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Chip cleaning and regeneration for electrochemical sensor arrays

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ABSTRACT

Sensing systems based on electrochemical detection have generated great interest because electronic readout may replace conventional optical readout in microarray. Moreover, they offer the possibility to avoid labelling for target molecules. A typical electrochemical array consists of many sensing sites. An ideal micro-fabricated sensor-chip should have the same measured values for all the equivalent sensing sites (or spots). To achieve high reliability in electrochemical measurements, high quality in functionalization of the electrodes surface is essential. Molecular probes are often immobilized by using alkanethiols onto gold electrodes. Applying effective cleaning methods on the chip is a fundamental requirement for the formation of densely-packed and stable self-assembly monolayers. However, the available well-known techniques for chip cleaning may not be so reliable. Furthermore, it could be necessary to recycle the chip for reuse. Also in this case, an effective recycling technique is required to re-obtain well cleaned sensing surfaces on the chip. This paper presents experimental results on the efficacy and efficiency of the available techniques for initial cleaning and further recycling of micro-fabricated chips. Piranha, plasma, reductive and oxidative cleaning methods were applied and the obtained results were critically compared. Some interesting results were attained by using commonly considered cleaning methodologies. This study outlines oxidative electrochemical cleaning and recycling as the more efficient cleaning procedure for electrochemical based sensor arrays.

1. Introduction

Chip arrays have drastically changed the way genetic analysis and research is performed. They enable the users to perform a huge number of analyses in parallel. For instance, deoxyribonucleic acid (DNA) microarrays allow highly parallel and low-cost analysis since they are fabricated with a large number of miniaturized detection sites on a single chip. They extract information from each of these sites after exposure to solutions containing the target DNA samples. Protein based biosensor arrays for proteomics, metabolomics, and secretomics are now under development. However, more work is required for investigating low-cost mass production of devices which may be easy to operate and, therefore, may be used outside of specialized laboratories. Solutions implementing direct electrical readout and avoiding molecular labelling would significantly enhance devices' portability, high-parallelism, on-site sensing and data processing. Complementary metal oxide semiconductor (CMOS) allows the integration of a large number of sensors on a single chip and requires only few electrical connections to the chip-outside, which significantly ease the device packaging. Big market players are now developing devices which integrate micro-fluidic units required for lab-on-chip operations, CMOS circuits required for molecular detection, signal processing units, and multiplexing addressing units on a single 'smart card'. First investigations on smart cards for sensing purposes appeared recently in literature [1]. The related microchip needs to integrate on-board all the required algorithms for cleaning, addressing, probe immobilization, detection, processing, and recycling. An important requirement for fully integrable and reusable microchip is the ability to generate and regenerate sensing surfaces without any harsh chemical treatment that may damage the sensitive areas or any other circuit into the chip. The electrodes' surface in each of the sensing sites has to be very clean for effective probes functionalization. For example, the capacitance label-free detection may be used for DNA hybridization and antibody–antigen binding. However, capacitance detection requires very stable sensing surfaces. Probes molecules are immobilized by means of densely-packed and stable self-assembly monolayers (SAM). Ultra-clean gold surfaces are necessary to form well anchored SAM onto the electrode surface [2]. Gold surfaces usually adsorb contaminants during post-processes, storage and transportation of chips. This contamination clearly affects the quality of the SAM and the efficiency of the sensor [3,4]. Henceforth, an effective pre-cleaning is an essential step for sensor-chip functionalization. Many techniques have been described in literature for gold surfaces cleaning. These techniques rely on the use of chemical treatments with strong Bronsted–Lowry acid, (for instance, sulphuric acid in piranha treatment) or the use of oxygen

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plasma. However, these treatments are difficult to be controlled and they have low repeatability. This could be a crucial drawback in multi-panel biochip detection. Techniques based on the application of bias potentials may be considered for cleaning and recycling metal electrodes. These techniques envisage a sufficient oxidative (positive) or reductive (negative) bias voltage for a certain time [5–13]. Application of an anodic potential sweep up to 1.2 V with a scan rate of 100 mV/s may be considered for gold electro-cleaning, too [14,15]. Therefore, the aim of this paper was to compare widely used cleaning and regeneration methodologies and to identify the best method for electrochemical micro-fabricated chip. The best cleaning method was identified by comparing the different above-mentioned protocols in generating highly stable and densely-packed alkanethiol SAM. The protocols were evaluated in terms of achieving high stability and high reproducibility of organic layers in spot-by-spot measurements with capacitance detection.

2. Experimental details

A simple biochip presenting gold electrodes onto silicon oxide wafer was developed to test the different cleaning and recycling methodologies. Various chips were treated with different methods and the cleaning results were evaluated in terms of SAM quality in capacitance measurements. Scanning Electron Microscope (SEM) analysis was carried out to understand the results of measurements and to check the chip damages.

2.1. Chemicals

Ethylene-glycol thiols (COOH- and OH-terminated) were provided by Prochimia, Poland. NaCl, Na_2HPO_4, KH_2PO_4, KCl, H_2O_2 (50%), and absolute ethanol were obtained from Sigma-Aldrich, Switzerland. Potassium ferrocyanide $K_4[Fe(CN)_6]$ was obtained from Merck, Germany, and H_2SO_4 (96%) from Carlo Erba, Italy. All the chemicals were used without further purification.

2.2. Chip layout

Each chip has five sensing areas and four contact pads for capacitance measurements (Fig. 1). Each sensing area has the diameter equal to 2.5 mm and contains interdigitated finger electrodes. Different chips were tested with different finger widths: 6 μm, 20 μm, 50 μm, 200 μm and 500 μm. The height of the fingers was 560 nm and the spacing was 6 μm for all the widths. Each chip contained electrode fingers of the same width. Non sensing areas were passivated by using silicon nitride. For the capacitance measurements, we added onto each sensing area a 10 μl solution of Phosphate Buffered Saline (PBS) (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) which completely covers the electrodes.

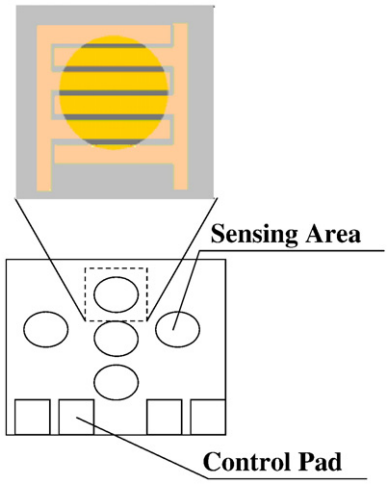

Fig. 1. Schematic drawing of the chip and the sensing spots for interdigitated geometry. Each finger has a length of 2 mm and variable width; the thickness is 560 nm and the distance between fingers is 6 μm. The five circles on the chip are the sensing spots (diameter 2 mm).

2.3. Piranha cleaning

The chips were dipped in piranha reagent. The reagent is obtained by a mixture of hydrogen peroxide (H_2O_2 — 50% of purity) and sulphuric acid (H_2SO_4) in the ratio of 1:3. Published protocols suggest keeping the reaction under temperature control at 60 °C, for 40 min. However, the piranha solution heats up to such a temperature for enough time because the mixing of peroxide with the acid is an exothermic reaction. Thus, to heat up the solution is not necessary in order to obtain an enough clean gold surface. After completion of piranha cleaning, the chips were rinsed with deionised water.

2.4. Plasma cleaning

In this cleaning process, the chips were kept in a plasma chamber. The system for generation of plasma was provided by Diener Electronics, Germany. The chamber has a volume equal to 2 l. The plasma cleaning was carried out in air. The samples were cleaned by applying 22 W, for a period of 2 min.

2.5. Electrochemical oxidative cleaning

In this case, the chips were kept in a three-electrode electrochemical cell. The electrochemical station was the model FRA 2 Autolab Type III, from EcoChimie. The oxidative cleaning process has been performed with a single pulse at + 1.4 V (DC pulse) for the duration of 30 s in PBS buffer.

2.6. Electrochemical reductive cleaning

In this case, as in the previous one, the chips were kept in a three-electrode electrochemical cell. The electrochemical station was the model FRA 2 Autolab Type III, from EcoChimie. However, differently by the previous case, the reductive cleaning has been performed by using a single pulse at − 1.4 V (DC pulse) for the duration of 30 s in PBS buffer.

2.7. Chip bio-functionalization

To test the feasibility of the different cleaning procedures, ethylene-glycol alkanethiols were deposited onto gold electrodes of the chips. These alkanethiols were used in the past for proteins' immobilization onto gold surfaces [16]. A mixture of two different alkanethiol molecules was employed to form the SAM. Both molecules were composed of a SH group for the molecules' immobilization on gold; an alkyl chain of 11 carbons to form an ordered layer, and a tri(ethylene-glycol) chain $(OCH_2CH_2)_3$, functionalized with a OH group or with a bit longer OCH_2–COOH group (Fig. 2). The tri(ethylene-glycol) part significantly improves the electrode polarization because, after equilibration, these functional groups form a stable hydrophilic layer that prevents solution

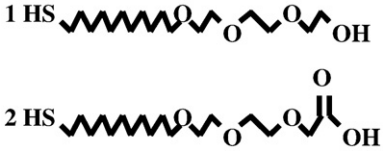

Fig. 2. The molecules used for SAM formation: (1) hydroxyl terminated, and (2) carboxyl terminated.