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► **To cite this version:**

Oumaima Zaki, Emmanuel Scorsone, Gaëlle Lissorgues, Lionel Rousseau. Development of pheromone-receptor based biosensors for the early detection of pest insects. Eurosensors XXXV Conference, Sep 2023, Lecce, Italy. cea-04256158

HAL Id: cea-04256158

<https://cea.hal.science/cea-04256158>

Submitted on 24 Oct 2023

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Development of pheromone-receptor based biosensors for the early detection of pest insects

Oumaima ZAKI ^{1,*}, Emmanuel SCORSONE ¹, Gaëlle LISSORGUES ² and Lionel ROUSSEAU²

¹ Université Paris-Saclay, CEA, List, F-91120, Palaiseau, France

² ESYCOM Lab UMR, CNRS 9007, ESIEE-Paris, Univ. Gustave Eiffel, Marne-la-Vallée, 77454, France

* Oumaima.zaki@cea.fr

† Presented at the Eurosensors XXXIV, Lecce, Italy, 10-13 September 2023.

Abstract:

Insects destroy approximately one third of the world's annual crop production. Their early detection enables the implementation of appropriate measures to prevent excessive infestation. Insects use species-specific chemical signals known as pheromones to communicate and attract their conspecifics. Interestingly, these pheromones are used to lure insects into traps for pest control purposes. Despite its effectiveness, this method is laborious and time-consuming as it requires frequent observations to identify and count the captures. As an alternative, the detection of pheromones is a promising solution for insect surveillance. In this context, we are developing biosensors based on pheromone receptors (PRs) immobilized on diamond-based capacitive micromachined ultrasonic transducers (CMUT) with the aim to achieve a highly selective detection of pest insect pheromones.

1. Introduction

Among a large number of technologies used for chemical transduction, gravimetric resonators are particularly suitable for odorant receptors (ORs) detection. They can operate at room temperature, allow label-free detection and can be manufactured at low cost. In this work, we are developing a diamond-based gravimetric resonator using (CMUT) technology. It consists of a thin boron doped diamond (BDD) membrane electrode suspended over a vacuum gap. This top electrode acts as a mechanical resonator while an underlying conductive layer acts as the bottom electrode. Variations of the resonant frequency of the membrane upon interactions of the PR immobilized over its surface with the target ligands (pheromones) produce measurable sensing signals with high sensitivity, which are indicative of the presence of the relevant insect in the field. CMUTs offer large chemically sensitive surface areas to increase the chance of interactions with the target molecules. Moreover, diamond's exceptional mechanical properties, such as its high Young's modulus (>1000 GPa) enables high frequency operation and therefore high sensitivity. Additionally, the carbon nature of the diamond surface allows robust C-C covalent immobilization of a wide range of receptors over its surface [1].

2. Materials and Methods

Firstly a fabrication process of the CMUT-type diamond transducer on a 4 inches silicon wafer was developed. In brief, a patterned sacrificial oxide layer was deposited on the silicon wafer via thermal oxidation. Then a 1.5 μm boron doped diamond (BDD) layer was deposited by microwave plasma assisted chemical vapor deposition (MPCVD) in a Seki Diamond AX6500 diamond growth reactor over the oxide layer. Through a number of steps consisting of photolithography followed by a DRIE aimed at etching the Si substrate and then removal of the sacrificial layer by gaseous HF etching, the diamond membrane was released, with a gap of 200 nm underneath.

Next, a protocol was optimized in order to immobilize the PRs over the diamond surface. The method consists in grafting a nitriloacetic acid with 3-COOH groups onto a BDD surface by an electrochemical procedure, followed by interaction of the NTA group with 6-His terminals on the protein via Ni-complex interactions. Since the PR was not available at an early stage in the project, the protocol was first optimized used Odorant Binding Proteins having the same 6-His Tags attached to them. Electrochemical impedance spectroscopy (EIS) was used to characterize this functionalization technique (Figure 1). Finally, PR from the fall armyworm, *Spodoptera frugiperda*, SfruOR13 has been produced successfully by cell-free expression and is currently being tested for PR detection.

3. Discussion

Fig.1 a shows a SEM image of a suspended diamond membrane obtained as part of the CMUT fabrication process. The resonance frequency of such membranes in water was measured by Laser Doppler vibration interferometry to be in the order of 125 to 160 kHz. Fig.1b, c shows the evolution of the transfer resistance of a BDD electrode upon the various steps of protein immobilization over its surface, measured by EIS. The presence of organic species on the surface of the electrode is correlated with R_t , as organic compounds can impede the accessibility of redox species in solution to the electrode surface. An increase in R_t after each step confirms the successful immobilization of the various chemical moieties over the surface. Work is now on progress to immobilize PR SfruOR13 in the same way in order to perform the first detection measurements.

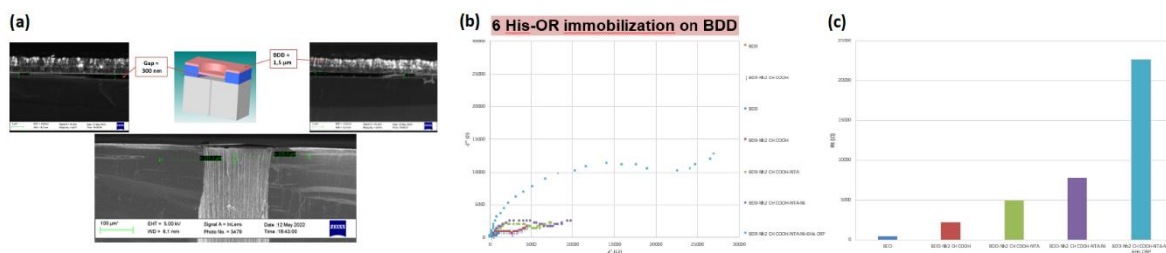


Figure 1: (a) SEM image of a cross section of a CMUT cell (b) Nyquist plots obtained in the frequency range from 100 kHz to 0.1 Hz at 0 V vs Ag/AgCl by applying 10 mV (AC mode) in 1 mM $[Fe(CN)_6]^{3/4-}$. (c) The evolution of electron transfer resistance (R_{et}) value after each immobilization step

Author Contributions: Methodology, Validation, Investigation, Writing-original draft, O.Z.; Conceptualization, Methodology, Validation, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition, E.S. and L.R. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the "Programme prioritaire de recherche – Cultiver et protéger autrement" under the "troisième programme d'investissements d'avenir (PIA3)" Pherosensor project.

Conflicts of Interest: "The authors declare no conflict of interest."

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