Ability of Acoustic Sensor Devices for Medical Analysis

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***Abstract*—There is an increasing demand to monitor course of disease or therapy success of certain diseases via determining concentrations of specific protein markers in blood. For such biomedical applications implying the detection of multiple proteins a cost-effective and rapid analysis system with small sample consumption is required. Biosensor packaging is a useful means to interface the biosensor with the surroundings while ensuring its integrity and performance. We recently developed surface acoustic wave (SAW) biosensor chips, which allow the label-free, sensitive and cost-effective detection of proteins. For that, single SAW devices first are embedded in polymer housings, however, the sensitive sensor area still remains accessible for surface modification. The sensor surface is modified with capture molecules binding specifically to the analyte. Unspecific binding reactions are prevented via intermediate hydrogel layers, such as dextran. Advantages of those SAW biosensor chips are simple handling, low consumption of chemicals used in the coating process and enabling parallel analysis of multiple analytes in one sample. Hence, when applying biosensor surface modifications to a packaging, it is essential that both the effect on the housing and the impact on the biosensor signal response in a subsequent assay are investigated. Our housed SAW chips enable a user-friendly, easy and rapid way to detect different clinical analytes.**

Keywords-SAW biosensors; array; point-of-care; cancer diagnosis

I. INTRODUCTION

In many cases, it is possible to diagnose diseases, such as cancer, via determining concentrations of several specific protein markers in blood to create a marker profile of the patient. Such a marker profile can also be used for monitoring the success of the therapy applied to cure those diseases. For example, therapy of breast cancer patients often has to be modified to achieve best possible chances for healing. In many cases, the critical time already lies within the first six weeks after therapy start [1]. For such Point of Care (POC) applications based on the detection of multiple proteins analytes in one sample a cost-effective and rapid analysis system with small sample consumption is required.

It has been shown by numerous publications [2] and patents [3] that biosensors allow rapid and accurate detection and determination of analyte concentrations in a comparatively easy way. Still, compared to the scientific output, the number of complete biosensor systems which are actually commercialized is rather small. One of the reasons could be that research projects often deal with optimization of individual biosensor system components only, but real system integration into a reliable instrumentation is neglected [4]. System integration includes, among others, the protection of the biosensor element in the system against external negative influences, while its mechanical, electrical, and biological integrity is ensured, for instance, accessibility to sample solutions. These requirements can be met with an appropriate biosensor packaging which provides both mechanical protection and suitable connections to the system's surroundings required for operation, such as a fluidic delivery system [5].

SAW biosensors allow the label-free, sensitive and cost-effective detection of biomolecules. They have been applied successfully to detect proteins, desoxyribonucleic acid (DNA) and bacteria [6]. For the detection of proteins the sensor surface is modified with capture molecules, specific analyte binding partner. Unspecific binding reactions were prevented by coupling the binding molecules via an intermediate hydrogel layer, such as dextran, on the sensor surface. This is a crucial issue for all biosensors based on label-free detection of analytes in serum samples due to the high protein background [7]. Capture molecules can be immobilized by amine coupling via reactive esters using a carbodiimide, e.g., 1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide (EDC), and N-hydroxysuccinimide (NHS). Due to its flexibility, relative ease of use, high coupling yields and robustness, the EDC/NHS coupling procedure is the most frequently employed immobilization method [8]. Therefore, if hydrogel layers supply amino groups instead of carboxyl groups, it might be useful to convert the amino groups to carboxyl groups prior to protein coupling, e.g., by means of dicarboxylic acid anhydrides [9], to obtain a higher coupling yield.

Our previous set-up was used successfully in several affinity binding experiments [10], however, it is not suited for medical applications: Aside from the SAW device itself it consists of non-disposable components making it inappropriate for a POC device.

We recently developed a SAW biosensor system consists of a SAW device embedded in a polymer housing, which is connected to an external flow injection analysis (FIA) system. Using acoustic biosensors, such as SAW biosensors, it additionally has to be considered that aside from mass change the change of viscoelasticity of the sensing layer (including layer thickness and penetration depth of the SAW) also contributes to the SAW biosensor signal response. The effect of the latter strongly depends on the morphology of the sensing layer and hence could be

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influenced by the surface modification procedure [11] [12]. To guarantee the suitability of a newly developed biosensor chip, after ensuring intactness of the materials involved, it is essential to test the effect of this procedure in a subsequent binding experiment. This will be shown in the following, by using the example of streptavidin/biotin assay.

II. MATERIALS AND METHODS

A. SAW device and fluidic set-up

The shear horizontal SAW resonator is based on a small (4 x 4 mm²) 36°YX-$LiTaO_3$ device with gold transducers and has a frequency of operation of 428.5 MHz. SAW measurements were performed in an oscillator circuit developed in-house with the SAW resonator as frequency-determining element. Difference frequencies relative to a permanently oscillating reference oscillator, featuring a constant frequency in the range of 434 MHz, were used as signal output. The frequency resolution was 1 Hz. Experiments were performed using a flow injection analysis (FIA) system equipped with two peristaltic pumps (Ismatec, Wertheim, Germany) and an injection valve (Besta-Technik, Wilhelmsfeld, Germany). PTFE tubes served as connections between single units and as sample loop. The SAW resonator was included by means of a flow cell which was designed as part of an electronic circuit board integrated in the oscillator unit. The SAW device was mounted upside down onto isolated contact pads on the electronic board and coupled capacitively. The milled flow channel in between the contact pads allowed the fluid to pass along the SAW path. Details of the previous measurement setup (Fig. 1) have been described earlier [10].

Our new developed SAW chips were mounted in a flow cell adapter, which connects the sensor device with FIA system (Fig. 2). The principle of measurement setup is the same as aforementioned.

B. Parylene coating

All SAW devices used in the following experiments were first coated with 0.1 µm parylene C to obtain a chemically homogeneous surface. This improves success and reproducibility of further preparation steps. Details of the process have been published earlier [10].

C. Covalent binding of hydrogel

Aminodextrane (AMD) with M_r 3.000 was used as intermediate hydrogel layer. The parylene C layer was activated by oxidation via plasma treatment and subsequent silanization with (3-glycidyloxypropyl)trimethoxysilane. After rinsing with acetone the sensors were treated overnight with an aqueous solution of hydrogel, c = 2 mg/ml. Then sensors were rinsed thoroughly with bidestilled water and dried.

D. Immobilization of biotinylated BSA (b-BSA)

The affinity system of streptavidin with biotin was used for exemplary measurements. The capture molecule b-BSA was immobilized on the AMD coated sensors. The amino groups were converted to carboxyl groups via glutaric anhydride dissolved in aqueous solution, c = 2 mg/µl. Details of the process have been published earlier [11]. Protein immobilization was performed on-line by means of the external FIA system. Phosphate-buffered saline (PBS) was used as carrier stream. First, the carboxylized surface was activated with a solution of 0.05 M N-hydroxy succinimide and 0.2 M N-(3-dimethylamino-propyl)-N'-ethylcarbodi-imide hydrochloride in bidistilled water and rinsed with PBS. Second, the surface was incubated with a solution of 1.8 µM b-BSA in acetate buffer, pH 5, and rinsed with PBS. Third, the remaining reactive groups on the surface were deactivated by flushing with a solution of 1 M ethanolamine, pH 8.5. After rinsing with PBS, the sensor was immediately used for the assay.

E. Streptavidin assay

Experiments were performed with PBS, pH 7.4, as carrier stream. The flow rate was set to 0.05 ml/min. Samples were loaded in the sample loop, injected into the carrier stream via the injection valve, and transported to the sensor. The injection interval was set to 60-300 s. After each injection the sensor was rinsed with PBS. Sensors with covalently immobilized b-BSA or BSA were used. Samples containing, c = 0 (1; 5; 10) µg/ml streptavidin were injected in the carrier stream.

III. RESULTS AND DISCUSSION

SAW biosensors enable label-free and direct detection of DNA, proteins, and bacteria in real-time. Aiming at multi-parameter analysis, we developed a disposable SAW biosensor chip based on an array-compatible biosensor housing which encapsulated a SAW sensor (Fig. 1 A) except for an opening in the area of the active sensing structure (Fig. 2 A) to permit common surface modification steps for immobilizing the sensing layer as described in section II.

The effects of the packaging of the newly developed SAW biosensor chips on the signal responses were compared to those of the previous SAW biosensor devices to investigate the suitability of the new housing regarding the biosensor's performance. Therefore, SAW measurements based on the affinity system streptavidin/biotin were performed with SAW biosensors integrated in the flow cell (Fig.1) and with newly developed SAW biosensor chips in the flow cell adapter (Fig.2) with the external FIA system. Biotinylated BSA was immobilized on the SAW sensor surface via an intermediate AMD layer, samples contained streptavidin. Each sensor was tested by injection of several concentrations of streptavidin, c = 0 (1; 5; 10) µg/ml. Exemplary signal responses are shown in Figure 3, the signals were reproducible (data not shown). Using a sensor with immobilized binding partner, i.e., b-BSA, the SAW signal response increases with higher concentrations of streptavidin until saturation is reached (Fig. 3A).

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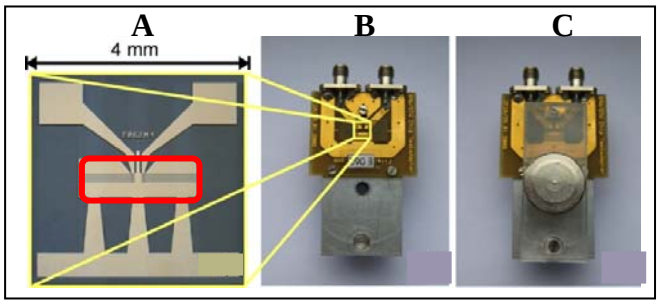

Figure 1. Previous set-up with inserting of the SAW device. SAW device (a), flow cell, open (b) and flow cell, closed.

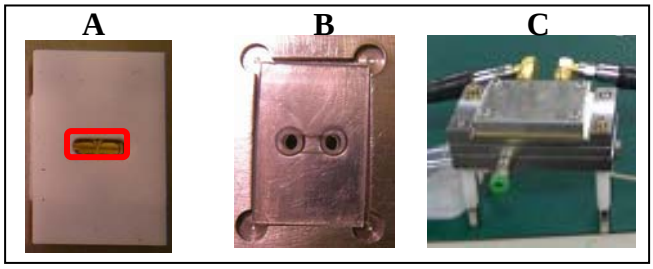

Figure 2. Current set-up with inserting of the SAW chip. SAW chip (SAW device embedded in a polymer housing) (A), cavity of flow cell adapter, open (B) and flow cell adapter, closed (C).

In this work, the ability of the use of SAW biosensor for specific protein detection was shown with measurements in our previous flow cell in general. Significant streptavidin signals were obtained when the corresponding binding partner, b-BSA, was immobilized on the SAW biosensor surface. Aside from being specific, direct and label-free, the protein binding is monitored time-resolved enabling the determination of kinetic parameters of the binding reaction. However, the SAW set-up with flow cell is not suitable for medical applications. It contains mainly non-disposable components which could lead to carry-over effects between different measurements. An additional disadvantage is the comparatively large dimensions of flow cell (and tubing) leading to a significant delay of signal response times and increase of sample volumes. In consequence, the flow cells are not array compatible.

Therefore, we investigated if inserting SAW devices in disposable polymer housings are not only enable simple handling and reduction of coating chemicals but also the signal response of streptavidin assay is comparable to those obtained with unpacked SAW devices. The signal response of SAW biosensor chips resulting from binding of different concentrations of streptavidin to b-BSA on the surface is significantly higher (Fig. 3 B) than obtained with the previous set-up (Fig. 3 A). We think that the increased signals obtained with SAW biosensor chips result from the different channel geometry of the two described setups. In consequence, this would apply the use of SAW biosensor chip system to develop a suitable platform for biomedical applications.

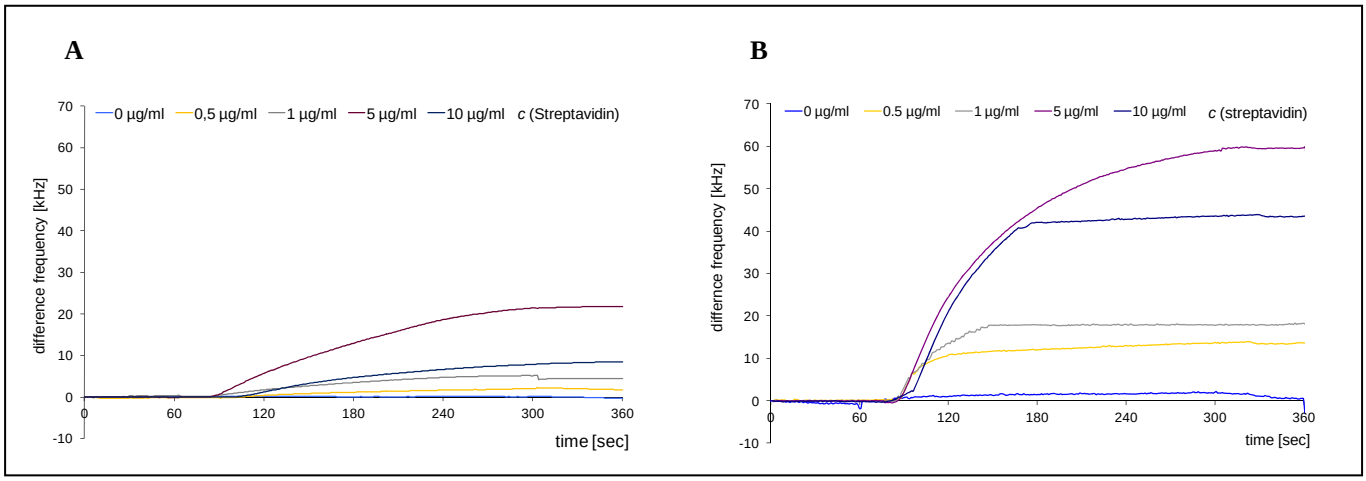

Figure 3. Streptavidin assay. Typical measurement curves of the SAW biosensor. Biotinylated BSA immobilized on sensor surface coated with AMD 3.000. Different concentrations of streptavidin (0 – 10 µg/ml) were used as analyte. (A) Previous set-up with SAW devices. (B) Actual set-up with housed SAW devices (B).

IV. CONCLUSIONS AND FUTURE WORK

We presented a set-up for analysis of future POC applications. In particular, this set-up offers a simple and cost-effective method for the detection of multiple protein analytes out of one sample, i.e., a protein marker profile. Such a profile could give a quick answer about the state of health of a patient (Figure 4) allowing an immediate adaptation of therapy. In the next step, the SAW biosensor chip array is optimized for biological applications with regard to real samples and required detection limits.

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In this work, we investigated that surface modification procedure established for unpacked SAW biosensors was successfully adapted to SAW biosensor chips in which the SAW devices were integrated in polymer housings. SAW biosensor signal responses obtained with streptavidin/biotin affinity system were not only similar to signals obtained with the previous setup, but even higher. Therefore, SAW biosensor chips permit a better biosensor efficiency. Our housed SAW chips will now used for medical applications, e.g., detection of different disease markers.

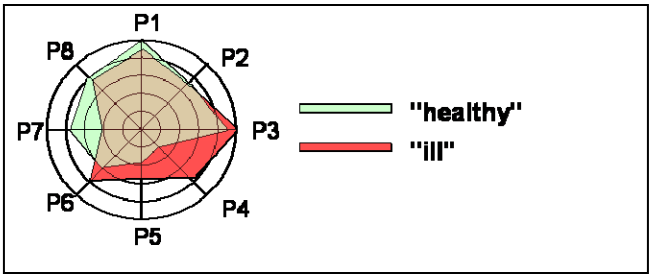

Figure 4. Model of a potential marker profile. Concentration of distinct proteins (here: 8) in serum can give quick information about state of health of patient.

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