Prototyping and Early Validation of an Integrated, Electrochemical and Mass Three-sensor Array for Dengue Detection

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Abstract: This paper presents the current progress towards a lab-on-chip biosensor for early dengue detection, consisting of an integrated sensor with dual-function working electrode that enables in-situ measurements of both electrochemical impedance spectroscopy (EIS) and quartz crystal microbalance (QCM) enclosed in a miniaturized 3D-printed package equipped with electrical contacts and sample fluid delivery to the quartz biosensor array. The sensors consist of an array of three 10 MHz IEQCM biosensors on a single quartz substrate. Early validation is performed for future dengue sensing application. We report the design, optimisation, and fabrication of the sensors, as well as early optimisation and validation of surface bioconjugation of antibodies. This lab-on-chip has the potential to provide accurate dengue detection due to its high sensitivity and dynamic range, as well as providing rapid and early dengue detection in point-of-care settings.

1 INTRODUCTION

Biosensors are analytical devices that integrate molecular recognition platforms with a physicochemical transducer to produce a single detection processing unit (Hong et al., 2012; Miserere & Merkoçi, 2015). Rapid point-of-care (PoC) biosensors are advantageous as they allow analyses to be performed in the field (Hu et al., 2016; Lisowski & Zarzycki, 2013; St John & Price, 2014). Commercial screen printed electrodes have been commonly employed by researchers for electrochemical dengue detection due to their simplicity, biocompatibility, cost-effectiveness, disposability, and flexibility of integration (Cecchetto et al., 2015; Parkash et al., 2014; Sinawang et al., 2016). Apart from SPGE, quartz crystal microbalance (QCM) has been used as a simple PoC device, which offers shorter analysis time, real-time monitoring and label-free detection (Omar & Fen, 2018). A 2017 study reported the detection of dengue NS1 antigens using modified bacterial cellulose nanocrystals (CN)-QCM (Pirich et al., 2017). Based on literature, while QCM devices excel in sensitivity, electrochemical sensors provide better selectivity.

Integration of these two mechanisms on a single device have also been done in the form of an electrochemical quartz crystal microbalance (EQCM). These devices produce high accuracy of measurements in biological and chemical systems (Yang et al., 2015; Yu et al., 2009). Basic EQCM sensors comprise of a quartz crystal microbalance sensor (QCM), a counter electrode and a reference electrode, integrated into a single measurement platform. The advantage of these integrated sensors is that it can measure both resonance frequency changes and electrochemical reactions in a single platform.

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This is done by using the top electrode as both the electrochemical working electrode and QCM terminal. An early demonstration of combined electrochemical and QCM sensors observed that the an integrated sensor enabled cross-validation of the measurement, increased the accuracy of detection, and reduced false positives and false negatives (Yu et al., 2009). However, existing EQCM systems are limited to laboratory-only settings due to the instruments' large footprint and handling complexities (Ashton, 2012; Srivastava et al., 2018).

We previously hypothesized that using dual-function sensors could increase sensor sensitivity to very low limits of detection, increase dynamic range of disease quantification, and provide higher diagnostic accuracy through cross-validation of parallel measurement techniques (A. Zainuddin et al., 2019). In this paper, we present our current progress towards a rapid point-of-care (PoC) device for early detection of dengue, based on integrated electrochemical and mass biosensor. This PoC device has an array of three identical sensors on a single quartz substrate. Each sensor is a three-electrode system which can perform *in-situ* measurements of the electrode's surface changes based on piezoelectric and electrochemical transductions (Figure 1(a, b)). This system is packaged in a portable unit for deployment at patient-side or in the field (Figure 1(c, d)). We envision this sensor to achieve ultralow limit of detection, dual-sensing cross-validation capability, portable size, short sample-to-analysis time, and parallelization of multiple assays.

2 DEVICE DESIGN, FABRICATION AND VALIDATION

2.1 Device Concept and Operation

The biosensor has two measurement mechanisms, namely mass and electrochemical sensing. The mass sensor, which is known as quartz crystal microbalance (QCM) are widely used as a non-destructive method to measure the changes in surface mass due to adsorption process, based on changes in the resonance frequency (Deng et al., 2018). The QCM consists of electrodes on the top and bottom (BE) sides of thin AT-cut quartz substrate. The electrochemical biosensor consists of a three-electrode system, which are working electrode (WE), counter electrode (CE) and reference electrode (CE). These electrodes are implemented as a planar device on top of thin AT-cut quartz substrate. This leads to the working electrode (top) having dual functions as electrochemical and QCM sensor in a single chip device. The quartz crystal substrate (diameter, d_q = 14 mm) consisted of an array of three 10 MHz integrated electrochemical quartz crystal microbalance (IEQCM) sensors as shown in Figure 1(a). The centre-to-centre distance of QCM electrodes was set to s = 6 mm to minimize frequency interference (A. Zainuddin et al., 2019). Figure 1(b) illustrates a two-electrode system consisting of WE and CE for both the cross-section and top view of the integrated electrochemical and QCM biosensor. The electrochemical sensor is placed within a well, which is made by silicone gasket to contain the liquid. Gold (Au) was selected for the working and counter electrodes as it is an inert (noble) electrode that has high resistance to oxidation (Pereira et al., 2011; Serafín et al., 2011) and has unique covalent bonding characteristics with thiol-based self-assembly monolayers (SAM) (A. A. Zainuddin et al., 2016) that enables simple antibody immobilization. The variables d_{WE}, w_{CE}, g and h_q indicate the diameter of working electrode, width of counter electrode, gap between electrodes and quartz thickness, respectively. To minimize the interference of electric field during electrochemical measurements and higher current density across electrodes, the w_{CE} and g were set to 1000 µm and 70 µm, respectively. The device is then interfaced with measuring instrumentation with a 3D-printed custom enclosed sensor packaging (Figure 1(c)). This 3D-printed packaging was developed for a single quartz crystal substrate with diameter of 14 mm.

2.2 IEQCM Sensor Fabrication

Sensor fabrication was performed at the XLIM Circuits Technology Center cleanroom in XLIM Research Institute, Université de Limoges. IEQCM sensors consists of 3 top/working electrodes (WE), a common counter electrode (CE), and 3 bottom electrodes (BE). The sensors were fabricated on a 14 mm (Ø) 168 µm thick AT-cut quartz crystal piezoelectric substrate (Great Microtama Industries, Indonesia) using standard lift-off lithography.

Fabrication begins with WE and CE on the top side of quartz, followed by the fabrication of BE at the bottom side of quartz. First, the substrate was cleaned with piranha solution, 30% H_2SO_4, 5% H_2O_2). Then, it was rinsed in ethanol and deionized water (DI) and dried in N_2 stream. Subsequently, the substrate was pre-baked at 120 °C for 300 s. The image reversal photoresist (Merck AZ5214E,

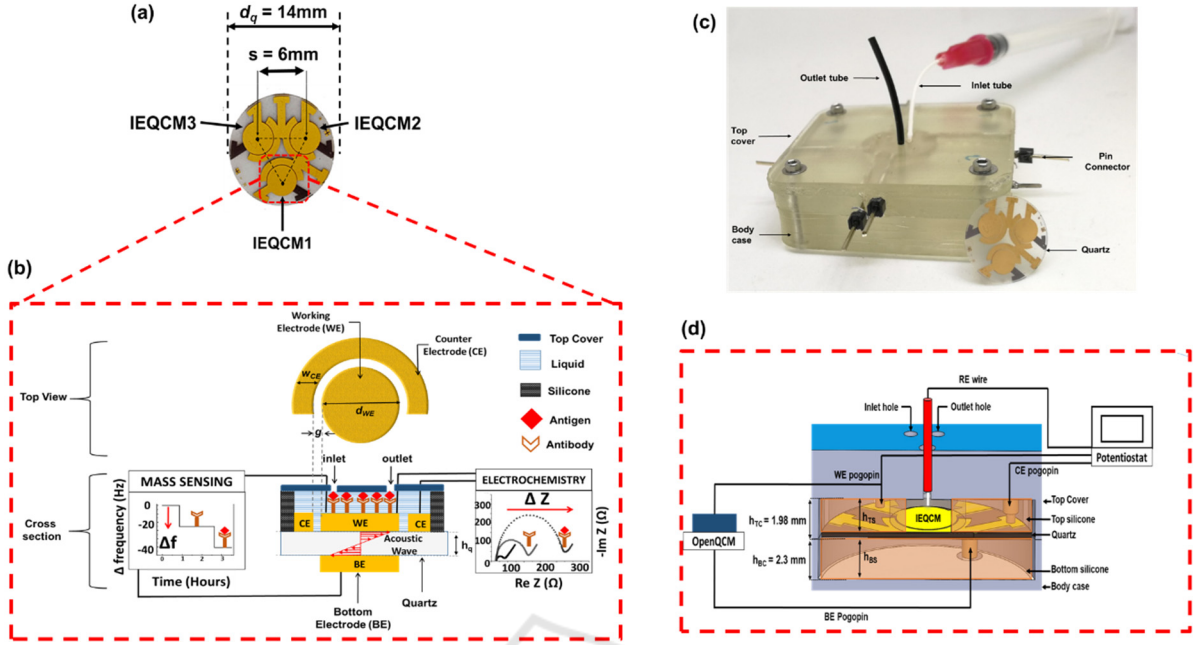

Figure 1: (a) Top view of the fabricated IEQCM sensor. (b) Conceptual illustration of the functional units of the integrated electrochemical and mass biosensor. (c) Custom 3D-printed enclosed sensor interfacing device. (d) Cross-sectional view of biosensor packaging. The height of top silicone area, h_{tc} was optimally set to 1.98 mm to prevent leakage. Pogo pins are used to form electrical contacts to the IEQCM quartz wafer. Laser-cut silicone (top and bottom) is used as hermetic gaskets and wells. Each unit of IEQCM has three holes with radius of 400 µm corresponding to the sample inlet and outlet, and a port for an external reference electrode (RE). The QCM measurements were done using a portable openQCM (Novatech, Italy) which displayed measurement of frequency versus time. The electrochemical measurements were done using three electrodes (WE, CE and RE), which were carried out using a potentiostat (Autolab PGSTAT128N).

MicroChemicals GmbH, Germany) was applied by spin-coating to a resulting thickness of 1.5 µm and baked at 105 °C for 60 s. Pattern was transferred onto the resist (exposure time: 3.5 s) using a Karl Suss MJB-3 or MJB-4 mask aligner. Prior to full exposure, the substrate was baked at 120 °C for 60 s, followed by the full exposure photolithography about 20 s. After the photoresist removal process with MF-26, Ti/Au electrode (20 nm/200 nm thick) was deposited using E-Beam evaporator (PLASSYS MEB 300). Finally, the release process (lift-off) was done with acetone to strip the remaining photoresist and define the electrodes. The same procedures were repeated to realize the BE at the bottom side of quartz substrate. The position of the BE relative to the WE are aligned with assistance of alignment markers on the top side of the quartz.

The disposable silver/silver chloride (Ag/AgCl) RE was fabricated through bleach immersion. Silver wire with diameter of 100 µm and minimum length of 3 cm was chosen so that it can be fitted into the reference electrode inlet port in the packaging module. The silver wire is first soldered to copper wired cables. The pure silver wire (99% Ag) is then immersed into undiluted household bleach (NaClO, 40 mg mL^{-1}) for up to 5 min (da Silva et al., 2014). All the wire interconnects will be described in the next section.

2.3 Sensor Packaging

A 3D-printed custom enclosure for the IEQCM biosensor was fabricated in this work. This sensor packaging would enable integration of electrical contacts and sample fluid delivery to the IEQCM sensor array. The packaging unit weighed 50 g, measured 80 mm × 32 mm × 12.4 mm (l × w × h), and was fabricated from a rapid 3D clear resin (Monocure 3D, New South Wales, Australia) formed using using a WDi7 DLP stereolithographic 3D printer (3D Synapsis, Malaysia). Print setting for the biosensor packaging is 50 microns per layer, with cure time of 22.4 s per layer. Printed units were rinsed using isopropyl alcohol and cleaned in an ultrasonic bath (Crest Ultrasonics Corp., NJ) to remove resin residuals, and post-processed under commercial nail UV lamp to cure underexposed coatings.

This packaging consists of two parts (top cover and body case). In the top cover, there are three different regions which correspond to three IEQCM

sensors in this system. Each region has three holes with radius of 400 µm corresponding to sample inlet and outlet, and a port for the external reference electrode. The top cover contains a slot with height h_{TC} = 1.98 mm to contain the silicone gasket. A circular silicone gasket (thickness h_{TS} = 2 mm, diameter 14 mm) is laser cut with three holes of diameter 5.5 mm as wells to contain liquid samples under test, which corresponds to the three IEQCM sensors in this system. This matching of h_{TC} and h_{TS} was optimized over several iterations to prevent sample leakages across the silicone gasket-top cover interface (see Figure 1(d)). Silicone was selected to ensure hermetic sealing and prevent mechanical stress on the quartz crystal.

For the (lower) body case, a circular silicone (thickness h_{BS} = 2 mm, diameter 14 mm) was used to support the quartz substrate, placed inside a slot with height h_{BC} = 2.3 mm. For electrical contact to the sensors, gold coated pogo pins (2.80 mm; Harwin Asia Pte Ltd, Singapore) were used due to its spring-loaded contacts that produced low mechanical stress on the quartz crystal surface. The pogo pins are distributed in radial symmetry (9 for top unit, 3 for bottom unit) to reduce mechanical stress over the quartz wafer. The top and bottom silicone gaskets are laser cut with holes that align with the pogo pins. The pogo pins are wired through the case by fine copper wires (100 µm diameters) and soldered to pin headers outside the case, for connections to external instrumentation. The top cover and the body case are then clamped together with bolts and nuts.

2.4 Surface Modification on IEQCM

Figure 3 shows the surface modification and immobilization process for the NS1 immunosensors. Bio-functionalization of mixed self-assembled monolayers (mix-SAMs) on gold working electrode surface was performed to immobilize the anti-NS1 IgG antibodies (ab138696, Abcam, Cambridge, UK). Before the mix-SAMs process, the bare IEQCM electrodes were cleaned with piranha solution, rinsed with a large amount of deionised water and dried with air blower pump. Polydimethylsiloxane (PDMS, 10:1 precursor-curing agent ratio; Sylgard 184, Dow Corning) slabs with punched liquid wells was used as a reversible mask to limit bio-functionalization reactions (SAM, EDC/NHS, Glycine) specifically to the working electrode.

The alkanethiols mix-SAMs was formed on the electrodes by immersing these electrodes for 24 h at 25°C in a mixed solution containing 1 mM 11-mercaptoundecanoic acid (for covalent anti-NS1 attachment) and 1 mM 6-mercaptohexanol (6COH) in ethanol. Alkanethiol SAM is formed on the WE due to formation of gold-thiol bonds. After the mix-SAMs preparation, the electrodes were washed with excess anhydrous ethanol and deionized water to remove any unbound molecules. It was followed by immersion of the WE in an aqueous solution containing 0.4 M N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) and 0.1 M N-hydroxysuccinimide (NHS) for 40 min, to activate the carboxylic acid terminated-group on the modified electrodes for NS1 antibody attachment. The electrodes were then washed with excess amounts of phosphate-buffered saline (PBS, pH 7.4) and dried with air blower pump. It was followed by the incubation of 50 µL of anti-NS1 solution (1 µg mL^{-1} in PBS) onto the electrodes surface. Following this incubation for 1 h, the remaining NHS esters were deactivated by addition of a 1 M ethanolamine solution for 5 min and the surface was thoroughly rinsed with deionized water. Finally, the modified mix-SAMs-NHS/EDC-anti-NS1 electrodes were immersed in 5 mM glycine solution (in PBS) for 30 min to block non-specific binding sites.

2.5 QCM and EIS Validation

Early validation was performed with endpoint measurements (i.e., not in continuous time) at each surface modification step. The measurement setup for the lab-on-chip biosensor is shown in Figure 1(d). Initially, the QCM measurements were done using a portable openQCM (Novatech, Italy) which displayed measurement of frequency versus time. The frequency changes were monitored by injecting 100 µL of phosphate-buffered saline (PBS) to the inlet and outlet of the packaging biosensor. Following injection of PBS into the chamber, the measurement is left to stabilize, and the last 200 s of the measurements is averaged to obtain frequency change values corresponding to the surface modification step.

The electrochemical measurements were performed on an Autolab PGSTAT128N potentiostat (Metrohm AG, Switzerland). In this work, 40 µl of 5 mM ferri-ferrocyanide ($[Fe(CN)_6]^{3-/4-}$) in PBS were used as redox probe ($K_3[Fe(CN)_6]$, Sigma Aldrich, St. Louis, MO). EIS measurements were recorded via AC potential of 5 mV amplitude in the frequency range from 1 MHz to 0.01 Hz at the optimized oxidation peak potential of 0.2 V and plotted over a Nyquist plot.

3 RESULTS AND DISCUSSIONS

3.1 Optimization of QCM Sensors

It was previously observed that quartz thickness of 168 µm with resonance frequencies of 10 MHz was used for biosensing (Pirich et al., 2017), and was also chosen for this work. The working (WE) and bottom (BE) electrodes' diameter were selected based on an optimal Q-factor resolution. This Q-factor corresponds to a greater mechanical energy stored by the resonator. Figure 2(a) indicates the measurements of resonance frequency for the working electrode of 7 varying diameters ranging from 200 µm to 6000 µm. Figure 2(c) shows the corresponding fabricated QCM sensors. From the results, all diameters of QCM, d_{we} showed a fundamental resonance frequency peak close to f_o = 10 MHz.

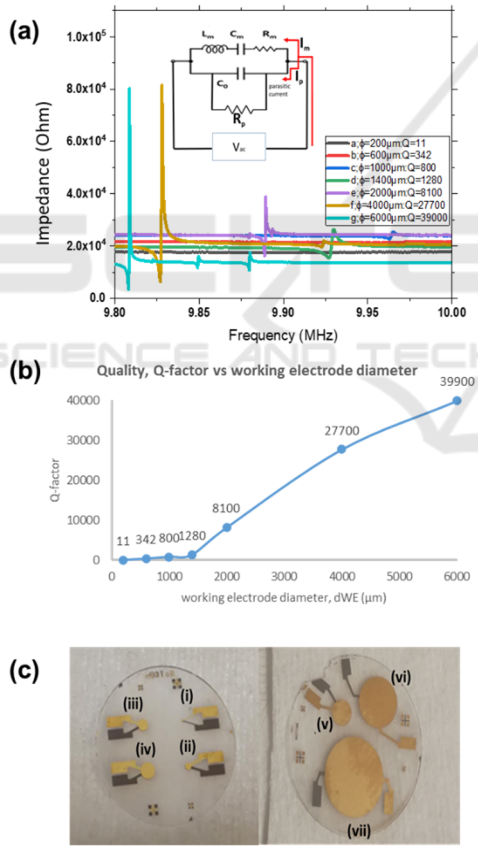

Figure 2: (a) Measurement of resonance frequency and Q-factor value at different diameter of QCM sensors. (Inset) A modified BVD equivalent circuit, where Rp was to represent an additional value for compensating parasitic capacitance. (b) Plot of Q-factor versus working electrode diameter, d_{WE}. (c) Fabricated QCM sensors for WE diameters (i) 200 µm, (ii) 600 µm, (iii) 1000 µm, (iv) 1400 µm, (v) 2000 µm, (vi) 4000 µm, (vii) 6000 µm.

According to the classical Butterworth-van-Dyke (BVD) circuit, the RLC parameters of the QCM equivalent circuit can be divided into motional components, R_m, L_m and C_m, as shown in Figure 2(a) (inset). These motional components are derived from the resonance operation of the QCM and an additional parallel static capacitor (C_0). The motional resistance (R_m) represents energy loss at resonance frequency. The motional impedance (L_m) and motional capacitance (C_m) represent the vibrating mass and coupling coefficient, respectively. C_0 contributes to the dielectric energy storage because the oscillation crystal is established in between the two electrodes (A. A. Zainuddin et al., 2018). R_p was added to represent an additional value for compensating parasitic capacitance. Taking these factors into

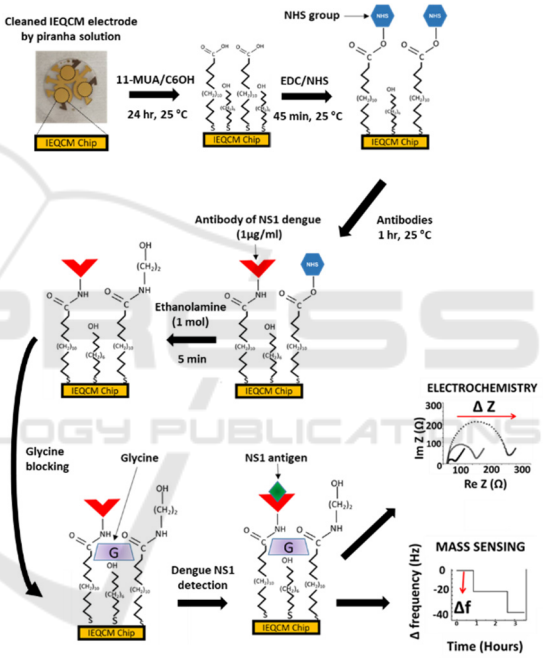

Figure 3: The modification and immobilization process for the NS1 immunosensor design. The mixed thiol-SAM structures, in which 11-mercaptoundecanoic acid serves as a linker layer for anti-NS1 antibodies attachment, and 6-mercaptohexanol functioned as spacers, is constructed onto a gold electrode surface. It is followed by the standard EDC/NHS amine conjugation which enables Anti-NS1 immobilization on this gold electrode. Glycine is used to block nonspecific sites. Samples containing dengue NS1 antigen is then introduced on the sensor interface. The faradaic impedance measurements are carried out using a redox probe in solution $[Fe(CN)_6]^{3-/4-}$. When NS1 antigens are bound to anti-NS1 antibodies, the change of impedance is measured by EIS through approximation of the diameter of the semi-circle of Re (Z), R_{ct}. The change of frequency is also evaluated from the reduction in resonance frequency of the propagated acoustic wave through the surface of the quartz using QCM.

account, widening the diameter of working electrode may enhance the Q-factor value. This is due a high resistance R_m, which caused significant reduction of current, I_m flowing through the RLC equivalent circuit of the QCM sensor. Therefore, a weak resonance frequency was observed due to the existence of a high parasitic current, I_p. This I_p was forced by the electric actuation signal flowing through the QCM parasitic capacitance C_0. Thus, this I_p caused small I_m flowing through the RLC equivalent circuit of the QCM (Waszczuk et al., 2011). Consequently, these smaller diameters (200 µm to 1400 µm) are not suitable to be used in the QCM in liquid sensing due to low Q factor.

The QCM diameters of 2000 µm, 4000 µm and 6000 µm corresponds to motional resistances $R_m = 1.51$ kΩ, $R_m = 315$ Ω and $R_m = 76.10$ Ω respectively. These R_m values correspond to higher Q-factors (Figure 2(b)), which is necessary for sensing application in fluids. The highest Q-factorvalue (39000) with the resonance frequency of 9.83 MHz is produced at diameter of 6000 µm. However, d_{WE} = 4000 µm was selected for our biosensor since it showed an optimal size with adequate Q-factor value (27700) while also allowing geometric fit into a radial array of 3 identical sensors on a single 14 mm quartz crystal substrate.

3.2 Electrochemical and Mass Detection for Validation of Surface Bio-Functionalization

Dengue is a mosquito-borne viral disease caused by the dengue virus, which is prevalent in most tropical and subtropical regions. Dengue disease is endemic in more than 100 countries from South East Asia, Western Pacific, Eastern Mediterranean, Africa to America (Cheah et al., 2014). About 400 million people are infected per year, of which 25% are symptomatic (febrile illness) and 75% are asymptomatic (Flores & O'Neill, 2018; Messina et al., 2015), resulting in an estimated 500,000 recorded cases of Dengue Hemorrhagic Fever (DHF) and 25,000 deaths occur per annum (Low et al., 2017). Early detection of dengue infection is very important for epidemiological strategies, clinical management and administering appropriate treatment to the patients. The urgency for early dengue detection presents a suitable test case for our biosensor.

Electrochemical impedance spectroscopy (EIS) is an electrochemical detection method that estimates impedance or current changes in electrode surface owing to surface reactions (Prakash et al., 2012). Deposition of antigens and antibodies on working electrode impede current flow to the electrode and electrolyte system. The use of Randles circuit can be implemented as a simple model to portray a solution resistance (R_S), a double layer capacitor or constant phase element (CPE) and a charge transfer resistance (R_{CT}). The value of CPE and R_{CT} are measured to show the changes in capacitance of electrode surface (Berggren et al., 1999). Warburg impedance (*W*) contribution to the overall impedance is negligible in our system, and thus excluded from the Randles circuit model used in our data acquisition (Figure 4(b)).

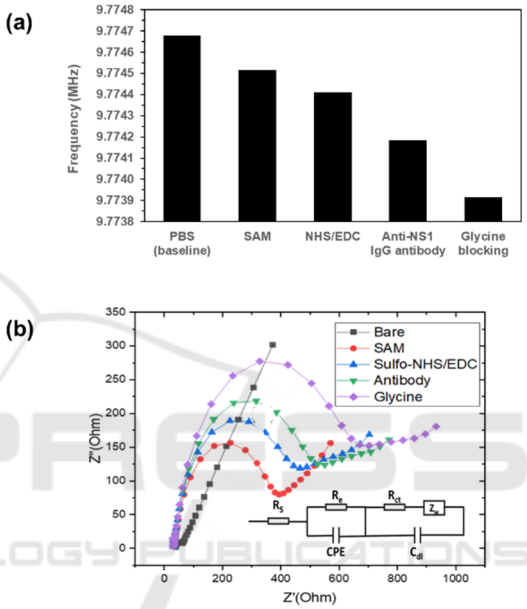

Figure 4: Change of frequency (a) and Nyquist impedance diagrams (b) of a surface functionalized IEQCM, layer-by-layer: bare electrode, SAM, NHS/EDC, dengue anti-NS1 capture antibody, and blocking agent glycine. Surface modification experiments were performed with n = 1.

The step-by-step monitoring of the surface functionalization of the WE are performed by EIS and QCM. Figure 4(a, b) shows the frequency change of the QCM measurement and Nyquist diagrams of EIS measurement following surface functionalization steps of the IEQCM: bare electrode (Au), SAM, SAM-EDC/NHS, SAM-EDC/NHS-antibody, SAM-EDC/NHS-antibody-glycine. In Figure 4(a), it can be observed that the resonant frequency is reduced for every phase of the functionalization steps, which corresponds to the added mass onto the surface of the bare gold electrode. The stepwise reduction of resonant frequency during each phase confirms the completion of each immobilization step. This is also confirmed by the increasing impedance (denoted by R_{CT} i.e., the diameter of the half circle) as indicated

by the Nyquist plot from the EIS measurement in Figure 4(b). The bare IEQCM exhibited a low resistance, suggesting a fast electron process of potassium ferrocyanide in PBS to the electrode surface.

4 CONCLUSIONS AND FUTURE WORKS

We report our progress in the development and validation of an integrated electrochemical quartz crystal microbalance (IEQCM) sensor arrays with mixed-SAM bio-recognition layer developed for point-of-care dengue detection. We demonstrated that this sensor could perform both as an electrochemical sensor and as a QCM sensor, enabling future developments for diagnostic cross-validation on a single platform. The biosensor is very promising with regards to its potential use at the point-of-care. The small footprint of the device, coupled with the portable openQCM instrumentation, allows a high degree of portability. While it is currently used with an AutoLab PGSTAT128N potentiostat which is too bulky for use in point-of-care settings, open-source portable potentiostats (Hoilett et al., 2020) may allow integration and development of portable and fully-automated IEQCM dual sensing devices in the near future. The sensor also allows to some degree of parallelized analysis due to its triple arrayed electrodes, further improving its capabilities for point-of-care use. Ongoing works on this project include development of miniaturized potentiostat for a portable integrated instrument, improved sensor fabrication process for integrated reference electrode and larger scale manufacturing, and validation works using real dengue patient samples.

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REFERENCES

Ashton, S. J. (2012). Differential Electrochemical Mass Spectrometry. In *Design, Construction and Research Application of a Differential Electrochemical Mass Spectrometer (DEMS)* (pp. 9–27). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30550-4_2

Berggren, C., St\a alhandske, P., Brundell, J., & Johansson, G. (1999). A feasibility study of a capacitive biosensor for direct detection of DNA hybridization. *Electroanalysis*, *11*(3), 156–160.

Cecchetto, J., Carvalho, F. C., Santos, A., Fernandes, F. C. B., & Bueno, P. R. (2015). An impedimetric biosensor to test neat serum for dengue diagnosis. *Sensors and Actuators B: Chemical*, *213*, 150–154. https://doi.org/10.1016/j.snb.2015.02.068

Cheah, W. K., Ng, K. S., Marzilawati, A. R., & Lum, L. C. (2014). A review of dengue research in malaysia. *The Medical Journal of Malaysia*, *69*, 59–67.

da Silva, E. T. S. G., Miserere, S., Kubota, L. T., & Merkoçi, A. (2014). Simple On-Plastic/Paper Inkjet-Printed Solid-State Ag/AgCl Pseudoreference Electrode. *Analytical Chemistry*, *86*(21), 10531–10534. https://doi.org/10.1021/ac503029q

Deng, F., Chen, W., Wang, J., & Wei, Z. (2018). Fabrication of a sensor array based on quartz crystal microbalance and the application in egg shelf life evaluation. *Sensors and Actuators B: Chemical*, *265*, 394–402. https://doi.org/10.1016/j.snb.2018.03.010

Flores, H. A., & O'Neill, S. L. (2018). Controlling vector-borne diseases by releasing modified mosquitoes. *Nature Reviews Microbiology*, *16*(8), 508–518. https://doi.org/10.1038/s41579-018-0025-0

Hoilett, O. S., Walker, J. F., Balash, B. M., Jaras, N. J., Boppana, S., & Linnes, J. C. (2020). KickStat: A Coin-Sized Potentiostat for High-Resolution Electrochemical Analysis. *Sensors*, *20*(8), 2407. https://doi.org/10.3390/s20082407

Hong, P., Li, W., & Li, J. (2012). Applications of aptasensors in clinical diagnostics. *Sensors*, *12*(2), 1181–1193.

Hu, J., Cui, X., Gong, Y., Xu, X., Gao, B., Wen, T., Lu, T. J., & Xu, F. (2016). Portable microfluidic and smartphone-based devices for monitoring of cardiovascular diseases at the point of care. *Biotechnology Advances*, *34*(3), 305–320.

Lisowski, P., & Zarzycki, P. K. (2013). Microfluidic paper-based analytical devices (μPADs) and micro total analysis systems (μTAS): Development, applications and future trends. *Chromatographia*, *76*(19–20), 1201–1214.

Low, J. G. H., Ooi, E. E., & Vasudevan, S. G. (2017). Current Status of Dengue Therapeutics Research and Development. *The Journal of Infectious Diseases*, *215*(suppl_2), S96–S102. https://doi.org/10.1093/infdis/jiw423

Messina, J. P., Brady, O. J., Pigott, D. M., Golding, N., Kraemer, M. U. G., Scott, T. W., Wint, G. R. W., Smith, D. L., & Hay, S. I. (2015). The many projected futures

of dengue. *Nature Reviews Microbiology*, *13*(4), 230–239. https://doi.org/10.1038/nrmicro3430

Miserere, S., & Merkoçi, A. (2015). Microfluidic Electrochemical Biosensors: Fabrication and Applications. In *Lab-on-a-Chip Devices and Micro-Total Analysis Systems* (pp. 141–160). Springer. http://link.springer.com/chapter/10.1007/978-3-319-08687-3_6

Omar, N. A. S., & Fen, Y. W. (2018). Recent development of SPR spectroscopy as potential method for diagnosis of dengue virus E-protein. *Sensor Review*, *38*(1), 106–116.

Parkash, O., Yean, C., & Shueb, R. (2014). Screen Printed Carbon Electrode Based Electrochemical Immunosensor for the Detection of Dengue NS1 Antigen. *Diagnostics*, *4*(4), 165–180. https://doi.org/10.3390/diagnostics4040165

Pereira, S. V., Bertolino, F. A., Fernández-Baldo, M. A., Messina, G. A., Salinas, E., Sanz, M. I., & Raba, J. (2011). A microfluidic device based on a screen-printed carbon electrode with electrodeposited gold nanoparticles for the detection of IgG anti-Trypanosoma cruzi antibodies. *Analyst*, *136*(22), 4745–4751.

Pirich, C. L., de Freitas, R. A., Torresi, R. M., Picheth, G. F., & Sierakowski, M. R. (2017). Piezoelectric immunochip coated with thin films of bacterial cellulose nanocrystals for dengue detection. *Biosensors and Bioelectronics*, *92*, 47–53.

Prakash, S., Pinti, M., & Bhushan, B. (2012). Theory, fabrication and applications of microfluidic and nanofluidic biosensors. *Phil. Trans. R. Soc. A*, *370*(1967), 2269–2303.

Serafín, V., Agüí, L., Yáñez-Sedeño, P., & Pingarrón, J. M. (2011). A novel hybrid platform for the preparation of disposable enzyme biosensors based on poly (3, 4-ethylenedioxythiophene) electrodeposition in an ionic liquid medium onto gold nanoparticles-modified screen-printed electrodes. *Journal of Electroanalytical Chemistry*, *656*(1), 152–158.

Sinawang, P. D., Rai, V., Ionescu, R. E., & Marks, R. S. (2016). Electrochemical lateral flow immunosensor for detection and quantification of dengue NS1 protein. *Biosensors and Bioelectronics*, *77*, 400–408. https://doi.org/10.1016/j.bios.2015.09.048

Srivastava, J., Kushwaha, A., & Singh, M. (2018). Imprinted Graphene-Starch Nanocomposite Matrix-Anchored EQCM Platform for Highly Selective Sensing of Epinephrine. *Nano*, *13*(11), 1850131. https://doi.org/10.1142/S179329201850131X

St John, A., & Price, C. P. (2014). Existing and emerging technologies for point-of-care testing. *The Clinical Biochemist Reviews*, *35*(3), 155.

Waszczuk, K., Piasecki, T., Nitsch, K., & Gotszalk, T. (2011). Application of piezoelectric tuning forks in liquid viscosity and density measurements. *Sensors and Actuators B: Chemical*, *160*(1), 517–523.

Yang, Y., Tu, Y., Wang, X., Pan, J., & Ding, Y. (2015). A label-free immunosensor for ultrasensitive detection of ketamine based on quartz crystal microbalance. *Sensors*, *15*(4), 8540–8549.

Yu, L., Huang, Y., Jin, X., Mason, A. J., & Zeng, X. (2009). Ionic liquid thin layer EQCM explosives sensor. *Sensors and Actuators B: Chemical*, *140*(2), 363–370.

Zainuddin, A. A., Nordin, A. N., Ab Rahim, R., & Mak, W. C. (2016). Modeling of a novel biosensor with integrated mass and electrochemical sensing capabilities. *Biomedical Engineering and Sciences (IECBES), 2016 IEEE EMBS Conference On*, 420–425. http://ieeexplore.ieee.org/abstract/document/7843485/

Zainuddin, A. A., Nordin, A. N., Rahim, R. A., Ralib, A. A. M., Khan, S., Guines, C., Chatras, M., & Pothier, A. (2018). Verification of Quartz Crystal Microbalance Array using Vector Network Analyzer and OpenQCM. *Indonesian Journal of Electrical Engineering and Computer Science*, *10*(1), 84–93. https://doi.org/10.11591/ijeecs.v10.i1.pp%p

Zainuddin, A., Nordin, A., Asri, M., Rahim, R., Guines, C., Chatras, M., Pothier, A., & Mak, W. (2019). Development of Integrated Electrochemical–QuartzCrystal Microbalance Biosensor Arrays: Towards Ultrasensitive, Multiplexed and Rapid Point-of-Care Dengue Detection: *Proceedings of the 12th International Joint Conference on Biomedical Engineering Systems and Technologies*, 220–227. https://doi.org/10.5220/0007523802200227