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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.

2.2. Laser-induced forward transfer

The LIFT experimental setup was reported in detail elsewhere (Duocastella et al., 2007) and it is briefly described in Supplementary information (S12).

For the realisation of the donor films three protein solutions were used. In particular, the wtbOBP and the dmbOBP were dissolved in Tris/HCl (10 mM, pH=8.0) with a final concentration in solution of 2.0 mg/ml, whereas the wtpOBP was dissolved in PBS (10 mM, pH=7.4) with a final concentration in solution of 1.5 mg/ml. The donor film was a mixture of one protein solution and glycerol (20 v/v) blade coated (15 μm thick, estimated by weight measurement) onto a Ti coated (50 nm thickness) glass microscope slide.

2.3. SAW biosensor system

The SAW devices were designed and fabricated on α-quartz substrates, along propagation direction with Euler angles (0°, 132.75°, 0°), as 2-port resonators operating at 392 MHz (see Supplementary information (S13)). The resonators were fabricated on the same quartz substrate and arranged in arrays consisting of 5 devices with total dimensions of 25×2.5 mm^2. An image of the SAW biosensor array is shown in Fig. 1(a). The SAW biosensor system is composed of five electronic oscillators with SAW resonators used as frequency control element in the feedback path. Three oscillators were used for the SAW devices coated with the three different types of OBPs and the remaining ones for reference uncoated resonators. Details on the electronics oscillators were previously reported (Di Pietrantonio et al., 2013). The photo of the system is shown in Fig. 1(b).

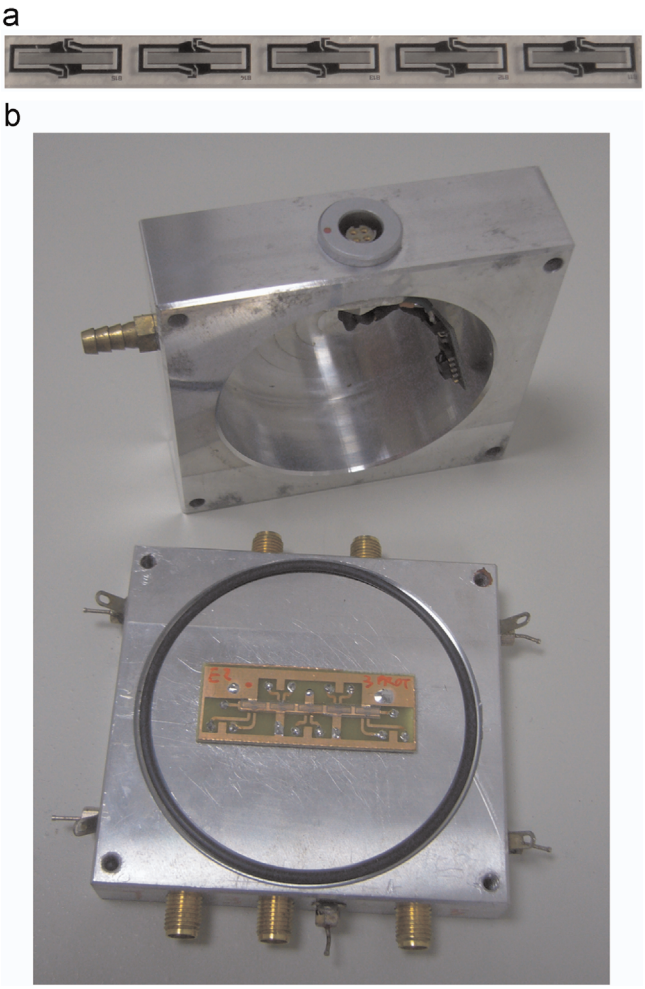

Fig. 1. Photo of the bio-electronic nose system: (a) array of SAW resonators; (b) bio-nose system with measurement chamber and conditioning electronics.

2.4. SAW electrical characterisation

SAW devices were electrically characterised before and after OBP depositions on a probe station (Wentworth) using microprobes (Picoprobe 40A-GSG-200-T) by GGB Industries Inc. The protein solutions were deposited by LIFT and, after the adhesion of the OBPs to the surface of the SAW resonator, they were rinsed with double distilled water to remove the unattached proteins or any residues from glycerol and salts, and dried under a soft nitrogen stream. The frequency response (S_{21} scatter parameter) was measured in both magnitude and phase formats using a Network Analyser (HP8753A). In particular, the frequency shift, the insertion losses, and the loaded quality factor Q of the SAW resonators were measured and compared before and after the protein depositions.

2.5. Sensor measurement setup

The SAW biosensor arrays were tested in nitrogen atmosphere upon exposure to concentrations of octenol and carvone vapours (each analyte was tested separately) with a total flux of 100 sccm. The measurement setup was described in detail in our previous work (Di Pietrantonio et al., 2013).

The SAW biosensor array responses, given by the frequency shifts of the controlled oscillators, were measured with a frequency counter (HP 536B) and a multiplexer module (Agilent 34980A and 34941A). All data were acquired using a custom LabVIEW® routine. The frequency of the reference device was measured and subtracted from the measurements of the coated sensors by using three mixers (Mini-CircuitsRMS-2MH). This differential configuration allowed the system to compensate the influence of humidity, temperature, and pressure on the sensor responses.

Prior to the odour measurements, the frequency baseline was obtained exposing the SAW biosensor array to a flux of pure nitrogen. Then, the odorant concentration was added to the system until saturation of the frequency response was reached. Finally, the biosensor array was exposed to nitrogen flux in order to reach again the initial frequency baseline. The performances of the system were evaluated considering the sensitivity, repeatability, and detection limit of the SAW biosensors. For each odorant, measurements at different concentrations were performed and the frequency shifts at saturation were recorded to evaluate the sensitivity. The repeatability was tested executing for each concentration four measurements in the same conditions. Finally, the detection limits were calculated considering a maximum noise level of 10 Hz. The obtained data were used to perform pattern recognition studies based on principal component analysis (PCA). In particular, the responses of the biosensors to five different concentrations of the odorants, each repeated four times, were considered. Data pre-processing for pattern recognition included the mean centring of the raw data Δf_{ij} (change in frequency of j-th sensor due to exposure of i-th odorant sample) by its mean value determined by averaging over all measured odorant samples, and the normalisation of mean-centred data using as weights the inverse of the sample variance. The PCAs were implemented by Matlab® using the singular value decomposition algorithm.

3. Results and discussion

3.1. OBP deposition by LIFT

As reported previously (Palla-Papavlu et al., 2014), the performance of the SAW sensors were mainly affected by the physical properties of the coatings. Thus, when applying protein containing solutions on the surface of the SAW sensors it is important to control parameters such as: i) laser fluence, ii) viscosity of the protein solution, and iii) droplet overlap.

However, as this work is focused on the direct application of the fabricated SAW biosensors, i.e. the detection of odorants for the food industry, the parameter optimisation studies for protein solution printing are briefly introduced.

Optical microscopy images of SAW resonators deposited by LIFT are shown in Fig. 2(a)–(d). When investigating the range of laser fluences for which well-defined features can be printed, it was found that only between 300 and 700 mJ/cm^2 neither splashing nor damage of the IDTs occurs. Individual droplets from solutions with 20% glycerol printed at 650 mJ/cm^2 onto the active area of a SAW resonator are presented in Fig. 2(a). The LIFT-ed droplets were transferred with high accuracy onto the SAW resonators and they have slightly corrugated shape. However, it was shown (Palla-Papavlu et al., 2014) that solutions with low viscosities, i.e. 1.9 mPa·s, although difficult to print are favourable to uniformly coating the active area of SAW resonators. The uniform coverage of the active area was achieved by changing the centre-to-centre distance between two successive droplets. In Fig. 2(b) an optical microscopy image of droplets that merge into pairs can be noticed. In Fig. 2(c) and (d) optical microscopy images of droplets that merge into continuous lines and cover the entire active area of the SAW resonators are presented. The uniform features, i.e. Fig. 2(c) and (d) appear for droplet overlaps (the ratio of the lengths between two neighbouring droplets to the individual droplet diameter) between 40% and 50%. Although the experimental conditions not being exactly the same as those in ref. (Palla-Papavlu et al., 2013), it can assumed that the dynamics taking place are essentially the same as those described in ref. (Palla-Papavlu et al., 2013). Printing of uniform continuous lines by LIFT and covering the entire active area of the SAW resonator can be explained in terms of the dynamics of needle-like jets which propagate along a gap between donor and receiver substrate (Duocastella et al., 2010; Palla-Papavlu et al., 2013).

3.2. OBP coating assessment by electrical characterisation of SAW resonators

The electrical characterisation of the SAW resonators provided a preliminary indication on the feasibility of the LIFT technique for the fabrication of SAW biosensors based on protein coatings. In fact, the functionality of SAW devices is significantly dependent on the mass and morphology of the sensible coating (McGill et al., 1998). In particular, the phase velocity, and hence the resonant frequency, and the insertion loss of SAW resonators are both dependent on the amount of the deposited proteins. Moreover, the uniform application of the proteins without the appearance of coffee ring effect minimises the SAW scattering and diffraction and ensures low degradation of the electrical characteristics of the resonators. Specificity, the Q-factor and the insertion loss of the SAW device will directly impact the phase noise and short-term frequency stability of the oscillator, and, hence, the sensitivity and resolution of the measurement system. As an example, the S_{21} amplitude responses of SAW resonators before and after the deposition by LIFT of the three used OBPs are shown in Fig. 3. The protein solutions were mixed with 20% of glycerol and deposited at a laser fluence of 650 mJ/cm^2. The decreases of resonance frequency were in the range between 85 kHz and 271 kHz and pointed out the change of the mass loading on the sensor surface due to the protein adhesion as indicated by the mass sensitivity equation reported by Grate and Klusty (1991). The behaviour of frequency responses was not significantly corrupted by the depositions, and the increase of insertion loss at the resonance frequencies was lower than 4 dB. After the coatings, the insertion losses at the resonance were below 15 dB and allowed the amplifier (Mini-Circuits MAR-8) to fulfil the *Barkhausen* conditions on the oscillator loop. The loaded Q-factor for the

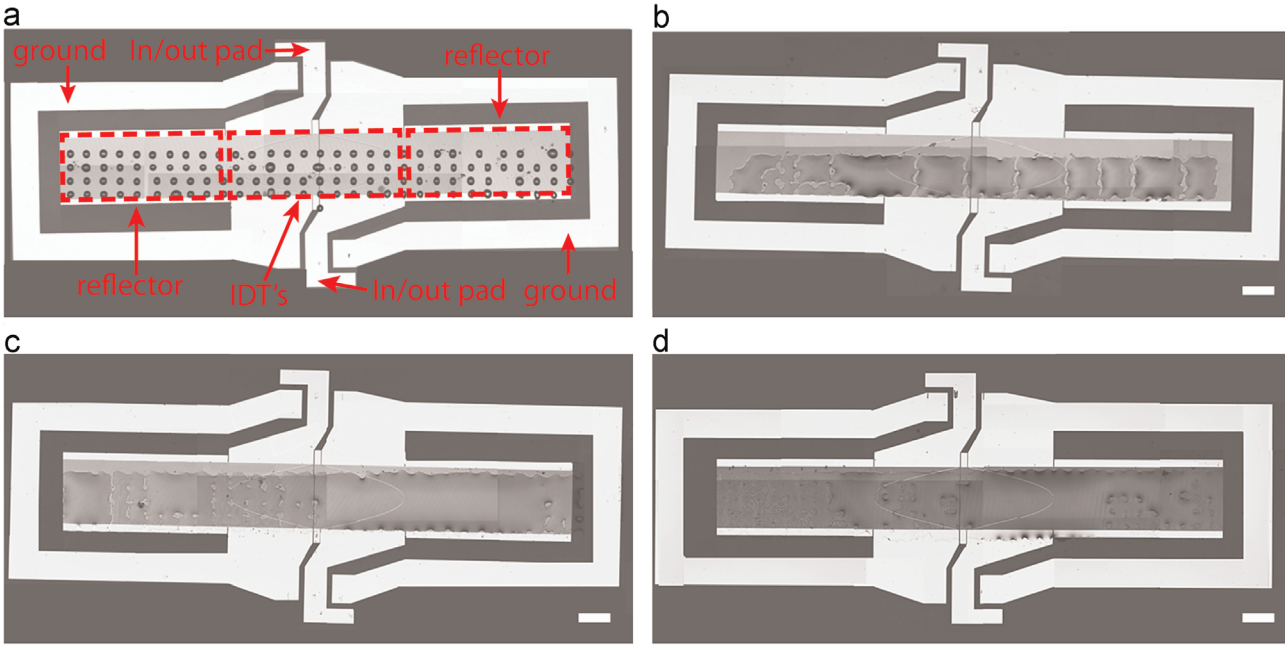

Fig. 2. Optical microscopy images of SAW devices printed by LIFT onto the active area of the SAW resonators (a) with individual droplets; (b)–(d) by overlapping individual droplets at different centre-to-centre distances (ratio of overlapped length between two neighbouring droplets to the individual droplet diameter): (b) 15% overlap, (c) 30% overlap, (d) 50% overlap.

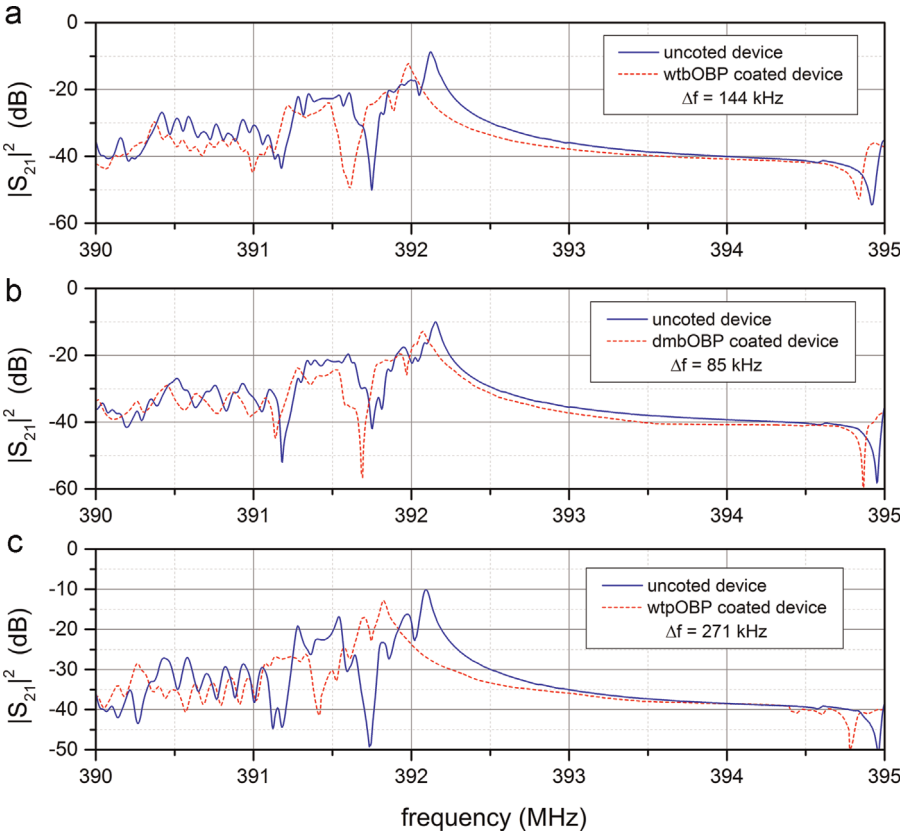

Fig. 3. Frequency response of the SAW resonators (amplitude of S_{21}) before (continuous line) and after (dotted line) deposition by LIFT of OBPs: (a) wtbOBP coated device, (b) dmbOBP coated device, and (c) wtpOBP coated device. The frequency shift is proportional to the mass loading of the protein on the SAW device.

uncoated devices was ~7500, while the obtained loaded Q-factors after protein depositions were greater than ~5500. These results provided a suitable frequency stability for the electronic oscillators and were in line with those obtained with drop casting depositions (Di Pietrantonio et al., 2013) and with those achieved for optimised depositions of polymers (Matatagui et al., 2011; McGill et al., 1998). These findings support the feasibility of the LIFT for the deposition of proteins on SAW resonators.

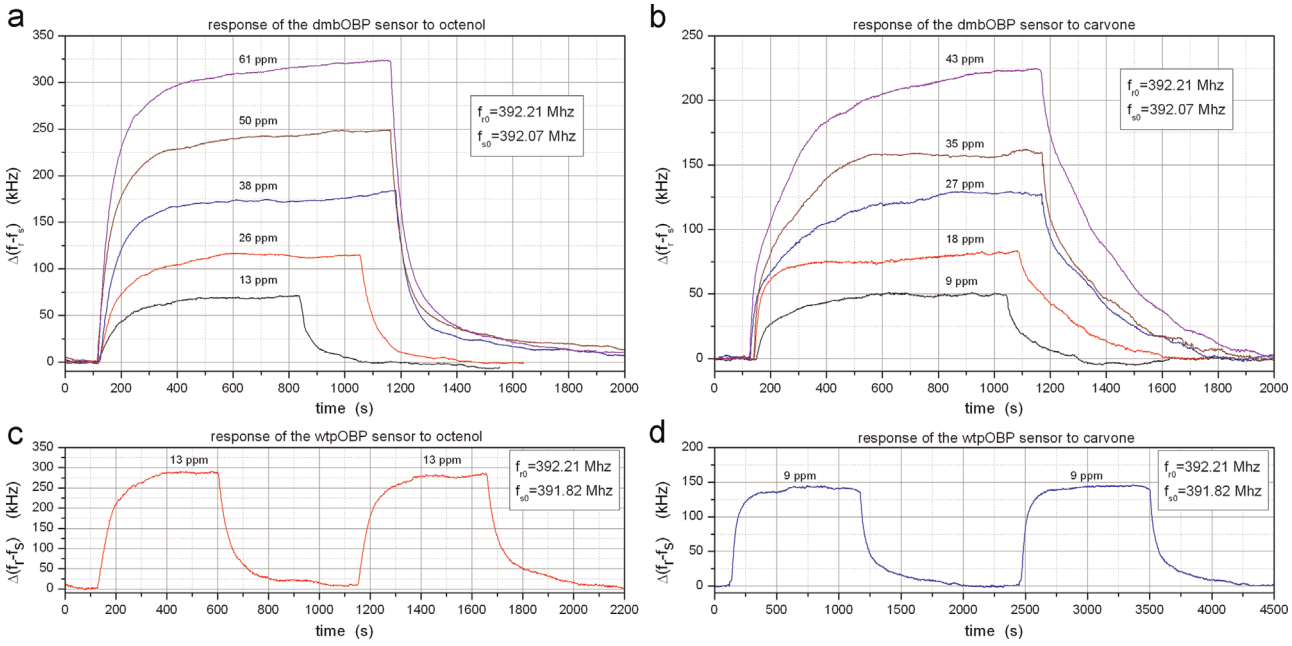

Fig. 4. Frequency response vs. time of two OBPs-based biosensors on the same array: (a) dmbOBP-based biosensor for different concentrations of octenol in the range from 13 ppm to 61 ppm; (b) dmbOBP-based biosensor for different concentrations of carvone in the range from 9 ppm to 43 ppm; (c) wtpOBP-based biosensor subjected to two cycles of 13 ppm of octenol; (d) wtpOBP-based biosensor subjected to two cycles of 9 ppm of carvone. The response is the difference between the reference and the OBPs-based biosensor frequency shifts, and f_{r0} and f_{s0} represent the original centre frequency of the reference and of the sensors, respectively.

3.3. Functionality test and biosensor measurements

The functionality of the proteins after the depositions with LIFT and the capabilities of the fabricated system were determined by exposing a SAW biosensors array to concentrations of octenol and carvone in nitrogen atmosphere and recording the differential frequency shifts as explained in Section 2.5.

The SAW biosensor system showed fast, remarkable, and reversible responses to the selected odorants. The real-time response of the dmbOBP coated SAW sensor for concentrations of octenol (Fig. 4(a)) and carvone (Fig. 4(b)), in the range from 13 ppm to 61 ppm and from 9 ppm to 43 ppm, are shown in Fig. 4. In addition, in Fig. 4 are presented the time response profiles and the short-term repeatability of the wtpOBP biosensor, implemented on the same array of the dmbOBP-based sensor, subjected to 2 cycles of on-off exposures of 13 ppm of octenol (Fig. 4(c)) and of 9 ppm of carvone (Fig. 4(d)). Fast response rise was observed for all used coating and odorants, and, in particular, for 13 ppm of octenol (Fig. 4(c)) and for 9 ppm of carvone (Fig. 4(d)), the responses of the wtpOBP sensor reached approximately 80% of the saturation value within 95 s. When the odorants were removed, the recovery times to return to 80% of the initial baseline values were within 190 s.

The response curves for biosensors coated with the three proteins showed a linear behaviour in the tested concentration ranges as reported in Fig. 5, where each point is the average of four independent measurements, while standard deviations are indicated as error bars. The relative standard deviations between responses obtained in the same conditions were within 5%, demonstrating a good repeatability.

For wtbOBP-based SAW biosensor, a sensitivity of 5.63 Hz/ppm to octenol was obtained with a calculated detection limit of 1.78 ppm. In the case of the dmbOBP-based SAW biosensor, the sensitivity and detection limit to octenol were 4.95 Hz/ppm and 2.02 ppm, respectively. Finally, for the wtpOBP-based SAW biosensor a sensitivity of 20.8 Hz/ppm was obtained and a detection limit of 0.48 ppm was calculated. The response curves to octenol from 13 ppm to 61 ppm are reported in Fig. 5(a). The response curves to carvone from 9 ppm to 43 ppm are reported in Fig. 5(b). Also in this case, wtpOBP-based SAW biosensor exhibited the best sensitivity (S=13.5 Hz/ppm) and detection limit (DL=0.74 ppm). The obtained sensitivity and detection limit for wtbOBP-based SAW biosensor and dmbOBP-based SAW biosensor were 7 Hz/ppm and 1.43 ppm, and 4.83 Hz/ppm and 2.07 ppm, respectively.

A comparison between the sensitivities of the three SAW biosensors towards the tested odorants is shown in Fig. 6(a). The highest sensitivity to octenol was obtained for the wtpOBP-based SAW biosensor. This result suggested that octenol molecule fits in the hydrophobic funnel of the wtpOBP better than the two bovine OBPs used in the other two SAW biosensors.

It should be noted how the wtbOBP-based SAW biosensor demonstrated the highest sensitivity to the carvone odorant, in respect to octenol. On the contrary, the wtpOBP-based SAW biosensor showed the highest sensitivity towards octenol. This result suggests that the proposed SAW biosensor array is able to discriminate between octenol and carvone, as confirmed by the PCA analysis.

The results of PCA are reported in Fig. 6(b) where the biplot of loadings and scores using the first two principal components (PCs) is shown. Octenol and carvone appeared in well-formed and separate clusters indicating that the two odorants under test can be clearly discriminated. Only minor deviations along the concentration profile were obtained as expected from the high linearity of the sensor response curves. Clusters were mainly aligned along PC1 (~93.4% variance) and the cumulative variance in the plane PC1–PC2 was exceeding over 99.4%. The contributions

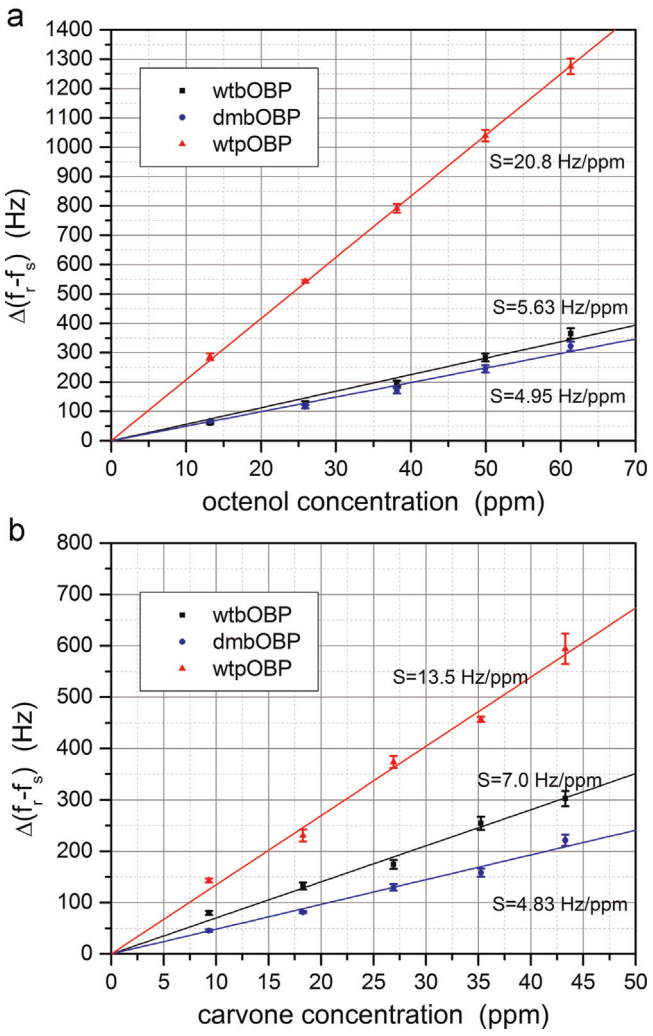

Fig. 5. (a) Response curves for wtbOBP, dmbOBP and wtpOBP based biosensors upon exposure to different concentrations of octenol; (b) response curves for wtbOBP, dmbOBP and wtpOBP based biosensors upon exposure to different concentrations of carvone. Error bars indicate the repeatability.

of the biosensors to the first two PCs are specified by the directions and lengths of the vectors reported in the biplot (Fig. 6(b)). In particular, the coefficients on PC2 for the loadings of the wtbOBP and the wtpOBP biosensors, that are opposite and almost equal, indicated how these sensors are able to discriminate between octenol and carvone.

This finding is a clear indication that the three different OBPs used in our system are characterised by different specificity towards different odorant molecules revealing the possibility of their simultaneous use for the design of a “bio-electronic nose”. The different protein selectivity towards different compounds may be ascribed to the presence of different structures of the funnels of the three proteins that are responsible of the selective binding. Docking experiments were designed to verify at molecular level the specific protein-compound interactions involved in the OBP binding events. Finally, the sensitivities and detection limits of the proposed biosensor system were similar to those obtained with drop casting depositions (Di Pietrantonio et al., 2013). Therefore, the obtained results indicate that LIFT deposition of OBPs is possible without significant modifications of their functionality, and with a much lower consumption of detecting agent.

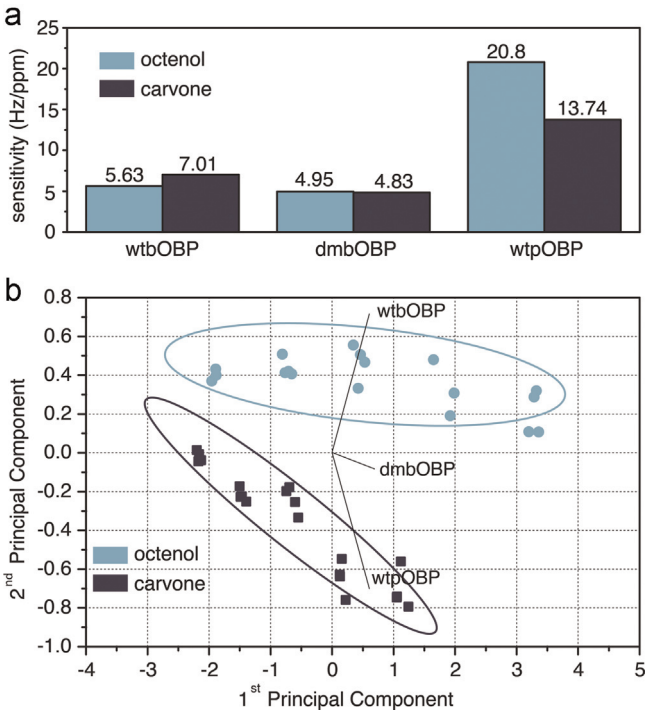

Fig. 6. (a) Comparison between sensitivities to octenol and carvone detection of the wtbOBP, dmbOBP and wtpOBP based biosensors; (b) biplot of loadings and scores using the first two principle components of the PCA analysis.

4. Conclusions

A "bio-electronic nose" based on SAW resonators and exploiting OBPs-based sensitive layers deposited by LIFT was fabricated and tested. After a careful optimisation of the experimental parameters such as the laser fluence, the viscosity of the protein solution, and the droplet overlap, only protein solutions mixed with 20% of glycerol were used for the fabrication of functional SAW biosensors capable of detecting specific odorants. The resonant frequency shifts of SAW resonators after the deposition of the biomolecules confirmed protein immobilisation on the Au/Al transducers. In particular, the low increases of insertion loss with a limited degradation of Q-factors of the resonators indicated the feasibility of LIFT for the deposition of proteins on SAW devices. The deposition of OBPs by LIFT without significant modifications of their functionality was established by the measurements performed with the fabricated OBPs-based SAW biosensors. The biosensors coated by LIFT showed sensitivities similar to those obtained with more conventional deposition methods (Di Pietrantonio et al., 2013), but with an easily reproducible process and with a much lower consumption of detecting agent.

Finally, the different sensitivities demonstrated by the three SAW biosensors and the results of PCA indicate the capability of the proposed "bio-electronic nose" to discriminate between octenol and carvone molecules, and the possible utilisation of this biosensor system for the assessment of food contamination by moulds or for the evaluation of indoor air quality in buildings.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.09.027.

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