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A Chemoemitter System Mimicking Chemical Communication in Insects

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Abstract

The first chemoemitter based on the concept of infochemical communication is presented emphasizing details on the microfabrication and functionality of its elements, particularly the biomicroreactor and the evaporator. The functionality of the integrated chemoemitter has been evidenced in electrophysiological assays using antennae from *Spodoptera littoralis* males for pheromone detection and quantification.
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Keywords: Electrophysiology; semiochemical signals; pheromone; microfabrication; evaporator; biomicroreactor; *Spodoptera littoralis*

1. Introduction

The pheromone communication of insects has evolved to become a complex scheme for encrypting and transferring messages. The aim of this study was to develop an artificial communication system based on functional equivalents of biological machinery that allow eusocial insects to exchange information [1]. A key component of such a system is a chemoemitter consisting of a micromachined evaporator that together with a biomicroreactor mimic the *S. littoralis* female pheromone biosynthesis.

2. Microfabrication

A glass-silicon microreactor was fabricated using established lithographic methods followed by deep reactive ion etching (DRIE) and anodic wafer bonding of the meander channel with rectangular cross section (dimensions 0.25x0.20x91.26 mm). The surfaces of connecting capillaries and meander were coated with anchored polyelectrolyte multilayer structure, in order to prevent adsorption of substrate, product and enzyme. Inside the microchannel, NTA-functionalized agarose beads were densely packed, and purified His_6-tagged acetyl transferase (*atf*) was immobilized on them to transform the substrate ((9Z,11E)-tetradecadienol) into the pheromone ((9Z,11E)-tetradecadienyl acetate) in the presence of acetyl-CoA.

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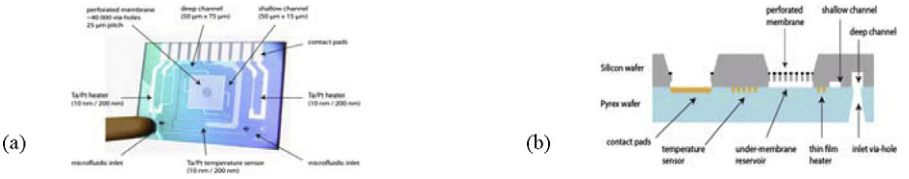

Fig. 1. (a) Evaporator image of the channel side; (b) schematic representation of its cross-section.

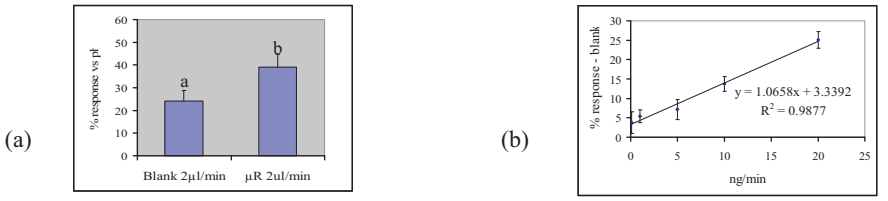

Fig. 2. (a) Electroantennographic detection of the pheromone produced by the microreactor and emitted by the evaporator; (b) Calibration curve of the flow rates *vs* EAG responses to (9Z,11E)-tetradecadienyl acetate evaporated from aq. soln.

The evaporator consists of a silicon membrane (5x5x0.04 mm) perforated with ~40,000 micromachined via-holes. Rectangular microfluidic channels deliver the mixture of predefined volatile compounds from the two inlets to the reservoir (375 nL) located under the membrane (Fig. 1).

Two thin-film Pt heaters and a 4-wire resistive temperature sensor are integrated in the evaporator and work in a PID loop to stabilize the temperature with a variation of 30 mK. The liquid passes through the membrane and evaporates from small droplets formed on the outlet of every via-hole (Fig. 1b).

3. Functionality of the chemoemitter

The activity of the biomicroreactor was determined and compared to that in batch by GC–MS. For a flow rate of 10 µL/min the results evidenced similar conversion in the biomicroreactor and in the batch assay (data not shown). The second step involved the integration of the whole chemoemitter system (the microreactor producing the pheromone plus the evaporator) with electroantennographic detection. The electroantennography (EAG) is a technique which records and quantifies the depolarization response of an insect antenna to a chemical stimulus [2]. The stimulus is carried from the chemoemitter to the antenna, fixed on two tungsten electrodes, by air flow. The mean response of ten male antennae to the sample emerging from the microreactor after partial conversion of the substrate into the pheromone was significantly higher (Student *t* test, $P<0.01$) than the blank at a flow rate of 2 µL/min (Fig. 2a). This is a prove of the chemoemitter functionality and the EAG suitability for product detection.

For quantification purposes, a calibration curve was made based on the amount of pheromone evaporated from an aq. soln. of the pheromone in DMSO at different flow rates (0.01–2 µL/min) (Fig. 2b). Substitution of the neat mean EAG response obtained with the microreactor in the curve provided a concentration of pheromone of 5 ng/µL, consistent with the GC–MS analysis of an aliquot of the pheromone solution emerging from the microreactor (3.2 ng/µL).

Acknowledgements

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