A new bio-nanochip sensor aids oral cancer detection

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Abstract

A diagnostic cytology-on-a-chip technique rapidly detects pre-malignant and malignant cells with high sensitivity and specificity

Oral squamous-cell carcinoma (OSCC) is a deadly and disfiguring disease that affects more than 200,000 people worldwide.¹⁻³ While potentially malignant lesions (PMLs) precede most oral cancers, only approximately 5% of these lesions progress. PMLs appear as white or red patches on the oral mucosa, and there is no definite indicator for which will develop into cancer.⁴⁻⁶ Unresolved lesions will typically undergo scalpel biopsy and histopathological evaluation for signs of epithelial dysplasia. Since the vast majority of these lesions are benign, this process is unnecessarily invasive and leads to financial, psychological, and physical costs. Noninvasive tools for assessing dysplasia and risk in PMLs are thus needed to improve early detection and reduce costs.

A variety of adjunctive autofluorescence, chemiluminescence, or dye-based tissue-staining techniques help visualize PMLs. However, since their sensitivity and specificity are insufficient for use in diagnosis, clinicians generally opt for assessment by biopsy.⁷ In contrast, cytological analysis is routinely used to diagnose a range of other malignancies,

including cervical, lung, urinary-tract, and other oral-cavity lesions.^{8,9} While this simple, noninvasive approach accurately detects atypical cells,¹⁰ the available oral test is expensive and examines only limited cellular features.¹¹ Here, we report our efforts toward a rapid oral-cytology assay that uses a novel bio-nanochip (BNC) sensor that combines the power of cytomorphometric analysis with quantification of tumor biomarkers.¹²

Our BNC sensor integrates multiple laboratory processes onto a microfluidic platform in three primary steps (see Figure 1). First, an oral-cytology suspension is delivered to the sensor using pressure-driven flow, where any cells larger than the membrane-pore size are retained on the membrane surface.^{12,13} The captured cells are then stained with fluorescent dyes and immunoreagents to distinguish the cytoplasm, nucleus, and cancer biomarkers. We selected epidermal growth-factor receptor (EGFR) as the targeted biomarker, since it is overexpressed and well characterized in OSCC and associated with aggressive phenotypes. Finally, the stained cells are subjected to a 3D fluorescence-microscopy scan of the membrane surface. This is followed by automated image analysis using open-source software^{14,15} with custom-written macros for quantitative intensity standardization and cellular contouring from the red, blue, and green channels.¹³ This permits concurrent analysis of surface-biomarker expression and cellular morphology using intensity and multiple key morphology parameters.

To assess the usefulness of our BNC technology, we launched a pilot study where the oral brush cytology specimens from 52 healthy and diseased patients were analyzed. We found an overall increase in nuclear size and a decrease in cellular size, yielding a significant elevation in the nuclear-to-cytoplasmic (N/C) ratio. The N/C ratio progressed from healthy mucosa (0.063) through PMLs with moderate to severe dysplasia (0.223, Student's T-test p value < 0.0001) to invasive OSCC (0.323, $p < 0.001$): see Figure 2. By further analysis of the outliers within the nuclear-area distributions, we identified another parameter to distinguish healthy epithelia from OSCC or dysplasia. We found that the intensity of emission from labeled EGFR increased significantly in diagnosed dysplasia (9.5, in arbitrary units, a.u.) and OSCC (11.8a.u.) compared to healthy epithelia (6.0a.u.): see Figure 2.

We then applied logistic regression, followed by receiver operating characteristic (ROC) analysis, to reveal which cellular markers (and in which combinations) were most effective in diagnosing OSCC. The N/C ratio and nuclear area exhibited the best performance characteristics with an area-under-the-curve (AUC) value of 0.93, followed by EGFR with 0.82. When these markers were combined, the AUC value was 0.94 with a projected 97% sensitivity. Additionally, the projected specificity for detection and classification of malignant and pre-malignant oral lesions was 93%. These results suggest that the combined panel has considerable potential for OSCC detection and diagnosis.^{12,13}

In summary, we demonstrated a novel, quantitative BNC approach to oral cytology that successfully identified malignant and pre-malignant lesions in less than 45 minutes.^{12,13} Both patients and clinicians would benefit greatly from this kind of accurate, noninvasive technique for point-of-care assessment and diagnosis of OSCC and PMLs. However, the greatest challenge for early detection lies in identification of PMLs with a high risk of tumor progression, i.e., discrimination of dysplastic from nondysplastic PMLs. Thus, our efforts are now focused on distinguishing among these PMLs, as well as other benign oral lesions, using a broader panel of tumor and dysplasia markers. A two-year international multisite clinical trial with 950 patients is currently underway. Also underway is a complementary 2200-patient trial that aims to validate the BNC device in the general population, where the prevalence of OSCC is lower.

Acknowledgments

We wish to thank Craig Murdoch and Paul Speight of the University of Sheffield (UK). We also wish to thank Rovers Medical Devices B. V. for their generous gift of Rovers® Orcellex® brushes. Funding and support from the National Institute of Dental and Craniofacial Research (grants RC2DE020785 and U01DE017793) and the Cancer Prevention Research Institute of Texas (RP101382-P01) are greatly acknowledged.

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Figure 1.

(A) A brush cytology sample is collected from the oral cavity and processed in a suspension for (B) analysis on the bio-nanochip platform for epidermal growth-factor receptor (EGFR) biomarker and morphometric characteristics of disease. (C) Fluorescence-microscopy images of cells stained with a cocktail of fluorescent dyes and antibody-based reagents such that the cytoplasm appears red (Phalloidin), the nucleus blue (4'-6-diamidino-2-phenylindole: DAPI), and EGFR is green (AlexaFluor®488). PMMA: Poly(methyl methacrylate).

Figure 2.

The nuclear-to-cytoplasm (N/C) ratio (blue) and mean intensity (in arbitrary units, a.u.) for EGFR biomarker (green) increase across patients groups from healthy to potentially malignant lesions (PML) to oral squamous-cell carcinomas (OSCCs).