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A surface acoustic wave bio-electronic nose for detection of volatile odorant molecules

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ABSTRACT

In this work, a "bio-electronic nose" for vapour phase detection of odorant molecules based on surface acoustic wave (SAW) resonators is presented. The biosensor system is composed of an array of five SAW resonators coated with three types of odorant-binding proteins (OBPs): the wild-type OBP from bovine (wtbOBP), a double-mutant of the OBP from bovine (dmbOBP), and the wild-type OBP from pig (wtpOBP). High resolution deposition of OBPs onto the active area of SAW resonators was implemented through laser-induced forward transfer (LIFT). The resonant frequency shifts of the SAW resonators after the deposition of the biomolecules confirmed the immobilisation of the proteins onto the Al/Au inter-digital transducers (IDTs). In addition, a low increase of insertion losses with a limited degradation of Q-factors is reported.

The "bio-electronic nose" fabricated by LIFT is tested in nitrogen upon exposure to separated concentrations of R-(−)-1-octen-3-ol (octenol) and R-(−)-carvone (carvone) vapours. The "bio-electronic nose" showed low detection limits for the tested compounds (i.e. 0.48 ppm for the detection of octenol, and 0.74 ppm for the detection of carvone). In addition, the bio-sensing system was able to discriminate the octenol molecules from the carvone molecules, making it pertinent for the assessment of food contamination by moulds, or for the evaluation of indoor air quality in buildings.

1. Introduction

The detection of contaminants in food is essential to avoid risks for humans. In recent years, the pressure from government authorities on food industries led to an increasing research effort for the development of new techniques for fast and cost-effective analysis of food contaminants (McGrath et al., 2013). In fact, traditional analytic techniques such as liquid and gas chromatography combined with mass spectrometry, require the sample pre-treatment and the use of highly trained personnel resulting both expensive and time-consuming (Arora et al., 2011). Although these methods allow for a correct identification and quantification of the contaminant compounds, they are not suitable for on-line measurements. In contrast, biosensors have the potential to allow cost-effective, fast and portable detection, which make possible on-line and real-time monitoring without extensive sample preparation (Van Dorst et al., 2010).

An interesting application for biosensors is the assessment of food quality based on the odour intensity evaluation of volatile compounds. In particular, a "bio-electronic nose", that mimics the biological olfactory system, has the capability to identify odorant molecules with high sensitivity and specificity, overcoming the low-selectivity of the well-known electronic noses reported in the literature (Sankaran et al., 2012). Several components of olfactory system, such as the olfactory receptors (Sung et al., 2006), olfactory neurons (Liu et al., 2006), and OBPs (D'Auria et al., 2004) have been investigated as probes to design biosensors based on electrochemical, calorimetric, optical and electro-acoustic devices (Di Pietrantonio et al., 2013; Ulmer et al., 1997).

In this work, the development of a bio-electronic nose based on SAW devices using OBPs as probes for the detection of specific odorant molecules in food is presented. The proposed sensing system exploits the high sensitivity and fast response time typical

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of SAW-based sensors (Ballantine et al., 1997) in combination with the adaptable selectivity of the OBPs (Ramoni et al., 2007).

SAW devices are based on the propagation of an acoustic wave generated and detected by IDTs fabricated on the surface of a piezoelectric substrate. Since the acoustic energy is strongly confined at the surface, SAW devices are very sensitive to changes in mass, viscosity, or conductivity on the surface of the sensor (Grate et al., 1996). In particular, adsorption and desorption of analytes from a thin sensitive layer coated on the substrate, give rise to changes in the characteristics of the propagation path affecting the velocity and the amplitude of the wave.

So far, only a limited number of studies on SAW devices using biologic molecules as sensing element to detect small molecules in vapour phase have been proposed (Hunt et al., 2003). A SAW resonator immuno-sensor array based on the monoclonal antibodies anti-RDX and monoclonal antibodies anti-TNT was reported (Lee et al., 2005). These authors demonstrate the detection of low vapour pressure plastic explosives containing nitro groups such as RDX and TNT. In a different work based on a SAW platform, the detection of cocaine vapours by anti-benzoylecgonine antibodies was proposed (Stubbs et al., 2005).

Vertebrate OBPs are small extracellular proteins belonging to the lipocalin superfamily (Briand et al., 2002; Dal Monte et al., 1993; Lobel et al., 2002; Spinelli et al., 1998). They have been supposed to play a role in receptorial events of odour detection by carrying, deactivating, and/or selecting odorant molecules (Blanchet et al., 1996; Herent et al., 1995; Tegoni et al., 1996). The reversibility of odorant–OBP binding with dissociation constants in the micromolar range (Hou et al., 2005) enables the utilisation of OBPs as specific elements in sensing systems (D'Auria et al., 2004; Hou et al., 2005).

In our previous work, we showed that OBPs are suitable for the implementation of biosensors based on solidly mounted resonators (Cannatà et al., 2012b). In particular, we demonstrated the capability to detect different odorant molecules with a OBP-based SAW biosensor system (Di Pietrantonio et al., 2013). The SAW biosensors were coated by drop-casting of OBP containing solutions on the active area of the SAW sensors (Di Pietrantonio et al., 2013).

However, the SAW sensors require a uniform active layer along the wave propagation path in order to prevent high attenuation and degradation of the Q-factor (Di Pietrantonio et al., 2013; Yunker et al., 2011). In addition, another major issue related to this type of sensors is the reproducibility of the deposition and the use of relatively large volumes of biomolecule solutions, which is expensive and can also lead to the appearance of the coffee ring effect (Yunker et al., 2011), which causes non-uniformity of the deposition.

An interesting alternative to conventional deposition techniques is LIFT. The feasibility of LIFT to print sensitive materials in solid phase i.e. polymer pixels onto electroacoustic devices has been already demonstrated (Cannatà et al., 2012a; Di Pietrantonio et al., 2012). In addition, in the case of biomolecules in liquid phase LIFT resolves issues common to most traditional methods: it allows printing small volumes of biomolecule solutions, and offers accurate positioning and repeatability of the printed patterns. Furthermore, a wide range of viscosities can be printed through LIFT, with minimal engineering of the printing solution properties, in contrast with other competing techniques, i.e. inkjet printing (Roth et al., 2004). The feasibility of LIFT for high resolution printing has been extensively demonstrated (Arnold et al., 2007), and in addition the mechanism responsible for droplet formation has been widely investigated by time-resolved imaging studies (Duocastella et al., 2010; Kuznetsov et al., 2012). Furthermore, LIFT has already been demonstrated to be feasible for printing sensitive materials such as biomolecules (Colina et al., 2005; Duocastella et al., 2008), biomolecule structures (Palla-Papavlu et al., 2011), and even cells (Doraiswamy et al., 2006) and microorganisms (Hopp et al., 2005).

In our recent work (Palla-Papavlu et al., 2014) we demonstrated the feasibility of LIFT for the uniform application of the wtbOBP containing solutions onto the active area of a SAW device.

The “bio-electronic nose” fabricated in this work is based on three SAW resonators coated through LIFT with three different OBPs, characterised by different binding specificity, plus an uncoated SAW device used as reference. The selected proteins are the wild-type OBP from bovine (wtbOBP), a double mutant of the OBP from bovine (dmbOBP), and the wild-type OBP from pig (wtpOBP). First, the capability of LIFT to uniformly apply the sensing layers along the SAW active area is analysed. Second, the SAW biosensor array is exposed to separated concentrations of octenol and carvone vapours, two odorant compounds largely used in food industry. Finally, the sensitivity and detection limits of the LIFT-ed arrays were determined and compared to those obtained with more conventional deposition methods.

The proposed “bio-electronic nose” represents the first approach towards biosensor systems for the assessment of food quality. Octenol is an eight carbon volatile compound that has been isolated from many natural sources like plants and fungi (Dijkstra and Wikén, 1976), and, it is produced by numerous species of moulds (Kaminski et al., 1974). Therefore, the evaluation of octenol concentrations could be used for the assessment of food contamination by moulds and fungi (Piotrowska et al., 2013). Actually, the detection and discrimination of octenol from other volatile compounds has many potential applications. The World Health Organization correlates the increased risk of respiratory symptoms and infections with damp and mouldy environments (World-Health-Organization, 2009) and among the highest reported concentration for a single volatile compound found in problem buildings was octenol (Morey et al., 1997). Lately, the neurotoxicity of low concentrations of octenol has been shown in a *Drosophila melanogaster* model (Inamdar et al., 2010) as well as in human embryonic stem cells (Inamdar et al., 2012) and murine splenic leukocytes (Gorham and Hokeness, 2012). Finally, Inamdar et al. demonstrated that octenol exerts toxicity via disruption of dopamine homeostasis and may represent a naturally occurring environmental agent involved in parkinsonism (Inamdar et al., 2013). Therefore, it is clear the importance of a low-cost system for the detection and discrimination of octenol in indoor air in buildings.

2. Material and methods

2.1. Odorant-binding proteins

Three different OBPs were used in this work: two OBPs from bovine and one OBP from pig. The purification procedure to obtain the proteins can be found in Supplementary information (S11).

The functionalities of the recombinant wtbOBP, dmbOBP and wtpOBP were determined by direct titrations using the fluorescent ligand 1-amino-anthracene (AMA). Specifically, 1.0 ml of 1.0 μM OBPs, in 20 mM Tris–HCl buffer pH 7.8, was incubated overnight at 4 °C in the presence of increasing concentrations of AMA (0.156–10 μM). Fluorescence emission spectra were recorded between 450 nm and 550 nm by an ISS K2 fluorometer (excitation and emission slits were set at 2.0 nm) at a fixed excitation wavelength of 380 nm. The formation of the AMA-OBPs complex was followed as the increase of the fluorescence emission intensity at 480 nm.