Electronic Supplementary Information

Self-assembled magnetic bead chains for sensitivity enhancement of microfluidic electrochemical biosensor platforms

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The supplementary information introduces the dry-film resist used in this work and the electrochemical characterization of the electrochemical microfluidic biosensors platform together with the employed magnetic beads.

The dry-film photoresist (DFR) technology

Material properties are crucially important for the design and fabrication of biosensor chips. Manufacturing, costs and functionality strongly depend on the chosen chip material. Biocompatibility, chemical and mechanical robustness as well as flexibility are key parameters for successful devices. An interesting technology for cheap and fast processing of polymeric microfluidic sensors is the DFR technology. In contrast to soft-lithographic materials like SU-8, DFR is supplied in form of a polymeric foil, packed between a polyester and a polyolefin foil. This shields the DFR from ambient oxygen. Application of DFRs to a substrate is done by lamination instead of spin-coating and subsequent structuring by lithography. DFR are available in different materials and foil thicknesses[1]. They provide excellent adhesion properties to various substrates and their uniform thickness enables to bridge gaps and form microfluidic channels. In this work, Pyralux® PC1025 (DuPont[TM], USA) in a thickness of 2.5 mil (64 µm) is used as the chip material for the biosensor fabrication.

The biosensor fabrication

As explained in the main paper, biosensor-chip processing is performed of wafer-level on a 50 µm thick polyimide-foil Pyralux® AP8525R (DuPont[TM], USA) as substrate (Fig. S1). Platinum electrodes are fabricated in a lift-off process and the integrated Ag/AgCl reference electrode is realized by electrodeposition of silver and subsequent chloridation. During fabrication of the reference electrode, the contact pads are shielded by a layer of adhesive foil. This reduces the silver-consumption during electrodeposition. Afterwards, the foil is removed without residuals to release the Pt contact pads. The microfluidic channel and the cover layer as

well as stabilizing layers on the backside are all made from Pyralux® in a thickness of 64 µm. A drop of 1% Teflon solution is dispensed into a predefined well. After solvent evaporation a thin Teflon layer is established that acts as a hydrophobic flow barrier during capillary filling. After sealing the chips with a cover out of pre-developed dry-film resist, the wafer is cut into chip duplicates, and hard backed for 3 hours at 145 °C.
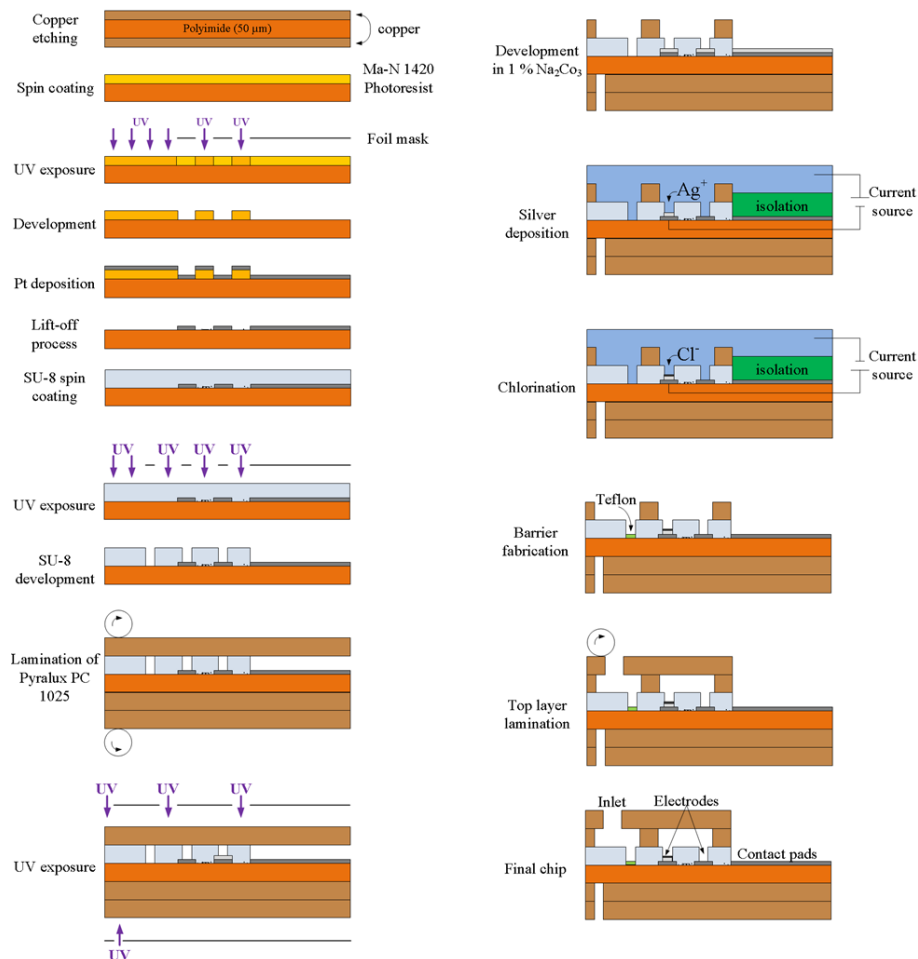

Figure S1: Shematic representation of the wafer level processing of the electrochemical microfluidic biosensor chip. Dicing is performed after the depicted process flow and before the final hardbake.

Boundary conditions for the design of soft-magnetic lattices

The fabrication of soft-magnetic lattices by lamination results in structures that can mainly be adjusted by the lattice spacing. Two counteracting effects play an important role here. The first is the distance between the lattice structure and the microfluidic channel. In most cases, this is defined by the thickness of the cover layer. The dry-film resist used in this work has a thickness of 64 µm. Soft-magnetic structures with small lattice spacings evoke magnetic field gradients with a small distance between maxima and minima of the magnetic flux. This results in a

reduced ability to evoke alternating magnetic fields in the microfluidic channel with sufficient strength for bead retention against fluid flow.
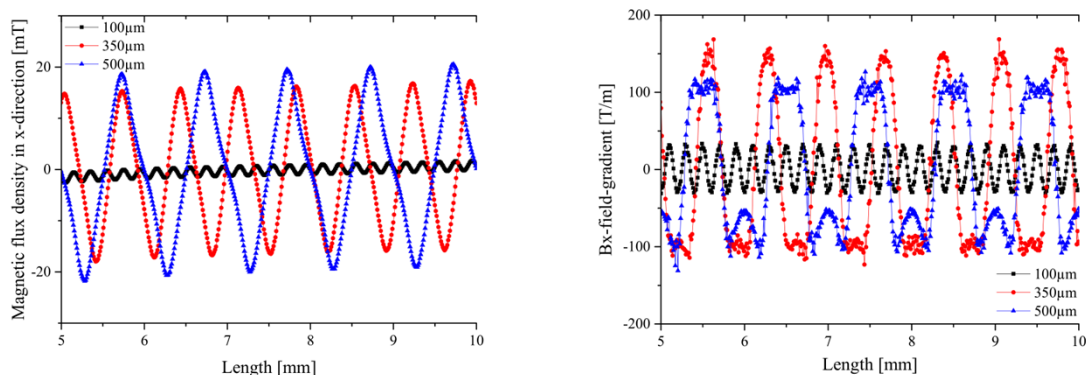
Figure S2: Simulations of the magnetic field profile along the microfluidic channel in a distance of 100 μm from the structure (approximating the distance between lattice structure and center of the microfluidic channel). Left: In comparison to the 100 μm lattice spacing (black), the 350 μm and 500 μm spacings evoke higher magnetic flux densities. Right: The gradient in the magnetic flux reveals that the 500 μm spacing is not having significantly higher flux gradients than the 350 μm lattice, but results in an elongated plateau with a high field gradient. The 350 μm lattice therefore is the optimal combination of high magnetic flux gradients and maximum density of those maxima.

Although large lattice spacings result in high magnetic field gradients even in increased distance to the lattice structure, they suffer from the increased distance in between the magnetic flux maxima and the resulting distance between magnetic bead chains. To capture a maximum of target molecules in the incubation time on chip, small distance between beads and analyte with improved diffusion is preferred. As a tradeoff, lattice spacings from 100 μm to 500 μm were investigated in this work, since these are provide high magnetic field gradients (see fig. S2) with low diffusion times (see table S3).

Table S3: Using Fick´s second law of diffusion the diffusion time for a defined length can be calculated. For a typical biomolecule with a mass of 30 kDa, the maximum time to reach the magnetic bead structure in the biosensor chip can be calculated.

| Lattice spacing [μm]	Maximum distance between target molecule and bead chain [μm]	Time for diffusion
100	50	15.63 sec
350	175	3.19 min
500	250	6.51 min

Flow dependency of the amperometric signal

For all amperometric measurements with our biosensor chip, a stable potential of 450 mV against an integrated Ag/AgCl reference electrode was applied to the Pt working electrode[2]. For comparability, the mean flow velocity is given instead of the volume flow (channel cross-section: 32000 μm^2). Variation of the flow velocity was determined with ultra-pure 0.1 M phosphate buffered saline (PBS), 40 mM glucose substrate in 0.1 M PBS and with 39 μM H_2O_2 in 0.1 M PBS (Fig. S2, left). Medium is pumped (syringe pump: PHD2000 Harvard Aparatus; syringes: Hamilton Company) through the microfluidic channel with a constant velocity and the amperometric signal was measured. Determination of the optimal flow velocity revealed almost no background signal for the glucose substrate. At the same time the H_2O_2 signal increases with the flow velocity. Due to limitations in the magnetic bead capture, the flow velocity was limited to a maximum of 1.5 mm sec^{-1}. At this velocity, a H_2O_2-calibration in a concentration range from 0.32 μM to 195 μM revealed a reproducible sensitivity of 6.04 nA mm^{-2} μM^{-1}(Fig. S2, right).
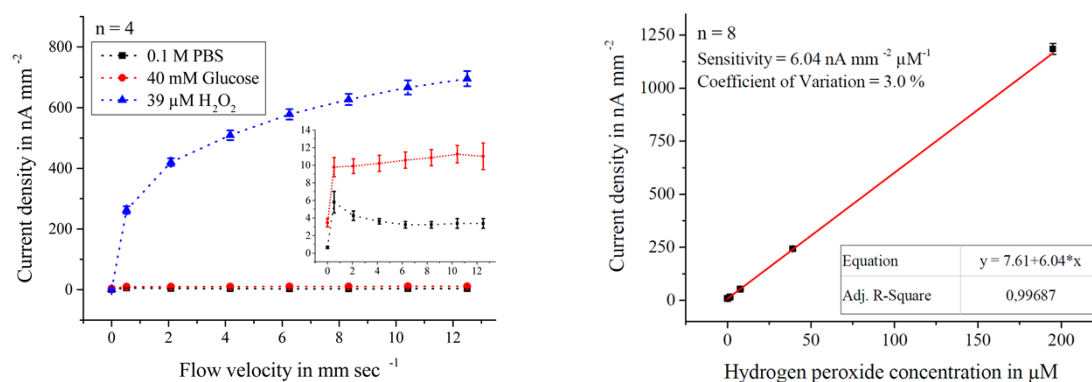
Figure S4: Left: measurement of the flow-rate dependency of the amperometric signal shows almost no glucose background compared to pure PBS. The hydrogen peroxide signal increases with the flow rate (left). Right: at the maximum flow rate for bead retention (1.5 mm sec^{-1}), the H_2O_2-sensitivity was found to be reproducible and linear with 6.04 nA mm^{-2} μM^{-1} (CV =3 %)

Re-usability of the chip

The biosensor chip detects enzymatically produced H_2O_2. Glucose oxidase (GOx) is used as the label, producing H_2O_2 from the supplied glucose substrate.[3] In order to determine an appropriate
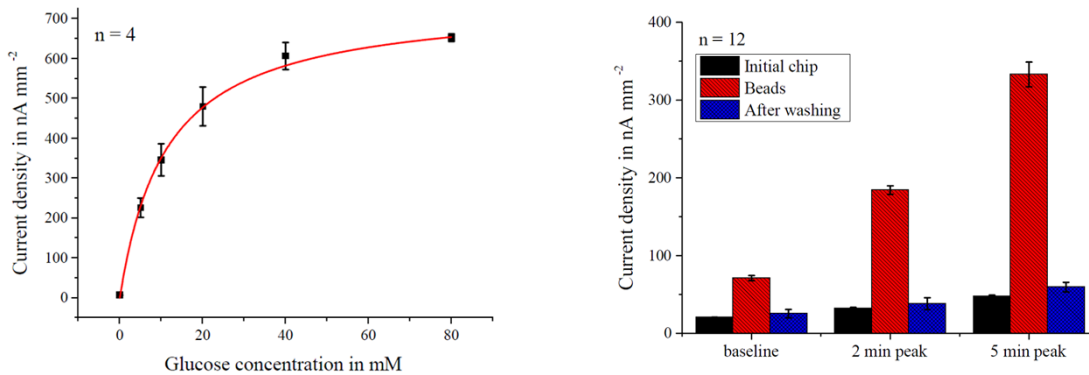
Figure S5: Left: Variation of the glucose concentration in the substrate. Saturation is already seen for 40 mM glucose. Right: baseline, 2 min and 5 min stop flow peak current densities shown for the initial chip, after bead introduction and after washing with 200 µl wash buffer. More than 95% of the signal is removed.

substrate, 3.5 µl of a 0.1 mg ml^{-1} GOx-labeled magnetic bead solution were introduced to the biosensor chips by capillary filling and the beads were magnetically captured by application of a permanent magnet (size: 20x10x4 mm³, Webcraft GmbH, Germany) underneath the micro-channel (monopolar setup). Different glucose concentrations were subsequently supplied at a mean flow velocity of 1.5 mm sec^{-1} (Fig. S3, left). Saturation of the continuous flow signal is reached at concentrations of 40 mM glucose in 0.1 M PBS. This concentration was therefore used as the substrate solution in all consecutive tests.

Washing of the biosensors was investigated after introduction of the same amount of beads into the micro-capillary utilizing a monopolar setup. Subsequent washing with 200 µl wash buffer (PBS with 1 % Tween20) at a flow rate of 50 µl min^{-1} removes more than 95 % of the signal. This proves that the magnetic beads in the microfluidic channel can be successfully washed out (Fig. S3, right). Re-usage of individual chips of the magnetic bead based biosensor is therefore possible.

Size distribution of magnetic beads:

For magnetic actuation mechanisms, the magnetization of the magnetic micro-beads is a key parameter. In order to predict the capture efficiency, mathematical models and calculations are based on the assumption of uniform sizes of the magnetic beads. In this work, magnetic beads with a nominal diameter of 1.43 µm (CV < 5%) (Microparticles GmbH, Germany) were used. The size distribution of these particles was independently measured with a coulter counter (Beckmann Coulter, USA) to validate the bead properties. The results perfectly match with the nominal diameter from the producer, although a higher CV of 11.97 % was found (Fig. S4).
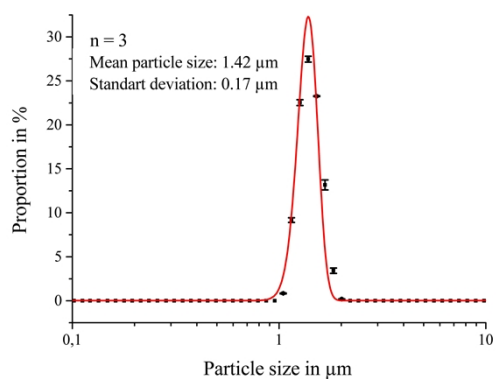
Figure S6: Particle size distribution of the 1.43 µm-sized magnetic beads (Microparticles GmbH, Germany). The graph summarizes measurements of thee individual samples.

References:

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