



Electrochemical restructuring of thin layer indium tin oxide electrode arrays for optimized (bio)electrocatalysis



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ABSTRACT

Bioelectrochemical research has elicited the potential of indium tin oxide (ITO) as a transparent electrode material for biological applications such as enzyme or cell coupled electrodes, especially in the context of integrated devices (multi-electrode arrays and lab-on-chip systems). Due to the miniaturized and multi-material nature of these devices, high sensitivity, cleaning and reuse tend to be difficult and are thus of pivotal interest. In this regard, our goal was to explore an electrochemical surface restructuring process based on a cyclic cathodic polarization in hydrochloric acid (E-HCl), to prepare ITO for electrochemical and bioelectrocatalytic purposes.

As studied by AFM, E-HCl shapes a columnar-like surface morphology by etching, thereby producing a fresh surface with an enlarged surface area. Contact angle measurements showed an increased wettability. XPS analysis of the O 1s signal implied a higher fraction of oxygen vacancy sites which are commonly linked to a higher conductivity, while the Sn/In ratio is not affected by the treatment. The restructuring process improved the interfacial electron-transfer of the ferri-/ferrocyanide and FAD/FADH₂ redox couple by at least the factor of two as measured by cyclic voltammetry and electrochemical impedance spectroscopy. Moreover, we demonstrate the applicability of the treatment and the sum of its effects on the bioelectrocatalysis of immobilized flavocytochrome P450 BM3. As measured by direct product quantification, the activity was increased more than twofold compared with the HCl control, clearly surpassing the effect which would be due to an increase in the surface area alone.

Overall, the combination of an improved interfacial electron-transfer, an increased surface area and the indication of a higher surface polarity and wettability make E-HCl restructuring suitable not only for ITO electrode cleaning and regeneration, but also for surface preparation and post-processing of bioelectrocatalytic electrodes within multi-electrode environments.

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1. Introduction

Indium tin oxide (ITO) belongs to one of the promising metal oxide semi-conductors that is mostly applied in optical devices, photovoltaic cells and luminescent displays due to its high conductance and transparency [1,2]. Beside these solid-state applications, ITO and related mixed metal oxides (e.g. fluorine or antimony doped tin oxide) are on the rise for use in electrochemical biosensors and bioelectronics, notably demonstrated for enzyme

electrodes [3–7] and multi-electrode array (MEA) based bio-electronic and photonic monitoring systems [8,9].

ITO is mostly applied in thin layer constructions. State of the art synthesis of ITO is *radio frequency* magnetron sputtering and a subsequent annealing procedure at high temperatures [10]. This allows layer thickness and durability to be controlled by process parameters while electrode layouts can be easily realized by photolithography [11]. Depending on the availability and cost of the sensors and MEAs thus produced, attempts are made to reuse them in experimental setups. In this context, surface cleanliness of the electrodes for electrochemical purposes is crucial for successful functionalization, high electron-transfer kinetics and sensitive measurements. Residual proteins and contaminants from previous

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treatments can be critical as well as inactivity due to prolonged storage of ITO MEAs in ambient air.

Several methods are available for surface cleaning, but only a few of them are compatible with integrated devices comprising multi-material and multi-electrode arrays consisting of sub-micrometer thin layer electrode structures. Strong cleaning processes including concentrated acids or bases, piranha solution, ultrasonication or plasma etching can compromise electrode integrity, especially if electrodes in an array vary in size and composition and should be treated separately. Moreover, array chambers, such as polystyrene cavities and bonding adhesive, favorable in giving rise to standard microplate format, are sensitive to elevated temperatures and organic solvents, which some cleaning procedures require [2,12]. Typically, as in the preparation of silicon wafer, multi-step cleaning is conducted to eliminate all contaminants, as in RCA treatment [13]. While there are also several electrochemical methods for cleaning gold electrodes [14], there seems to be no general method for ITO.

Here we propose an easy and quick cyclic cathodic polarization of ITO in 1 M HCl solution (E-HCl), as a restructuring and cleaning process to improve (bio)electrochemical electrode response. While some studies investigated aspects of the electrochemical reactions occurring during a cathodic polarization [15–17] – possible benefits and surface effects with regard to redox chemistry have not yet been evaluated. In addition to analyses of changes in morphology and surface composition, we investigate the electrochemical performance of treated ITO using the redox probes ferri-/ferrocyanide and the organic flavin cofactor FAD. We also show the influence of the E-HCl treatment on the efficiency of bioelectrocatalysis in a cytochrome P450 BM3 test system. P450 BM3 is a multi-domain redox enzyme involved in hydroxylation of long fatty acids. It transfers electrons from NADPH to its bound cofactors FAD, FMN and heme. As shown previously, it can also be activated by artificial electron supply without NADPH, if immobilized on ITO electrodes [8].

2. Experimental

2.1. Electrochemical cell design

Fabrication and design of the multi-electrode array that was used in electrochemical measurements were already described previously [18]. ITO electrodes were produced by photolithography with an ITO deposition by RF/DC (85 W/350 W) reactive magnetron sputtering. The array consists of 3×3 ITO working electrodes ($A_{(WE)} = 3.73 \text{ mm}^2$, thickness: 500 nm) and platinum counter electrodes ($A_{(CE)} = 33.57 \text{ mm}^2$, thickness: 500 nm ITO + 100 nm platinum) on a borofloat substrate. Reaction chambers were made of polystyrene derived from 96-well microtiter plates (Greiner Bio-One) and bonded with epoxy resin (EPO-TEK 302-3 M, Epoxy Technologies) to the electrode array. For potentiometric measurements a Ag/AgCl/1 M KCl bar reference electrode (CH Instruments) was implemented. Electrodes were contacted via self-developed adapter board.

2.2. RCA-1-like and E-HCl electrode cleaning procedure

Indium tin oxide electrodes were pre-cleaned with a solution of $\text{NH}_4\text{OH}(25\%)/\text{H}_2\text{O}_2(30\%)/\text{H}_2\text{O}$ (1:1:5 (v/v/v)) for 5 min at 50 °C. Afterwards ITO electrodes were potentiometrically cycled two times in 1 M HCl-solution (Merck KGaA) starting at 0 V with vertices at -1 V and 0 V and a scan rate of 50 mV/s. The treated electrodes were rinsed with ultrapure water to remove the HCl solution and stored in ultrapure water until further use.

2.3. Electrochemical characterization and analysis

Cyclic voltammograms were recorded using an IVIUM compactstat (IVIUM Technologies). The ITO electrode was set as working electrode. Platinum and Ag/AgCl/1 M KCl were used as counter and reference electrode, respectively. The measuring solution consisted of 0.5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/0.5 \text{ mM } \text{K}_4[\text{Fe}(\text{CN})_6]$ (Sigma Aldrich) or 1 mM FAD (Sigma Aldrich) in 0.5 M KCl.

Electrochemical impedance spectroscopy was carried out using a SI 1260 (Solartron Analytical, USA) impedance analyzer. The measured frequency range was 1 MHz to 0.1 Hz, whereby 10 points were recorded per decade with an integration time of 1.0 s. The measurement solution consisted of 0.1 M KCl (\pm) 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/5 \text{ mM } \text{K}_4[\text{Fe}(\text{CN})_6]$.

Impedance data recorded in 0.1 M KCl were analyzed by an equivalent circuit model containing a constant phase element in series with an ohmic resistance (CPE-Rs). Impedance data recorded in presence of ferri-/ferrocyanide were analyzed by Randles equivalent circuit model in which the capacitor was replaced by a constant phase element because of planar solid disk electrode configuration. Fitting protocol was Nelder-Mead method CLNS written in a MATLAB script as described previously [18].

2.4. AFM, QCM and contact angle analysis

The morphology of indium tin oxide surface was imaged in air using a NanoWizard 3 atomic force microscope (JPK Instruments AG, Germany) with a RTESPA cantilever (MPP-11120-10, Bruker, USA). Roughness (RMS) and surface area were calculated using Gwyddion 2.46. Layer height of indium tin oxide electrode was analyzed in contact mode using a DNP-10 cantilever.

QCM analysis was carried out as described previously [8]. Gold coated 6 MHz quartz crystals (Kurt. J. Lesker, Germany) were sputtered with a 300 nm ITO layer and analyzed with an Agilent 4194 A high precision impedance analyzer (Agilent Technologies, Germany). Frequency shifts were determined by a self-developed LabView software and mass alteration was calculated according to Sauerbrey [19]. Real surface area of the gold coated quartz crystals was analyzed by stripping voltammetry [20] and used for correction of the conversion of mass into layer height alteration, assuming a indium tin oxide density of 7.12 g cm^{-3} .

Static Contact angle measurements were conducted by the sessile drop technique with a 2.5 μL droplet of ultrapure water at room temperature (298 K). Surface-droplet interaction was imaged using a high-resolution Canon EOS100d camera. The contact angle was analyzed from the images using ImageJ 1.51j8.

2.5. XPS analysis

In order to study the surface composition during the electrochemical treatment, photoelectron spectra were recorded with a VG ESCALAB 220 iXLS spectrometer. The x-ray source was non-monochromatized Al K_{α} . During the experiment the base pressure was 1×10^{-8} mbar. Detail spectra of relevant core levels were scanned four times at a pass energy of 10 eV with a step width of 0.1 eV and a dwell time of 300 ms. The detail spectra were corrected by subtraction of excitation satellites before peak fitting due to the non-monochromatized Al anode. Data analysis was performed by UNIFIT 2015 [21]. The background of the detail spectra was fitted using a combination of polynomial and Shirley background. Peak shapes were simulated combining Lorentzian and Gaussian functions by convolution (Voigt profile) according to literature [22]. The binding energy scale was calibrated to the C 1s signal of adventitious hydrocarbons at 285.0 eV.

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