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A disposable and cost efficient microfluidic device for the rapid chip-based electrical detection of DNA

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ABSTRACT

Requirements for a point-of-care device are an easy and robust read-out and – above all – a simple handling. We integrated an established robust electrical read-out for DNA-chips into a microfluidic device, thereby creating an automated analysis system that combines the necessary steps for a chip-based analysis. It is based on the electrical detection of biotin-labeled DNA in a gap between two microstructured electrodes on the surface of a DNA-chip. The biotin serves as binding molecule for streptavidin-conjugated horseradish peroxidase. A following enzyme-induced silver deposition bridges the gap by a conductive layer. The miniaturized chip gives the possibility to realize a durable system suitable for point-of-care applications.

To enable an initial automation, all corresponding process steps were executed in a miniaturized silicone flow cell. The required defined temperatures for the hybridization and the washing steps can be adjusted by a heating foil.

This paper characterizes the performance of the flow cell based system in terms of reaction speed and analysis time, sensitivity as well as specificity, and the comparison to a conventional system, without flow cell. These first steps of automation and integration will help to realize a laboratory-independent bioanalytical tool, for the use outside of specialized laboratories for fast analysis of different chemical and biological applications.

1. Introduction

Since its introduction the microarray technology is used in many different fields exploiting the advantages of biochips, such as high throughput screening, high specificity, miniaturization, and so on (Schena et al., 1995). In principle, short single stranded DNA, so-called capture molecules, become immobilized on a planar chip surface like glass, silicon or polymeric materials (Oh et al., 2006). Later the sample is delivered to the microarray surface (Diehl et al., 2001).

In the last years many endeavors have been made to further develop the microarray technology. Different techniques were used to integrate biochips (Dittrich et al., 2006; Morais et al., 2007). Acoustic piezo elements (Toegl et al., 2003), centrifuges (Peytavi et al., 2005), rotators (Pappaert et al., 2003), and electric fields (Edman et al., 1997) are utilized to induce an additional movement of the sample solution on the chip to overcome the diffusion limitation of the capture-target molecule interaction. Furthermore microfluidic applications are a promising complementation of microarray technology, especially for the on-site analysis. Microfluidics is an enabling technology behind a whole new class of miniaturized analysis systems for chemical and biological applications (Haeberle and Zengerle, 2007). All the benefits of microfluidics and miniaturization such as smaller sample requirement, reduced reagent consumption, decreased analysis time, higher levels of throughput, and automation can be realized in such applications (Abgrall and Gué, 2007).

Combining microfluidics and biochips aims to improve binding efficiency, and to maximize the speed of the individual process steps on a biochip (Pappaert et al., 2006). However, the expenses for the analysis of a test rise unnecessarily by the additional instrumentation that usually leads to higher costs and efforts.

The detection of biomolecules on a biochip is nowadays mainly performed using fluorescent dyes (Schena et al., 1998). Disadvantages (like photo bleaching and quenching of the fluorescent dyes) can be overcome by using quantum dots (Han et al., 2001). Nowadays, different working groups could successfully demonstrate the miniaturization of the mostly sophisticated fluorescence equipment (Kang et al., 2007; Basabe-Desmonts et al., 2008; Myers and

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Lee, 2008). But, the developed handheld and point-of-care systems are limited in terms of their sensitivity and robustness. Due to the difficulties, the use of biomolecule detection via fluorescence is usually limited to specialized laboratories (Festag et al., 2005).

Here we introduce a disposable and cost efficient microfluidic device for the rapid nanoparticle-based electrical detection of DNA (Möller et al., 2005). We focused on this technology because of its robustness, simplicity and the potential to refine it to a mobile, disposable and cost efficient device for on-site testing.

Therefore, the DNA-chip with electrical detection was integrated into a custom-made flow cell. This work describes for the first time the integration of the enzyme-induced deposition of silver nanoparticles that do not fade or bleach for the specific and sensitive chip-based detection of DNA in a microfluidic system.

Developing point-of-care applications it is important to focus on flexible, inexpensive, and reliable devices with a simple handling. Since photolithographic methods allow microstructuring of nearly any channel geometry and extremely small sizes the use of other structuring technologies like molding seems not suitable anymore in terms of microstructure geometry (Abgrall and Gué, 2007).

Our approach is based on the idea to use alternative techniques that can be used to produce cost efficient and robust fluidic systems that guarantee an easy exchange of all main components of the flow cell. The exchange of the essential parts ensures that no cross-contamination can occur, thereby avoiding false positive signals through cross-contamination. Further the integrating of the DNA-chip into the flow cell reduces the analysis time remarkably and improves the sensitivity of the system.

The results of these investigations represent an initial step for the development of a fully automated system, in order to realize a transportable analysis of biomolecules for the chip-based electrical detection of DNA.

2. Experimental

2.1. Materials

Screen printed DNA-chips were obtained from Heraeus Sensor Technology GmbH, Hanau, Germany. The streptavidin-horseradish-peroxidase-polymer was purchased from Sigma (Streptavidin-Peroxidase Polymer, Ultrasensitive, Sigma-Aldrich, Germany). Silver enhancement kit for enzyme-induced silver deposition was supplied by Nanoprobes (EnzMet kit, Nanoprobes Inc., Yaphank, NY). Microarray printing buffer was obtained from ArrayIt (Micro Spotting Solution Plus, TeleChem International, Sunnyvale, CA). Chemicals for phosphate buffer solution (PBS pH 7.4), saline buffered sodium citrate (SSC pH 7), and sodium dodecyl sulphate (SDS) were ordered from Merck (Merck KGaA, Darmstadt, Germany). Tween20, hydroquinone and silver acetate were also ordered from Sigma.

2.2. Microarray printing

The DNA-chips were chemically modified with (3-glycidyloxypropyl)-trimethoxysilane (GOPS) (Wong and Krull, 2005) for the binding of amino-modified single stranded (ss) capture-DNA molecules. First, the chips were cleaned by sonication for 5 min each with acetone, ethanol, and water. After a drying step by nitrogen the chips were modified in a 10 mM GOPS solution in dry toluene for 7 h at 70 °C. Finally they were washed 2 × 5 min with toluene, ethanol, and water each (Möller et al., 2000).

The deposited capture DNA was modified with a C6-Aminolink on the 5′ or 3′ 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 monolayer and the 5′ or 3′-amino modification of the DNA (Lamture et al., 1994). The capture-sequences were spotted by a non-contact Nanoplotter from GeSIM (Gesellschaft fuer Silizium-Mikrosysteme mbH, Großerkmannsdorf, Germany). Beside a positive (already biotin-labeled) and a negative control, we immobilized a complementary sequence, a sequence containing 1 mismatch, and a sequence containing 3 mismatches. The concentration of the spotted capture DNA in the microarray printing buffer was 10 μM.

2.3. Hybridization and enzyme-induced silver deposition

A microstructured glass chip with screen printed electrodes and 42 measurement points (electrode gaps) serves as microarray platform (Schüler et al., 2009). The capture molecules are immobilized in the gap between the electrodes. After the specific binding event of biotinylated target molecules, a streptavidin-horseradish-peroxidase-polymer is bound in the electrode gap (Diamandis and Christopoulos, 1991). A following enzyme-induced deposition of silver nanoparticles leads to bridging the electrode gap by a conductive silver layer (Möller et al., 2005; Schüler et al., 2008). Finally, the increase of the conductivity over the electrode gap can be measured by a DC measurement (Urban et al., 2003). Due to the easy DC measurement the required equipment is reduced to a minimum.

Prior work describes the use of the chip-based electrical detection of biomolecules without flow cell (Möller et al., 2008). Following this approach will be denoted as “conventional” system.

Different from the conventional system the best specificity for the hybridization of the used biotin-labeled target-DNA sequences was achieved in 0.5× SSC buffer and 0.1% SDS hybridization buffer at 42 °C. The hybridization was performed in the microfluidic device using a tubing pump with a planetary drive at a flow rate of 600 μl/min in 3 s intervals for 3 min. A following washing step in 0.2× SSC for 1 min (in continuous flow) removed unbound DNA to avoid false positive signals. The streptavidin-horseradish-peroxidase-polymer was diluted 1:1000 in PBS buffer and 0.05% Tween20. This buffer was also used for the washing step, in continuous flow, after a 3 min incubation step. The binding of the enzyme was also carried out at the same pump parameters as for the hybridization. After binding of the streptavidin-enzyme conjugate, the three solutions (A, B, C) of silver enhancement kit (EnzMet) from Nanoprobes (Nanoprobes Inc., Yaphank, NY) were introduced to the chip surface in ratio 1:1:1 (as given by the manufacturer). The reaction was stopped after 3 min to avoid unspecific silver deposition. Before enzyme-induced silver deposition the flow cell was rinsed with deionised water to remove any chloride from the PBS buffer. Fig. 1 shows the principle steps of the detection system.

2.4. Instrumental setup

The flow cell consists of a polycarbonate base containing microfluidic connections and an embedded thermal management to adjust the optimal environment for the biomolecular recognition reactions. To guarantee impermeability of the flow cell a custom-made PDMS (polydimethylsiloxane) component seals the reaction chamber (Sia and Whitesides, 2003). Additionally, the PDMS seal carries meandering structures (Fig. 4d). The DNA-chip serves as cover to close the reaction chamber whereby, in combination with the PDMS seal, the formed microfluidic channel guides all solutions over all measurement points on the chip (Fig. 4e). A specially developed read-out device allows online measurement of the electrical signal.

To move liquid through the flow cell a flexible tube pump IPC-N-4 (Ismatec Laboratoriumstechnik GmbH, Wertheim-Mondfeld, Germany) with a RS232 remote control and flow rates between 0.0004 and 11 ml/min, depending on the used tube diameter, was used. A PC running a custom build Delphi program capable of changing flow rate and switching between clockwise and counter