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Screen printing as cost-efficient fabrication method for DNA-chips with electrical readout for detection of viral DNA

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ABSTRACT

The fast development in the field of DNA analytics is driven by the need for cost-effective and high-throughput methods for the detection of biomolecules. The detection of DNA using metal nanoparticles as labels is an interesting alternative to the standard fluorescence technique. Fluorescence is highly sensitive and broadly established, but shows limitations, for example instability of the signal and the requirement for sophisticated and high-cost equipment. A recently developed approach realizes a method for the electrical detection of DNA, based on the induction of silver nanoparticles growth in microelectrode gaps on the surface of a DNA-chip. This breakthrough towards robust and cost-effective detection was still hampered by the need for microstructured (and therefore expensive) substrates. We demonstrate that it is possible to utilize screen printed electrode structures for a chip-based electrical DNA detection. The electrode structures were produced on a glass substrate which made an additional optical readout possible. The screen printed structures show the required precision and are compatible with the applied biochemical protocols. A comparison with chip substrates produced by standard photolithography showed the same sensitivity and specificity for the screen printed chips. Screen printing of electrode structures for DNA-chip with electrical detection offers an interesting and cost-efficient possibility to produce DNA-chips with microstructured electrodes.

1. Introduction

The identification and analysis of biomolecules is an important task especially in fields of life science, food technology, forensic and environmental research (Schena et al., 1998). During the last decade DNA-chips could show the potential as analytical tool in these areas. First of all the requirements for high-throughput, specific reactions and high sensitivity could be fulfilled by microarray technology (Wang, 2000; Schena, 2003). Nowadays various microarray-based approaches are used to detect binding events of biomolecules on surfaces of so-called biochips (Choi et al., 2007; Marquette et al., 2008; Warsinke, 2008). Thereby, simple detection methods that require only simple instrumentation and readout techniques are of growing interest (Cheung et al., 1999; Heller, 2002). Furthermore, nanoparticles demonstrated their potential as alternative labels to the standard labelling of oligonucleotides by fluorescent dyes (Mirkin et al., 1996; Csaki et al., 2002). Nanoparticles allow for a variety of detection schemes for bioanalytical investigations, such as optical, electrical, or electrochemical approaches (Gonzalez-Garcia et al., 2000; Wang et al., 2001; Albers et al., 2003; Drummond et al., 2003; Fritzsche and Taton, 2003). They promise to overcome the cost-related problems of fluorescence detection, which requires rather complicate and thereby expensive instrumentation, which is not easily adaptable for point-of-care applications with decentralized (and cost-effective) equipment needs (Fritzsche, 2001). Approaches using the conductive properties of metal nanoparticles offer simple and cost-effective detection units (Park et al., 2000, 2002). Despite their analytical performances these systems are still at the stage of proof-of-concept and further developments are needed for real life diagnostic applications (Marquette et al., 2006). Especially the requirement for electrode gap structures with micrometer precision makes the production of the chip expensive (Avramescu et al., 2002; Marquette et al., 2006). The precision is mainly determined by the process of capture-DNA immobilization, which happens usually by spotting methods with final spot sizes of 100–200 μm and a positioning precision of about 10 μm. The gaps themselves are usually in the lower micrometer range of 1–50 μm.

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Screen printing technique, possibly one of the oldest forms of graphic art reproduction, is distinguished mainly to other methods of microstructuring by the way of film (material) deposition. The technique has a typical resolution of about 50–150 μm.

Screen printing technology is widely used for large-scale fabrication of disposable biosensors with several advantages including low cost, versatility, and miniaturization. Thereby modern sensors can be integrated in portable systems, an important requirement of analytical methods for on-site testing (Tudorache and Bala, 2007). The design and fabrication of screen printed electrodes, including microelectrodes and chemically modified electrodes expand the possibility of direct implementation of laboratory-developed screen printed electrodes in real life applications. One prominent example is the personal glucose biosensor used by diabetics (Matthews et al., 1987; Nagata et al., 1995). A thick-film biosensor can be produced on different substrates such as alumina, ceramics, PVC, gold, iron, etc., with printed conducting electrode structures consisting of carbon ink/paste, or platinum, or other metal paste (Dequaire and Heller, 2002; Susmel et al., 2003; Liao and Chou, 2006; Luo et al., 2006; de Albuquerque and Ferreira, 2007).

Thereby it is in principle compatible with any detection scheme that needs microstructured electrodes. In the following, the principles of the electrical detection as well as possible applications are described.

For the electrical readout chips with 42 electrode gaps were manufactured by screen printing. The screen printed electrodes consist of gold or platinum. Each electrode gap on the chip is a measurement point for biomolecular interactions on the chip. Each gap is modified with a specific capture sequence. In a second step biotin-labelled target-DNA hybridizes to the complementary capture-molecules on the chip surface. Due to a streptavidin modification the enzyme horseradish peroxidase (HRP) can subsequently bind between the electrodes (Möller et al., 2005). A specific deposition of silver nanoparticles driven by the enzymatic activity leads to a bridging of the electrode gap by a conductive metallic layer (Mayer et al., 2000; Willner et al., 2007). The increase of the conductivity over the electrode gap is measured afterwards by a custom-made readout device.

In this work the applicability, sensitivity, and specificity of screen printed electrodes is compared to electrodes produced by standard photolithography. To demonstrate the potential of this system cytomegalovirus (CVM) DNA is detected on the chip. The CMV belongs to the family of human herpesvirus and has one of the largest known viral genomes (Chambers et al., 1999). Especially immuno-debilitated individuals and pregnant women (e.g. 10% of infected newborns with CMV exhibit permanent mental retardation and auditory damage) are affected (Ibanez et al., 2008). During the past decades microarrays showed great potential for the analysis of viral DNA. Due to the developments in the field of miniaturization, multiplexing, and automation of chip-based detection of biomolecules, microarray technology is starting to replace traditional determination methods such as virus isolation and serological diagnosis (Zheng et al., 2008). By using viral cultures from infected patients DNA microarrays can even monitor viral load of CMV. In the presented work a short biotin-labelled fragment of CMV DNA was used for the detection. The chips were readout electrically as well as optically after the silver deposition. DNA concentrations as low as 500 fM were detectable on the electrical DNA-chip.

The presented results demonstrate the application of screen printing as a cost efficient (and potentially large scale) production method for the realization of microstructured chips for the electrical DNA detection based on nanoparticle labelling and subsequent site-specific silver deposition.

2. Experimental

2.1. Materials and reagents

DNA-oligonucleotides (both capture and target probes) were obtained from Operon (Operon Biotechnologies GmbH, Cologne, Germany). For our investigations we used a positive (already biotin labelled) control (5′-NH_2-C6-CAT AGA ATC AAG GAG CAC ATG CTG AAA AAA-biotin-3′), a complementary capture sequence (5′-NH_2-C6-TTT TTT CAG CAT GTG CTC CTT GAT TCT ATG-3′), a sequence containing 1 mismatch (5′-NH_2-C6-TTT TTT CAG CAT G**G**G CTC CTT GAT TCT ATG-3′), a sequence containing 3 mismatches (5′-NH_2-C6-TTT TTT CAG CAT **TAT** CTC CTT GAT TCT ATG-3′), and a negative control (5′-ACT GAC TGA CTG ACT GAC TGA CTG GGC GGC GAC CT-NH_2-C7-3′). The biotin-labelled target-DNA (5′-biotin-CAT AGA ATC AAG GAG CAC ATG CTG AAA AAA-3′) had only a biotin modification. The streptavidin conjugated horseradish peroxidase was purchased from Sigma (Streptavidin-Peroxidase Polymer, Ultrasensitive, Sigma–Aldrich, Taufkirchen, Germany). Silver enhancement kit for enzyme induced silver deposition was supplied by Nanoprobes (EnzMet® kit, Nanoprobes Inc., Yaphank, NY, USA). Microarray printing buffer was obtained from ArrayIt (Micro Spotting Solution Plus, TeleChem International, Sunnyvale, USA). Chemicals for phosphate buffer solution (PBS pH 7,4), saline sodium citrate (SSC pH 7), and sodium dodecyl sulphate (SDS) were ordered by Merck (Merck KGaA, Darmstadt, Germany). Tween20, hydroquinone and silver acetate were also ordered by Sigma. The organo silane (3-glycidyloxypropyl)-trimethoxysilane used for the surface modification of the DNA-chips was purchased from ABCR (ABCR GmbH, Karlsruhe, Germany).

2.2. Preparation of the electrode structures

The metallic microelectrode structures of the biochips, a square at 0.5 in. size, were printed on 50 mm × 50 mm glass samples (Fig. 1). For the analysis of different probes each chip contains 42 measurement points (electrode gaps) on the surface. Due to the resolution of the fabrication method the electrode gaps on the chips have a width of about 50 μm. Glass substrates were washed and tempered prior to screen printing. Electrode and conducting path structures were realized using low firing Au and Pt (W.C. Heraeus GmbH, Hanau, Germany). Depending on the composition of the electrode structures a gold or platinum paste was used for screen printing. The platinum resinate paste consists of an organic platinum compound with a low content of noble metal. By a burn-in process the organic compound starts to decompose. Thereby, a thin platinum layer remains. For the gold electrodes a low burn-in gold paste was used. The control biochips were made by standard photolithography on silicon wafer.

2.3. Chip-based electrical detection of DNA

Screen printed substrates were chemically modified with (3-glycidyloxypropyl)-trimethoxysilane (GOPS) for the binding of amino-modified single stranded (ss) capture-DNA molecules (Wong and Krull, 2005). The screen printed chips were cleaned by sonication for 5 min each with acetone, ethanol, and water. After a drying step by nitrogen the entire chip surface was epoxy modified, by immersing the chips in a 10 mM GOPS solution in dry toluene for 7 h at 70 °C. In the end they were washed 2× 5 min each with toluene, ethanol, and water.

The deposited capture DNA was modified with a C6-Aminolink on the 5ĭ or 3ĭ end. These modified molecules were attached to the epoxy (introduced by GOPS) modified surface. The attachment occurs by a secondary amine formation between the epoxysilane