Biosensor for rapid detection of SARS-CoV-2 in real-world samples

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***Abstract*—The current COVID-19 pandemic has become a worldwide problem with more than 169 million people infected by May 2021. Here we demonstrate a unique technology, based on the quartz crystal microbalance method, for the rapid detection of SARS-CoV-2. This biosensor fulfils all of the many requirements for the rapid detection of SARS-CoV-2 in complex samples. This is achieved by a tailored antifouling surface post-modified with antibodies against SARS-CoV-2 nucleocapsid protein (N). The A-QCM profits from absence of sample pre-treatment and utilizes the natural properties of N protein, which forms complexes with vRNA. Thanks to this, the clinically relevant LOD of 6.7×10³ PFU/mL was reached using one-step detection assay. The A-QCM biosensor was also validated with clinical samples (i.e. nasopharyngeal swabs) with full agreement with qRT-PCR. The A-QCM biosensor was also utilized for the presence of SARS-CoV-2 in surface swabs in means of public transport.**

Keywords—biosensor, SARS-CoV-2, polymer brush, QCM, A-QCM, rapid detection, antifouling

I. Introduction

The Coronavirus disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has paralysed the entire world. Since the first reported case in December 2019 in Wuhan, China, the virus has spread worldwide with 215 million cases and 4.5 million deaths reported by September 2021 [1]. SARS-CoV-2 is a positive single stranded RNA virus that contains 4 main structural proteins: spike protein, envelope protein, membrane protein, and nucleocapsid protein (N-protein). The two main approaches available in fight against the pandemic are (1) vaccination, and (2) identification and isolation of infected individuals. The vaccination approach does not consider mutational escapes and the length of gained immunity is still unknown. For the latter approach, the detection of SARS-CoV-2 is primarily based on sensitive molecular methods. The most common of these methods, qRT-PCR, is time-consuming (~ 4 hours), demands sample transport, and highly-qualified personnel and advanced laboratory equipment [2]. In comparison, lateral flow immunoassays (LFIAs), often named as antigenic test, provide fast, simple and cheap detection of SARS-CoV-2. However, their detection format provides only a qualitative result with a lower sensitivity [3]. There is still an urgent need for sensitive, rapid test, and POC (point-of-care) test with minimal sample pre-treatment. Here we present a unique biosensing technology, A-QCM (Antifouling Quartz Crystal Microbalance), which enables SARS-CoV-2 detection directly in complex samples in a short time (~ 20 minutes) without any sample pre-treatment (Figure 1).

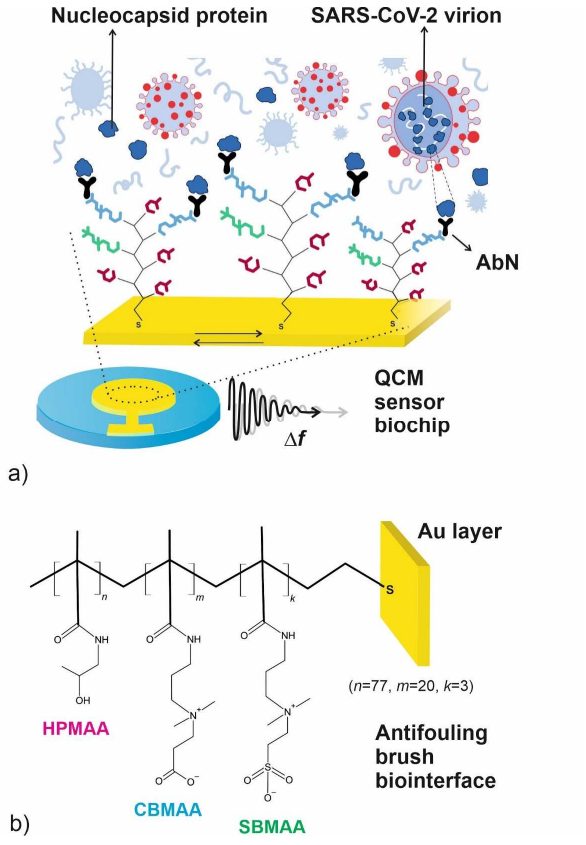

Fig. 1. A: One step A-QCM detection assay scheme based on an antifouling polymer brush biointerface. The antifouling brush biointerface is post-modified with a tailored human cell-produced antibody to SARS-CoV-2 nucleocapsid protein that forms complexes with vRNA. B: The antifouling polymer brush architecture is prepared from random terpolymer of carboxybetaine methacrylamide (CBMAA), N-(2-hydroxypropyl) methacrylamide (HPMAA), and sulfobetaine methacrylamide (SBMAA).

II. Experimental Methods

A. The QCM

The A-QCM device was developed at the Institute of Physics (Prague, Czech Republic). AT cut quartz resonators (KRYSTALY, Hradec Králové, Czech Republic) with nominal frequency of shear-thickness oscillations of 10 MHz were modified by visco-elastic coatings to sense the bio substances of interest in a fluid environment [4]. The fluid circuit incorporating injection, pumping, thermal regulation, and sensing moduli was developed in the institute based on the openQCM Q-1 (Novaetech, Italy) fluid cell.

B. Modification and Functionalization of Gold-coated Crystal Surfaces

Polymer brush layers were "glued" to the gold electrodes of the quartz crystals using a layer of alkenthiols (SAM – self assembled monolayer). Polymer brush coatings were prepared using a modified procedure (SI-ATRP) as described previously [5]. The polymer chains were synthesized as a terpolymer with random distribution of N-(2-hydroxypropyl) methacrylamide (HPMAA), carboxybetaine methacrylamide (CBMAA), and sulfobetaine methacrylamide (SBMAA), with optimized ratios in the polymerization feed of 77 mol%, 20 mol%, and 3 mol%, respectively. The thickness of the polymer brush was measured as 70 ± 10 nm in wet environment by spectroscopic ellipsometry. The polymer brush was then modified (using NHS/EDC chemistry) with human cell-expressed antibody against N-protein of SARS-CoV-2 (AbN).

C. SARS-CoV-2 Cultivation

Virus infection experiments were performed in biosafety laboratories (level 2 and 3). SARS-CoV-2 (SARS-CoV-2/human/Czech Republic/951/2020, EPI_ISL_414477, isolated from a clinical sample at The National Institute of Public Health Centre for Epidemiology and Microbiology, Prague, Czech Republic), kindly provided by Dr. Jan Weber, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic, was passaged in Vero E6 cells (African green monkey kidney cells) up to six times before its use in this study. Vero E6 cells were cultured at 37 °C and 5 % CO_2 in DMEM low glucose with supplementation. The titre of the virus was measured by plaque assay.

D. One-step Detection Assay

The samples (SARS-CoV-2 virus, Hepatitis A Virus, *Escherichia coli* and swabs) were analysed in real-time using crystals with immobilized antibodies from the previous step. A stable flow rate of 30 µL/min was used. All the coatings were rinsed with PBS buffer for 10 min. Afterwards, target samples were added and left to react for 10 min. Finally, all the coatings were rinsed with PBS buffer for 10 min. The amount of detected mass was calculated from the sensor response in PBS buffer before and after the application of the sample.

III. Results

A. Verification of Antifouling Properties of the Polymer Brush

The main compound of the functional biochip is unique antifouling polymer brush biointerface, which is composed from 3 types of monomers, each of which has a proper role in the polymer brush architecture. The HPMAA creates a non-ionic antifouling background. The CBMAA provide carboxy-groups for the post-modification with functional biomolecules, i.e. antibodies [6]. The incorporation of the monomer SBMAA ensures a permanent charge that allows for preserving the net charge electroneutrality after the post-modification step (i.e., the immobilization of antibody).

The antifouling properties were verified in several ways: i) The surface without antibody post-modification was exposed to different types of clinical samples (SARS-CoV-2 negative) – oropharyngeal, nasopharyngeal and rectal swabs and swabs from internal surface. ii) The surface with abN was exposed to samples spiked with high concentration of different pathogens – *Escherichia coli* (10^6 CFU/mL, heat-killed) and Hepatitis A Virus (10 µg/mL, inactivated viral antigen). iii) The surface without antibody post-modification was exposed to samples spiked with SARS-CoV-2. iv) The surface with anti-*E.coli* antibody post-modification was exposed to samples spiked with SARS-CoV-2. In all cases we did not observe a significant resonant frequency shift (when compared resonance frequency before and after sample exposure). Thus, the antifouling properties enable further analysis in complex media.

B. Detection of SARS-CoV-2

The biochip surface with a polymer brush functionalized with abN was exposed to samples (cell culture medium) containing SARS-CoV-2. The concentration of virus ranged between 10^2 to 10^6 PFU/mL. The one step assay format was used without additional washing or signal amplification steps. Figure 2 shows the resulting calibration curve. Based on the noise of the signal the LOD was determined as 6.7×10^3 PFU/mL (LOD = mean - standard error of the mean). In following experiments, the mass bounded to biochip was examined with mass spectroscopy and qRT-PCR. The presence of bound N-protein was verified by mass spectroscopy. SARS-CoV-2 N-protein was detected as one of the most copious proteins, as well as subunits of antibodies which were used for post-modification. The analysis of bounded mass by qRT-PCR showed the presence of vRNA, which further encourage the natural property of N-protein, which forms N- protein/vRNA complexes.

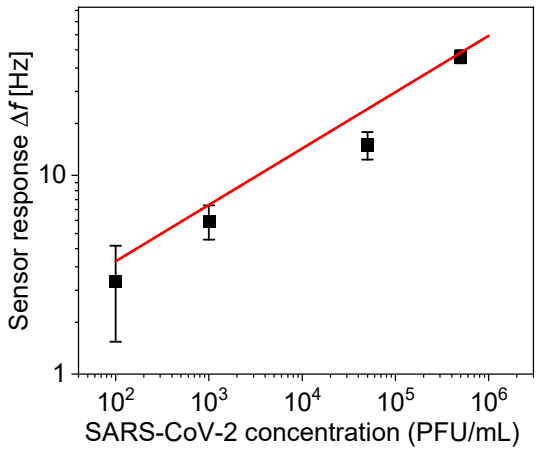

Fig. 2. Established calibration curve of A-QCM biosensor for SARS-CoV-2 detection in cell culture medium (log-log scale).

C. *Comparison A-QCM Technology with qRT-PCR in Crude Clinical Samples*

The developed A-QCM biosensor was then employed for the analysis of a set real-world clinical samples, i.e. nasopharyngeal swabs, which are most used in praxis for qRT-PCR analysis. Three patient samples were evaluated as positive and three as negative samples with qRT-PCR. We found a complete match between both methods when using a cut-off value of $\Delta f = \mu - 3\times\sigma$ = -8,5 Hz (Fig. 3). When comparing the quantitative signal of both methods, the results show only partial agreement with qRT-PCR, which is due to the fact, that qRT-PCR focuses on the number of viral vRNA copies, while the A-QCM is focused on the detection of the viral N-protein.

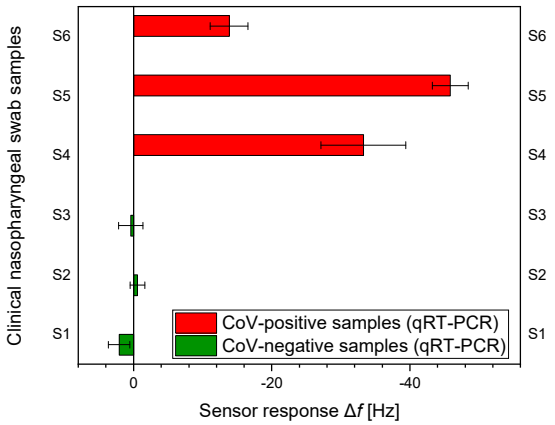

Fig. 3. A-QCM detection of qRT-PCR positive and negative clinical nasopharyngeal swab samples. The difference between A-QCM signal for qRT-PCR positive and negative clinical nasopharyngeal swab samples was statistically significant.

D. *Study of Surface Swabs from Means of Public Transport*

One of the advantages of implementation of A-QCM technology is the independence of the biological medium matrix. Therefore, we implemented the A-QCM technology for an extensive study of surface swabs from public means of transport. Based on a pilot study, we selected places related to public transport in Prague: buses, trams, subway cars and subway platforms. 492 samples were collected in total, which were analysed by both A-QCM and by qRT-PCR. The results of both methods are summarized in Table 1 and Fig. 4.

All samples that were determined as positive and borderline positive were subjected to culture analysis. Virus replication was not confirmed in any of the tested samples. Thus, it is highly probable that material contained in these samples was just a residual amount without infectious potential.

TABLE I. *SUMMARIZED RESULTS FROM EACH PLACE OF SAMPLING*

	Total number of samples	Positive		Borderline positive		Negative	
		qRT-PCR	A-QCM	qRT-PCR	A-QCM	qRT-PCR	A-QCM
Buses	154	1	2	7	8	146	144
Trams	160	0	2	5	17	155	141
Subway cars	126	0	1	8	10	118	115
Subway platforms	52	1	0	1	3	50	49

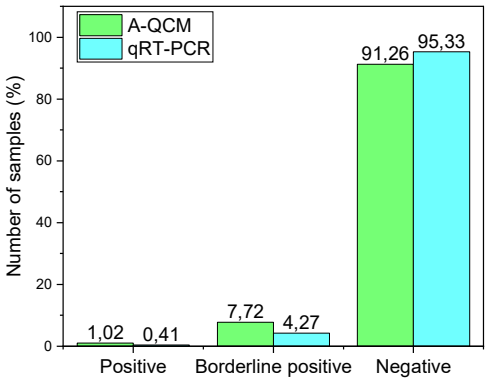

Fig. 4 Summarized results from all examined swabs (all places of sampling together).

IV. CONCLUSION

With the combination of a unique polymer brush coating, tailored human cell-produced antibodies, and the quartz crystal microbalance method, the A-QCM biosensing technology accurately detects SARS-CoV-2 in complex samples within a short time. The absence of sample pre-treatment simplifies the detection assay, enabling natural protein behaviour that forms complexes with vRNA and increases the sensitivity. The A-QCM technology was successfully validated in crude clinical samples and swabs from frequented places.

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REFERENCES

[1] World Health Organization, "COVID-19 Weekly Epidemiological Update," 2021. [Online]. Available: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-august-2021.

[2] D. K. W. Chu, Y. Pan, S. M. S. Cheng, K. P. Y. Hui, P. Krishnan, Y. Liu, D. Y. M. Ng, C. K. C. Wan, P. Yang, Q. Wang, M. Peiris and L. L. M. Poon, "Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia," *Clin. Chem.*, vol. 66, no. 4, pp. 549–555, Apr. 2020.

[3] G. C. Mak, P. K. Cheng, S. S. Lau, K. K. Wong, C. S. Lau, E. T. Lam, R. C. Chan and D. N. Tsang, "Evaluation of rapid antigen test for detection of SARS-CoV-2 virus," *J. Clin. Virol.*, vol. 129, no. June, p. 104500, 2020.

[4] M. V Voinova, M. Rodahl, M. Jonson and B. Kasemo, "Viscoelastic acoustic response of layered polymer films at fluid-solid interfaces: continuum mechanics approach," *Phys. Scr.*, vol. 59, no. 5, p. 391, 1999.

[5] I. Víšová, B. Smolková, M. Uzhytchak, M. Vrabcová, Y. Zhigunova, M. Houska, F. Surman, A. de los Santos Pereira, O. Lunov, A. Dejneka and H. Vaisocherová-Lísalová, "Modulation of Living Cell Behavior with Ultra-Low Fouling Polymer Brush Interfaces," *Macromol. Biosci.*, vol. 20, no. 3, p. 1900351, Mar. 2020.

[6] H. Vaisocherová-Lísalová, F. Surman, I. Víšová, M. Vala, T. Špringer, M. L. Ermini, H. Šípová, P. Šedivák, M. Houska, T. Riedel, O. Pop-Georgievski, E. Brynda and J. Homola, "Copolymer Brush-Based Ultralow-Fouling Biorecognition Surface Platform for Food Safety," *Anal. Chem.*, vol. 88, no. 21, pp. 10533–10539, Nov. 2016.