecting the oral cavity and merely 50% of individuals manage to survive the initial 5 years subsequent to diagnosis [1, 2]. This circumstance predominantly attributed to the frequent late-stage detection of OSCC whereas early detection leads to around an 80% survival rate for patients over five years [1, 3]. OSCC frequently arise after the onset of oral potentially malignant disorders (OPMD) which comprises a diverse spectrum of conditions, such as leukoplakia, erythroplakia, proliferative verrucous leukoplakia, lichen planus, oral submucous fibrosis, and actinic keratosis. Though the existence of OPMD heightens the risk of lip or oral cavity cancer, the complete trajectory or mechanism of OSCC remains somewhat elusive and only a portion of those with OPMD progress to malignancy [2, 4].

Screening for early diagnosis of OPMD and OSCC has been encouraged in an effort to lower the death rates from oral cancer. Programs for screening play a part in referral for biopsies, histological analysis, and other diagnostic procedures. The process of finding asymptomatic people who most likely have a disease and using testing to separate them from others who might not is known as screening. The visual and tactile oral examination, is the most often used screening procedure. Additionally, many non-invasive, supplementary diagnostic techniques such as vital staining, oral cytology, and light-based assays have been documented in the literature for the purpose of screening OSCC [5–7]. Among these tests, oral cytology swiftly carried out in an outpatient setting, ensures minimal or no discomfort for the patient and doesn't necessitate anesthesia. It's an easy to use, minimally invasive procedure that shows promise [2, 8, 9]. Furthermore, oral cytology is evolving beyond a screening tool, offering molecular insights into OSCC progression and prognosis. Recent studies highlight its potential for non-invasive risk assessment through DNA image cytometry, micro-RNA profiling, and DNA methylation analysis [10, 11].

To collect samples from the oral mucosa, various brushes with different physical properties and diverse manufacturing purposes are used. The Oral CDx® brush (CDX Laboratories Inc., Suffern, NY, USA) utilizes computer-assisted analysis to identify abnormal cells across all layers of the oral mucosa epithelium. The Oral CDx® cytologic test has demonstrated a high degree of sensitivity and specificity in identifying dysplastic epithelial changes in clinically high-risk lesions [12]. The Oracellex® brush (Rovers Medical Devices B.V., Oss, the Netherlands), when combined with liquid-based cytology

(LBC), produces high-quality samples with excellent cellularity and well-preserved cell morphology. It has shown high sensitivity (95.6%) for detecting malignancies [3]. These brushes are specifically designed and used for oral brush biopsy. However, the availability of these specialized brushes is relatively limited [13].

Considering the availability of various brush types, the most commonly used brushes for oral mucosa sampling have been reported to be cylindrical-shaped brushes. These brushes feature a slender, flexible stem with soft, tapered nylon bristles [14] and are primarily used in Papanicolaou (Pap) smear tests for sampling epithelium from the endocervix. Additionally, triangular-shaped brushes, mainly designed for medical purposes, have semi-circular, soft, and flexible bristles that radiate outwards [15] and are also used for brush biopsies [16]. Beyond these specialized brushes, studies have reported the use of toothbrushes for oral mucosa sampling, highlighting that these easily accessible products can be especially useful as a cost-effective option and could be advantageous in areas with limited resources [17].

In this study, we aimed to compare the most easily accessible cell sampling devices efficiency in oral cytology practice. The interdental brushes, are affordable, easily accessible tools produced for oral hygiene purposes, were tested for the first time for brush biopsy sampling in the present study. The null hypothesis of the present study is that there is no statistically significant difference in the effectiveness of sampling oral mucosa among three different types of brushes.

## Methods

### Study population, sample size

The participants, comprising healthy volunteers including dental nurses, undergraduate, and graduate dental students from Bahçeşehir University Dental Hospital, were selected for the study. Informed consent was obtained from all volunteers, and data were anonymized by assigning a unique study number to each participant. A thorough anamnesis and clinical examination was performed and cytological material of the study population was collected in the department of Oral and Maxillofacial Radiology. Inclusion and exclusion criteria were adhered to when selecting patients for the study. Subjects who were older than eighteen were eligible to participate in the study. On the other hand, people with a history of oral cancer, systemic illness, or oral mucosal lesions were excluded from the study.

Based on the power analysis conducted using the results of the pilot study ( $n = 40$ ), a minimum sample size of  $n = 75$  participants was determined to achieve 80% power (power = 0.80) at a 5% significance level ( $\alpha = 0.05$ ) for detecting statistical significance in the integrity levels

between groups. This calculation was based on an effect size (d) of 0.3187 and a standard deviation of 0.8. Sample size estimations were performed using G\*Power 3.1.9.4 (Franz Faul, Universität Kiel, Kiel, Germany).

#### **Institutional review board statement**

The study protocol was approved by the Bahçeşehir University Ethics committee (project number 2023–21/05). The study was performed in accordance with the tenets of the 1964 Helsinki Declaration and its later amendments.

#### **Informed consent statement**

Informed consent was obtained from all subjects involved in the study.

#### **Cytological material collection**

To minimize operator-related variability, all brush biopsies were performed by a single examiner (E.Ç) who has seven years of experience in Oral and Maxillofacial Radiology. Before the oral brush biopsy technique, a saline mouth rinse was employed to remove debris. Before getting buccal cells, all patients were asked to wait at least one hour to eat or drink. Sample collection performed using three types of brushes;

Cell Sampling Device 1 (CSD1): A medical brush with a slender, flexible stem and soft, tapered nylon bristles (Cytobrush Plus GT, Medscand Medical AB, Sweden).

Cell Sampling Device 2 (CSD2): A medical brush with semi-circular, soft, and flexible bristles that radiate outward (CervexBrush, Rovers, Oss, Netherlands).

Cell sampling-Interdental Brush (CSIB): Interdental Brush- TePe Original Interdental Brush size 6-medium (TePe, Malmö, Sweden).

The head of the brushes was placed tangentially to the buccal mucosa, then ten rotations and a sweeping motion were performed. Subsequently, the collected cell samples were spread on slides by rolling with a brush and fixed using a methylated spirit. Every brush biopsy was performed on the left or right buccal mucosa, avoiding the collection of samples from the same location.

#### **Cytologic evaluation**

The specimens were sent to an oral pathologist with over twenty years of experience. The conventional cytological evaluation has been carried out using standard procedures. Routinely the pathologist stained the cell samples with Pap stain. Subsequently, the slides were examined under a light microscope at magnifications of 10X, 40X, and 100X (Fig. 1).

The evaluation criteria included:

- **Cellularity:** defined as the number and quality of the collected cells, assessed by analyzing the number, distribution, and homogeneity of cells (1. mild, 2. moderate, 3. severe).
- **Depth of the epithelial layer:** deepest epithelial layer from which the collected cells are taken (1. superficial/intermediate, 2. parabasal/basal),
- **Cellular integrity:** refers to the general condition and structural preservation of the cells obtained from a sample (1. inadequate, 2. barely adequate, 3. adequate).

#### **Statistical analysis**

Data analysis was conducted using IBM SPSS V23. The Friedman test was employed to compare sample collection capacities based on brush types, and multiple comparisons were examined using the Dunn test. Significance values have been adjusted by the Bonferroni correction for multiple tests. Data are presented as frequencies,

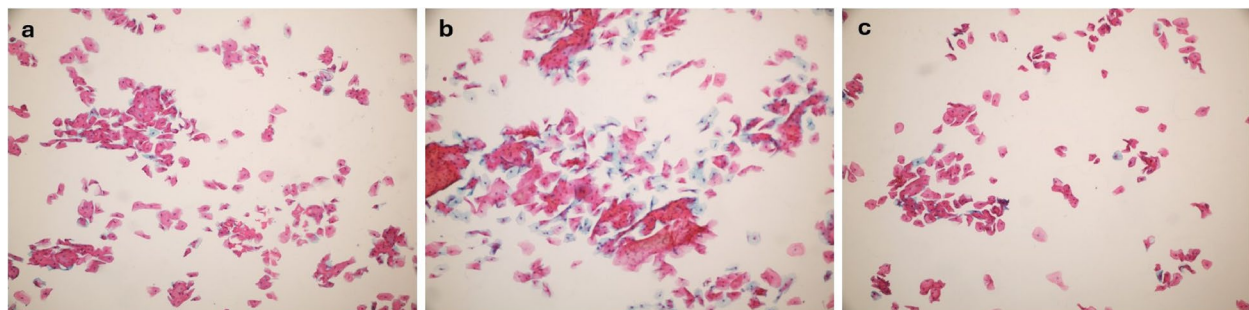


**Fig. 1** Photomicrograph depicting cellular samples collected using (a) Cell Sampling Device 1, (b) Cell Sampling Device 2, and (c) Cell Sampling-Interdental Brush through oral cytology ( $\times 100$  magnification): **a** Moderate cellularity with adequate structural integrity; **b** Severe cellularity with superficial and parabasal cells; **c** Mild cellularity and barely adequate integrity

percentages, means, standard deviations, and Friedman test results including mean ranks, chi-square values, and degrees of freedom. The significance level was set at  $p < 0.05$ .

## Results

### Descriptive statistics

Of the 99 cases included in the study, 59.6% were female, and 40.4% were male. The prevalence of smoking was determined to be 43.4%, while the rate of alcohol consumption was also 43.4%. The mean age was  $24.3 (\pm 4.5)$  years, with age distribution ranging from 18 to 46 years.

### Cellularity values by brush types

Mild cellularity was obtained at a rate of 3% for CSD1, 9.1% for CSIB, and 9.1% for CSD2 (Table 1). When the cellularity values were examined according to brush types, no statistically significant difference was found among them ( $p = 0.205$ ) (Table 2).

### Depth of the epithelial layer values by brush types

The parabasal/basal depth of the epithelial layer was 1% for the CSD1 and 4% for the CSD2. Samples collected with the CSIB showed only superficial/intermediate cells (Table 1). The Friedman test for the depth of the epithelial layer revealed a statistically significant difference ( $p = 0.039$ ) (Table 2), but no significant differences were found in pairwise comparisons.

**Table 1** Frequencies and percentages based on the cellularity, integrity, and depth of the epithelial layer in samples collected using tested brushes

|                               | Brush Types | Mild       | Moderate                 | Severe          |
|-------------------------------|-------------|------------|--------------------------|-----------------|
| Cellularity                   | CSD1        | 3 (3)      | 60 (60.6)                | 36 (36.4)       |
|                               | CSD2        | 9 (9.1)    | 55 (55.6)                | 35 (35.4)       |
|                               | CSIB        | 9 (9.1)    | 56 (56.6)                | 34 (34.3)       |
|                               |             | Inadequate | Barely Adequate          | Adequate        |
| Integrity                     | CSD1        | -          | 52 (52.5)                | 47 (47.5)       |
|                               | CSD2        | -          | 61 (61.6)                | 38 (38.4)       |
|                               | CSIB        | 30 (30.3)  | 44 (44.4)                | 25 (25.3)       |
|                               |             |            | Superficial/Intermediate | Parabasal/Basal |
| Depth of the epithelial layer | CSD1        |            | 98 (99)                  | 1 (1)           |
|                               | CSD2        |            | 95 (96)                  | 4 (4)           |
|                               | CSIB        |            | 99 (100)                 | -               |

\* Data presented as n (%)

CSD1 Cell sampling device 1, CSD2 Cell sampling device 2, CSIB: Cell sampling-interdental brush

**Table 2** Comparison of the tested brushes performance based on the cellularity, integrity, and depth of the epithelial layer parameters

|                               | Brush Types | Mean Rank | Chi-square ( $\chi^2$ ) | df | p*      |
|-------------------------------|-------------|-----------|-------------------------|----|---------|
| Cellularity                   | CSD1        | 2.08      | 3.16                    | 2  | 0.205   |
|                               | CSD2        | 1.97      |                         |    |         |
|                               | CSIB        | 1.95      |                         |    |         |
| Integrity                     | CSD1        | 2.25      | 33.25                   | 2  | < 0.001 |
|                               | CSD2        | 2.12      |                         |    |         |
|                               | CSIB        | 1.64      |                         |    |         |
| Depth of the epithelial layer | CSD1        | 1.99      | 6.5                     | 2  | 0.039   |
|                               | CSD2        | 2.04      |                         |    |         |
|                               | CSIB        | 1.97      |                         |    |         |

Analysis performed using Friedman's test, df: degrees of freedom

CSD1 Cell sampling device 1, CSD2 Cell sampling device 2, CSIB Cell sampling-interdental brush

### Integrity values by brush types

Upon analyzing the integrity values in relation to the various brush types, a statistically significant difference was observed ( $p < 0.001$ ). The rates of adequate integrity differed among the brushes, with CSD1 achieving a rate of 47.5%, CSIB at 25.3%, and CSD2 at 38.4%. Among the samples collected, 30.3% obtained via the CSIB exhibited inadequate integrity. Conversely, neither CSD2 nor CSD1 yielded any inadequate samples (Table 1). The distributions derived from the employment of CSIB indicated a lesser incidence of samples falling within the adequate/barely adequate category when compared to the other instruments. In contrast, there was no discernible distinction between CSD1 and CSD2, both of which demonstrated a higher prevalence of samples within the adequate/barely adequate categories (Table 1). Pairwise comparison of the brushes for integrity reveals a statistically significant difference between CSIB and both CSD1 and CSD2 (Table 3).

**Table 3** Variation in depth of the epithelial layer across different brush types

|           | Pairwise Comparison | Mean Rank Difference | P*    |
|-----------|---------------------|----------------------|-------|
| Integrity | CSIB—CSD1           | 0.61                 | 0.001 |
|           | CSIB—CSD2           | 0.48                 | 0.002 |
|           | CSD2—CSD1           | 0.13                 | 1.000 |

\* Pairwise comparisons conducted using the Dunn test. The significance level is 0.05. Significance values have been adjusted by the Bonferroni correction for multiple tests

CSD1: Cell sampling device 1, CSD2: Cell sampling device 2, CSIB: Cell sampling-interdental brush

## Discussion

Over the past three decades, there has been a growing emphasis on cancer prevention and early detection globally. Screening programs have become an essential part of healthcare systems, particularly in high-income countries, aiming to detect cancer at an asymptomatic stage and ultimately reduce mortality [18, 19]. The standard screening method typically involves a systematic visual examination under bright lighting and palpation of the oral cavity and neck. However, the effectiveness of these programs is often limited by the insufficient ability of many general practitioners, family doctors, and even dentists to recognize early signs of carcinoma, primarily due to inadequate training or experience [1, 20].

Given these challenges, the integration of simple, non-invasive, and accessible cytological sampling methods into routine clinical practice is critical. Oral cancer screening relies heavily on frontline healthcare providers who may have limited access to specialized oral medicine services, particularly in underserved regions. Brush cytology offers a promising approach to enhance early detection at the primary care level by providing an easy-to-use tool for sample collection. However, increasing availability and optimizing post-collection preservation methods, such as LBC, remain crucial for ensuring the reliability and clinical applicability of these samples.

In recent years, several additional methods and techniques have been introduced and even marketed as supplements to visual oral cavity examinations. These aids utilize technologies that employ different wavelengths of light, cytological methods such as Oral CDX and LBC, techniques for detecting saliva proteins, miRNA analysis, and DNA quantification methods [20].

Oral cytology is increasingly being recognized as more than just a screening tool; it has the potential to provide valuable molecular insights into OSCC progression and prognosis. Recent studies have demonstrated that cytological samples can be analyzed using DNA image cytometry, microRNA profiling, and DNA methylation patterns, offering a non-invasive approach to identifying high-risk lesions [10, 11]. For instance, Feulgen-based DNA cytometry has been utilized to assess DNA ploidy in oral leukoplakia, revealing a strong correlation between aneuploidy and the risk of malignant transformation [21]. Similarly, LBC, when combined with AgNOR staining, has shown improved diagnostic accuracy for OSCC [11]. The integration of gene expression profiling—such as AngiomiR-31 as a marker for angiogenesis—has further expanded the scope of cytological analysis [22]. These molecular advancements indicate that oral brush cytology could eventually evolve into a tool for both cellular morphology evaluation and biomarker-based cancer risk assessment. Additionally, LBC

approaches, which involve detecting circulating tumor DNA, exosomes, and microRNAs in saliva and blood, are being investigated for non-invasive cancer monitoring. Given the accessibility of oral cytology, future studies could explore how brush biopsy samples could complement LBC techniques, making OSCC screening more effective and widely applicable [10].

The oral brush biopsy, characterized by exfoliative sampling of the oral mucosa, offers a standardized, user-friendly, and accurate approach with substantial potential for life-saving outcomes. Compared to excisional biopsies, it is minimally invasive and typically painless, factors that contribute to its high patient acceptance [8, 9]. Also implication of this technique doesn't require specialized training, can be done by front line practitioners and dentists [1]. Furthermore, incorporating additional techniques such as DNA-image cytometry [23] or Raman microspectroscopy [24] has led to reduced false positive diagnoses when combined with oral cytology. Collectively, these findings emphasize the potential of the oral brush biopsy as a valuable screening tool for oral lesions.

Various types of brushes are utilized for both conventional and LBC applications. Two brushes have been specifically designed for oral cytology procedures. The Oral CDx<sup>®</sup> Brush Biopsy (CDX Laboratories Inc., Suffern, NY, USA) is tailored for oral use, that facilitate sampling from deeper layers of the oral epithelium [25]. Meanwhile, the Orcellex<sup>®</sup> Brush (Rovers Medical Devices B.V., Oss, the Netherlands) is characterized by a uniquely designed head with five-segmented high-density fibers [26]. The brushes designed for cervical smears, including the Cyto-brush Plus GT (Medscand Medical AB, Sweden) and Cervexbrush<sup>®</sup> (Rovers, Oss, Netherlands), have also been employed for oral cytology. These brushes are primarily designed for pap-smear tests to sample epithelial cells from the cervix and endocervix [16]. In the current study, we employed two of brushes used for medical purposes along with the one type of interdental brush to collect cytological samples from the oral mucosa. Notably, interdental brushes were evaluated for cytological sampling for the first time in this research.

For effective cytological sampling using a brush, it is essential to have bristles that are suitable for the intraoral mucosal structure. These bristles should enable transfer of epithelial cells while preserving their integrity and facilitate collection of an optimal number of cells [13]. Brushes under investigation exhibited similar performance in terms of cellularity and the depth of the epithelial layer, suggesting that, for these measures, the choice of brush type may not significantly impact the outcomes of the sampling procedure. However, the examination of integrity values by brush types revealed a statistically significant difference. Notably, the CSIB demonstrated

a lower success in transporting cells without damaging their integrity compared to the other brushes. This finding raises intriguing questions about the potential influence of brush type on the integrity of sampled cells, possibly attributed to different physical characteristics of the brushes and/or their bristles. Stiffness may be a determinant selectable for interdental brushes, which was the medium used in the present study. One study reported that the stiff brush (Orcellex) had better performance in terms of sensitivity in detecting certain lesions (95.6%) compared to the nylon brush (Cytobrush GT) (93.8%) [3]. The Orcellex Brush features a specially designed head composed of five-segmented high-density fibers. The Oral CDx Brush Biopsy was designed for oral use with stiff bristles, enabling sampling from deeper epithelial layers of the epithelium. However, the clinical utility of these devices remains contentious due to issues of limited availability and high cost [13].

Our study revealed inadequate cellularity rates of 3% for CSD1, 9.1% for CSIB, and 9.1% for CSD2. These results align with Hayama et al.'s study, which reported 6.8% of conventional smears as hypocellular and uninterpretable. Another study evaluating cervical cytology samples demonstrated unsatisfactory cervical cytology rates ranging from 2 to 9% with the Cytobrush and 3–15% with the CervexBrush [27]. In the study by Navone et al. (2007), where Cytobrush was used for conventional cytological examination of 89 suspected lesions, 12.4% of the samples were found to be inadequate [28]. While the methodologies of the mentioned studies differ, the percentages of inadequate samples were comparable to our results.

Another important finding of the present study was the similar performance in collecting cells from the basal/parabasal layer among the tested brushes. Epithelial dysplasia typically initiates in basal layers (stratum germinatum) and extends through all layers of the epithelium. To detect deeper epithelial abnormalities, the brushes must effectively collect cells from the basal/parabasal layer. In the oral cavity, cytology has been of limited use due to the collection of superficial cells and the presence of the keratin layer. Consequently, deeper epithelial abnormalities often go undetected [29]. In this study, parabasal/basal cells were obtained at rates of 0%, 1%, and 4% for CSIB, CSD1, and CSD2, respectively. Kujan et al. (2006) reported that 12% of oral brush biopsy samples from normal mucosa displayed cells from the basal/parabasal layer [16]. In contrast, Reboiras-López et al. (2012) reported that none of the samples acquired with Cytobrush, dermatological curette, or Oral CDx Brush Kit displayed basal cells [30]. Our results similarly showed very low percentages of basal/parabasal layer cells for all tested instruments. These findings highlight the need to

improve the sampling depth of brushes, as this feature is of paramount importance for the early detection of epithelial dysplasia.

The oral brush biopsy technique is still not considered a substitute for scalpel biopsy, and histopathological evaluation of full-thickness tissue remains the gold standard for diagnosis [1, 29, 31]. The main reason for this is the fact that sensitivity and specificity in detecting dysplasia with oral brush biopsy have been reported to be highly variable, with sensitivity ranging from 43.5% to 100% and specificity from 23.5% to 100% [29, 32]. A recent systematic review conducted by Walsh et al. (2021) reported that oral cytology has a sensitivity of 0.90 (95% CI, 0.82–0.94) and a specificity of 0.94 (95% CI, 0.88–0.97) [2]. The limited sensitivity observed in conventional exfoliative cytology is primarily due to the inability of cytological tools to collect samples from the deeper layers of oral lesions. Additionally, false-negative results are often linked to the presence of obscuring factors, including blood, inflammation, mucus, and necrotic debris (Alsarraaf, 2018). On the other hand, biomarker-based approaches, including LBC techniques, DNA image cytometry [33], and molecular profiling [34], have been found to enhance diagnostic accuracy. Comparative studies have demonstrated a considerable increase in sensitivity and specificity with the use of the LBC, which offers clearer cell morphology and a more efficient evaluation process [35–37]. The pooled sensitivity and specificity of the OralCDx brush biopsy were reported as 86% (95% CI, 81–90) and 81% (95% CI, 78–85), respectively, while DNA image cytometry demonstrated a higher pooled sensitivity of 89% (95% CI, 83–94) and a specificity of 99% (95% CI, 97–100) [33]. Differential gene expression in OSCC and normal oral mucosa cells using DNA microarray analysis of brush biopsy samples was found to be significantly high, and the measurement of ATP6 V1 C1 expression levels is a highly sensitive (81.25%) and specific (93.75%) diagnostic method [34].

Introducing the use of interdental brushes for oral cytology could potentially expand the use of the technique. For reasons such as being easily accessible, affordable, and the fact that the interdental brush has different types suitable for different areas in the mouth, it can provide better access to different areas of the oral mucosa compared to cytobrush or cervexbrush. However, the examination of integrity values by brush types yielded a statistically significant difference. Notably, the CSIB demonstrated a lower success to transport cells without damaging their integrity compared to the other brushes. This finding raises intriguing questions about the potential influence of brush type on the integrity of sampled cells. There was no study found in the literature that utilized the interdental brush for intraoral cytological

examination. The considerable accessibility and affordability of the interdental brush is the main reason for the inclusion of this type of brush in this study. Future investigations could delve into possible factors that contribute to these observed results.

It is essential to acknowledge the study's limitations, including a relatively small sample size, the sampling of only one region of the oral mucosa, and the inclusion of only healthy subjects. All brush biopsies were performed by a single operator which may introduce potential observer bias. The inclusion of DNA quantification as an evaluation criterion could establish a connection between the study and molecular biomarker analysis. Additionally, the condition of the tissue under evaluation should be considered, taking into account that mucosal alterations, such as hyperkeratinization, have been shown to affect sampling depth [38]. However, the ability of brushes to collect cells efficiently when applied to oral mucosal pathologies remains uncertain, which may affect the generalizability of the results. Therefore, the sampling efficiency of different brushes should also be evaluated in OPMDs and malignant lesions to determine their diagnostic accuracy in oral cytology. Future research should explore the effects of different types of brushes, technical improvements, and consider potential confounding variables. Efforts should be made to enhance the accessibility and effectiveness of sampling and processing techniques in brush cytology, aiming to establish best practices for increasing the detection of oral cancer in its early stages.

## Conclusion

This pilot study explored the potential of interdental brushes as an alternative sampling tool for oral cytology. The findings demonstrated that interdental brushes collected high-cellularity samples and achieved a comparable epithelial sampling depth to medically designed cytology brushes. However, cellular integrity was lower, which may limit their diagnostic reliability unless further improvements in technique or brush design are implemented.

While our study presents promising preliminary results, further research is needed to optimize interdental brush-based cytology by refining sample collection techniques, improving cellular integrity, and evaluating its diagnostic performance in lesional mucosa. Future studies should also explore molecular biomarker integration to enhance the diagnostic accuracy of cytological screening.

## Abbreviations

|      |                                      |
|------|--------------------------------------|
| OSCC | Oral Squamous Cell Carcinoma         |
| OPMD | Oral Potentially Malignant Disorders |
| CSD1 | Cell Sampling Device 1               |
| CSD2 | Cell Sampling Device 2               |
| CSIB | Cell sampling-Interdental Brush      |

|       |                        |
|-------|------------------------|
| MiRNA | Micro-ribonucleic acid |
| DNA   | Deoxyribonucleic acid  |
| LBC   | Liquid-based Cytology  |

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Not applicable.

## Authors' contributions

Conceptualization: E.Ç, E.A.S. Formal analysis: E.Ç, M.Ö. Investigation: E.Ç, M.Ö, M.S.T. Resources: E.Ç, M.Ö. Data curation: E.Ç, M.Ö, M.S.T. Writing- original draft preparation: E.Ç. Writing- review and editing: E.A.S, M.Ö, M.S.T. Visualization, supervision: E.A.S. All authors read and approved the final manuscript.

## Funding

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## Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Bahçeşehir University Ethics committee (project number 2023–21/05). The study adhered to the tenets of the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all subjects involved in the study.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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