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Polysaccharide microarrays with a CMOS based signal detection unit

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ABSTRACT

Microarray based test assays have become increasingly important tools in diagnostics for fast multi-parameter detection especially where sample volumes are limited. We present here a simple procedure to create polysaccharide microarrays, which can be used to analyze antibodies using an integrated, complementary metal-oxide-semiconductor (CMOS) based electric signal readout process. To accomplish this chips are used which consist of an array of silicon photodiodes and where different types of polysaccharides from the bacteria *Streptococcus pneumoniae* are printed on the (silicon dioxide) chip surface. Typical amounts of polysaccharide deposited in the printing process are around 12 attomol/spot. In a subsequent reaction step the polysaccharide microarrays were used for the measurement of IgG antibody concentrations in human blood sera using either chemiluminescence or fluorescence based detection. To understand the device performance the influence of surface density of the immobilized polysaccharide molecules and other parameters on the assay performance are investigated. The dynamic measurement range of the sensor is shown to reach over more than 3 decades of concentration and covers the whole physiologically relevant range for the analysis of antibodies against a large panel of pneumococcal polysaccharides.

1. Introduction

A major trend in medicine goes towards personalized therapeutics and consequently the demand rises for reliable, fast and economic diagnostic tools. In the field of proteomics most standard clinical diagnostic techniques like turbidimetry only measure one factor at a time (Salden et al., 1988) and have limited sensitivity (Roberts et al., 2001). A more sensitive (and also routinely used) method is the enzyme-linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1971; Wernette et al., 2003). Excellent sensitivity, variability and a for many applications sufficient reproducibility have made ELISA processes one of the standard methods used today in biomedical analysis. Drawbacks of the standard ELISA process are the rather laborious procedures, which require significant amount of time during sample preparation and analysis. In addition, the effort required for the analysis scales directly with the number of parameters to be tested (Engvall and Perlmann, 1971; Nahm and Goldblatt, 2002). However, in many cases analysis of a multiplicity of factors is crucial for a comprehensive diagnosis. Accordingly parallel detection of a series of parameters would reduce time, cost and ease requirements on sample volume. Point-of-care diagnostics requires high degrees of automation of sample processing and analysis to ensure a high test precision in clinical daily routine. Microarrays are promising diagnostic devices to fulfill these specifications: Due to the high integration density many parameters can be tested in parallel. Short diffusion path lengths and small geometries provide rapid time to result and require only small sample volumes (Squires et al., 2008). Additionally, microarrays can be easily incorporated into lab-on-chip devices equipped with liquid guiding structures to provide simplified and reproducible test handling.

Although microarrays have widely been used in the fields of genetics (Bonetta, 2006; Schena et al., 1995; Schenk et al., 2009) and proteomics (Joos and Bachmann, 2009; MacBeath, 2002; Michaud et al., 2003), they are not yet routinely used to study carbohydrate–protein interactions. Carbohydrates, or more precisely polysaccharides (Ps) are a component of bacterial capsules of various pathogens (e.g. *Haemophilus influenzae*, *Neisseria meningitidis*, *Salmonella typhi* and *Streptococcus pneumoniae*). Thus it is an important task in clinical diagnostics to measure antibody titers against polysaccharide structures (Wang, 2007; Wang et al., 2002). In addition, the analysis of such systems might

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help to fully understand host-microorganism interactions. Inspired by the success of chip-based assays, carbohydrate microarrays are regarded as a promising tool in the emerging field of glycomics (Kiessling and Cairo, 2002; Wang et al., 2002). They are used for a broad range of applications, e.g. analysis of cell–sugar interactions, screening of binding inhibitors or enzyme activities.

Several different procedures to create carbohydrate microarrays have been presented in the literature (Blixt et al., 2008, 2004; Horlacher and Seeberger, 2008; Wang et al., 2002): typically substrates are modified with reactive groups which bind specifically to functional linkers synthesized to the carbohydrate structures. Examples for surface activation reagents are NHS-esters (Blixt et al., 2004), epoxides (Park et al., 2007) (both working with amide linkers), maleimides (thiols) (Ratner et al., 2004) or photoreactive groups (Pei et al., 2007). Obviously, each of these methods requires supplemental processing steps and modifies the carbohydrate structure causing changes in the conformation of the biomolecule and thus can lead to alteration or even destruction of the type-specific epitopes (Biagini et al., 2003; Pickering et al., 2007). The group of Wang had successfully adsorbed unmodified polysaccharides onto nitrocellulose coated glass slides (Wang et al., 2002). However, these coatings also tend to exhibit unspecific adsorption of serum proteins on the surface which might result in high background signals, which compromises the lower detection limit of the test (Jones, 1999; Kusnezow and Hoheisel, 2003; Ramsden, 1994). Recently Marchese et al. (2009) reported a multiplex electrochemiluminescence-based detection assay where different types of pneumococcal polysaccharides were adsorbed on untreated carbon electrodes.

In our manuscript we describe a simple and versatile pathway for creating polysaccharide microarrays by spotting unmodified polysaccharides on a silicon dioxide passivation layer of a semiconductor chip. The chip itself consists of an array of 32 addressable photodiodes with integrated amplifiers (photodiode-type active pixel configuration [APS]). With this setup the photodiodes are highly sensitive (Fossum, 1997) and allow the detection of very low intensities of light generated in e.g. chemiluminescence reactions. Compared to conventional detection systems such as fluorescence scanners the concept of placing the detection unit, the photodiode in very close proximity to the light emitting reaction provides a strongly increased sensor sensitivity. In such photochip approaches the detector is only nanometers away from the light emitting process instead of milli- or centimeters as in conventional scanners, e.g. microarray fluorescence readers or electrochemiluminescence plate readers (e.g. MSD® sector imager) (Miao, 2008). In addition the size of the measurement apparatus is considerably reduced by integration of the detection unit. As the light intensity (i.e. number of photons per photodiode area and time) drops off quadratically with the distance between light source and detector, the distance between them becomes very important.

As a model assay we analyzed a panel of IgG antibodies against different types of polysaccharides in human blood serum. We used pneumococcal polysaccharides (PnPs) which are contained in the outer shell of the bacterium *S. pneumonia* (Wernette et al., 2003). As some persons show insufficient response to inoculation against this bacterium (Elkayam et al., 2007; Goldacker et al., 2007), the determination of a patient's vaccination status is an important task in clinical diagnostics. In the following the process to bio-functionalize the chips is presented. The influence of capture molecule density at the sensor interface with respect to assay performance and the suitability of these biosensors for quantifiable antibody concentration measurements in human serum are analyzed.

2. Materials and methods

2.1. Pneumococcal polysaccharides and antibodies

All pneumococcal polysaccharides used are part of the commercially available vaccine Pneumovax® 23 (Sweeney et al., 2000), which is manufactured by Merck & Co., Inc., USA. Labeling of PnPs type 14 with the amino-modified dye Dy647 from Dyomics (Jena, Germany) was performed according to standard protocols (Hermanson, 1996). Unbound dye was removed by centrifugation in a Microcon centrifugal filter device with a 10 kg/mol cutoff obtained from Millipore (Billerica, MA, USA). Conjugation of secondary antibody anti-human-IgG-F(ab′)$_2$ fragment antibody from Sigma–Aldrich (Munich, Germany) with NHS-ester biotin was done in our laboratory according to standard protocols (Hermanson, 1996). Streptavidin-Cy5 from GE Healthcare (Munich, Germany) and streptavidin conjugated horseradish peroxidase (strep-HRP) were bought from Invitrogen (Eggenstein, Germany).

2.2. CMOS chip production and readout

The photochip was fabricated in a standard mixed signal 0.5 μm complementary metal-oxide-semiconductor (CMOS) technology process (Fossum, 1997; Wu et al., 2004) by Micronas GmbH, Freiburg, Germany. Each pixel of the chip consists of a silicon photodiode and an integrated active amplifier and can be addressed individually. Since there is no overlying polysilicon layer the photodiode-type APS pixels have a high quantum efficiency. The n-well/p-substrate photodiode is operated in reverse bias and the photocurrent is integrated in the pn-junction's capacitance (C_{pn} = 14 fF). The 32 photodiodes on the chip have diameters of 190 μm and are arranged in a 4 × 8-grid with 500 μm pitch center-to-center. The diodes are covered with thermally grown silicon dioxide with a thickness of about 300 nm. The light generated by the chemiluminescence reaction with luminol (λ = 428 nm) induces a photocurrent of a few femtoampere in the photodiodes. This photocurrent and an additional dark current (thermally induced) discharge the capacitance of the pn-junction as soon as the reset transistor is switched off. The resulting potential drop is amplified and buffered by the pixel's addressable amplifier (gain externally set to 10) and readout with a conventional notebook equipped with a measurement card for analog-to-digital conversion.

2.3. Detection of enhanced chemiluminescence reactions

For enhanced chemiluminescence (ECL) reactions the Supersignal West Pico Kit from Perbio Science (Bonn, Germany) was used. The two components of the kit (hydrogen peroxide and buffered luminol) were mixed together directly before the measurement. For signal readout each pixel of the photochip was serially addressed 15 s after the chips were incubated with the ECL solution and the course of the voltage was recorded for 0.1 s. The recorded data was analyzed by calculating the slope of the capacitance discharge of the photodiode over time. For analysis, these signals were then corrected by subtracting the dark current of each photodiode (PBS-T-BSA-buffer was applied) which had been recorded previous to the chemiluminescence measurement.

2.4. Printing polysaccharide microarrays

The chips were washed in toluene in an ultrasonic bath at 40 °C for 5 min. Additional rinsing steps with toluene were followed by drying of the chips under nitrogen flow. All microarrays were created by using the piezo-actuated contactless printer SciFlexarrayer™ S5 from Scienion AG (Berlin, Germany). This print