

# A NEMS approach to specific and sensitive DNA/protein biomarker sensors for early detection of oral cancer in raw saliva

Winy Tan<sup>1</sup>, Na Li<sup>1</sup>, Yang Li<sup>2</sup>, Xiaofeng Zhou<sup>2</sup>, David T. Wong<sup>2</sup>, Chih-Ming Ho<sup>1</sup>

<sup>1</sup>Henry Samueli School of Engineering and Applied Science

<sup>2</sup>School of Dentistry

University of California, Los Angeles

420 Westwood Plaza

Los Angeles, CA 90025

Oral cancer affects 350,000 people worldwide. Using a patient-based genomic wide search we have found salivary IL-8 (mRNA and protein) to be a biomarker capable of discrimination between oral cancer patients and normal subjects. Our immediate goal is to develop a NEMS-based biosensor capable of detecting IL-8 mRNA and protein in saliva to enable the early detection of oral cancer in raw saliva. Fast and accurate methods to diagnose the early and most treatable stages of cancer have the most value, however they are not yet available. We have recently developed specific and sensitive DNA/RNA and protein sensors that are able to detect the oral cancer biomarker Interleukin-8 (IL-8) in raw saliva without the need for amplification of target molecules. The DNA/RNA and protein sensors implement NEMS surface modification methods including covalent coupling chemistry, self-assembled monolayers (SAMs), and immobilization of specific capture probes using streptavidin-biotin binding. DNA/RNA is amperometrically detected using an enzyme mediated electrochemical reaction. The DNA/RNA sensor for IL-8 mRNA has 1 fM sensitivity (equivalent to 1500 ssDNA molecules); this level of sensitivity is an improvement over most available DNA sensors by at least 3 orders of magnitude. There is also a 90% reduction in required sample volume. IL-8 protein on the other hand, is optically detected using specific fluorescence probes. IL-8 protein is detected at a sensitivity of 100 pg/ml (~12pM) with a large detection range spanning four orders of magnitude (pg/ml-μg/ml). The sample volumes used for IL-8 protein detection are 6 times smaller than conventional protein detection methods. Both the DNA/RNA and protein sensors correctly identified oral cancer patients from healthy individuals by detection of IL-8 mRNA and IL-8 protein concentrations in raw saliva, respectively. Quantification of salivary IL-8 protein in oral cancer patients is also possible. These sensors will be integrated onto a single miniaturized fluidic platform that provides complex biosignatures for accurate oral cancer diagnosis.

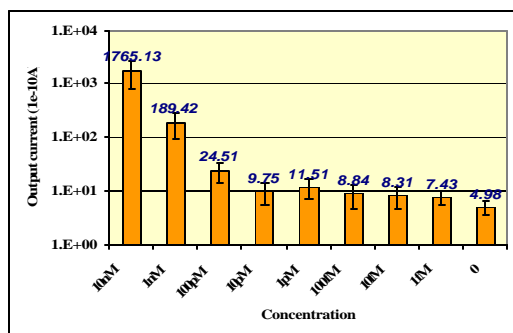


Fig. 1 IL-8 ssDNA detection

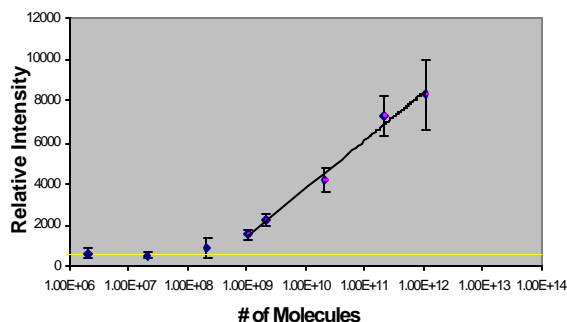


Fig. 2 Standard curve for IL-8 protein detection

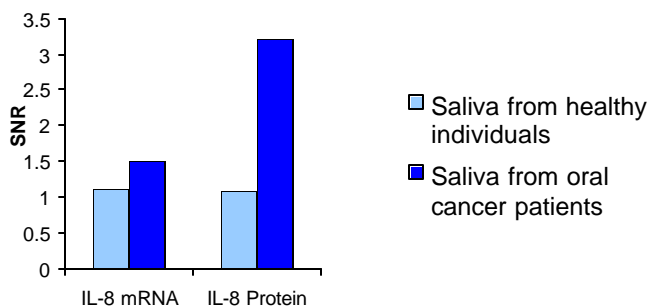


Fig. 3 IL-8 mRNA detection and IL-8 protein detection in raw patient saliva

**Acknowledgements:** This work is supported by a grant from the National Institute of Dental and Craniofacial Research (UO1 DE15018).

**Reference:**

1. De Wildt, R. T., Mundy, C. R., Gorick, B. D. & Tomlinson, I. M. (2000). Antibody arrays for high-throughput screening of antibody-antigen interactions. *Nature Biotechnology* 18, 989-994.
2. Hashimoto, K., Ito, K. & Ishimori, Y. (1994). Sequence-specific gene detection with a gold electrode modified with DNA probes and an electrochemically active dye. *Analytical Chemistry* 66, 3830-3833.
3. MacBeath, G. & Schreiber, S. L. (2000). Printing proteins as microarrays for high-throughput function determination. *Science* 289, 1706-1763.
4. St. John, M., Li, Y., Zhou, X., Denny, P., Ho, C.-M., Montemagno, C. D., Shi, W., Qi, F., Wu, B., Jordan, R. C. K., Sinha, U., Park, N.-H., Abemayor, E. & Wong, D. T. W. (2004). IL-6 and IL-8: Potential biomarkers for oral cavity and oropharyngeal SCCA. *Archives of Otolaryngology/Head & Neck Surgery*. *in press*.