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Label-free and reagentless capacitive aptasensor for thrombin

Hsin-Ju Chen[a], Richie L.C. Chen[a], Bo-Chuan Hsieh[a], Hsien-Yi Hsiao[a], Yi Kung[a], Yung-Te Hou[a], Tzong-Jih Cheng[a,b,*]

[a] Department of Bio-Industrial Mechatronics Engineering, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan
[b] Department of Biomedical Engineering, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

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ABSTRACT

This investigation develops a label-free and reagentless aptasensor, based on a capacitive transducer with simple face-to-face electrode pairs. The electrode pairs of the transducer are composed of a gold electrode and an indium tin oxide film with micrometer separation with a double-side polyethylene terephthalate tape. Aptamers and 1-dodecanethiol are modified to form a self-assembled monolayer (SAM) on the gold electrode surfaces, and function as bio-recognition elements and preventers of non-specific protein binding, respectively. Electrochemical characterization results indicate that the SAM also forms an effective insulating layer, which is sufficient for capacitive sensing. The feasibility of the capacitive biosensor is validated using thrombin as a model analyte. The ultra-small value changes of capacitance originating from thrombin binding with the aptamers modified on the biosensor were measured with a home-made capacitance measuring circuit based on switched capacitor (SC) technology. The developed biosensor has detection limits of 1 pM and 10 pM of thrombin in phosphate buffered saline and mimic serum solution, respectively. The linear range for thrombin detection in human serum solution is from 10 pM to 1 μM, with a regression coefficient of 0.98. Additionally, the proposed aptasensor does not have significant levels of non-specific binding of bovine serum albumin and human serum albumin. Accordingly, the combination of SC and SAM bringing capacitive transduction at the forefront of ultrasensitive label-free and reagentless biosensing devices, particularly for point-of-care clinical analysis, which adopts small numbers of biological samples with low analyte concentrations.

1. Introduction

The ideal operating mode of biosensors is single-step testing, which requires excellent specificity and sensitivity. The sensitivity of a biosensor is the combination of the transducer sensitivity and enhancing power of sensitivity in one or more modified bio-recognition layers. Enhancement of sensitivity and detection limit of bio-recognizing layers in biosensors has mainly been achieved with label-based sensing approaches in modified bio-molecular layer(s) of biosensors. Fluorescence and chemiluminescence are more common measures than radioactivity for directly detecting a specific target in biosensors. Enzymatic tag measurement is another popular label-based method for indirectly enhancing biosensor sensitivity, but is currently hampered by the high cost and activity stability of labeled enzyme(s), the need for additional reagents and the extended test procedures.

Label-free biosensors are typically classified by transducer type as reagent-based and reagentless platforms. The traditional reagentless and label-free bio-sensing platforms include optic (such as the market-leading product Surface Plasmon Resonance, SPR) (Wu et al., 2013), and the optical interference spectrum on functionalized porous scaffolds (Urmann et al., 2015), gravimetry (e.g., Quartz Crystal Microbalance, QCM) (Seo et al., 2008) and electrochemistry (Sun et al., 2017; Trindade and Dutra, 2018). Increasing the sensitivity of the high-density bio-recognition layer in SPR results in reagentless & label-free sensors, whereas QCM has inherent ultra-sensitive transducer performance. The high cost of both SPR- and QCM-based reagentless & label-free biosensors impedes their potential for widespread utilization. Researchers have started to consider electrochemically analytical technologies to develop reagentless or label-free biosensors, since those are simple, low-cost and robust in electrode-type transducers as well as instruments. Potentimetry (field-effect transistor, FET) (Goda and Miyahara, 2013; Chen et al., 2016), voltammetry (Schoukroun-Barnes et al., 2016; Tran et al., 2017), electrochemical impedance spectroscopy (EIS) on electrodes modified with the reversible redox couples (Schoukroun-Barnes et al., 2016; Kurzątkowska et al., 2015) or nanoparticles (Eissa and Zourob, 2017) tags.

* Corresponding author at: Department of Bio-Industrial Mechatronics Engineering, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan.
E-mail address: tzongjih@ntu.edu.tw (T.-J. Cheng).

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EIS has widespread applications in physic-chemically characterizing solid/liquid interfaces on functionalized electrode surfaces, and in transducing signals of bio-sensing events (Muñoz et al., 2017). The EIS implemented in biosensors is not only in common Faradaic EIS (impedimetric) as relately sensitive transducers, but also in non-Faradaic EIS (capacitive) approaches. Faradaic EIS is typically applied in a solution required with redox couples (e.g., ferri/ferrocyanide) reagent, and is not a reliable method for performing surface modification steps such as DNA hybridization/dehybridization (Vogt et al., 2016) or DNA aptamer/protein combination (Bogomolova et al., 2009) at thiolated gold electrodes, even if a stable redox couple is available as an alternative. Non-Faradaic EIS without redox couples reagent have improved reagentless and simple procedures for constructing biosensors based on molecule-recognition.

Capacitive sensors provide diverse benefits in a wide range of fields, due to characteristics such as low cost, fast response, non-invasiveness and flexibility in electrode design (Huang et al., 1989; Xie et al., 1990). Although capacitive sensing technology is extensively employed in measuring of physical parameters, capacitive biosensors were not invented until the late 1980s (Newman et al., 1986). The sensing principle of capacitive biosensors is based on changes in dielectric properties, charge distribution, dimension, shape and conductivity that result from an analyte/recognition molecule (such as antigen/antibody) complex formed on the electrode surfaces. Furthermore, a capacitive biosensor can be built by immobilizing bio-recognition elements and measuring the changes to dielectric properties when an analyte binds (Berggren et al., 2001). The capacitance between a pair of electrodes can then be described using the basic equation $C = \varepsilon\varepsilon_o \frac{A}{d}$, where ε denotes the dielectric constant of the medium between a pair of plates; ε_o represents the permittivity of free space (8.85 pF/m); A indicates the area of the electrodes, and d is the distance between the two electrodes. Therefore, a change in the dielectric properties of the material between a pair of electrodes leads to a change in capacitance, which is correlated with the bound molecules and amount captured by bio-recognition elements on the electrode surface.

Capacitive biosensors have been widely utilized in detection of cellular behavior (Ehret et al., 1997), bacteria (Wannapob et al., 2010), DNA hybridization (Berggren et al., 1999), antibodies (Taylor et al., 1991), biomarkers (Qureshi et al., 2010) and chemical compounds (Kitsara et al., 2007). Many capacitive biosensors have been reported as impedimetric biosensors, because both the resistive and capacitive properties can be analyzed with electrochemical impedance spectroscopy (EIS) (Guan et al., 2004). EIS measurements in non-faradaic process are label-free, and allow for the investigation of capacitance; however, the limited sensitivity needs to be improved in the faradaic process with addition of a redox couple (i.e. not reagentless) (Pänke et al., 2008). Additionally, many works have used interdigitated electrodes to improve the sensitivity of capacitive measurements by increasing the ratio of sensor surface to distance between the electrodes (Moreno-Hagelsieb et al., 2007; Quershi et al., 2009; Radke and Alocilja, 2005). The apparent limitation of this approach is that the electric field distribution within the geometry of interdigitated electrodes is inhomogeneous (Rahman et al., 2010). Additionally, the penetration depth of the electric field is not concentrated around the analyte, thus reducing the sensing performance of the capacitive biosensors.

Another critical issue for constructing capacitive biosensors is the fabrication of the insulating layer on the electrodes. If the insulating layer is not sufficiently insulated, then the influence of Faradic currents lowers or eliminates the capacitive signal (Berggren et al., 2001). Furthermore, the material and thickness of insulating layer is also important. In order to detect the capacitance change resulting from analyte binding, the insulating layer should be as thin as possible (Gebbert et al., 1992). Since the bio-recognition layers anchored on the capacitive biosensors are not sufficiently insulating and compact, some researchers have selected other materials such as metal oxides (Moreno-Hagelsieb et al., 2009; Olthuis et al., 1997), silicon oxides (de Vasconcelos et al., 2009) or polymers (Li et al., 2004; Wu et al., 2005) as the insulating layer. Nevertheless, few studies have assessed the insulating effect of insulating layers and reported self-assembled monolayers (SAM) as insulators on capacitive biosensors.

An organized SAM is a single layer of molecules on a substrate in which the molecules exhibit a high degree of orientation, molecular order and packing (Finklea, 2006). Previous studies have recognized that SAMs can be employed as the dielectric of capacitors with nanoscale thickness (Boulas et al., 1996; Haag et al., 1999; Rampi et al., 1998). SAMs of thiols, sulfides and disulfides on gold electrodes have been widely explored, and long chain alkanethiols are known to form well-organized insulating structures on gold substrates (Porter et al., 1987). Consequently, the ultrathin insulating layer formed by a SAM may be appropriate for developing capacitive biosensors.

Capacitive biosensors are label-free, and are promising for developing hand-held devices and point-of-care applications. However, traditional micro-fabricated capacitive sensors, such as those based on interdigitated electrodes, face the challenges such as delicate manufacture and non-uniform distribution of the electric field (Tsoutia et al., 2011). Furthermore, EIS for capacitance measurements typically requires additional reagents of redox species to enhance sensitivity. To provide an alternative method of developing a capacitive biosensor, this work proposes straightforward electrode design with a pair of (face-to-face planar) parallel plate electrodes. The electric field between the electrode pair becomes stronger, so that the presence of biomolecules can significantly change the sensing capacitance. Capacitance sensing can be achieved by directly measuring the stored charge at fixed voltage, the interface impedance. Switched capacitor (SC) technology can obtain minute capacitance value based on a differential readout voltage between sensing and reference capacitors (Ghafar-Zadeh and Sawan, 2010).

In this investigation, the electrode pair is constructed with a gold electrode from compact discs (CDs) and indium tin oxide (ITO) film as the opposing electrode. A double-side coated tape is utilized as a thin insulating spacer to reduce the distance between the electrode pair, and thus improve the system sensitivity. Aptamers and long-chain alkanethiols are immobilized on the gold electrode through self-assembly, and formed the insulating layer with a thickness of a few nanometers. The insulating effects of SAM on the gold electrodes are investigated using cyclic voltammetry and EIS. Aptamers, which are oligonucleotide or peptide molecules that bind to a specific target molecule, have potential as biosensing materials with outstanding specificity in various detection schemes (Navani and Li, 2006; Willner and Zayats, 2007). Meanwhile, aptamers with high affinity of binding and high specificity of target recognition (Cho et al., 2009) are appropriate for protein detection. Aptamers are also easily and reproducibly synthesized, and are easy to store, in contrast with antibodies (Mascini, 2008). Thrombin was adopted as the target analyte to validate the proposed capacitive aptasensor employing an electrode pair. The capacitance change originated from thrombin binding with aptamers was measured with SC technology with a homemade capacitance measuring circuit.

2. Material and methods

2.1. Apparatus

Cyclic voltammetric and electrochemical impedance determination were performed with the GEPS-module and FRA-module of Autolab PGSTAT-30 (Eco Chemie B.V., Utrecht, Netherlands). The capacitive transducer (capacitance-to-digital converter) was homemade.

2.2. Reagents and solutions

Sulfuric acid (H_2SO_4), sodium chloride (NaCl), potassium chloride

(KCl), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium hexacyanoferrate (II) trihydrate (ferrocyanide, $K_4Fe(CN)_6$), tri-potassium hexacyanoferrate (III) (ferricyanide, $K_3Fe(CN)_6$) and 99% ethyl alcohol (C_2H_6O) were received from Nacalai Tesque, Inc. (Kyoto, Japan). Thiolated thrombin binding aptamer with polyT (10) tail (5′-HS-$(CH_2)_6$ -T TTT TTT TTT GGT TGG TGT GGT TGG-3′) was synthesized by Purigo Biotech, Inc. (Taipei, Taiwan). 70% nitric acid (HNO_3) was purchased from Labscan, Asia Co, Ltd. (Thailand). Bovine serum albumin (BSA) was purchased from Arcos organics (Geel, Belgium). 1-dodecanethiol (DDT, $CH_3(CH_2)_{11}SH$), human α-thrombin (factor IIa, EC number: 3.4.21.5) and human serum albumin (HSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Normal human serum was purchased from Jackson ImmunoResearch Laboratories, Inc. (Pennsylvania, USA). Archival gold CD-R was purchased from Delkin Devices, Inc. (San Diego, USA). Double coated tape on a polyethylene terephthalate (PET) polyester carrier (9628FL, thickness 0.05 mm) was purchased from 3 M Co. (Taipei, Taiwan). Indium tin oxide (ITO) film was purchased from STK corp. (Taipei, Taiwan). The buffer or solutions used in this study were prepared as follows: a phosphate buffered saline (PBS) buffer (1X PBS, pH 7.4) and blocking buffer (1 mM DDT in 1X PBS containing 75% ethyl alcohol in final concentration). All chemicals were of analytical grade, and were applied without further purification.

2.3. Fabrication of aptamer-modified gold CDtrodes

A framework of disposable strip based on gold CDtrode (Fig. S1) and ITO electrode was fabricated as an electrode pair (Fig. 1). The double-coated PET tape was stuck on gold CDtrodes to define the reaction area (12.6 mm^2) for further immobilization. (Fig. S2) Immobilization steps for each condition of CDtrodes are described as follows. Each electrode was washed with PBS buffer between steps. In the treatments of the reaction area of CDtrode, 10 µL of 1 µM thrombin binding aptamer was deposited on the electrode area for 16 h at 25 °C. 10 µL of 1 mM DDT solution was dropped on the aptamer-treated CDtrode for 1 h at 25 °C, and then washed with PBS buffer and dried.

2.4. Characterization of modified CDtrodes by cyclic voltammetry and electrochemical impedance spectroscopy

The immobilization step of modified CDtrodes was performed as described above. The CDtrodes were divided into groups *a–c* to investigate the insulating effects of different modification. Group *a* CDtrodes were fabricated without either aptamer or DDT (Au). The Group *b* CDtrodes were fabricated with aptamer but without DDT (Au/Aptamer). The group *c* CDtrodes were fabricated with both aptamer and DDT (Au/Aptamer/DDT). Cyclic voltammetry was performed on all groups of Cdtrodes, with 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 1 × PBS buffer (pH 7.4) from − 200 mV to + 500 mV at a scan rate of 50 mV/s. Electrochemical impedance spectroscopy was performed with 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 1 × PBS buffer (pH 7.4). The frequency range was from 0.1 Hz to 100 kHz with 10 mV sinusoidal signal at 0.23 V. All potentials were reported with respect to an Ag/AgCl reference electrode, and the counter-electrode was a Pt wire.

2.5. Determination of thrombin

The specific recognition for thrombin was performed by adding 10 µL of definite concentration of thrombin on the modified CDtrode for 1 h at 25 °C. The CDtorde was then washed with PBS buffer to eliminate the unbounded substances. The capacitive measurement was conducted in a PBS buffer with an electrode pair by a homemade capacitance sensing circuit.

2.6. Data analysis

Each experiment was performed thrice using three different CDtrodes to test the reproducibility. All data were derived as mean signals ± standard deviation (S.D.). Differences between groups were evaluated by the two-tailed Student's *t*-test, where $p \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Characterizations of different modified CDtrodes by electrochemistry

Ultra-sensitive capacitive sensing platforms are based on

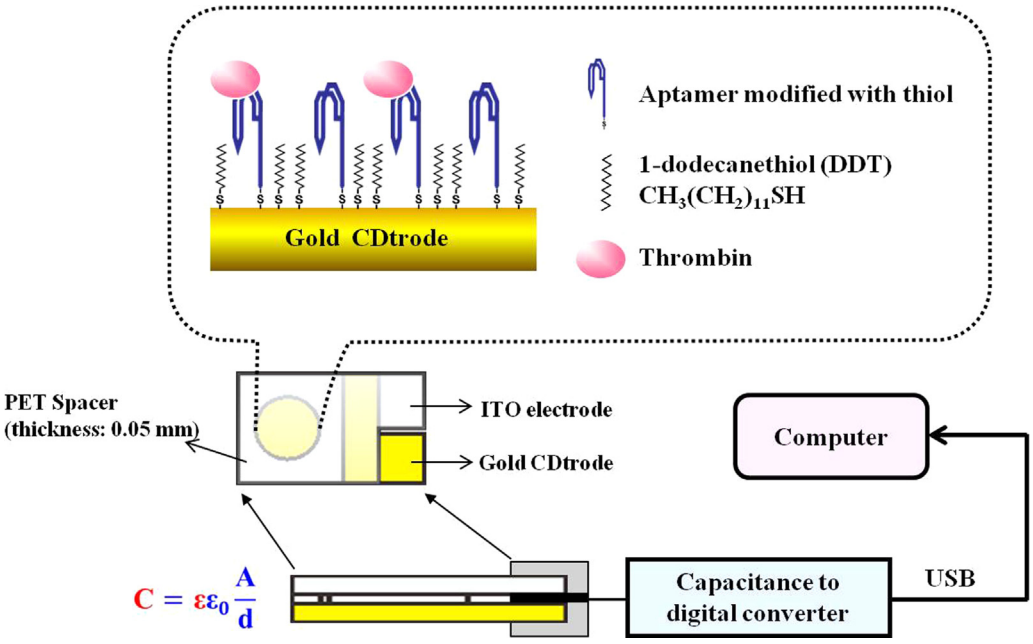

Fig. 1. Schematic illustration of the capacitive aptasensor for thrombin detection.

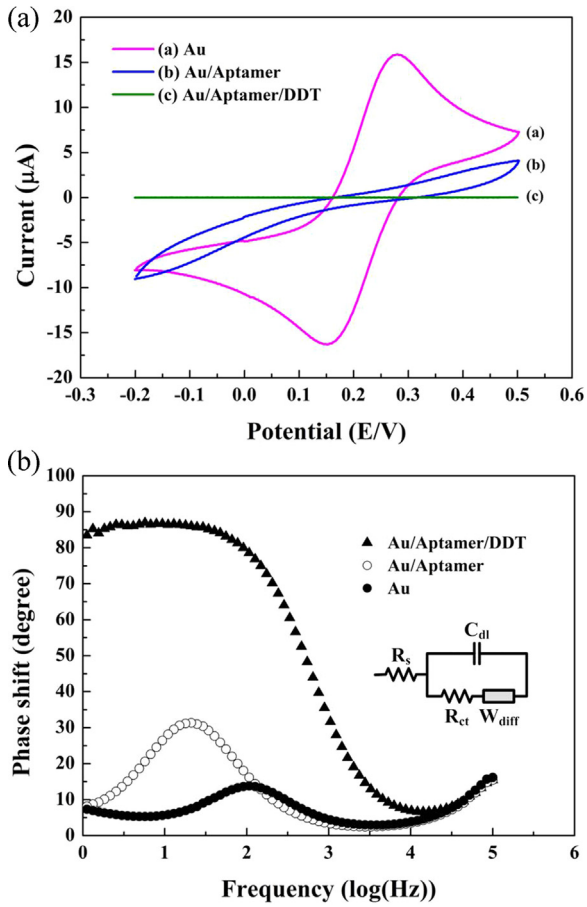

Fig. 2. Electrochemical characterization of modified CDtrodes. (a) cyclic voltammograms for gold CDtrodes with different modifications and (b) Bode phase plot of gold CDtrodes with different modifications. All data was recorded in 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ with 1 × PBS buffer (pH 7.4). Cyclic voltammetry was performed from −200 mV to + 500 mV (vs. Ag/AgCl) at a scan rate of 50 mV/s. Electrochemical impedance spectra was acquired at 0.23 V (vs. Ag/AgCl).

combination of sensitive capacitive sensor and instrumentation (circuit). An excellent insulating layer on the prepared electrode is essential for developing a sensitive capacitive biosensor. In this study, aptamer and DDT were immobilized on gold CDtrodes through self-assembly to form an ultra-thin insulating layer (several nanometers). The insulating effect of the modified CDtrodes was thus assessed using cyclic voltammetry and electrochemical impedance spectroscopy.

The electrical properties of SAMs adopted in electrochemical systems consist of both electronic and ionic modes of conduction. Cyclic voltammetry has been employed to test SAMs for electron tunneling, as long as a redox center is isolated from the outer part of the SAM (Alleman et al., 1996; Richardson et al., 1995). An electrochemically reversible and one-electron redox couple $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ was preferentially selected to examine the quality of the SAMs formed on the gold CDtrodes. Two obvious peaks representing the reduction of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ and reoxidation of $Fe(CN)_6^{4-}$ were observed for the bare Au (curve "*a*" in Fig. 2a). The voltammetric responses of the electrodes modified with aptamer (curve "*b*") were significantly smaller than those of bare Au (curve "*a*"). This is contributed by the immobilization of aptamer reducing the penetration of redox couples. Furthermore, co-immobilizing the electrode with DDT (curve "*c*") further improves the insulation, because the resulting profile has no redox current peaks, and the current response is insignificant (< 10 nA). The current response is much lower in curve *c* than in either curve *a* or *b*, and is mostly capacitive non-Faradaic current. This experimental result indicates that the SAMs of aptamer and DDT form an effective insulating layer on the gold CDtrodes for developing capacitive biosensors. Thus, the gold sheets, which are inexpensively and conveniently prepared from CDs, are compliant with requirement for formatting SAMs.

To further investigate the insulating effect of SAMs for aptamer and DDT, EIS was also performed to evaluate the formation of SAMs on the gold CDtrodes through the change in the phase angle. As shown in Fig. 2b, the SAM for Au/Aptamer/DDT has better insulating properties than that for Au and Au/Aptamer, due to the significantly greater phase shift of Au/Aptamer/DDT, which thus acts as pure capacitor. The experimental data also reveal that the polarizing current was consumed by the non-Faradic process from the typical equivalent circuit (Randles circuit, the inset of Fig. 2b), which comprises the uncompensated resistance of the electrolyte (R_s), in series with the capacitance of the dielectric layer (C_{dl}) and the charge-transfer resistance (R_{ct}). An additional component, the Warburg impedance (Z_w) connected in series with R_{ct}, accounts for the diffusion of ions from the bulk electrolyte to the electrode interface. This SAM is an ideal dielectric material, because a phase shift of 90° over the low-to-medium frequency range ($1\ Hz < f < 10^3$ Hz) means that current leakage does not occur at defect sites (Boubour and Lennox, 2000). Even though the phase shift of the SAM co-formed by aptamer and DDT at about 87° is a sign that the SAM behaves like a capacitor contaminated by a tiny resistive component (resistor) associated with current leakage at minor defect sites, the SAM can still could be assumed to behave like an ideal capacitor. These results indicate that the aptamer and DDT effectively co-form a thick insulating layer on a gold CDtrode surface, and the constructed electrode acts almost like a capacitive sensor.

3.2. Feasibility of the capacitive aptasensor with switched capacitor technology

The next issue to be addressed after verifying the insulating properties of SAMs is measuring the minute capacitance change caused by thrombin binding with aptamer. Many previous works have reported capacitive biosensors as impedimetric biosensors. Even though no equivalent model is guaranteed to fit all experimental data the electrochemical impedance spectrum is often analyzed by an equivalent circuit such as Fig. 2b (inset) to interpret the change of the impedance elements as a function of the solution composition. The relationship between capacitance and impedance is given by the equation $C = -\frac{1}{2\pi f Z_{im}}$, where f denotes the working frequency (Hz), and Z_{im} denotes the imaginary part of impedance. The capacitance value can be directly determined with the equation above. Although non-Faradic impedance can be undertaken without any redox probe, Faradic impedimetric affinity sensors have higher sensitivity in general. However, EIS investigates only the electrical features of interfacial phenomenon on the electrodes, and does not provide sufficient information for minute capacitance change.

Capacitance measurement in electronics has three main approaches. The SC method is faster and simpler than the bridge and resonance methods, and its ultra-low detection limit is the most attractive for transducers employed in ultra-sensitive biosensors. Therefore, this work develops a method to measure the total capacitance between the electrodes in a pair (Fig. S3) using SC technology as used in solid electronics. The measured capacitance between the electrode pair (C_S) was connected between the excitation source and the Σ−Δ (charge balancing) modulator input. Both the sensing capacitor ($C_{S,}$) and the internal reference capacitor ($C_{Ref,}$) were switched at a fixed sampling rate to be charged. The charges were then transmitted to the integrator to calculate their respective capacitances. A comparator checked the integrator output, and represented the ratio between C_S and C_{Ref}. A minute capacitance change could thus be measured. The preliminary performance of the SC circuit prototype indicates that its resolution can

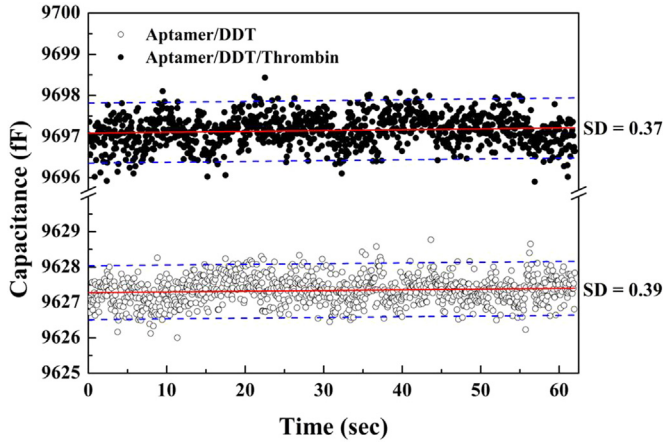

Fig. 3. Feasibility test of the proposed aptasensor. The developed system was used to detect 1 μM thrombin. Data was continuously recorded for 62 s (1000 data points) in 1 × PBS buffer (pH 7.4). The dash line shows the prediction intervals at 95% confidence level.

be as small as 0.1 fF, with a full-scale (changing) capacitance range 10 pF. These sensing performances were already filled with need of this study, even though it is not yet optimized for enhancement. Moreover, the circuit module can be applied to real-time biosensing because its sampling rate is less than 0.1 s. The prototype has a 2-wire, I^2C-compatible serial interface, and can operate with a single power supply 5.0 V that is very easy to implant with micro-controllers, but does not need additional interface parts, such as AD/DA converters.

The capacitance of Aptamer/DDT (aptamer and DDT co-modified CDtrode) and Aptamer/DDT/Thrombin (thrombin binding with aptamer) were continuously recorded for 62 s with a preliminary homemade capacitance measuring circuit based on SC technology, with the measurement results illustrated in Fig. 3. The electrical model of this biosensor indicates that the sensing capacitance C_S is equal to series of C_I, C_{Ins} and C_{Thr}, but without any resistor (as shown in Fig. S3). The interfacial capacitance, capacitance caused by thrombin binding and capacitance across the insulating layer are given as C_I, C_{Thr} and C_{Ins}, respectively. Thrombin binding with aptamer modified on SAMs of the aptasensor raised the capacitance by about 70 fF (from 9627.3 fF to 9697.1 fF). This result demonstrates that the presence of thrombin is detectable with the developed aptasensor based on capacitance change measured by a SC-based capacitance metric module. Additionally, the proposed capacitive aptasensor was stable for real-time thrombin detection, since both systemic drifts were insignificant (only about 0.1 fF/min) for control (Aptamer/DDT) and sensing (Aptamer/DDT/Thrombin) conditions.

This investigation confirms recognition between thrombin and aptamer, as well as sensing effectiveness by capacitive measurement. However, physic-chemical mechanism of how thrombin/aptamer interaction affects the capacitance of sensor is outside the scope of this work. Previous research results can elucidate this sensing principle. For a complex protein molecule, the presence of ionizable acidic and basic amino acid side chains lead to positive and negative charges in the protein structure (Pethig and Kell, 1987). These charges can be moved upon exposure to an electrical field, resulting in a dipole moment. The rise in the value of capacitance may be attributed to the thrombin possessed dipole moment. Furthermore, the thrombin-aptamer complex became larger in molecular size than aptamer alone, so probably also had a larger effective dipole moment.

3.3. Specificity of the aptamer-based strip for thrombin detection

This investigation adopted two proteins with different concentrations to confirm the specificity of the aptamer-based strip. Fig. 4 shows

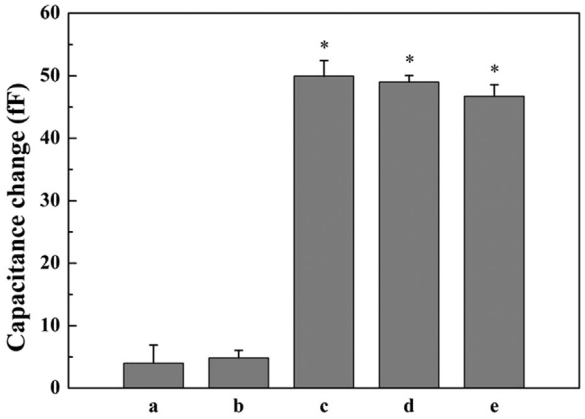

Fig. 4. Specificity of the assay. (a) 10^{-6} M BSA, (b)10^{-6} M HSA, (c) 10^{-9} M thrombin, (d) 10^{-6} M BSA and 10^{-9} M thrombin and (e) 10^{-6} M HSA and 10^{-9} M thrombin. *P < 0.001 vs. groups a–b.

that even higher concentrations (10^{-6} M) of BSA and of HSA do not exhibit apparent capacitance change. In contrast, significant responses were obtained with 10^{-9} M thrombin and mixtures of thrombin and other proteins. The presence of BSA and HSA did insignificantly affect the sensing performance of the aptasensor. Therefore, the specific thrombin/aptamer recognition and excellent blocking performance of 1-dodecanethiol SAM indicate that the system may be highly selective or insensitive to nonspecific binding, respectively. Additionally, this plot implies that the resolution and detection limit of the proposed aptasensor can reach sub-nM levels for thrombin in biological matrix, such as serum. The concentration of HAS is about 500–750 times of thrombin (1–2 μM) in blood, and the result indicates the sensor performs well specificity in a condition of 1000 for HAS-to-thrombin ratio with excellent detection limit below nM. When the sensor used in clinical practices, its availability will be achieved via dilution of blood or serum samples with 1000 times.

3.4. Determination of thrombin

The sensing performances of the proposed capacitive aptasensor to thrombin were respectively investigated in PBS buffer and serum conditions. As given in Fig. 5, the response ranges of the capacitive sensing system cover at least 5 orders of thrombin concentrations for both conditions. The sensitivity and detection limits in linear detection ranges were 1.6 times and 1-order lower in the buffer condition than in

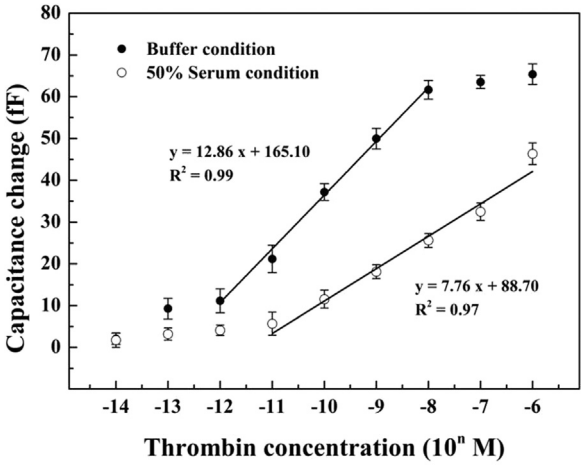

Fig. 5. Detection of thrombin by capacitive measurement. Results obtained with 50% serum samples spiked with different concentrations of thrombin and comparison with the same concentrations tested in PBS buffer.

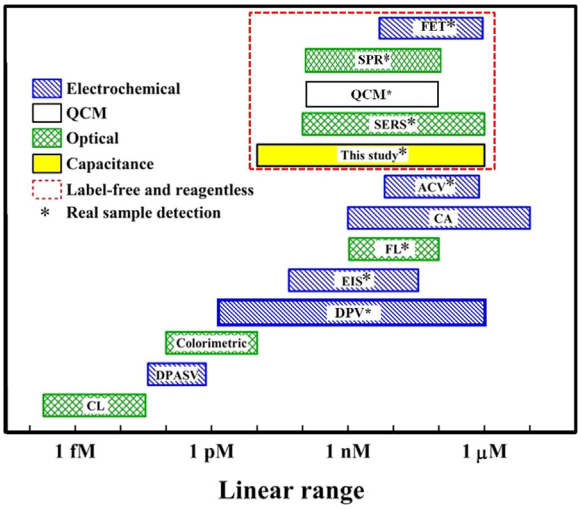

Fig. 6. Detection limit and linear range of different aptasensors for thrombin detection. The linear range (x-axis) is in logarithmic scale. CL: chemiluminescence; DPASV: differential pulse anodic stripping voltammetric; EIS: electrochemical impedance spectroscopy; FET: field-effect transistor; DPV: differential pulse voltammetry; SERS: surface-enhanced Raman scattering; FL: fluorescence; CA: chronoamperometric; ACV: alternating-current voltammetry; QCM: quartz crystal microbalance; SPR: surface plasmon resonance.

the serum condition, respectively. In buffer condition, the developed capacitive biosensor had excellent sensing performance with a pM-level detection limit, at least 4-order detection range and good linearity (0.99) for thrombin. This capacitive biosensor showed significantly better sensing performance than label-free and reagentless biosensors using SPR in both buffer and biological matrix conditions (Mani et al., 2011; Polonschii et al., 2010; H.-Y. Bai et al., 2013; Y. Bai et al., 2013), QCM (Seo et al., 2008; Evtugyn et al., 2008) and FET (Goda and Miyahara, 2013) based on the straightforward principle of thrombin/apatmer recognition without any additional amplification strategies (presented in Fig. 6 and Table S1). These experimental results indicate that the proposed capacitive sensor platform performs significantly better than label-free sensors in constructing proteins biosensor(s) as previous mentioned.

The aptasensor was then applied in a mimic serum obtained from whole blood after coagulation, to which enriching proteins were added to simulate the human physical condition. Although a biological matrix effect was observed, leading to the signal decrease in serum conditions, the capacitive biosensor still exhibited high sensitivity and good selectivity with the linear range for thrombin concentration from 10 pM to 1 μM (Fig. 5). Although some studies found that various time/reagents consuming accumulation/amplification strategies improved detection limits finer than pM levels (Zhang et al., 2009; Chen et al., 2010; Li et al., 2010; H.-Y. Bai et al., 2013; Y. Bai et al., 2013), the developed biosensors might be more useful for end-user if they have linear detection ranges that sufficiently cover required specifications in clinics, and need no additional process procedures. Analytical results show that the proposed aptamer-based capacitive biosensor can be directly employed to clinical applications, because thrombin is produced in nM concentrations during the initial phase of blood coagulation (Butenas and Mann, 2002).

Fig. 6 (details in Table S1) compares the thrombin detection of the proposed and other aptasensors. The proposed biosensor in biological matrix solution has significantly better limit of detection (LOD) (10 pM) and linear detection range (10 pM to 10 μM) than all label-free and reagentless aptasensors. The LODs of most electrochemical or optical thromin biosensor were at nM and pM levels in case without and with amplifications, such as nano-gold-particles, respectively. The superior performance of aptasensors with sub-pM-levels of detection limits at the detection limit is highly dependent on the nano-particle dual-amplification (chemiluminescence, CL) or time-accumulation (Colorimetric and DPASV) strategies. The proposed capacitive thrombin biosensor intrinsically has LOD at pM level, and requires no amplification strategies, owing to the use of SC technology enabling ultra-low capacitance measurement. Moreover, it has a wider linear detection range than all of others, as revealed in Fig. 6. This good performance can be attributed to the extremely large number (> 20 bits) of digital quantization in the developed capacitive measuring sub-system using the SC technology.

Biosensor researchers have recently begun adopting capacitive sensor to develop label-free and reagentless biosensors. The traditional transducers of choice for developing label-free and reagentless biosensors are SPR, QCM and FET, because they are well established in instrumentation and easy to work with. Aptasensors based on SPR or QCM may be restricted by their sensing principles of reflective index or mass changes, respectively. Both sensors are insensitive to small target molecules, possibly leading to poor detection ability at sub-nM levels, and loss of superiority in label-free biosensing applications. Although aptasensors based on tiny FETs require only a few test samples, they are only sensitive to the intrinsic electric charge of biomolecules, and have medium detection limits and detection ranges, whereas the proposed capacitive aptasensor has excellent sensing performance. Although SER has the same sensing performance as SPR and QCM is as well as simple preparation of sensing strips, the expensive non-real-time instrumentation makes it unattractive for biosensor researchers. Additionally, the proposed capacitive biosensor has much better sensing performance in terms of detection limit and linear detection range than the other label-free and reagentless aptasensors. The proposed capacitive biosensor has advantages for practical applications because it simplifies test/analytical steps, operations in real time, and has low cost, thus making it useful for developing biosensors. These properties among others make the proposed capacitive biosensor an attractive alternative to other label-free and reagentless biosensors including SPRs, QCMs and FETs.

4. Conclusions

This study demonstrates an innovative and cost-effective approach for label-free and reagentless determination of thrombin without any need for amplification strategies, based on a capacitive sensor integrating electrode pairs with switched capacitor technology. The simple and ultrasensitive capacitive aptasensor is fabricated by the SAMs of aptamers and 1-dodecanethiol onto gold electrode surfaces. Experimental results of electrochemistry (voltammetry and EIS) confirm the excellent recognition capacity of aptamer/thrombin molecules and effective electric insulating function suitable for determining capacitance. The aptasensor for thrombin was validated in serum conditions, and demonstrated good linearity (r^2 = 0.98) over a wide concentration range (10 pM to 1 μM). We conclude that the developed platform is superior to current methodologies used in label-free and reagentless biosensors, due to its sensitivity, simplicity, stability and lower cost, and thus is very promising for the design of versatile portable biosensing platforms, allowing for real-time detection and quantification of analytes in a simple and reliable manner. Moreover, the improvement of geometry of electrode pairs should be done to achieve higher area-to-distance ratio of capacitor to enhance sensor detection limit. The feasibility of the sensing platform to small molecules will be pursued in future work.

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Declaration-of-competing-interests

None

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2019.02.025.

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