Sensors and Actuators B 192 (2014) 369–377

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Preparation of surface acoustic wave odor sensors by laser-induced forward transfer

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ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form
27 September 2013
Accepted 20 October 2013
Available online 15 November 2013

Keywords:
Laser-induced forward transfer
LIFT
Surface acoustic wave
SAW biosensors
Odorant binding protein
wtbOBP

ABSTRACT

In this study it is presented the development of surface acoustic wave (SAW) biosensors for odor detection in the food industry. The SAW biosensors are coated by laser-induced forward transfer (LIFT) with wild-type bovine odorant binding protein (wtbOBP) solutions containing 20% and 50% glycerol. Optical microscopy investigations revealed that individual droplets could be printed from 50% glycerol solutions with a high resolution. Further investigations proved that despite the lower resolution of the 20% glycerol printed droplets it is possible to achieve higher uniformity in the coverage of the entire active area of the SAW biosensors. In addition, it is shown that the surface density of the wtbOBP LIFT-ed layer is four times higher than in the case of pipette deposition. Finally, the functionality of the SAW biosensors was investigated by testing the biosensors upon exposure to various concentrations of octenol vapors. A high sensitivity, i.e. of 5 Hz/ppm and detection limit in the low ppm range was obtained, lower that what has previously been reported with conventional methods.

1. Introduction

Pollution control, recognizing environmental conditions such as hazardous agents or detection of pathogens, pesticide and drug residues in food are topics of great interest receiving increasing attention in both developed and developing countries [1]. The interest of olfactory systems for such purposes is obvious, since they are able to accurately recognize and discriminate between odorant volatile species with high sensitivity and specificity. Biosensors show great potential for the detection of volatile species, with important advantages, like miniaturization, fast response, and minimal sample preparation [2]. Olfactory systems based on electro-acoustic devices, such as SAW devices, can discriminate between odorant volatile species only if a large number of sensors are used in conjunction with pattern recognition techniques. In fact, sensors coated with polymers show a low selectivity. In particular, SAW biosensors seem to be a powerful tool to measure small concentrations of volatile compounds since they can overcome the intrinsic low-selectivity of polymer coated SAW chemical sensors.

SAW sensors are based on the generation and detection of acoustic waves at the surface of a piezoelectric crystal. The acoustic energy is strongly confined at the surface of the device and is independent of the thickness of the substrate. SAWs are very sensitive to changes in mass, viscosity, or conductivity on the sensor's surface [3]. Recently, a great interest is directed toward the development of SAW devices based on biological molecules as sensing material able to detect low concentrations of molecules in air [4]. An interesting application is the use of SAW devices as odor sensors with odorant binding proteins (OBPs) as sensing element. OBPs are small extracellular proteins which belong to the lipocalin super-family [5,6]. They have an important role in odor detection by carrying, deactivating, and/or selecting the odorant molecules.

An important issue with SAW sensors is the uniform application of the sensing layer along the wave propagation path, in order to prevent high attenuation. The sensitivity of SAW resonators is dependent on the amount of odor binding to the protein overlayer and also by the SAW's inherent ability to respond to the physical changes in the overlaying film. Therefore, it is very important to precisely place the protein coating, and moreover for the

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http://dx.doi.org/10.1016/j.snb.2013.10.082

coating to have the correct thickness and to completely cover, i.e. without voids the active area of the sensor. The most common method of applying small amounts of biomolecules to the surface of the SAW devices is by a simple technique, i.e. pipette or droplet method which does not require elaborated optimization [7]. Another method for applying sensing layers on SAWs is spin coating. However, the main disadvantage of these methods is the use of relatively large volumes of biomolecule solutions, which on one hand is very expensive, and on the other can lead to the appearance of the coffee ring effect when drying, due to the long evaporation time of a high amount of solvent [8]. In addition, in order to realize a SAW sensor array which is capable of detecting multiple analytes, i.e. to reach high selectivity, the spin coating process cannot be used.

An interesting alternative to such conventional techniques is laser-induced forward transfer (LIFT). The feasibility of LIFT to print sensitive materials in solid phase, i.e. polymer pixels onto electroacoustic devices has been already demonstrated [9,10]. 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 being required, in clear contrast with other competing techniques, like inkjet printing [11].

In LIFT, a laser beam is focused through a transparent support onto the backside of an absorbing thin film [12–14] coated with a film of the solution of the material to be transferred (donor film). Each pulse promotes the transfer of a small fraction of the solution onto a receiver substrate that is usually placed parallel and facing the thin film at a short distance (between a few tens and several hundreds of μm). A pattern of the transferred material can be "written" on the substrate with multiple shots by translating both donor and receiver substrates respect to the laser beam.

The feasibility of LIFT for high resolution printing has been extensively demonstrated [15–22], and in addition the mechanism responsible for droplet formation has been widely investigated by time-resolved imaging studies [23–27]. Furthermore, LIFT has already been demonstrated to be feasible for printing such sensitive materials as biomolecules [15–20,28], biomolecule structures [13], and even cells [14,21] and microorganisms [22]. Until recently, most of the attention on LIFT research had been focused on regular droplet morphology [14–20].

In this work it is investigated the possibility of depositing biomolecule containing solutions, i.e. wild type bovine odorant binding proteins through LIFT onto the active area of SAW devices for applications in odor detection. LIFT it is not only an appropriate technique for individual droplet deposition aiming applications such as microarrays, but also entire area deposition with a very high lateral resolution for applications such as biosensor fabrication. The performance of LIFT for the uniform application of the sensing layer along the device active area is analyzed. In addition, it is proven the feasibility of the technique for odor sensing through the fabrication of a functional SAW sensor capable of detecting specific odors with sensitivity and detection limit similar to those which can be obtained with more conventional methods, but with a much lower consumption of detecting agent.

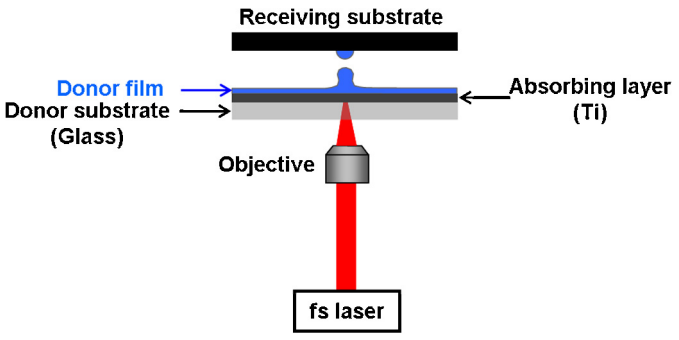

Fig. 1. Scheme of the LIFT experimental setup.

2. Materials and methods

2.1. Laser printing on SAW sensors

The scheme of the LIFT experimental setup is shown in Fig. 1 [29]. LIFT consists in laser printing of small quantities of material from a donor thin film onto a receiver substrate. The donor film is a liquid, i.e. a mixture of protein solution and glycerol (20% and 50%, v/v) blade coated (15 μm thick, estimated by weight measurement) onto a Ti coated (50 nm thickness) glass microscope slide. The protein solution is wild type bovine odorant-binding protein (wtbOBP) [30] dissolved in Tris/HCl (10 mM, pH 8.0), with a final concentration in solution of 2 mg/mL. The surface tension and the viscosity of the protein solution mixed with 50% glycerol is 30 mN/m and 6.5 mPa s, respectively, and the corresponding values for the 20% glycerol solution are 24 mN/m and 1.9 mPa s, respectively.

The transfer mechanism in the case of LIFT of individual droplets is similar to the behavior of cavitation bubbles near the surface of a bulk fluid. The laser beam irradiates the Ti layer producing a high-pressure vapor bubble which expands and displaces the liquid downwards. Once it reached its maximum, the bubble collapses and a jet develops. This jet continues to advance, becomes thinner and after some time breaks due to surface tension effects [23–27].

The laser used for the transfer is a diode pumped Yb:YKW laser system from Amplitude Systems, s-Pulse, 1027 nm wavelength, 450 fs pulse duration, and 1 Hz repetition rate. The laser beam is focused through a microscope objective (50×, NA 0.55, WD 13 mm) onto the backside of the donor film. The donor is placed parallel to the receiving substrate on a motorized translation stage.

The receiving substrates are 2-port SAW resonators [9] which consist in two inter-digital transducers (IDTs) arranged between two grating reflectors, all of them deposited on α-quartz substrates (ST-cut, × propagation) (Fig. 2). The metallic electrodes are made of Al (100 nm thickness) coated with Au (2 nm thickness), which promotes protein adhesion [31]. Both the Au and Al films are grown by radio frequency magnetron sputtering from a 99.9% and 99.999% pure Au and Al target, in an Ar atmosphere. The patterns are transferred by photolithography using a poly(methyl-metacrylate) resist exposed by deep UV radiation, and lift-off procedure to remove the unexposed resist. Each IDT consists of 76 couples with a finger width of 2 μm and a metallization ratio of 0.5, resulting in a wavelength of 8 μm. The maximum finger overlap is 450 μm and IDTs are shaped with a Gaussian apodization in order to minimize spurious transverse modes. The number of reflecting fingers for each grating is 300 and the cavity length is 1278 μm. The operating frequency of the resonators results to be approximately 392 MHz.

The donor and the receiver substrates are separated by a gap of 100 μm through polyimide spacers. The computer controlled stage allows the creation of different features, i.e. from an array of individual droplets to lines and areas simply by varying the spacing between the droplets (center-to-center distances from consecutive droplets). All transfer experiments are carried out in air at ambient temperature.