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Label-free impedimetric biosensor for thrombin using the thrombin-binding aptamer as receptor

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Abstract. This study presents the further establishment of impedimetric biosensors with aptamers as receptors. Aptamers are short single-stranded oligonucleotides which bind analytes with a specific region of their 3D structure. Electrical impedance spectroscopy is a sensitive method for analyzing changes on the electrode surface, e.g. caused by receptor-ligand-interactions. Fast and inexpensive prototyping of electrodes on the basis of commercially available compact discs having a 24 carat gold reflective layer was investigated. Electrode structures (CDtrodes [1]) in the range from few millimetres down to 100 microns were realized. The well-studied thrombin-binding aptamer (TBA) was used as receptor for characterizing these micro- and macro-electrodes. The impedance signal showed a linear correlation for concentrations of thrombin between 1.0 nM to 100 nM. This range corresponds well with most of the references and may be useful for the point-of-care testing (POCT).

1. Introduction

In this work, an aptamer-based biosensor for the detection of the clinical relevant protein thrombin was developed. This biosensor consists of electrodes (CDtrodes) prepared from CDs [1] as transducer and the thrombin-binding aptamer (TBA) [2] as receptor for the detection of thrombin.

Thrombin is a protein of importance which regulates many processes in inflammation and tissue repair at the blood vessel wall. Its concentration in blood is normally around 0.01 nM. TBA itself has a well-known structure and was investigated in several previous biosensor studies, such as electrochemical gold nanoparticles [3], microfabricated thin film electrodes [4], and electrochemiluminescence [5].

The demand for convenient methods of detecting and measuring specific proteins in biological and environmental samples has motivated the development of commercial biosensors. One of the main difficulties, however, is the instability of the biological recognition element. Aptamers are well-known for their excellent stability. This is why aptamers can contribute to producing stable biosensors.

Our work concentrates on the characterization of this biosensor using immobilized streptavidin and the biotinylated aptamer as sensitive layer on the electrodes. It also contributes to the development of impedimetric biosensors, which are portable, inexpensive, provide rapid response, and can be manufactured with ease.

2. Material and methods

Recordable compact discs were used as an inexpensive and abundant material for preparing electrodes. CDs typically consist of four layers of materials:

- polycarbonate base for providing mechanical stability
- polymer layer which will be sensitized during the recording process
- metallic reflective layer with thickness of about 100 nm
- polymeric lacquer film for protecting the underlying metal layer

In this work we used CDs from SONY having a 24 carat gold layer. For removing the lacquer film, the CDs were submerged in nitric acid (50%) for 5 min. After this procedure, the CD exposed a clean gold surface which does not require an abrasive cleaning process, such as polishing, or the piranha method typically used. The surface was rinsed with ethanol and then hydrophilized under UV. Afterwards the gold surface was sprayed with positive photoresist lacquer for photolithographic wet-etching of the various electrode patterns (etchant: 4 g KI and 1 g I₂ in 40 mL water for 5 min [1]).

We developed two electrode patterns for biosensor fabrication. The first pattern (Figure 1A) has a fixed measurement chamber glued on the electrodes. The samples are pipetted in and out between the measurements. Volumes of 50 μ L to 400 μ L can be used. The second pattern is the interdigitated finger electrode (IDE) (Figure 1B) which uses a flow-through channel chamber for the electrolyte (Figure 1C). Samples of 15 μ L to 90 μ L can be applied.

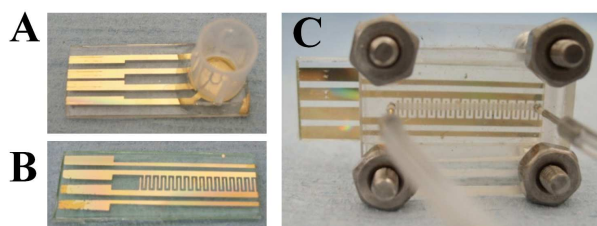


Figure 1. Photographs of electrode pattern with the glued measurement chamber (A) and the IDE pattern (B) which uses a self-made flow-through channel chamber in PDMS (C).

The surface of the clean gold electrode was treated with thiol for creating a self-assembled monolayer. We chose cysteamine (20 mM for 2h) for the initial layer and glutaraldehyde (300 mM for 2h) for the protein binding, according to the procedures described by Ferriera et al. [6]. Streptavidin, 1 μ g/mL diluted in PBS-buffer, was then immobilized via the aldehyde groups (at 4°C overnight). After blocking the non-specific binding sites with bovine serum albumin (BSA, 1.0 mg/mL in PBS for 1 h) we immobilized the biotinylated aptamer TBA (1 μ M diluted in TA-buffer) for 1 h. The TA-buffer consisted of 20mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂. The pH-value was adjusted to 7.4. TBA, which we used in this work, was a generous gift from the working group of Mrs Strehlitz, UFZ, Leipzig [2].

The measurement procedure was as follows: A baseline was established using the TA-buffer solution. Thrombin diluted in the TA-buffer was applied for ten minutes, after which the unbound thrombin was washed with the TA-buffer. Upon reaching a stable line, regeneration solution (2 M sodium chloride in water) was applied for two minutes.

Electrochemical impedance spectroscopy (EIS) was performed using a BioLogic Science Instruments Model SP-150 in the frequency range from 100 mHz to 200 kHz. As electrolyte we used TA-buffer with 5 mM potassium hexacyanoferrate(II) and 5 mM potassium hexacyanoferrate(III). Frequency response of the impedance was expressed as Nyquist plot and modelled using a Randles circuit consisting of an active electrolyte resistance R_s in series with the parallel combination of the double-layer capacitance C_{dl} and the impedance of a Faradaic reaction. The impedance of the Faradaic reaction consisted of an active charge transfer resistance R_{ct} and a specific electrochemical element of diffusion W , which is the well-known Warburg element.

For some experiments the aptamer was heat treated according to Tombelli, et al. [7] before the immobilization step. This heat treatment consisted of heating the aptamers in TA-buffer to 90°C for one minute, and then cooling the solution on ice for 10 minutes.

3. Results and discussion

Preliminary thrombin-binding experiments resulted in Nyquist plots that showed noticeable changes in the real and imaginary parts of the impedance. Higher sensitivity was observed at lower frequencies, in agreement with our earlier observation of the Bode impedance curve. In order to record the thrombin binding kinetics with the biosensor we considered measuring the impedance at a fixed frequency. Figure 2 shows the time course during a thrombin binding measurement (111 nM) at four different frequencies (10 Hz to 100 kHz). The figure illustrates a typical binding and dissociation curve that is comparable to most of the biosensors. Discontinuities in the curves were a result of pipetting the solutions in and out of the chamber.

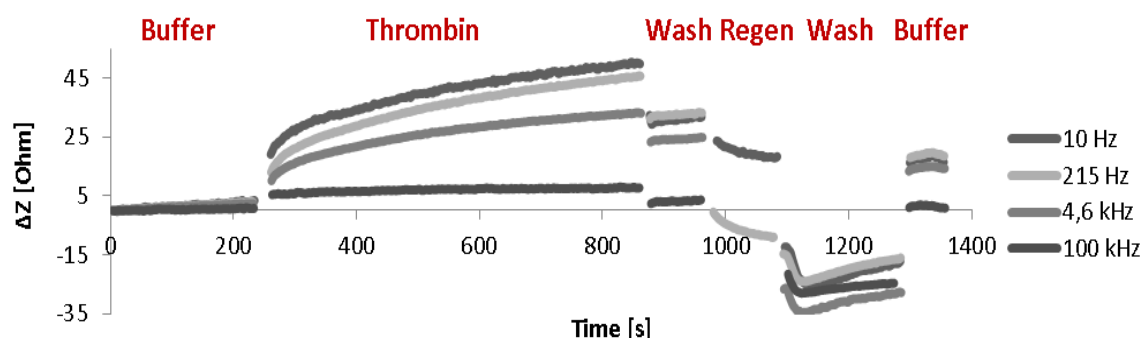


Figure 2. Thrombin binding measurement across a range of frequencies.

In order to determine the specificity of the biosensor we compared specific binding to thrombin and to 1.0 mg/mL BSA as the negative control at the fixed frequency of 10 Hz. The biosensor showed good specific binding to thrombin while the negative control produced a similar response to that of only TA-buffer. The sensitivity of TBA for thrombin was investigated in the range from 1.0 nM to 330 nM (Figure 3). There was a strong correlation between the concentrations of thrombin and the total change in impedance at 10 Hz after 10 minutes.

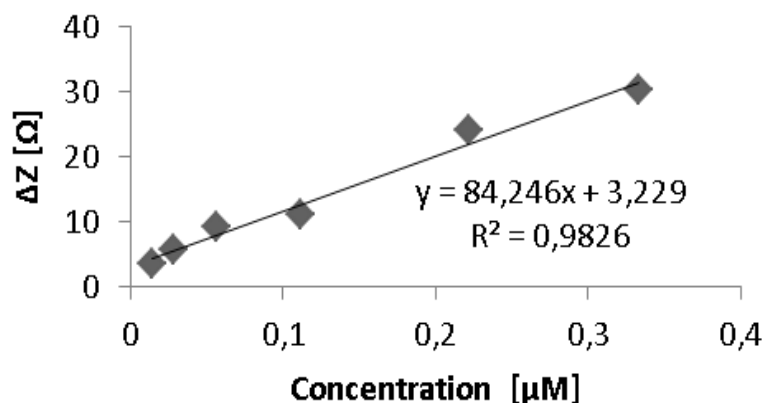


Figure 3. Correlations between the changes in the impedance and various concentrations of thrombin.

Tombelli, et al. [7] concluded that heat treatment of the aptamers influences intramolecular folding. The sDNA unfolds so that the biotin label and the recognition structure are available for interaction with streptavidin and the ligand. We performed the same heat treatment with our TBA and tested the sensitivity to thrombin and the stability of the biosensor. The aptamers which were not heat-treated showed higher sensitivity to thrombin. In contrast, the heat-treated aptamer were more stable (see Figure 4). The measurements consisted of thrombin binding (concentration: 111 nM) for ten minutes followed by the regeneration solution for one to two minutes followed by another thrombin binding test. Differences in impedance were measured at 10 Hz after 10 minutes binding. Detection cycle number 7 was treated as an outlier due to storage of the biosensor at 4°C overnight. The standard deviation of the other cycles was found to be around 15%.

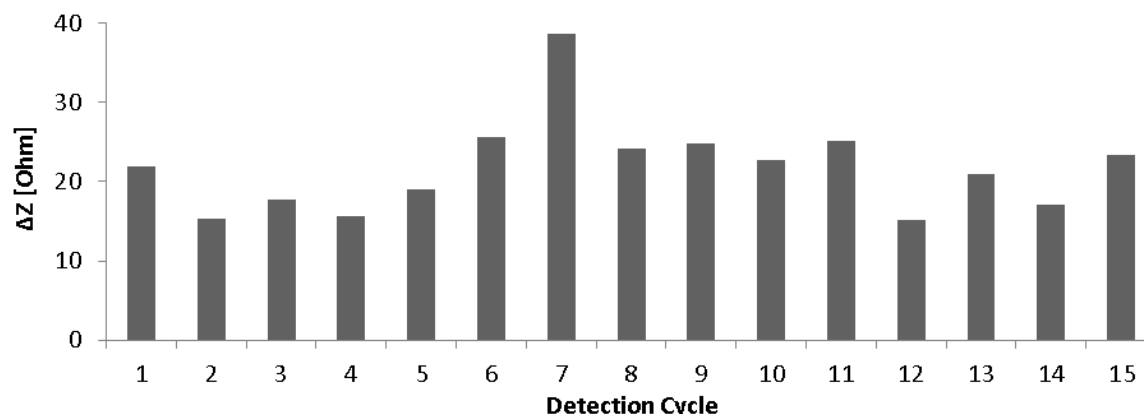


Figure 4. Regeneration of the heat-treated aptamers.

4. Discussions

In blood, plasma clots begin to form when thrombin concentrations reach a level of 5 nM, with the highest concentrations reaching 100 nM to 200 nM approximately ten minutes after clot formation. Thrombin is typically present in the blood in the 0.01 nM range. Low nanomolar concentrations of thrombin are generated early in haemostasis, and high range in patients suffering from diseases known to be associated with coagulation abnormalities. Therefore further studies are required to improve the sensitivity of this biosensor for thrombin-related diseases.

5. Conclusions

In this work we present inexpensive, stable, and easily producible biosensors for the purpose of thrombin detection. The electrodes showed good selectivity towards thrombin and were insensitive to BSA. The sensitivity of the electrodes to thrombin was not quite as good as some other results reported in literature earlier, but the detection limit was nevertheless in the clinical relevant range of 1 nM to 100 nM.

Regeneration of the biosensor was successful at least up to 15 times for electrodes with the heat-treated aptamers. This heat treatment, however, was found to deteriorate the sensitivity of the aptamer for thrombin detection. The efficiency of our label-free impedimetric biosensor was compared to measurements using the same aptamer and the quartz-crystal-microbalance (QCM) and the BIAffinity system, which is based on RIfS technique for detection of molecular interaction. These investigations achieved similar sensitivity and linear ranges (results not shown).

The advantage of the proposed biosensor is that the inexpensive electrode materials allow single-use applications. The fixed frequency of 10 Hz is in a range, for which inexpensive impedance systems can be realized with ease. This may overcome the low sensitivity, at least for POCT-applications. The deviations of our biosensor were about 15%. Similar values can be found for such simple systems. Nevertheless, more effort is needed to overcome this limitation before commercialization.

References

- [1] Angnes L, Richter E M, Augelli MA and Kume G H 2000 *Anal. Chem.* **72** 5503.
- [2] Strehlitz B, Nikolaus N and Stoltenburg R 2008 *Sensors* **8** 4296.
- [3] Li L, Zhao H, Chen Z, Mu X and Guo L 2011 *Sens. Actuat. B* **157** 189.
- [4] Cai H, Lee T M-H and Hsing I M 2006 *Sens. Actuat. B* **114** 433.
- [5] Fang L Y, Lu Z Z, Wei H and Wang E 2008 *Anal. Chim. Acta* **628** 80.
- [6] Ferreira C, Fugivara C, Barrazo S, Suegama P, Yamanaka H and Benedetti A 2009 *J. Electroanal. Chem.* **634** 111.
- [7] Tombelli S, Mascini M and Turner A P 2002 *Biosens. Bioelectr.* **17** 929.