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Piezoresistive Membrane Surface Stress Sensors for Characterization of Breath Samples of Head & Neck Cancer Patients

Hans Peter Lang [1,*], Frédéric Loizeau [2], Agnès Hiou [3], Jean-Paul Rivals [3], Pedro Romero [3], Terunobu Akiyama [2], Christoph Gerber [1] and Ernst Meyer [1]

[1] Department of Physics, University of Basel, Swiss Nano Institute, Klingelbergstrasse 82, CH-4056 Basel, Switzerland; E-Mails: Christoph.Gerber@unibas.ch (C.G.); Ernst.Meyer@unibas.ch (E.M.)

[2] SAMLAB, EPFL Neuchâtel, CH-2002 Neuchâtel, Switzerland; E-Mails: frederic.loizeau@epfl.ch (F.L.); terunobu.akiyama@epfl.ch (T.A.)

[3] Ludwig Institute for Cancer Research, University of Lausanne, CH-1066 Epalinges, Switzerland; E-Mails: Agnes.Hiou@chuv.ch (A.H.); Jean-Paul.Rivals@chuv.ch (J.-P.R.); Pedro.Romero@hospvd.ch (P.R.)

* Author to whom correspondence should be addressed; E-Mail: Hans-Peter.Lang@unibas.ch; Tel.: +41-61-267-3769; Fax: +41-61-267-3784.

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Abstract: For many diseases, where a particular organ is affected, chemical by-products are found in the patient's exhaled breath. Breath analysis is often done using gas chromatography and mass spectrometry, but interpretation of results is difficult and time-consuming. We performed characterization of patients' exhaled breath samples by an electronic nose technique based on an array of nanomechanical membrane sensors. Each membrane is coated with a different polymer layer. By pumping the exhaled breath into a measurement chamber, volatile organic compounds present in patients' breath diffuse into the polymer layers and deform the membranes by changes in surface stress. The bending of the membranes is measured piezoresistively and the signals are converted to voltages. The sensor deflection pattern allows to characterize the condition of the patient. In a clinical study, we investigated breath samples from head and neck cancer patients and healthy control persons. Evaluation using principal component analysis (PCA) allowed clear distinction between the two groups. As head and neck cancer can be completely removed by surgery, the breath of cured patients was investigated after surgery again and the results were similar to those of the healthy control group indicating that surgery was successful.

Keywords: piezoresistive membrane sensors; surface stress sensor; nanomechanical sensor; electronic nose; breath analysis; head and neck cancer

1. Introduction

Cancer is a disease where cells are growing in an uncontrolled way forming a tumor, invading and destroying adjacent healthy tissues and organs. Cancerous cells spread to other locations in the body via lymph or blood vessels to form metastases, the most common cause of cancer-related death in patients with solid tumors.

Head and neck squamous cell carcinoma (HNSCC) is the fifth most important cancer type worldwide. HNSCC is highly curable if detected early. However, second primary tumors and local recurrences are a major challenge, the latter being the most common cause of treatment failure and disease-related death. Early detection of HNSCC and identification of residual or recurrent disease in treated patients allow early therapeutic intervention and may result in a survival advantage. Diagnosis is normally performed by endoscopy and taking a biopsy of suspect lesions. We propose here a non-invasive diagnostic technique based on detection of volatile organic compounds (VOCs) in exhaled breath using a technology based on the atomic force microscope [1]. Such VOCs are associated with the occurrence of certain cancer types, in particular head and neck cancer. VOCs related to diseases like diabetes mellitus and uraemia in breath were reported to be detectable easily using polymer-coated nanomechanical cantilever arrays [2]. Detection of VOCs in gas phase using polymer-coated cantilever arrays is based on a characteristic cantilever bending response pattern, allowing to distinguish different VOCs. Moreover, polymer-coated sensors can be reproducibly regenerated by purging them with dry nitrogen gas [3]. We have further developed this technique by using piezoresistive, nanomechanical membrane surface-stress sensor (MSS) arrays implemented in a portable device.

2. Experimental Section

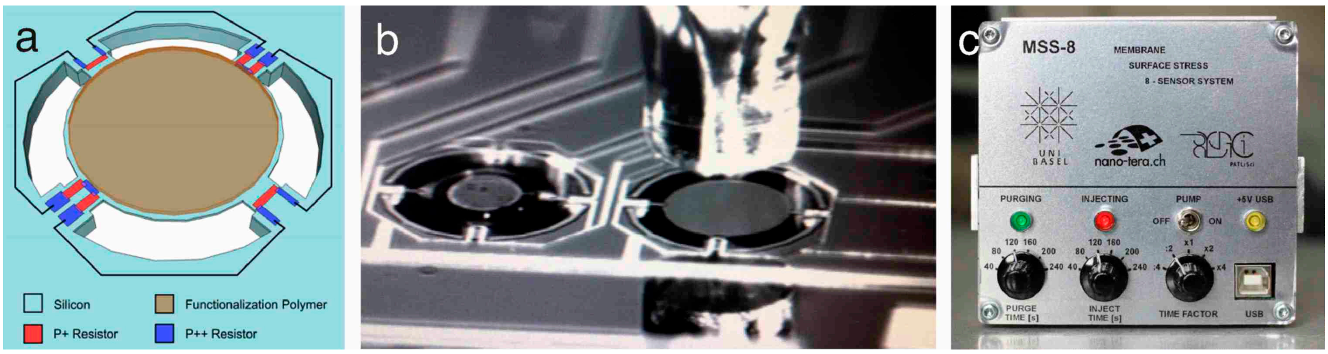

Figure 1. (**a**) Graphical representation of a silicon membrane suspended by four constricted beams with integrated piezoresistors connected in a Wheatstone bridge. The membrane is coated with a polymer that responds to surrounding molecules by swelling; (**b**) Functionalization of MSS using inkjet spotting of polymer solutions; (**c**) Portable universal serial bus powered compact measurement device with pumping system for gaseous samples, signal readout and data acquisition (10 cm × 10 cm × 16 cm).

The sensor bending is usually determined via optical beam deflection. However, for the sake of compactness, we have applied piezoresistive readout of the sensors. Medical applications favor the routine use of a compact, small-sized, portable and non-invasive device. A prototype was used to examine patients' exhaled breath samples in search for VOC patterns associated with head and neck cancer. Membrane-type surface stress sensors (MSS) arranged in arrays for molecular detection in gaseous phase have been microfabricated from silicon-on-insulator substrates and structured by deep reactive ion etching. The round membranes have a diameter of 500 µm and a thickness of 2.5 µm and are suspended by four sensing beams with integrated p-type piezoresistors, representing a full Wheatstone bridge (Figure 1a). The p-doped piezoresistors have been fabricated using two distinct doping processes (ion diffusion through Boron Silica Glass and implantation). The latter method features shallow resistors, which are very sensitive to surface stress changes.

The membranes have been coated with a thin (<1 µm) polymer layer using inkjet spotting. VOCs present in the breath sample diffuse into the polymer layer in a way characteristic for each polymer and produce bending of the membrane. As 8 different polymers were used, a characteristic bending pattern of the membranes is generated on exposure to individual patient's breath samples. The polymers applied are CMC = carboxy methyl cellulose, PEO = poly-(2-ethyl-2-oxazoline), PEGMEMA = polyethylene glycol methyl ether methacrylate macromer, HPC = hydroxypropyl cellulose, PAA-AA = poly(acrylic acid)-acetic acid, PVPy = poly(vinylpyridine), PIB = butyl rubber, PEI = polyethylenimine.

3. Results and Discussion

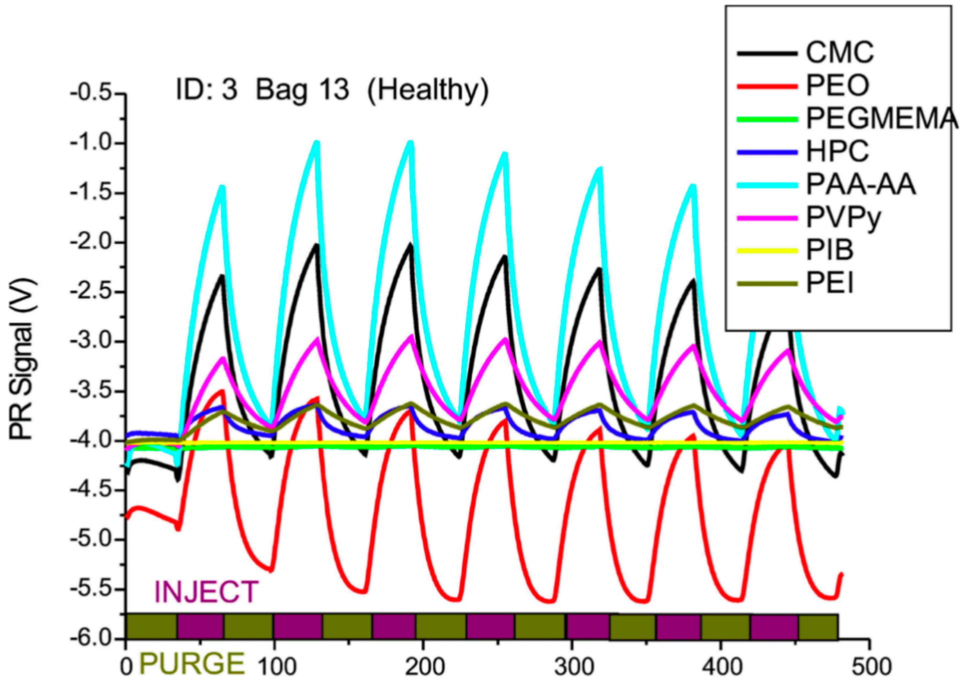

Figure 2. Piezoresistive (PR) membrane response curves upon injection with patients' breath samples and purging with dry nitrogen. Injection and purging duration: 30 seconds, flow rate 15 mL/min.

In a clinical study, we investigated breath samples from HNSCC patients and healthy donors (smokers) in a double blind trial. The patient inclusion criteria were based on histologically confirmed carcinoma at a comparable stage. The patients were selected from the same age groups. Patients and donors were asked to breathe into a 1 Liter Tedlar bag. The bags were then stored at 4 °C until analysis. Each breath sample has been measured 7 times (Figure 2), whereby the first injection-purge cycle has been discarded to avoid influence of previous measurements.

The deflection values after 10, 15, 20 and 25 seconds after beginning of each injection were subtracted from the value at the beginning of the injection (0 seconds) to reduce the influence of possible drifts in the measurement. These 4 differential values obtained for each membrane sensor were processed using principal component analysis (PCA) to be represented in a two-dimensional plot showing one dot for each breath sample measurement, i.e. one injection - purging cycle (Figure 3).

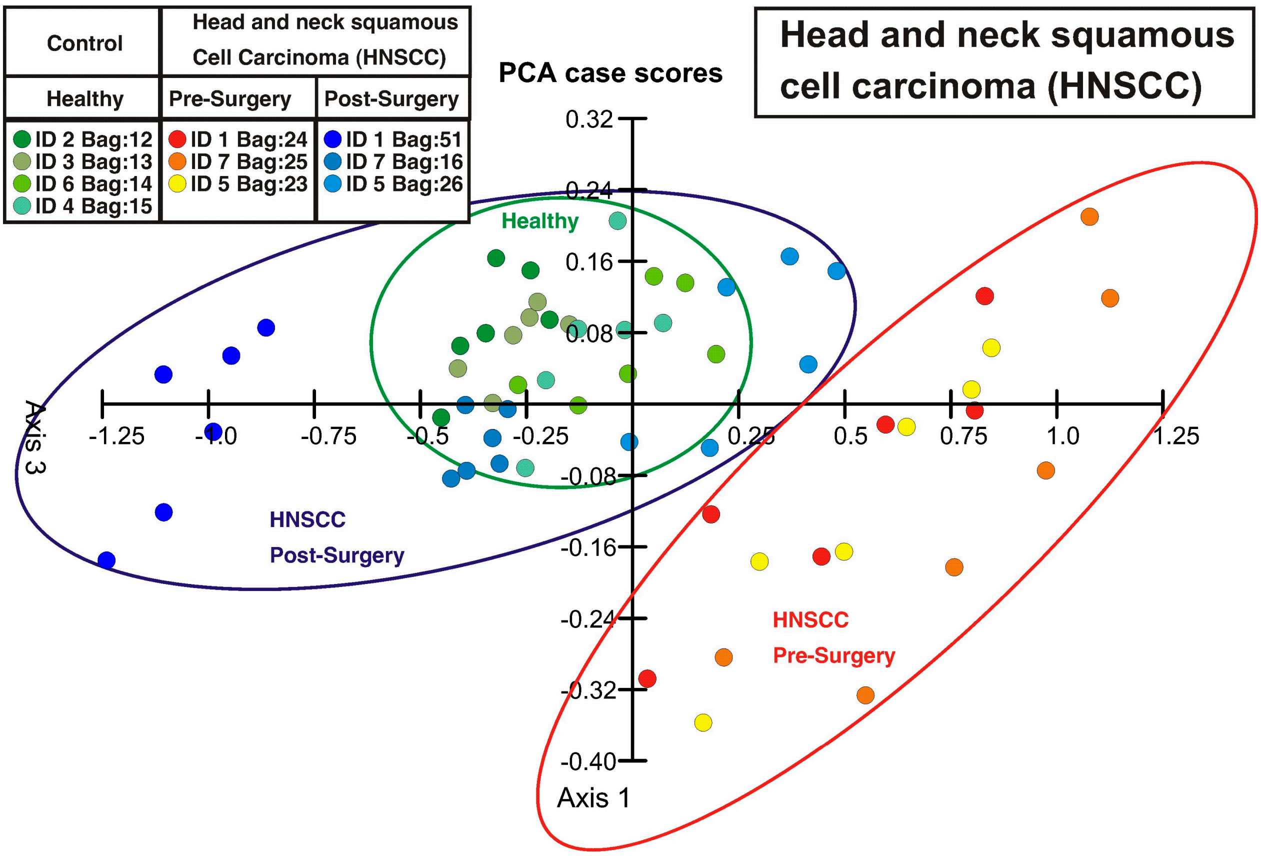

Figure 3. Principal Component Analysis (PCA) plot showing three distinct clusters (indicated with ellipses) that represent healthy control persons, HNSCC patients before surgery and HNSCC patients after surgery, i.e. after removal of the tumor by operation. The points of the HNSCC patients after surgery are at a similar location in the PCA plot as those from the healthy persons and differ clearly from the points of the HNSCC patients before surgery, indicating that the removal of the tumor has been successful.

To emphasize the distinction capability of the method, hierarchical tree analysis (Unweighted Pair Group Method with Arithmetic Mean, UPGMA) was performed. In this method, the data are analyzed by calculating the Euclidian distance between vectors consisting of data points and their closest neighbors. Figure 4 shows the UPGMA diagram of the data. Breath measurements of NHSCC patients before surgery are clearly different from measurements of healthy control persons and cured NHSCC patients after surgery, demonstrating the success of surgery.

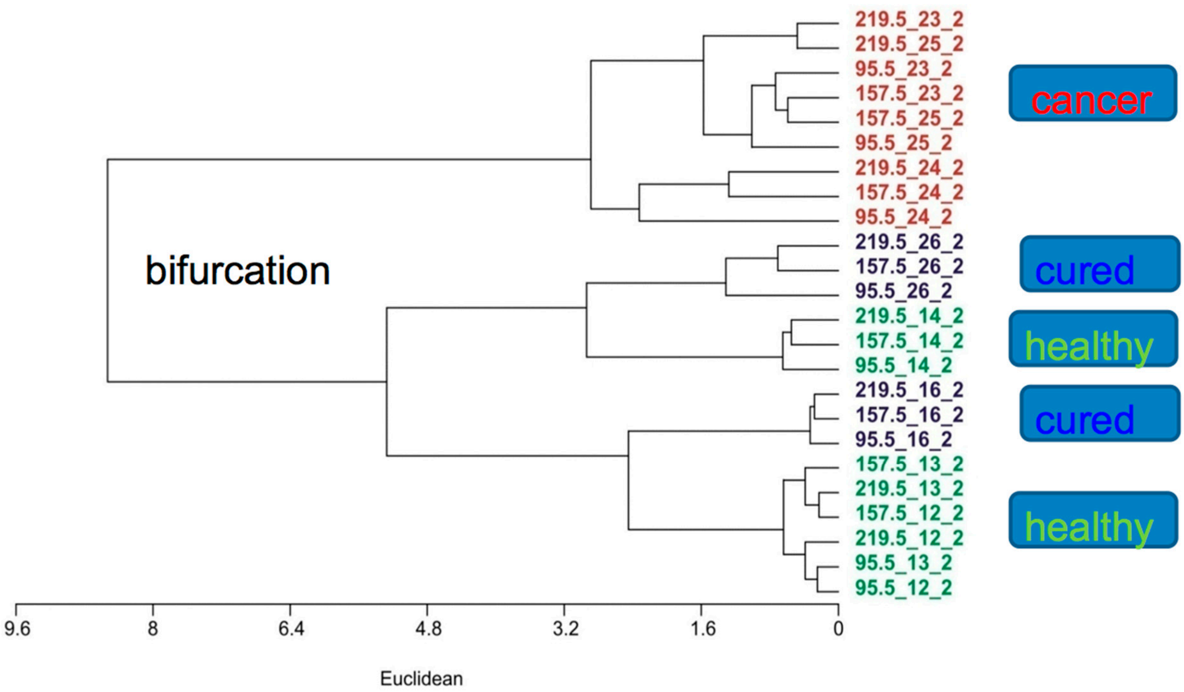

Figure 4. The UPGMA diagram (dendrogram) shows bifurcations for distinct distances between pairs of measurements implying that the datasets from cancer patients (HNSSC) before surgery are clearly different from healthy control persons and cured NHSCC patients after surgery. Number labels indicate individual injection - purge cycles.

Other evidence that VOC profiles in exhaled breath can be used to detect diseases has been shown by Phillips et al. for lung and breast cancer [4]. A pilot study of analysis of air exhaled by HNSSC patients using an array of 5 gold nanoparticle sensors and gas chromatography has shown promising results [5].

4. Conclusions

Early detection of primary tumors and of recurrences after surgical removal of the primary tumor is crucial for patients with HNSSC. Invasive analyses, e.g., endoscopies, give clear indication on the treatment success, but are a hassle for the patient. Detecting the VOCs in exhaled breath represents a non-invasive method to follow the success of a treatment/surgery. We have shown that MSS are capable to distinguish HNSSC patients before surgery from healthy control persons and HNSSC patients after surgery by monitoring VOCs in patients' breath samples. The measurement device used is portable and powered by a laptop computer's universal serial bus port.

Detecting VOCs associated with cancer growth will ultimately lead to a simple, easily performable and non-invasive screening technique that can be used in conjunction with, or as alternative to standard more invasive techniques. The technique could eventually be adapted to other pathologies affecting the respiratory tract.

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Author Contributions

Hans Peter Lang conceived, performed and evaluated the experiments, Frédéric Loizeau and Terunobu Akiyama fabricated and provided the MSS sensors, Agnès Hiou, Jean-Paul Rivals, Pedro Romero conducted the clinical study at the CHUV Lausanne and provided the patients' breath samples, Christoph Gerber, Hans Peter Lang and Ernst Meyer proposed the method and were involved in manuscript writing.

Conflicts of Interest

The authors declare no conflict of interest.

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