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Nanolithography and subnanomolecular interactions for biomimetic sensors

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Dedicated to Prof. Dr. Heinz Hoffman on the occassion of his 70th birthday

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Abstract

Template-directed moulding of polymer thin films is performed to generate artificial receptors directly on pre-coated piezoacoustic devices and interdigital capacitors (IDCs). This generally applicable approach is useful for the fabrication of robust nano- and microstructured sensor coatings. Exemplary label-free detection results of yeast and mammalian cells are shown with these biomimetic receptors allowing even single cell detection. Grid electrodes were applied to quartz crystal microbalances (QCM) screening the dielectric properties of viable *Saccharomyces cerevisiae* and unusual non-gravimetric frequency shifts are observed. Autocorrelation analysis of capacitive responses to yeast cells yields characteristic time constants related to cell trapping by the sensor surface.

Keywords: Molecular imprinting; Viruses; Yeast cells; Erythrocytes; Quartz crystal microbalance; Interdigital capacitor

1. Introduction

Template-directed synthesis of synthetic receptors from highly crosslinked polymers is an innovative and generally applicable technique [1]. The synthesized materials are usually termed molecularly imprinted polymers (MIPs) [2]. The recognition sites in the bulk of these materials are formed by self-organizing principles during the polymerization process. The cavities in the polymer bulk remaining after the dissolution or evaporation of the small organic molecules in the polymer are rather rigid due to the high crosslinking and thus do not collapse. The selective chemical recognition of the templating species by re-incorporation is achieved by a geometrical fit in combination with a pronounced adhesion to the cavities based on non-covalent phenomena as electrostatics, hydrogen bonding, van-der-Waals and hydrophobic forces. The geometrical dimensions range from diameters of molecules to micrometers characterizing cells. The selective interactions between analyte and sensor surface, however, are in any case due to subnanomolecular interactions. These MIPs are most often applied in analytical sciences for solid phase extraction or chromatographic separation. Now, an increasing number of publications are applying this self-organization technology as an effective strategy to create receptors for chemical sensing [3,4].

Our work is motivated by the possibility to extend the imprinting concept with small organic molecules towards the recognition of biological specimen. Bioimprinting with bulky specimen as templates, such as viruses or cells, can rationally only be performed on the polymer surface due to diffusion limitations [5]. We therefore developed a surface imprinting concept of thin films based on a stamping procedure to mould the polymer surfaces during the polymer curing [6]. This

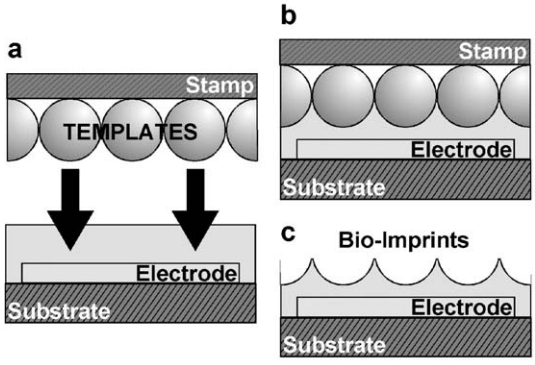

Fig. 1. Schematic illustration of surface imprinting using biotemplates directly on a pre-coated transducer.

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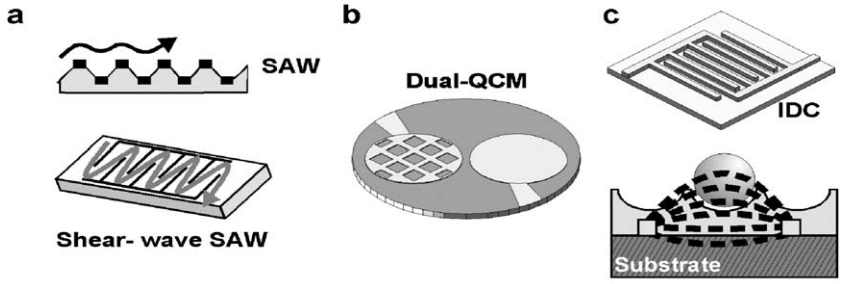

Fig. 2. Schemes of transducers applied for biomimetic sensing. (a) QCMs with two screen-printed channels, a conventional and a grid electrode. (b) High frequency SAWs for gas and liquid sensing are shown. (c) IDCs for dielectric measurements.

technique is related to soft- and imprint-lithography and is generally applicable to biological templates spanning the nano- and micrometer scale [7].

2. Experimental

Materials for surface imprinting are prepared by polymerizing a monomer solution until reaching the gel point. Polymers synthesized by either radical polymerization or polyaddition can be applied for this process [8]. The results presented were obtained with polyurethane composed of bisphenol A, 4,4′ diisocyanatodiphenylmethane (30% trifunctional) and phloroglucinol as cross-linker. The purified compounds were used as received from MERCK. The gel-like pre-polymer is redissolved in tetrahydrofurane and diluted in an appropriate concentration for the transducer coating. Thin films of these pre-polymers are formed by either spin or drop-coating of the transducer. To perform the surface imprinting, a stamp covered with a layer of purified templates is prepared and pressed onto the polymerizing thin film during the curing (Fig. 1a and b). The critical point is to remove the stamp from the cured polymer layers without peeling-off the sensor layer. This is possible since stamp and surface of the sensor layer are slightly separated by the bulky bio-templates. The aqueous solution can wet the void between thin film and stamp making possible a non-destructive lift-off. The templates remaining on the polymer surface are removed by brief rinsing in an ultrasonic bath (Fig. 1c). To minimize a covalent embedding of templating structures reactive isocyanato groups are either blocked by brief exposure to humid air or by blocking agents with amino groups.

Mass producible and highly sensitive transducers are used for the sensing experiments, quartz crystal microbalances (QCMs), surface acoustic wave resonators (SAWs) and interdigital capacitors (IDCs). Two or three gold electrodes were screen printed on a single quartz substrate to fabricate a QCM with a fundamental resonance frequency of 10 MHz. The multi-electrode structure is used for differential measurements to compensate for temperature, viscosity and pressure fluctuations. Electrodes facing the aqueous solution have larger diameter than the electrodes oriented to the gas phase to minimize conductivity effects [9]. In contrast to this electrode geometry of QCMs, we fabricated grid electrodes facing the aqueous phase to increase the stray-field influence. This allows us to monitor dielectric properties of the enriched analytes (Fig. 2a). Additionally, high frequency shear wave SAWs were applied for ultra-sensitive mass resolution in the picogram range. The $LiTaO_3$ SAWs had a fundamental resonance frequency of 428 MHz. Here, surface acoustic shear waves are used avoiding the high damping observed for Rayleigh waves [10].

Piezoacoustic measurements are performed with home-built oscillator circuits and a network analyzer. A precision LCR meter is used for impedance and capacitance measurements

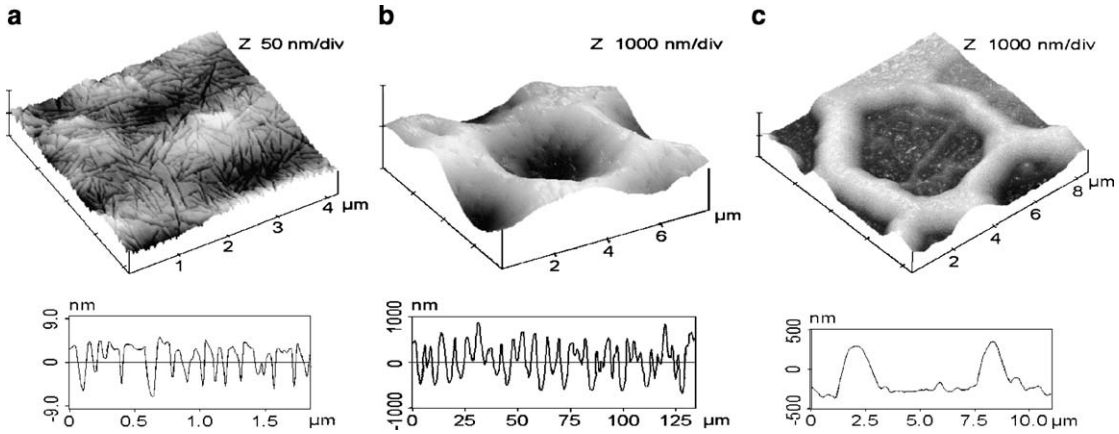

Fig. 3. AFM micrographs and section analyses of molded polyurethane thin films by surface imprinting (Digital Instruments Nanoscope III atomic force microscope). (a) The TMV MIP is imprinted with a sub-monolayer of viruses. The virus imprint depth corresponds to half the TMV diameter. (b) A single *S. cerevisiae* imprint from a packed sensor layer is shown. Around 20% of the yeast diameter moulds the polyurethane layer. (c) The red blood cell imprint has a lower mechanical stiffness due to the missing cell wall and thus rather shallow imprint depths are obtained compared to yeast imprints.