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BIOMIMETIC DIAMOND MEMS SENSORS BASED ON ODORANT-BINDING PROTEINS: SENSORS VALIDATION THROUGH AN AUTONOMOUS ELECTRONIC SYSTEM

Maira Possas-Abreu¹, Lionel Rousseau¹, Farbod Ghassemi¹, Gaelle Lissorgues¹, Massiel Habchi², Emmanuel Scorsone², Khasim Cal³, Krishna Persaud³

¹ESYCOM, ESIEE-Paris, Cité Descartes BP99, 93162 Noisy-le-Grand, France

²CEA, LIST, Diamond Sensor Laboratory, 91191, Gif-sur-Yvette, France

³University of Manchester, School of Chemical Engineering, Oxford Road, Manchester, UK

ABSTRACT

Odorant binding proteins (OBPs) have been considered one of the best candidates for conceiving a biosensor to detect biochemical molecules and for odor sensor applications. In the same way, MEMS sensors for chemical and biological applications proved to be a good option for applications requiring high sensitivity. In this context, micro-cantilever sensor is one of the most promising micromechanical sensor. In this paper a grafting method, compatible with MEMS technology, was used for grafting porcine OBPs on polycrystalline diamond cantilevers. These sensors have been tested, for the first time, in combination with an autonomous dedicated electronic system developed to automatically detect sensors response signals. Electronic detection of binding 2-isobutyl-3-methoxypyrazine (IBMP) to OBP was demonstrated.

Index Terms—Odorant Binding Proteins; Diamond micro-cantilever; Sensor read-out; Sensor Array; MEMS

1. INTRODUCTION

The OBPs are soluble proteins of transportation of odorant molecules. Many studies defend that in vertebrates, they contribute to the transport of odorous molecules through the mucus to the cilia of the olfactory neurons [1, 2]. Odorant Binding Proteins are able to bind diverse odorants and present high conformational stability which are attractive characteristics for the design of label free biosensors in particular for the detection of small volatile organic compounds [3, 4].

The most part of existing biosensors have been developed combining bio-receptors with different transducers like quartz crystal microbalance (QCM), surface acoustic wave (SAW) sensors, electrochemical sensors, etc. Micro-cantilever based sensors offer many advantages for odor detection, since they can be miniaturized (and are suitable for parallelization into arrays) and can achieve high sensitivity [5,6]. However, the most important challenge on using micro-cantilevers as bio-sensors transducers for odor detection (e-nose applications) is related to the need of reliable actuation and readout techniques.

Optical techniques are the most commonly-used method for measure cantilever bending or dynamic movement. They are conventionally used in atomic force microscopy (AFM). The main challenges of optical readout technologies are high cost, surface preparation, and optical alignment and adjustment requirements. Optical external read-out is also susceptible of interfere with bio-molecules in cantilever surface. In addition, integrated optical readout makes cantilever fabrication more complex, requiring waveguide light integration and alignment. In contrast, piezoresistive strain-sensing techniques provide benefits over optical in readout size and the ability to work in light-sensitive environments or use opaque materials.

With regard to sensor material for bio-interfaces, synthetic diamond has been recognized for many years for being an interesting bio-sensing interface because of the wide range of possibilities to covalently immobilize bio-receptors onto its carbon based surface and for the long term stability of carbon sp³ [7]. Previous works [8] have shown the feasibility of grafting OBPs on polycrystalline diamond cantilevers. However, the use of OBP-grafted micro-cantilevers together with a dedicated electronic read-out has never been demonstrated. Indeed, such a device would require the integration of the read-out component, which is, generally, not compatible with grafting techniques.

In this paper, we investigate a new approach consisting of grafting chemically the OBPs onto synthetic diamond micro-cantilevers at low temperatures, allowing the integration of electronic components in the transducer. Electronic detection of binding 2-isobutyl-3-methoxypyrazine (IBMP) to OBP is demonstrated in order to validate our approach.

2. MATERIALS AND METHODS

2.1. Diamond Cantilever Fabrication

Micro-cantilevers have been conceived to work on dynamic mode. In this mode, the analyte causes a shift in the cantilever's resonance frequency due to the increased mass loading. The response of the sensors is monitored by integrated piezoresistors.

Diamond micro-cantilevers were fabricated according to a process described in previous works [9,10]. Cantilever dimensions were $310\text{ }\mu\text{m} \times 140\text{ }\mu\text{m}$ (Figure 1). The cantilever geometry led to a resonance frequency of typically $f=85000\text{ Hz}$ and a Q-factor of 600 in air. Diamond micro-cantilevers are cleaned before grafting the OBPs.

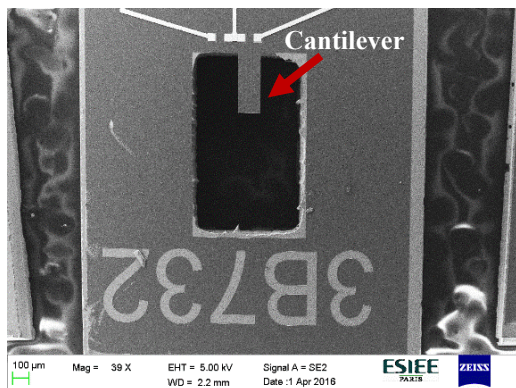


Figure 1: SEM image of a diamond micro-cantilever

2.2. OBPs Immobilization

All chemicals used for OBP grafting were purchased from Sigma-Aldrich, France. Here the method consisted of the activation of the diazonium salt [11]. This activation is done by forming the diazonium function N_2^+ from an aromatic primary amine (phenylenediamine: $\text{NH}_2\text{-}\phi\text{-NH}_2$ in this case), then to create a radical cycle ϕ which can react with the surface of the nanodiamonds in the presence of the C-C covalent bonds.

We first proceed to the *in situ* formation of nitrous acid (HNO_2) by adding sodium nitrite NaNO_2 in an acid medium (HCl (0,5 M)). Subsequently, the nitrous acid solution will react with phenylenediamine ($\text{NH}_2\text{-}\phi\text{-NH}_2$) for the formation of the hydrophilic function diazonium N_2^+ , which can be removed in gaseous form, causing the radical phenyl to form. In our case, 4-aminobenzoic acid ($\text{NH}_2\text{-}\phi\text{-COOH}$) is used in order to complete the grafting by the immobilization of OBPs on the surface of the polycrystalline diamonds. OBPs solution (1.0 mg/mL in 20 mM PBS, $\text{pH}=8.00$) are used as received (preparation is described in [8]). Cantilevers are exposed to it for 2 hours. Again the substrates were rinsed thoroughly with deionized water and stored in PBS solution.

The main difference between this OBP-grafting technique in diamond surface and other techniques presented on the literature [8] is the fact that hydrogenation steps at high temperatures are not needed. Indeed, it requires only low temperatures ($<4^\circ\text{C}$) for its preparation. For this reason, piezoresistive gauges integrated in diamond cantilever can be preserved, and electronic detection is possible.

2.3. Gas cell and electronic system

The sensor chips were placed in a customized gas analysis cell, which consists on stainless steel chamber containing

eight sensor cavities with a gas inlet/outlet (Figure 2). The sensors are organized radially on a holding part (represented in green) below which lies a piezoelectric cell used to bring each micro cantilever to its main resonance frequency.

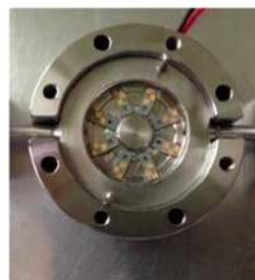


Figure 2: A photography of the gas cell and eight sensors placed inside

A low noise and highly reconfigurable electronic system has been designed as well as a dedicated human-machine interface. The main board is composed of a micro-controller, a signal generation circuit to excite the sensors around their resonance frequencies and an analog processing chain to extract the amplitude of each sensor response. An image of the complete system is showed on Figure 3.

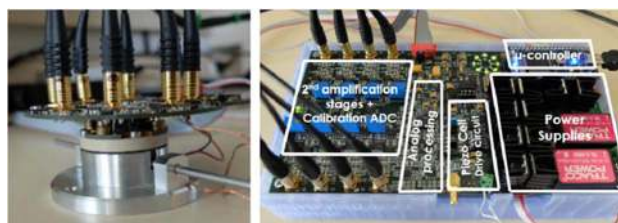


Figure 3: Gas analysis cell and electronic system

3. RESULTS AND DISCUSSION

3.1. Fluorescence imaging

First confirmation of protein immobilization was obtained by Fluorescence imaging. After the last step of OBPs grafting, a first sample was immersed in a fluorescein solution then rinsed vigorously with deionized water.

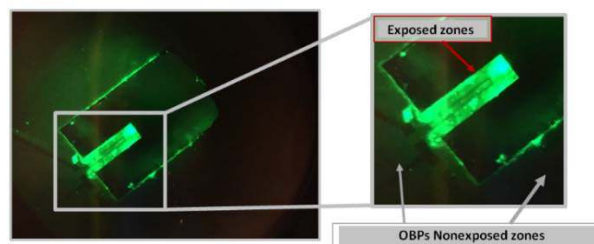


Figure 4: Fluorescence microscopy picture observed at $\lambda_{exc}=550\text{ nm}$ and $\lambda_{em}=570\text{ nm}$ of diamond cantilever functionalized with OBP immobilization after immersion in a fluorescein solution.

Fluorescein has the ability to bind the OBP thus offering the possibility to visualize under UV excitation the presence of OBPs on the diamond surface.

3.2. Grafting validation through electronic system measurements

In order to verify the presence of OBPs grafted on the sensors, the 2-isobutyl-3-methoxypyrazine (IBMP) was used as reference gas due to its excellent affinity with porcine OBP [13].

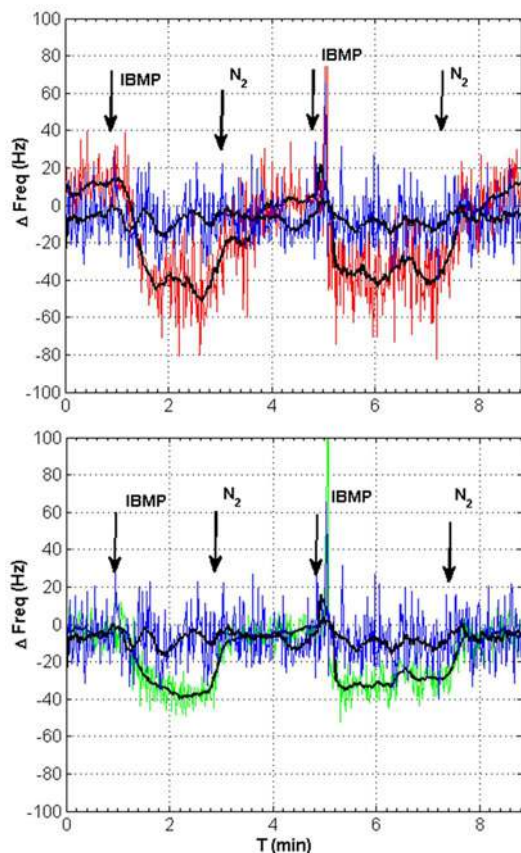


Figure 5: Response of two OBP-grafted diamond micro-cantilevers and two references to water vapor and IBMP

Results of Figure 5 presents the response of two OBP grafted sensors (red and green) compared with two non-functionalized cantilevers (blue). Here nitrogen is used as reference gas. The response of our sensors for saturated IBMP atmosphere is around 40 Hz. Considering only the mass effect hypothesis of this detection, we can estimate the presence of 10^8 IBMP molecules bound to immobilized OBPs.

4. CONCLUSION

We reported on the grafting of porcine OBP onto diamond micro-cantilevers using a different chemical approach

enabling the use of integrated components and offering the possibility to use a dedicated electronic system. Surface immobilization of the proteins was validated by fluorescence imaging. These sensors have been tested, for the first time, in combination with an autonomous dedicated electronic system developed to automatically detect sensor response signals. This is highly promising for further general investigations of ligand binding properties of OBPs and for the development of reliable biosensors that may be used in vapor sensing applications as well as electronic nose applications.

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