Sensors and Actuators B 178 (2013) 19–25

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Sensitive and reproducible detection of cardiac troponin I in human plasma using a surface acoustic wave immunosensor

Joonhyung Lee[a], Yeolho Lee[a], Jin-Young Park[a], Hyejung Seo[a], Taehan Lee[a], Woochang Lee[a], Sang Kyu Kim[a], Young Ki Hahn[a], Jae yeon Jung[a], Sunkook Kim[b], Youn-Suk Choi[a,*], Soo Suk Lee[a,**]

[a] Bio Research Center, Samsung Advanced Institute of Technology, Samsung Electronics Co., Ltd., San #14-1, Nongseo-dong, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea
[b] Department of Electronics and Radio Engineering, Institute for Laser Engineering, Kyung Hee University, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea

ARTICLE INFO

Article history:
Received 6 September 2012
Received in revised form
22 November 2012
Accepted 23 November 2012
Available online 16 December 2012

Keywords:
Biosensor
Surface acoustic wave
Cardiac troponin I
Gold staining
Internal reference

ABSTRACT

We present a sensitive and reproducible surface acoustic wave (SAW) immunosensor that uses an internal reference sensor. A sandwich immunoassay was performed on the working sensing area of the SAW sensor that is able to specifically capture and detect cardiac troponin I (cTnI), while an immunoassay comprising rabbit IgG and anti-rabbit IgG was carried out on the reference sensing area. The analyte in human plasma was captured on gold nanoparticles (AuNPs) that were conjugated in advance with detection antibody. Introduction of these complexes as well as AuNPs conjugated with anti-rabbit IgG into the capture antibody-immobilized working sensor surface and its adjacent rabbit IgG-immobilized reference sensor surface resulted in a classic AuNP-based immunoassay format on both sensing areas. In order to achieve signal enhancement, a gold staining method was performed. Normalized sensor response (a ratio of working sensor response to reference sensor response) due to gold staining varied as a function of applied cTnI concentration. Furthermore, introduction of the internal reference sensor with the normalization technique reduced the coefficient of variation (CV) of the assay by a factor of 4–12, improving reproducibility of the SAW immunosensor. To validate the accuracy of the SAW system, correlation with a commercial system (Centaur®XP, Siemens) was investigated using the plasma of 21 patients and exhibited good agreement with a correlation coefficient (r) of 0.986 and a slope of 0.8 for SAW/Centaur®XP.

1. Introduction

Detection of proteins in a sensitive and reproducible manner is essential for clinical application [1–3]. Most common biosensing platforms require the use of labels, such as fluorophores or radiolabels. However, these techniques require long sample preparation times and additional costs due to the labeling process [4–6]. Label-free techniques, such as surface plasmon resonance (SPR) [7,8], quartz crystal microbalance (QCM) [9,10], and surface acoustic wave (SAW) [11,12], have been developed to alleviate this concern. SAW sensor has been widely investigated and used to detect a variety of target analytes due to its high sensitivity, low cost, and reliability. In particular, the guided shear horizontal (SH)-SAW sensor (also known as a Love wave sensor) that consists of a SH-SAW substrate with an overlayer having a lower shear wave velocity has been shown to be one of the most promising platforms for biosensor applications due to its high sensitivity and stability in the liquid phase [13–15]. The role of the overlayer is to trap acoustic energy near the sensing surface, thus yielding high detection response to any physical perturbation on the surface, such as changes in mass density, mechanical stiffness, viscosity, pressure or temperature. Furthermore, the overlayer can also protect interdigital transducer (IDT) electrodes from the liquid environment. Various dielectric materials, such as silicon dioxide (SiO_2) [16,17], zinc oxide (ZnO) [18], and polymers [19], can be used as waveguide material. In many studies, silicon dioxide (SiO_2) has been widely used as the guiding layer due to its low acoustic loss, high mechanical and chemical resistance, and ease of functionalization with biomolecules. The principle of operation of the SAW sensor is that mass loading on the guiding layer, results in a surface acoustic velocity change, which is detected as a frequency shift or a phase shift of a surface acoustic wave. Therefore, the frequency shift or the phase shift of the SAW sensor is proportional to the accumulation mass on the guiding layer surface [13,14]. In a previous study, we demonstrated that a Love wave SAW biosensor in combination with gold staining signal enhancement strategy exhibited high sensitivity [20]. However, although the gold staining method has been shown to enhance signals, it can result in low

* Corresponding author. Tel.: +82 31 280 1839; fax: +82 31 280 6816.
** Corresponding author. Tel.: +82 31 280 6947; fax: +82 31 280 6816.
E-mail addresses: ysuk.choi@samsung.com (Y.-S. Choi), soosuk@samsung.com (S.S. Lee).

0925-4005/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2012.11.082

uniformity and reproducibility due to non-linear growth of the gold nanoparticles. Furthermore, a method to overcome background interference due to human serum proteins was required for better reproducibility.

In order to improve reproducibility, an internal reference sensor has been introduced into various biosensing platforms [21,22]. This enables differential or normalized data acquisition from the working sensor signal and reference sensor signal, which compensates any drift or noise and discriminates against non-specific binding. Also, normalization (working sensor response divided by reference sensor response) can be used to suppress disturbances that are known to similarly influence both the working sensor signal and reference sensor signal.

Cardiac markers play an essential role in the diagnosis, prognosis, monitoring, and risk stratification of suspected heart attack patients [23,24]. For example, cardiac markers have been used as an important factor in ruling in or ruling out acute myocardial infarction (AMI), the world's leading cause of morbidity and mortality. Among currently used cardiac markers, the detection of cardiac troponins (cardiac troponin I (cTnI) and cardiac troponin T (cTnT)) is regarded as the biochemical "gold standard" for diagnosis of AMI due to excellent specificity and sensitivity. Cardiac troponins remain elevated for 4–10 days after the onset of AMI, allowing a suitable window for diagnosis [25,26].

This study describes a highly sensitive 200 MHz Love wave SAW biosensor that can achieve detection of cardiac troponin I in human plasma below the clinical cut-off level (0.06–1.5 ng/mL) of cTnI. The Love wave SAW sensor is composed of the working sensing area and its adjacent reference sensing area. An immunoassay in combination with gold staining was used as a basic detection methodology. Capture antibody of cTnI and rabbit IgG were adsorbed onto the working sensing and reference sensing areas, respectively. cTnI in human plasma was captured on gold nanoparticles (AuNPs) conjugated with the detection antibody of cTnI. Introduction of this cardiac marker-AuNP complex as well as anti-rabbit IgG conjugated AuNP to the capture antibody-immobilized surface and then to the rabbit IgG-immobilized surface sequentially resulted in an immunoassay on both surfaces, where subsequent catalyzed deposition of gold onto AuNP led to signal enhancement. In this fashion, we significantly increased the sensitivity of the assay by reducing the limit of detection (LOD) of the cardiac marker below the clinical cut-off level. Furthermore, reproducibility of the assay was significantly improved by introducing an internal reference sensor adjacent to the cTnI working sensor onto a single substrate and then by normalizing the working sensor signal to the reference sensor signal. In order to understand the role of the internal reference sensor in decreasing the coefficient of variation (CV) of the sensor signal, which was attributed to improving reproducibility of the sensor, platinum and rabbit IgG were coated onto the working sensor surface as well as onto the reference sensor surface. Then, CVs of normalized signals (a ratio of working sensor signal to reference sensor signal) due to platinum and rabbit IgG adsorption were compared with those of each sensor signal. Next, in order to investigate the normalization minimizes plasma protein interference, the assay described above was performed with four different non-patient plasma samples spiked with the same concentration of cTnI (25 ng/mL). Given that the CVs of the normalized signal were less than those in the working sensor signal, normalization played an essential role in compensating any drift or noise due to plasma protein interference. We also compared concentrations of 21 patient plasma samples determined by the SAW system with those using a commercialized system (Centaur®XP; Siemens, Erlangen, Germany). An acceptable agreement between the two methods was observed over the clinical range of cTnI concentration (300 pg/mL to 3.0 ng/mL).

2. Materials and methods

2.1. Materials

Cardiac troponin complex was purchased from Fitzgerald (Acton, MA). Three cTnI monoclonal antibodies were obtained from Hytest (Turku, Finland). Rabbit IgG and anti-rabbit IgG were purchased from Sigma–Aldrich (St. Louis, MO). AuNPs with a 30-nm diameter were obtained from BBI (Cardiff, UK). Bovine serum albumin (BSA) and trimethoxy(octadecyl) silane were purchased from Sigma–Aldrich (St. Louis, MO), as were gold (III) chloride trihydrate and hydroxylamine hydrochloride. PBS buffer was obtained from Invitrogen (Carlsbad, CA). Phosphate buffer (PB) consisted of sodium phosphate monobasic and sodium phosphate dibasic that were purchased from Sigma–Aldrich. Normal and cTnI clinical patient human plasma samples were obtained from SCIPAC (Sittingbourne, UK).

2.2. Design and fabrication of the SAW sensor

3000 Å thick aluminum interdigital transducer (IDT) electrodes deposited by sputtering were patterned onto a 36°YX-$LiTaO_3$ substrate (Yamaju Ceramics Co., Ltd., Anada-Cho Seto, Japan) using conventional photolithography. As shown in Fig. 1, the SAW sensor area has a working sensor and its adjacent reference sensor. The input and output IDT electrodes of each sensor consisted of 72 finger pairs with an electrode width of 5.0 μm to obtain 200 MHz center frequency. The length of the delay line is 2 mm and the distance between the working sensor and the reference sensor is 0.8 mm. A 5.2-μm-thick SiO_2 guiding layer was deposited onto the IDT patterned substrate by plasma-enhanced chemical vapor deposition (P-500; Applied Materials, Santa Clara, CA), followed by wet etching with buffered oxide etchant to open the contact pads for electric connection. After dicing the fabricated wafer, the SAW sensors were mounted on custom-made printed circuit board (PCB) to form a dual-type SAW sensor, followed by aluminum wire bonding to establish an electric connection.

2.3. Measurement system

A SAW sensing system was comprised of fluidic and detection modules, as depicted in Fig. 2, used to obtain real-time sensor information during the assay. The mass loading on SAW sensor was monitored by tracking the phase change of surface acoustic waves at the central frequency of 200 MHz with a HP8753ES network analyzer (Hewlett-Packard Company, Palo Alto, CA). The Fourier transform and time gating function of the network analyzer were used to minimize unwanted interference from electromagnetic feed-through and reflected waves. A path controller that connected the network analyzer with the SAW sensor was used to perform continuous and repetitive measurements of phase signals in the working and reference sensors. The flow cell was constructed with a peristaltic pump (ISM597; ISMATEC, Glattbrugg, Switzerland), a custom-made fluidic block, and a U-type silicon gasket. To exclude effects of temperature change on the SAW sensor signal, a temperature controller was installed below the chamber to maintain the temperature at 25 °C. All signal acquisition and network analyzer manipulation were automatically conducted via custom-built control software executed on a PC.

2.4. Platinum coating on the sensor surface

Before a sensor was loaded onto a holder within a Cressington 208 sputter coater (Cressington Scientific Instruments, Ltd., Watford, UK), the initial phase signals of the working and reference

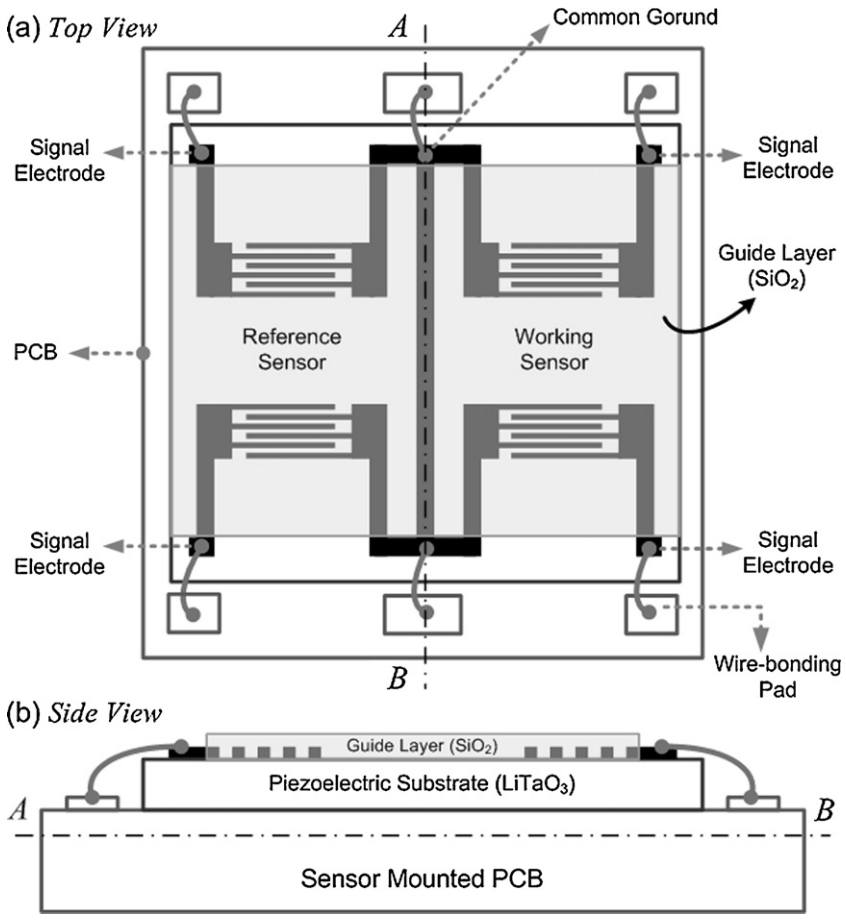

Fig. 1. (a) Top and (b) cross-sectional views of the dual-type Love wave SAW sensor.

sensors were measured. The sensor was then sputtered with 1.5 nm of platinum (for 30 s at 20 mA and 0.085 mBar) in the sputter coater, followed by phase measurement. Again, the sensor was reloaded and sputtered with Pt for 30 s in the sputter coater, followed by final phase measurement.

2.5. Antibody conjugation to gold nanoparticles

Each antibody (cTnI detection antibody and anti-rabbit IgG (10 μL of 1 mg/mL)) was added to 1 mL of 30 nm colloidal AuNPs, followed by incubation at room temperature for 30 min. To block

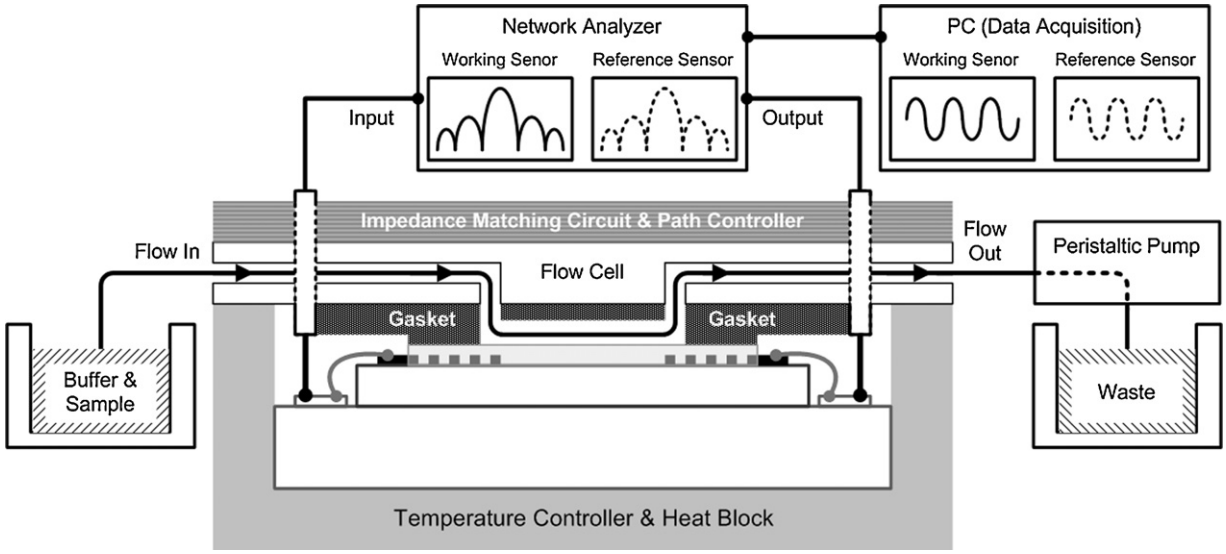

Fig. 2. Diagram of the fluidic cell.

unreacted sites on the AuNP surface, 0.1 mL of 1% BSA in deionized water was introduced to the AuNP-antibody mixture. After additional incubation at room temperature for 30 min, the mixture was centrifuged at 13,000 rpm for 10 min at 4 °C. Supernatant was removed, and the antibody-conjugated AuNPs were dispersed in 0.5 mL of PBS solution (0.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, pH 7.4) containing 0.1% BSA. All conditions, including the concentration of antibody, gold particle and buffer were determined that the absorbance at 580 nm did not decrease after conjugation procedure.

2.6. Immunoassay with gold staining

The silicon dioxide (SiO_2) sensing area was sequentially rinsed with deionized water and absolute ethanol and then dried under nitrogen gas. The chip was next placed in a UV/ozone chamber (144AX-220; Jelight Company, Inc., Irvine, CA) for 10 min. The chip was incubated in a solution of 5% (vol./vol.) fresh trimethoxy(octadecyl) silane in toluene with 0.5% *n*-butylamine as a catalyst for 1 h, followed by rinsing with toluene for 2 min and drying under nitrogen gas. The silanized sensor was then baked in an oven at 110 °C for 1 h, followed by rinsing with toluene for 2 min and drying under nitrogen gas.

Two different cTnI antibodies (19C7, MF4, 100 μg/mL in phosphate buffer (PB), 7.4 pH) specific to different epitopes of cTnI were immobilized on the working sensing area of the guiding layer for 1 h. It is known that a combination of the two chosen antibodies as capture antibody is not sensitive to proteolytic degradation and/or the presence of heparin and autoantibodies [27–29]. The 19C7 capture antibody and MF4 capture antibody were chosen for specificity to the stable region and to the carboxyterminal region of cTnI, respectively. Rabbit IgG (100 μg/mL in phosphate buffer (PB), 7.4 pH) was also immobilized non-specifically on the reference sensing area of the guiding layer for 1 h. Hence, each immobilization layer can be formed with a custom-made dispenser that is able to release drop by drop and able to control the quantity of one drop. In order to avoid evaporation, the dispensing process was performed inside a humidity chamber. Afterward, unreacted sites on the sensing area were blocked with 5% BSA in PB solution.

50 μL of heparinized human plasma containing cTnI was mixed with 50 μL of its specific antibody-AuNP conjugate as well as anti-rabbit IgG-AuNP conjugates for 3 min, allowing the cTnI to bind to its specific antibody-AuNP conjugate. These complexes were first introduced to the working sensing surface and then to the reference sensing surface and allowed to bind the immobilized capture antibody and rabbit IgG, respectively. After washing with PBS solution for 1 min, the gold staining solution that consisted of 50 μL of gold (|||) chloride trihydrate (10 mM) and 50 μL of hydroxylamine hydrochloride (20 mM) was incubated with AuNPs on the sensor surfaces for 2 min, which resulted in catalyzed deposition of gold onto the AuNPs captured on the sensing surfaces. The sensing surfaces were then rinsed again with PBS solution for 1 min.

All experiments were performed with heparinized human plasma that was spiked with the cTnI. In order to quantify the original concentration of the analyte, human plasma sample was analyzed five times with Centaur®XP whose detection limit was 6 pg/mL. Since the plasma contained less than 6 pg/mL of cTnI, it was assumed that the cTnI concentration of the blank sample was 5 pg/mL.

3. Results and discussion

3.1. Detection of cardiac markers via gold staining

Fig. 3 highlights the immunoassay procedure on both the working sensing surface and the reference sensing surface used in this study, including immobilization of capture antibodies and rabbit IgG, complex formation between antibody-AuNP conjugates and cardiac marker in human plasma, capture of these complexes to the immobilized antibodies (cTnI capture antibody, rabbit IgG), and subsequent gold staining-based signal enhancement strategy. The capture antibodies and rabbit IgG were immobilized non-specifically on the SiO_2 guiding layer of the working sensor and its adjacent reference sensor, respectively. The cardiac troponin I

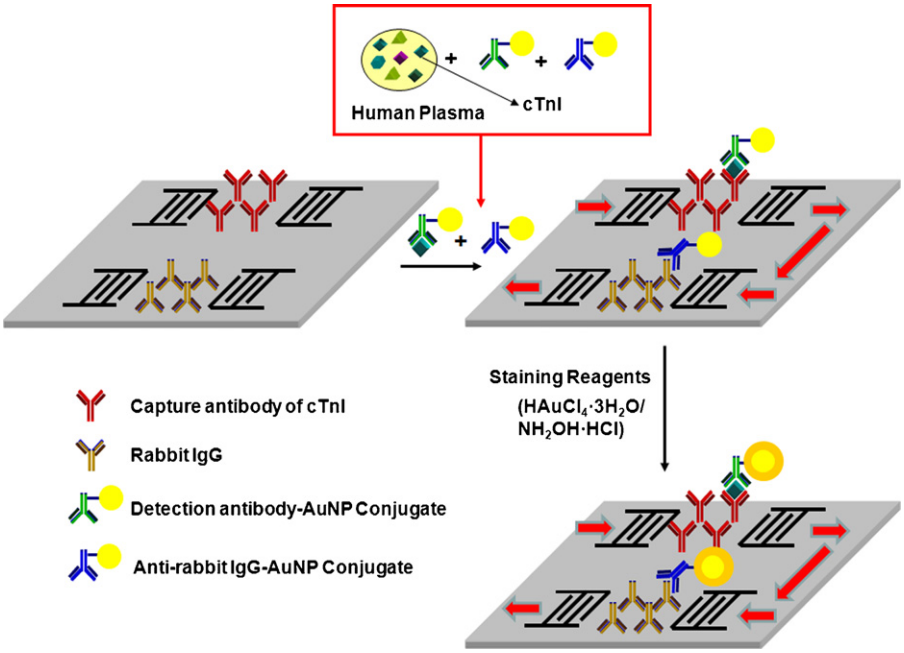

Fig. 3. Schematic of the immunoassay format utilized in this study in combination with gold staining on a dual-type SAW sensor. Red arrows show direction of flow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

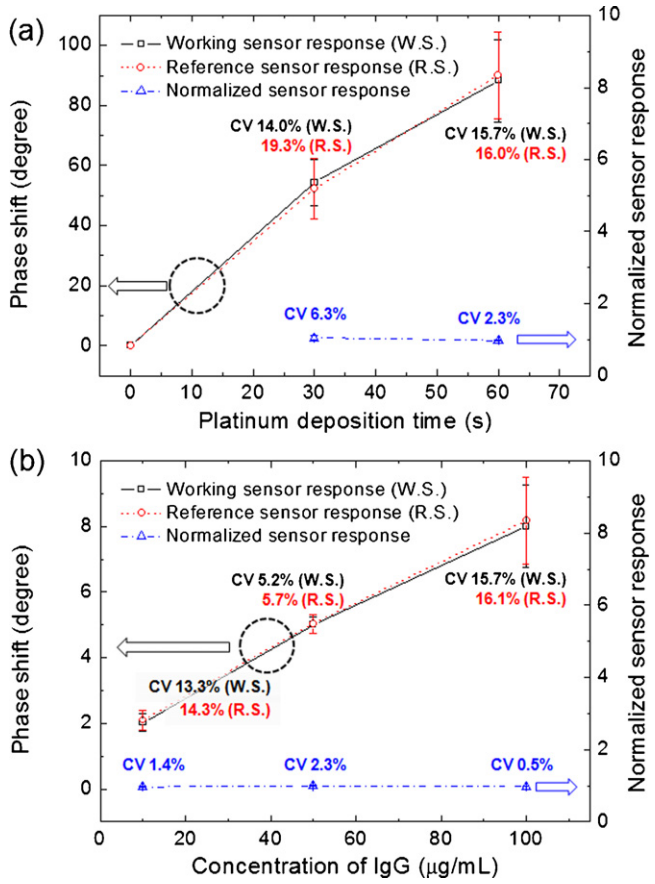

Fig. 4. CV (coefficient of variation) comparison of normalized sensor response with working and reference sensor responses upon adsorption of (a) platinum and (b) rabbit IgG.

present in human plasma was captured on antibody-AuNP conjugates, which then bound to the immobilized capture antibodies. Anti-rabbit IgG-AuNP conjugates bound to the immobilized rabbit IgG. Then, introduction of gold staining solution that consisted of gold (III) chloride trihydrate and hydroxylamine hydrochloride resulted in catalyzed deposition of gold onto the captured AuNPs on both sensor surfaces.

3.2. Adsorption of platinum and rabbit IgG onto sensor surface

Fig. 4(a) shows working sensor response, reference sensor response, and normalized sensor response to platinum coating on the working sensor as well as the reference sensor as a function of Pt deposition time. Pt was deposited on trimethoxy(octadecyl) silane-coated working and reference sensing areas. We investigated how the normalization process (a ratio of working sensor response to reference sensor response) plays a role in decreasing the coefficient of variation (CV) of the sensor signal, which is attributed to improving reproducibility of the sensor. Average and CV values were calculated with five sensors. At 30 s, the CVs of the working sensor response and the reference sensor response were 14.0% and 19.3%, respectively, while that of the normalized sensor response was 6.3%. At 60 s, the CVs of the working sensor response and the reference sensor response were 15.7% and 16.0%, respectively, and that of the normalized sensor response was decreased to 2.3%.

We also performed measurement of working sensor response, reference sensor response, and normalized sensor response to rabbit IgG adsorption on the working sensor as well as the reference sensor, as shown in Fig. 4(b). The IgG was adsorbed on trimethoxy(octadecyl) silane-coated working and reference sensing areas. Measurements were performed five times for each concentration of the rabbit IgG (10, 50 and 100 μg/mL). Averages and CVs were again calculated. For 10 μg/mL, the CVs of the working sensor response and the reference sensor response were 13.3% and 14.3%, respectively. The CV of the normalized sensor response was 1.4%. For 50 μg/mL, the CVs of the working sensor response and of the reference sensor response were 5.2% and 5.7%, respectively. The CV of the normalized sensor response was 2.3%. For 100 μg/mL, the CVs of the working sensor response and of the reference sensor response were 15.7% and 16.1%, respectively. The CV of the normalized sensor response was 0.5%. The CVs of the normalized signal due to platinum deposition and rabbit IgG adsorption onto both the working sensor surface and reference sensor surface were lower than that in each sensor signal. Hence, we demonstrate the potential of the normalization process to enable reproducible detection of target molecules. However, it is still necessary to investigate the CV of the assay used in this study can also be improved by introduction of the internal reference sensor, improving reproducibility of the SAW immunosensor.

3.3. Reproducibility of normalized sensor response to cardiac troponin I in the SAW system

We investigated the dependence of normalized sensor response due to gold staining on applied cardiac troponin I (cTnI) concentration. Seven measurements were carried out for each concentration of cTnI, and the results are displayed in Fig. 5(a). As concentration of cTnI increased from 5 pg/mL to 25 ng/mL, the normalized and working sensor responses due to gold staining also increased. We also compared CV of normalized sensor response with that of working sensor response. The CV of the normalized sensor response was in the range of 0.4–2.3%, which was lower than that of the working sensor response in the range of 3.1–9.0%. In this assay, high standard deviation or CV of the working sensor response mainly results from background noise and non-linear growth of the gold nanoparticle due to gold staining. Therefore, introduction of the internal reference sensor reduced the CV of the assay by a factor of 4–12, improving reproducibility of the SAW immunosensor. This demonstrated that normalization screened out background noise by manipulating data and minimized non-uniformity in the gold staining process by suppressing the disturbances to both the working sensor signal and the reference sensor signal.

The standard curve for the assay was determined using a five-parameter logistic model on MasterPlex curve fitting software (Hitachi Solutions, San Francisco, CA), and the normalized signals and working sensor signals were converted into concentrations using standard curves. The limit of detection (LOD) of the SAW immunosensor with/without the internal reference sensor were determined as 6.2 pg/mL and 9.7 pg/mL, respectively, according to the definition in NCCLS guideline EP17-A, where LOD is affected by the standard deviation of the blank sample measurement as well as a low sample concentration ($LOD = \mu_B + 1.645\,\sigma_B + 1.645\,\sigma_S$. μ_B and σ_B are the mean and standard deviation of the blank measurements, σ_S is the standard deviation of the population of the low sample measurements). This indicates that the introduction of the internal reference sensor with the normalization process also improved LOD of the SAW immunosensor. The LOD was far below the clinical cut-off level of cTnI (0.06–1.5 ng/mL) [23,24]. Hence, it was confirmed that the SAW biosensor was able to perform sensitive and reproducible detection of cardiac troponin I.

A major concern inherent in protein detection assays is potential background interference from other biomolecules and chemical species in human plasma. Provided that a target cardiac marker was present in human plasma, it was important to assess the impact of human plasma proteins on our detection strategy. Four different

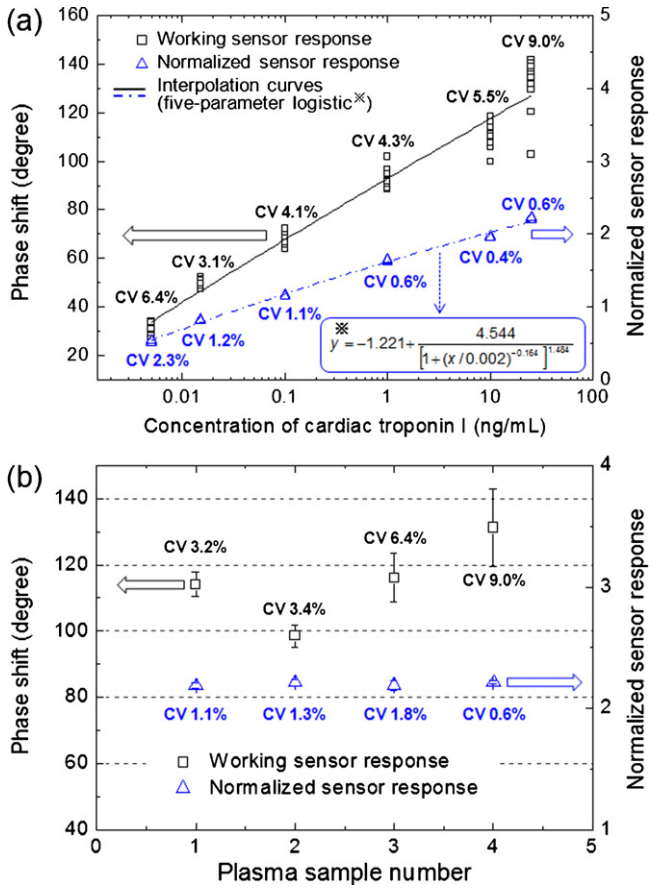

Fig. 5. (a) Variation of the normalized and working sensor responses due to gold staining with cardiac marker concentration in the dual-type sensor chip system. Measurements were performed seven times for each concentration of cardiac markers (5 pg/mL to 25 ng/mL). (b) CVs of the normalized and working sensor responses due to gold staining with the four different non-patient plasma samples (<6 pg/mL) spiked with the same concentration of cTnI (25 ng/mL). Five measurements were carried out for each plasma sample.

non-patient plasma samples (<6 pg/mL) spiked with the same concentration of cTnI (25 ng/mL) were assayed as described above. We compared the CVs of normalized sensor signals with observations in working sensor signals. Five measurements were carried out for each plasma sample. Theoretically, provided that the concentration of analyte is the same, the biosensor should be able to produce the same signal. As shown in Fig. 5(b), the CV of the working sensor signals was 12.4% higher than that of each working sensor signal in the range of 3.2–8.9%, indicating that background interference from plasma proteins in different non-patient plasma obviously has an effect on our detection assay. However, the CV of the normalized sensor signal was 1.4% less than that of the working sensor signals by a factor of 9. Therefore, by introducing the internal reference sensor, normalization compensated any background noise due to plasma protein interference.

3.4. Method comparison between the SAW immunosensor and a commercial system

For the purpose of accuracy validation of the SAW immunosensor, correlation with a commercial system (Centaur®XP) was also investigated in a blind fashion using the same plasma samples of the 21 patients. Normalized signals of the 21 patient samples were converted into concentrations using the standard curve determined using a five-parameter logistic model as shown in Fig. 5(a). Also, concentrations of the same 21 patient samples

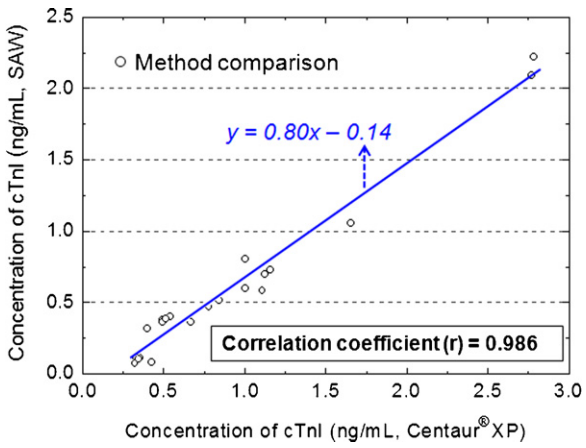

Fig. 6. Method comparison test with 21 patient plasma samples. The SAW system and a commercialized system (Centaur®XP; Siemens, Erlangen, Germany) showed good agreement over the clinical range of cTnI concentration (300 pg/mL to 3.0 ng/mL).

were measured using a commercial system (Centaur®XP). Then, we made a plot (Fig. 6) of concentrations measured by the SAW immunosensor versus those measured by the commercial system (Centaur®XP) over the same plasma samples of the 21 patients. An acceptable agreement between the two methods was observed over the clinical range of TnI concentration (300 pg/mL to 3.0 ng/mL). As shown in Fig. 6, the TnI concentrations from the two methods corresponded well and showed a significant correlation with a correlation coefficient (r) of 0.986 and a slope of 0.8 for SAW/Centaur®XP in Deming regression analysis. In the regression, the proportional bias (slope = 0.8) observed was acceptable, since each assay was developed with different methods, standards and calibrants [30,31].

4. Conclusions

In conclusion, we demonstrated a surface acoustic wave (SAW) immunosensor, which uses an internal reference sensor in combination with a gold staining signal enhancement strategy, detected cardiac troponin I in a sensitive and reproducible manner. It was confirmed that normalization (a ratio of working sensor response to reference sensor response) screened out background interference by manipulating data and minimized non-uniformity in the gold staining process by suppressing disturbances to both the working sensor signal and reference sensor signal. We also showed that the normalized sensor response depended on cardiac marker concentration. Our future efforts will be focused on extending this platform to detection of other disease markers present in body fluids, such as blood and plasma. Due to its size, sensitivity and reliability, we expect this platform will be useful in development of devices for point-of-care diagnostics.

Acknowledgement

This work was supported by the Bio Research Center at Samsung Advanced Institute of Technology.

References

[1] G.H. Wu, R.H. Datar, K.M. Hansen, T. Thundat, R.J. Cote, A. Majumdar, Bioassay of prostate-specific antigen (PSA) using microcantilevers, Nature Biotechnology 19 (2001) 856–860.
[2] C.A. Savran, S.M. Knudsen, A.D. Ellington, S.R. Manalis, Micromechanical detection of proteins using aptamer-based receptor molecules, Analytical Chemistry 76 (2004) 3194–3198.

[3] J. Lee, K. Icoz, A. Roberts, A.D. Ellington, C.A. Savran, Diffractometric detection of proteins using microbead-based rolling circle amplification, Analytical Chemistry 82 (2010) 197–202.
[4] L.T. Yang, C.W. Fung, E.J. Cho, A.D. Ellington, Real-time rolling circle amplification for protein detection, Analytical Chemistry 79 (2007) 3320–3329.
[5] G. Acharya, C.L. Chang, D.D. Doorneweerd, E. Vlashi, W.A. Henne, L.C. Hartmann, P.S. Low, C.A. Savran, Immunomagnetic diffractometry for diagnostic serum markers, Journal of the American Chemical Society 129 (2007) 15824–15829.
[6] S.M. Knudsen, J. Lee, A.D. Ellington, C.A. Savran, Ribozyme-mediated signal augmentation on a mass-sensitive biosensor, Journal of the American Chemical Society 128 (2006) 15936–15937.
[7] L.A. Lyon, M.D. Musick, M.J. Natan, Colloidal Au-enhanced surface plasmon resonance immunosensing, Analytical Chemistry 70 (1998) 5117–5183.
[8] S.D. Soelberg, R.C. Stevens, A.P. Limaye, C.E. Furlong, Surface plasmon resonance detection using antibody-linked magnetic nanoparticles for analyte capture, purification, concentration, and signal amplification, Analytical Chemistry 81 (2009) 2357–2363.
[9] W.A. Henne, D.D. Doorneweerd, J. Lee, P.S. Low, C.A. Savran, Detection of folate binding protein with enhanced sensitivity using a functionalized quartz crystal microbalance sensor, Analytical Chemistry 78 (2006) 4880–4884.
[10] J. Lee, J. Jang, D. Akin, C.A. Savran, R. Bashir, Real-time detection of airborne viruses on a mass-sensitive device, Applied Physics Letters 93 (2008) 013901–013903.
[11] H.J. Lee, K. Namkoong, E.C. Cho, C. Ko, J.C. Park, S.S. Lee, Surface acoustic wave immunosensor for real-time detection of hepatitis B surface antibodies in whole blood samples, Biosensors and Bioelectronics 24 (2009) 3120–3125.
[12] K. Länge, B.E. Rapp, M. Rapp, Surface acoustic wave biosensors: a review, Analytical and Bioanalytical Chemistry 391 (2008) 1509–1519.
[13] E. Gizeli, A.C. Stevenson, N.J. Goddard, C.R. Lowe, Acoustic Loveplate sensors: comparison with other acoustic devices utilizing surface SH waves, Sensors and Actuators B 13–14 (1993) 638–639.
[14] E. Gizeli, A.C. Stevenson, N.J. Goddard, C.R. Lowe, A novel Loveplate acoustic sensor utilizing polymer overlayers, IEEE Transactions on UFFC 39 (1992) 657–659.
[15] A.C. Stevenson, E. Gizeli, N.J. Goddard, C.R. Lowe, Acoustic Love plate sensors: a theoretical model for the optimization of the surface mass sensitivity, Sensors and Actuators B: Chemical 13–14 (1993) 635–637.
[16] G. Kovacs, A. Venema, Theoretical comparison of sensitivities of acoustic shear-wave modes for (bio)chemical sensing in liquids, Applied Physics Letters 61 (1992) 639–641.
[17] G. Kovacs, M.J. Vellekoop, R. Haueis, G.W. Lubking, A. Venema, A Love-wave sensor for (bio)chemical sensing in liquids, Sensors and Actuators A 43 (1994) 38–43.
[18] Y.Q. Fu, J.K. Luo, X.Y. Du, A.J. Flewitt, Y. Li, G.H. Markx, A.J. Walton, W.I. Milne, Recent developments on ZnO films for acoustic wave based bio-sensing and microfluidic applications: a review, Sensors and Actuators B: Chemical 143 (2010) 606–619.
[19] F. Bender, R. Cernosek, F. Josse, Love-wave biosensors using cross-linked polymer waveguides on $LiTaO_3$ substrates, Electronics Letters 36 (2000) 1672–1673.
[20] J. Lee, Y. Choi, Y. Lee, H. Lee, J.N. Lee, S.K. Kim, K.Y. Han, E.C. Cho, J.C. Park, S.S. Lee, Sensitive and simultaneous detection of cardiac markers in human serum using surface acoustic wave immunosensor, Analytical Chemistry 83 (2011) 8629–8635.
[21] V.C. Martins, F.A. Cardoso, J. Germano, S. Cardoso, L. Sousa, M. Piedade, P.P. Freitas, L.P. Fonseca, Femtomolar limit of detection with a magnetoresistive biochip, Biosensors and Bioelectronics 24 (2009) 2690–2695.
[22] A.D. Taylor, H. Vaisocherov, J. Deeds, S. DeGrasse, S. Jiang, Tetrodotoxin detection by a surface plasmon resonance sensor in pufferfish matrices and urine, Journal of Sensors (2011), Article ID: 601704.
[23] P.A. Kavsak, A.R. MacRae, M. Yema, A.S. Jeffe, Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury, Clinical Chemistry 55 (2009) 573–577.
[24] M. Mohammed, M.Y. Desmulliez, Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review, Lab on a Chip 11 (2011) 569–595.
[25] Z. Yang, D.M. Zhou, Cardiac markers and their point-of-care testing for diagnosis of acute myocardial infarction, Clinical Biochemistry 39 (2006) 771–780.
[26] S.F. Melanson, D.A. Morrow, P. Jarolim, Earlier detection of myocardial injury in a preliminary evaluation using a new troponin I assay with improved sensitivity, American Journal of Clinical Pathology 128 (2007) 282–286.
[27] S. Eriksson, H. Halenius, K. Pulkki, J. Hellman, K. Pettersson, Negative interference in cardiac troponin I immunoassays by circulating troponin autoantibodies, Clinical Chemistry 51 (2005) 839–847.
[28] S. Eriksson, M. Junikka, P. Laitinen, M. Majamaa-Voltti, H. Alfthan, K. Pettersson, Negative interference in cardiac troponin I immunoassays from a frequently occurring serum and plasma component, Clinical Chemistry 49 (2003) 1095–1104.
[29] S. Eriksson, T. Ilva, C. Becker, J. Lund, P. Porela, K. Pulkki, L.M. Voipio-Pulkki, K. Pettersson, Comparison of cardiac troponin I immunoassays variably affected by circulating autoantibodies, Clinical Chemistry 51 (2005) 848–855.
[30] E. Kuhn, T. Addona, H. Keshishian, M. Burgess, D.R. Mani, R.T. Lee, M.S. Sabatine, R.E. Gerszten, S.A. Carr, Developing multiplexed assays for troponin I and interleukin-33 in plasma by peptide immunoaffinity enrichment and targeted mass spectrometry, Clinical Chemistry 55 (2009) 1108–1117.
[31] J. Liu, G. Li, Application of biosensors for diagnostic analysis and bioprocess monitoring, Sensors and Actuators B: Chemical 65 (2000) 26–31.

Biographies

Joonhyung Lee completed his PhD in chemical engineering at Purdue University (2009). Since 2009, he has been working as a research staff at Samsung Advanced Institute of Technology. His research interests are focusing on development of label-free mass-sensitive sensor, electrochemical sensor, and optical sensor.

Yeolho Lee received his PhD at Pohang University of Science and Technology (PSTECH) in 2008. His research interests focus on piezoelectric transducers, especially piezoelectric bio sensors.

Jin-Young Park completed PhD in department of chemistry at Pohang University of Science and Technology and is working at Samsung Advanced Institute of Technology since 2010. Her main research fields are the electrochemical biosensors based on self-assembled monolayer employing Fourier-transform electrochemical impedance spectroscopy.

Hyejung Seo is a research staff member, POCT In Vitro Diagnostics Group, Samsung Electronics. She received her PhD from Pohang University of Science and Technology (POSTECH), Pohang, Korea in 2011. Her research interests include development of biosensors and influence of nanobubbles on molecular binding.

Taehan Lee completed MS in biosensors at Korea Research Institute of Bioscience and Biotechnology (KRIBB) campus of University of Science and Technology (UST).

Woochang Lee is a research staff member of Samsung Advanced Institute of Technology (SAIT), Samsung Electronics Co., Ltd. Before joining SAIT, he was a post-doctoral scholar of Michael L. Roukes group in California Institute of Technology. During his PhD, he won a graduate research award of Korean Institute of Chemical Engineers. His research interests are biomarker detection on chip, micro-/nano-fabrication technology for sensing device, and polymer surface modification in the field of bionanoscience.

Sang Kyu Kim studied nanobiotechnology, and completed his PhD at University of Science and Technology in South Korea. He is a staff member at Samsung Advanced Institute of Technology. His research interests are focusing on the development of assay for point of care testing.

Young Ki Hahn is a researcher of Bio Research Center, SAIT (Samsung Advanced Institute of Technology). He obtained his PhD degree in Bio and Brain Engineering of KAIST in 2011. His thesis was concerned with the isomagnetophoresis-based immunoassay system and current research focuses on developing point-of-care technology platforms for diagnosis of cancers, myocardial infarction, and various diseases.

Jae yeon Jung received BS degree and PhD degree in the department of biosystems and biomaterials science and engineering from Seoul National University, in 2005, and 2010, respectively. From September 2010, she is working at Samsung Electronics Co., Ltd., as a research staff member. Her research interest covers diagnostic biosensor systems for point-of care application.

Sunkook Kim received the PhD degree in electrical and computer engineering from Purdue University at West Lafayette, in 2009. He is currently an assistant professor with electronics and radio engineering department, Kyung Hee University. His research focuses on 2D layered semiconducting transistor, the conventional oxide/Si thin-film-transistor, an electrical bio-sensor, and flexible OLED display. In addition, his interests include the field of an augmented human research. He has authored or coauthored over 60 refereed journal and conference in these area.

Youn-Suk Choi completed his PhD in bio and brain engineering at KAIST and is a research staff member at the Samsung Advanced Institute of Technology. His main research fields are lab-on-a-chip, bio-MEMS, bio-sensor, electroporation, and computational fluid dynamics.

Soo Suk Lee, PhD in chemistry at the Pohang University of Science and Technology (1998), is a vice president and a research group leader of point of care in vitro diagnostics group at Samsung Advanced Institute of Technology, Korea. His major research activities are development of biosensors, surface chemistry, synthesis of organic molecules and analysis of circulating tumor cell, etc.