Silicon based electrical biosensor technology for mobile diagnostics

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Abstract:

The need for mobile diagnostics increases in particular with the changing requirements of the health market and its medical applications. It is of increasing importance that physicians or in future also patients are able to execute even complex analyzing tests at the point of care on their own. Therefore mobile analysis systems have to provide easy to handle and rapid tests with required sensitivity for related applications. Referring to this the silicon based biochip platform of the Fraunhofer ISIT is applicable for a wide range of immunological based tests to meet the needs of mobile diagnostics.

The fully automated biosensor system is able to run an analysis of e.g. a Hepatitis-C Virus (HCV) infection with only microliters of blood sample. The entire procedure from sample collection to result presentation takes just a few minutes, while a central laboratory needs approximately four hours for the same test. The system comprises a disposable cartridge equipped with fluidic structures and the integrated electrical biochip with a gold microelectrode array. Next to easy sample collection and automated sample dilution the "single electrode redox cycling" for amperometric signal amplification during electrochemical detection is an important method. The generation of the multi-parametric read-out of the results is performed by special electronics in a mobile diagnostic device, which also processes the required reagents by pumps and valves through the biochip cartridge. Overall the system combines micro system technology and modern methods of molecular biology to enable highly sophisticated biosensors to fulfil the requirements of the point-of-care diagnostics.

Key words: Biochip, Biosensor-System, Point-of-Care-Diagnostics, Cartridge, Lab-on-Chip.

Introduction

More than 150 million people are chronically infected with hepatitis C virus (HCV) and unlike hepatitis A virus and hepatitis B virus there is no vaccine available for HCV [1]. The diagnosis of HCV infection is important because most infections are caused by transmission by undiagnosed and symptom-free carriers, or by contaminated blood transfusions or transplants. An easy-to-use, mobile and inexpensive detection system would therefore be advantageous and valuable for routine HCV testing in blood banks and clinical laboratories.

Current state-of-the-art antibody-based methods can identify patients carrying HCV by their immune response. Therefore an immunoassay is carried out to detect anti-HCV antibodies in the blood. An alternative to standard colorimetric enzyme-linked immunosorbent assays (ELISAs) is the electrical biochip platform that uses "single electrode redox cycling" and detects anti-Core antibodies in the blood [2]. In combination with advanced lab-on-chip functionality, this technology has been improved by the addition of two additional antigens to the biochip. The integration of the HCV proteins Core, NS3, and NS4A in one test shows the increase of the test correctness, which was demonstrated by measuring of an anti-HCV antibody response in diluted serum or whole blood samples in less than 15 minutes [3]. A special biochip cartridge allows direct sample collection and dilution in a fully automated analysis system. The portability of the system makes it suitable to improve diagnostics in small hospitals, doctors' offices or even directly at the patient's home. It is of increasing importance that physicians or in future also patients are able to execute even complex analyzing tests at the point of care on their own. Therefore the presented electrical biosensor system illustrates an increased level of integration. The integrated biochip cartridge system requires only mechanical and electrical interfaces to the device and provides easy to handle and rapid tests with required sensitivity for related applications. Referring to this the system is applicable for a wide range of immunological based tests to meet the needs of mobile diagnostics.

Electrical biochip technology

The disposable silicon-based chips were fabricated in the cleanrooms of the Fraunhofer Institute for Silicon Technology in Itzehoe, Germany. The production of the chips with a dimension of 8 ×10 mm^2 was carried out on 8-inch silicon wafer technology. The chips shown in figure 1 provide 16 gold electrode positions with a diameter of 350 µm, each. The electrical interface is realized by one row of contact pads with a pitch of 0.5 mm, allowing the use of commercial connectors.
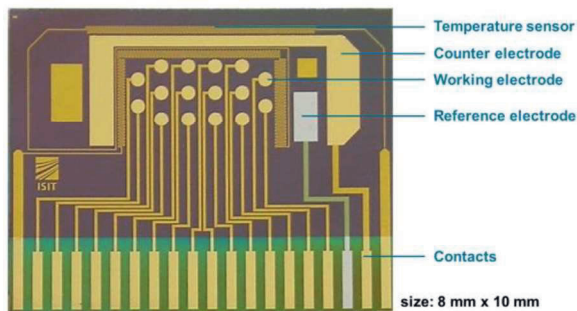

Fig. 1. Electrical biochip with an array of 16 gold electrodes.

During fabrication the wafers were thermally oxidized to a depth of 650 nm for insulation. The gold electrodes comprised vacuum-deposited stacks of 20 nm tantalum as a bonding agent and a final 150 nm upper gold layer. A gold counter-electrode and an iridium oxide reference electrode were also integrated into the chip, structured by photolithography and lift-off technology. The conducting paths were passivated against the wet samples using a combination of 400 nm silicon nitride and a 2 µm structured photoresist. Only the electrodes and the contact pad areas were opened by dry etching. This combination of the hydrophilic electrode surface and surrounding hydrophobic passivation optimized the dispensing and immobilizing of the capture molecules [3].

The capture molecules were applied to the twelve central gold electrodes using a piezo-driven micro-dispensing device from GeSiM GmbH (Großerkmannsdorf, Germany) as shown in figure 2. Human IgG was used as a positive control (300 µg/mL in PBS) and BSA as a negative control (100 µg/mL in PBS) spotted on the chip at three positions each. The deposition of 26 droplets represents 10 nL per array electrode. The HCV Core antigen (10 µg/mL in PBS with 3 M urea) was deposited at positions 4 and 7, the NS3 antigen (280 µg/mL in PBS with 3 M urea) at positions 5 and 8, and the NS4A antigen (80 µg/mL in PBS with 3 M urea) at positions 6 and 9. The antigens were initially diluted 1:2.5 in PBS. Positions 1–3 were designated the "positive control" and positions

10–12 the "negative control", because they were used for mathematical standardization. The immobilization of these capture molecules was achieved by thiol–gold interaction and hydrophobic adsorption. The spotted chips were incubated for 2.5 hours at room temperature in a humidity chamber. After washing with PBS-T, the chips were blocked with BSA (0.5 mg/mL in PBS) for 20 minutes, washed with deionized water, and dried under a vacuum.
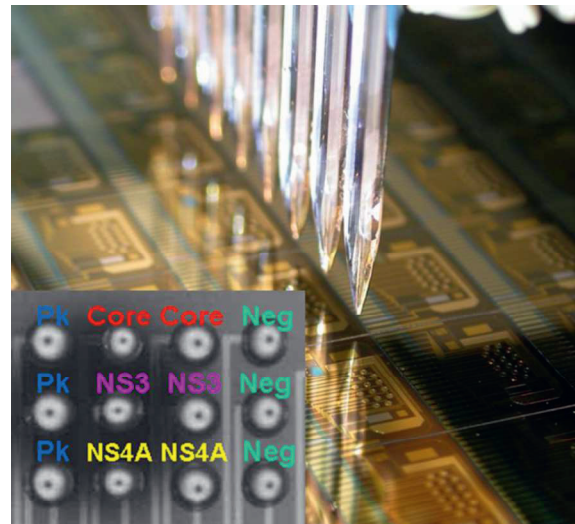

Fig. 2. Array format of the biochip with capture antigen pattern and spotting tips of the piezo-driven micro-dispensing device.

Biochip cartridge and automated device

The spotted biochips were housed in a polycarbonate microfluidic cartridge. A flow-through cell allowed the samples and reagents to be passed successively over the biochips in the cartridge. The cartridge also contained a sample collector and a dilution reservoir together with fluid channels and five ports connecting the biochips to the measurement device.

Fig. 3. Sample collection with the biochip cartridge and point-of-care reader system.

The device provides additional connection to a disposable reagent container for buffer, enzyme conjugate, substrate and waste. By a special biochip cartridge adapter the automated analysis via two miniaturized peristaltic pumps (Prolatec GmbH, Radebeul, Germany) and six pinch solenoid valves (Sirai®, Bussero, Italy) was performed. The total size of the measurement device represented in figure 3 was 144×250×244 mm^3. An integrated multichannel potentiostat was used to change the reaction potential of each electrode between 200 mV and –350 mV and to measure the resulting currents in a range of ±200 nA. The electronic components controlled the assay procedure and also the pump, valves, and temperature regulation unit. The biochip cartridge adapter realized simultaneously the connection to the multichannel potentiostat, the controlled heating unit and the cartridge to the fluidic system of the automated device.

Integrated biochip cartridge systems

The integrated biochip cartridge is designed to realize the same assay procedure as the previous described system, but with an increased level of integration. That means the system consists of a modified fluid system with a reagent module and a biochip cartridge module, where the measurement electronics is equal to the reader system of figure 3. Like illustrated in figure 4 the biochip cartridge module is equipped with functionalities for sample collection, biochip integration and a waste containment. Next to that a venting membrane enables flow through of the sample and the reagents over the biochip and a special valve to provide optimal stop flow condition in the final measurement step. The reagent module contains the assay reagents, which are stored in and processed through the fluid system in the automated device [3]. This integrated system has a direct fluid interface between the two modules sketched in figure 4.
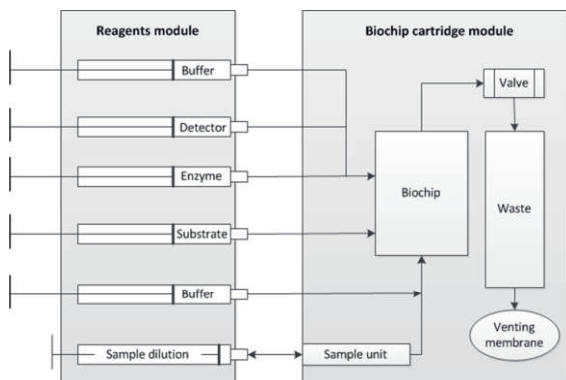

Fig. 4. Scheme of the integrated system with reagents and biochip cartridge modules.

The automated processing of the sample, buffer, enzyme conjugate and substrate is performed by syringe pump functionality driven by six individual controlled DC motors (DR. FRITZ FAULHABER GMBH & CO. KG, Schönaich, Germany). Figure 5 shows a first prototype of the integrated system with control circuit and syringe pumps, which are connected directly to the biochip cartridge through a septum interface.

Fig. 5. Prototype of the integrated biochip cartridge system including reagent processing unit by disposable syringes.

Analysis procedure on electrical biochips

The assays of both systems, the first described automated one and the more integrated one, were configured and controlled by the proprietary software "MCDDE" (Fraunhofer Institute of Silicon Technology, Itzehoe, Germany). It provides a user interface to adjust the action of valves, pumps, and heating to realize an automated assay procedure. Next to the control of the fluid and temperature functions the software transmit the measurement data from the device to the visualisation and evaluation program OriginPro 8G (OriginLab Corporation, Northampton, MA, USA).

The 15-minute HCV test on the electrical biochip was carried out by pipetting 2 µL of serum into the cartridge sample collector. Once inserted into the measurement device, the sample was diluted automatically 1:250 with buffer. Whole blood samples were taken from a droplet on the fingertip into the cartridge sample collector by capillary transfer. Spotted biochips were already integrated into the cartridges, which were connected to the corresponding fluidic, electrical, and thermal systems. All steps were carried out with the chip temperature of 38°C. Goat anti-human β-galactosidase was diluted 1:400 in PBS-T containing MgCl2, trehalose, and BSA, and the substrate p-APG was provided at a concentration of 1 mg/mL. The diluted samples were passed across the chip and incubated for 8 minutes to allow anti-HCV antibodies to bind the HCV target antigens on the electrodes. The bound antibodies and

human IgG control were enzyme-labeled afterwards for a further 3 minutes, before the position-specific generation of the electro-chemically active substrate 4-hydroxyaniline from p-APG was used to provide the readout by single electrode redox cycling in a stop flow mode. A further 4 minutes were necessary for all dilution, reaction, and washing steps in between the other reaction steps [2].

The production of 4-hydroxyaniline was measured by switching the gold electrodes on the biochip between +200 mV and −350 mV with a frequency of 1 Hz. Current measurements were taken 120 milliseconds after switching, resulting in position-specific increasing oxidation and reduction currents. The magnitude of these oxidation and reduction currents was summarized to create a current slope for each chip position, beginning 1 second after stop flow mode. Finally, linear regression analysis of a 6-second time period yielded target concentration-dependent slopes in the nA/min range.

A mean value was generated for each triplicate of the positive and negative control or duplicate of the target antigens on each individual chip. A standardized procedure was carried out by setting the negative control values (chip position 10–12) to 0, then adjusting the positive control and HCV values accordingly. The positive control was set to 1,000 nA/min and the HCV signals were normalized relative to this value.

Results

The performance of the biochip system was first demonstrated by the comparison to a standard ELISA running in a microtiter plate and optical detection. Therefore 71 serum samples were analysed on the biochip with electrical readout as well as on the standard ELISA with optical readout by using of the same antigens and antibodies for the assays [3].
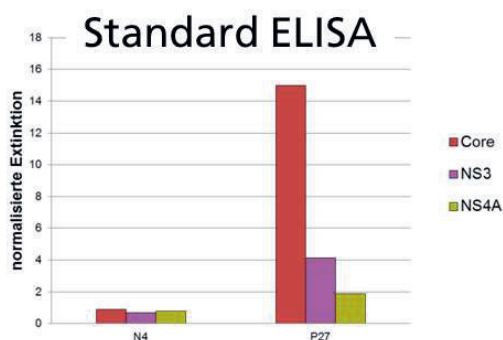

Fig. 6. Exemplary negative sample and positive sample result of the standard ELISA system.
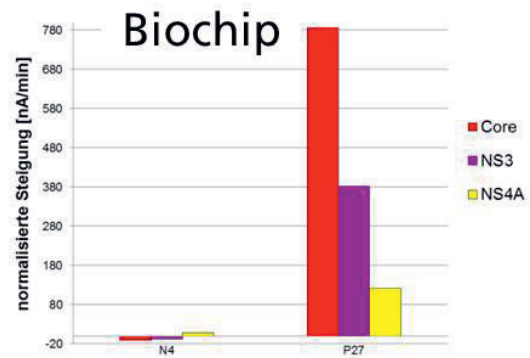

Fig. 7. Exemplary negative sample and positive sample result of the electrical biochip system.

Figure 6 and 7 show two example results of each detection method. Because of the different scale of each method it illustrates just the better signal to noise ratio of the biochip system. This is especially caused by the very low background signal of the method with the electrical readout. The results of the analysis of 32 positive and 39 negative serum samples show a better test correctness of the biochip. As summarized in table 1 this is realized in combination with a significant reduction of the test duration of a factor of 16 compared to the standard ELISA.

Tab. 1: Result of the analysis of 32 positive HCV samples and 39 negative HCV samples

HCV antibody detection	Standard ELISA	Biochip
Correctness of the test	88,7%	94,4%
Duration of the test	4 h	15 min

For point-of-care applications it is essential that no further sample preparation is needed after collection. Therefore the cartridge-based biochip system was tested with realistic whole blood samples. HCV-negative blood was sampled by drawing 2 μL into the cartridge from a droplet on a fingertip (Figure 3). After closing the collector, the cartridge was inserted into the measurement device. The test was carried out as described previously for serum samples, using HCV-negative serum as a negative control and 2 μL of HCV-negative whole blood spiked with 1 μL of positive serum as a positive serum control. The presence of red blood cells did not affect the performance of the electrical biochips or the automated analysis in the cartridge.

To analyze the performance of the biochip system with the increased level of integration, which works with a modified fluid system, the HCV assay of the first study were performed in both systems. For each test a measurement of a positive serum sample was used. The results are illustrated in figure 8 and 9 without standardization procedure, to get the possibility of detailed data evaluation. The following figure 8 shows the measurement result of the biochip system, which was already used for the previous comparative study. It shows very low signals on the negative control positions and reasonable test results on each target position.
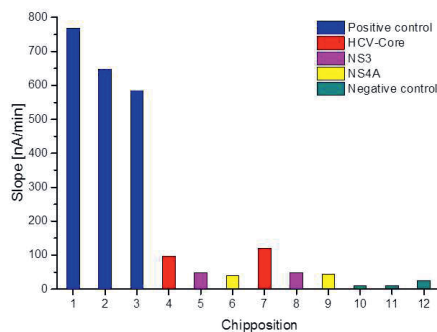

Fig. 8.   Result of a positive sample detected by the automated reader system and biochip cartridge.

The measurement data of the integrated biochip cartridge version is presented in figure 9 and demonstrated higher individual signals, but also higher signals on the negative control positions.
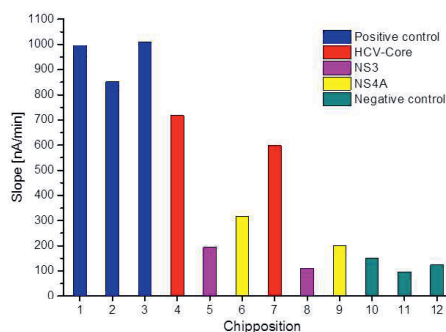

Fig. 9.   Result of a positive sample detected by the automated reader and the integrated biochip cartridge system.

These background signals caused by the modified fluid system generated in total somewhat worse results compared to the established biochip system with fluid processing insight the device.

Conclusion

The developed point-of-care diagnostic systems based on electrical biochips allow fully automated and sensitive detection of anti-HCV antibodies in serum or whole blood samples within 15 minutes. A robust assay design is combined with direct sample collection into the cartridge, thus avoiding syringes, cannulas, and further pipetting steps. Only 2 µL of serum or whole blood is needed to detect a HCV infection and automated dilution by the system achieves reproducible results. The signal-to-noise ratio and assay correctness is better than in the microtiter plate ELISA. But especially the reduced assay duration to less than 15 minutes makes the system suitable for a point-of-care approach. The further development of the integrated cartridge system with two disposable modules show in its first demonstration promising results, which have to be optimized regarding reduction of background signals. But in direction of mobile point-of-care diagnostics where the patients are able to execute even complex analyzing on their own, it is essential to provide a simple and maintenance-free system. By some adjustments in the combination of the pump and valve functions during the stop-flow detection step it will be possible to achieve better results, to enable highly sophisticated biosensor systems which fulfil the requirements of the medical point-of-care diagnostics.

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