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Supplemental information

A bacterial cellulose-based and low-cost electrochemical biosensor for ultrasensitive detection of SARS-CoV-2

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SUPPLEMENTAL FIGURES AND TABLES

Table S1: Estimated costs for each fabrication step of the BC-based biosensor. The cost to produce our biosensor was estimated at US$3.50 taking into account the chemicals and materials used to produce it (conductive inks, BC, G-PEG, ACE2, BSA, Nafion) and considering a lab-based production of 100 test units per batch, which requires mg/mL scale of the commercially available chemicals. Note that, based on the materials and methods used to produce the electrochemical tests, the technology is highly scalable and, thus, the cost per biosensor is likely to go down when scaled up.

Fabrication step	**Materials used**	**Cost per sensor (US$)**
Manufacturing the screen-printed electrodes	BC, conductive carbon and Ag/AgCl inks	0.25
ACE2-functionalization	G-PEG, EDAC, NHS, ACE2	3.23
Coating materials	BSA, Nafion	0.02
TOTAL (US$)		**3.50**

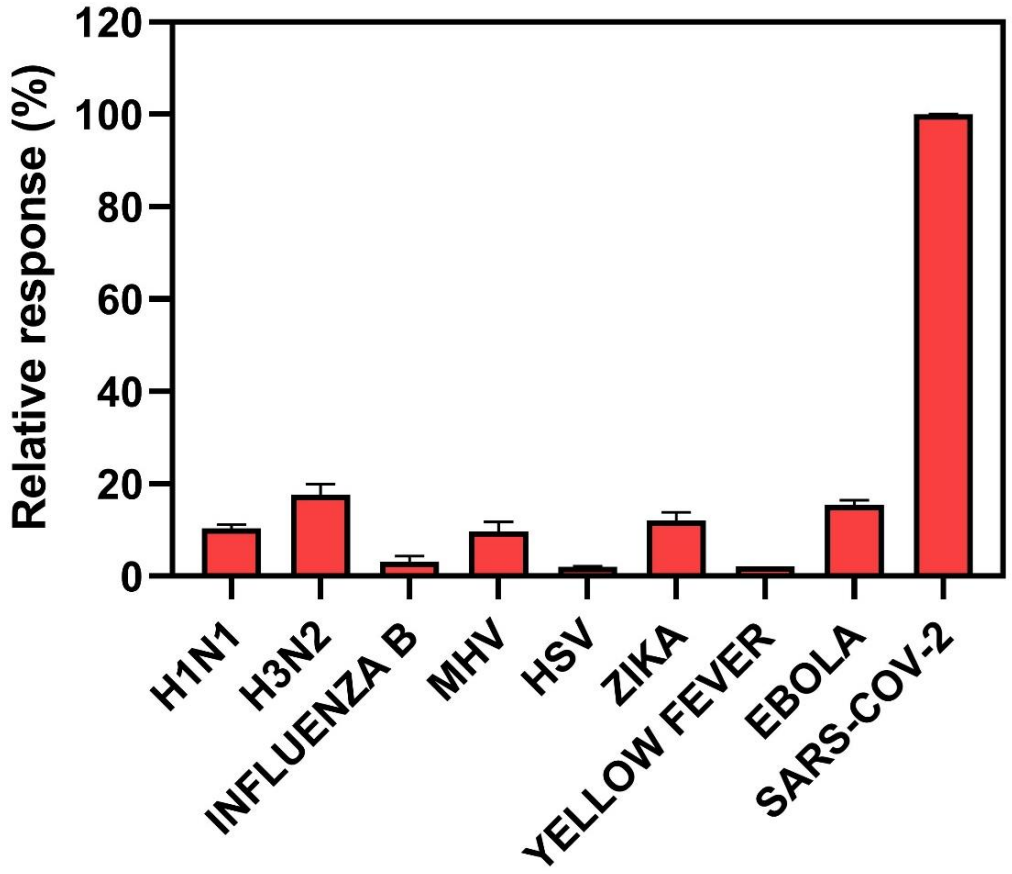

Figure S1: Selectivity studies of the biosensor. Optimal conditions for each virus were used for cross-reactivity assays. The viruses were: influenza A virus (H1N1), strain A/California/2009; influenza A virus (H3N2), A/Nicaragua; Influenza B – B/Colorado; MHV – mouse hepatitis virus; HSV2 – herpes simplex virus-2; and SARS-CoV-2, all at 10^5 PFU mL^{-1}. Antigenic preparations [heat-inactivated Zika (1.1×10^7 copies μL^{-1}), yellow fever (1.8×10^4 copies μL^{-1}), and gamma-irradiated Ebola (1.1×10^7 copies μL^{-1})]. All experiments were carried out in triplicate (n=3) using 0.1 mol L^{-1} PBS for 300 seconds of analysis. The error bars correspond to the standard deviation. 10 µL of each virus or antigenic preparation was incubated for 7 minutes on the biosensor surface before the potentiometric measurements were made.

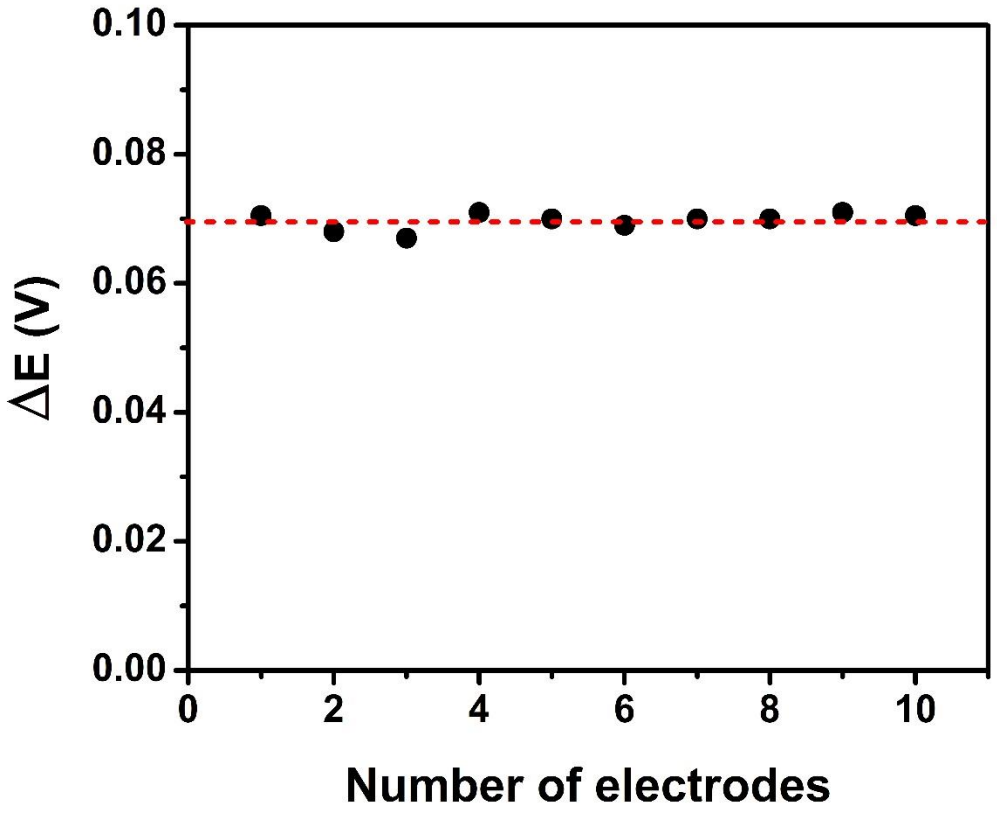

Figure S2: Reproducibility studies. Plot showing potential difference (ΔE) obtained for 10 biosensors when incubated with $1x10^{1}$ copies μL^{-1} of SARS-CoV-2 prepared in VTM medium. A volume of 10 µL of each virus was incubated on the biosensor surface for 7 minutes before the potentiometric measurements were made. The relative standard deviation (RSD) was 3.78% in these assays.

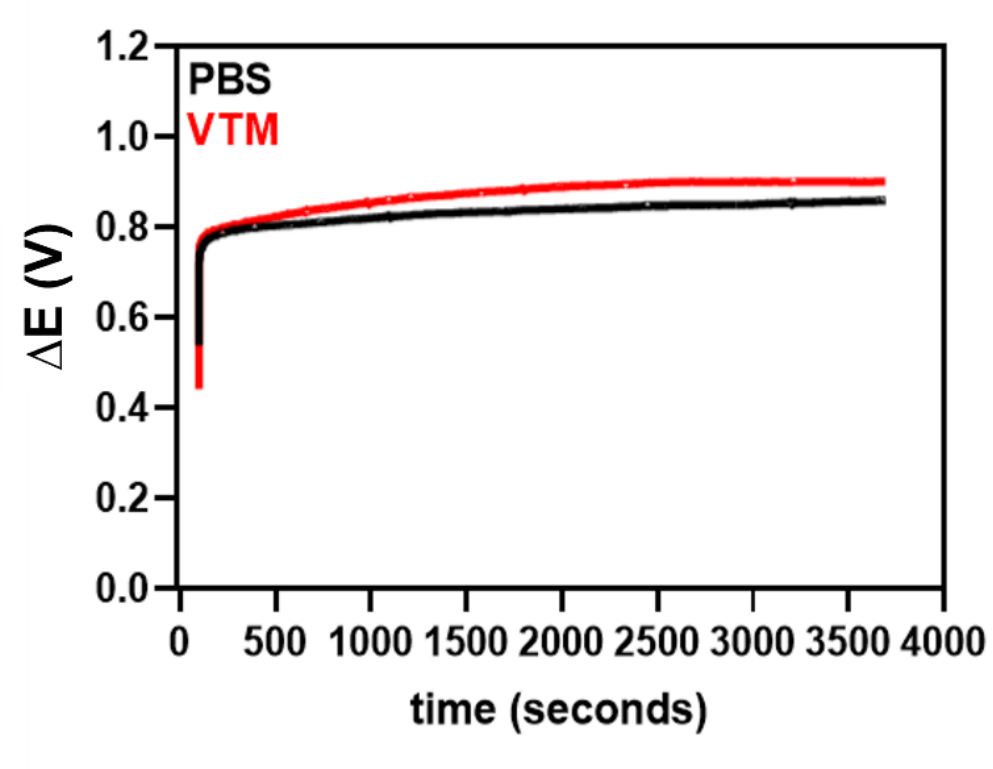

Figure S3: Potential stability of the biosensor. Biosensors were tested for stability for 1 hour using 0.1 mol L^{-1} PBS as a blank sample (black line) and with VTM as a blank sample (red line) to evaluate the best medium for sample analysis. Note that PBS presented a stable response after the first 60 s, whereas VTM presented a drift potential response over a long period of use (>500s).

Table S2: Comparative analysis of 15 NP/OP clinical samples using the biosensor (ΔE) and RT-PCR (Ct). We tested 15 positive samples consisting of 5 original SARS-COV-2 strains and 10 delta variant samples. Note that the delta variant samples provided higher ΔE responses than the original SARS-CoV-2 sample for similar Ct values. These heat-inactivated COVID-19 delta variant specimens with linked Ct values were obtained from residual clinical samples of patients at the Hospital of the University of Pennsylvania under the IRB protocol 814859.

ID Sample	ΔE (V)	Ct
SARS-COV-2 (8)	0.084	22.8
SARS-COV-2 (27)	0.081	24.2
SARS-COV-2 (20)	0.079	25.3
SARS-COV-2 (30)	0.073	26.1
SARS-COV-2 (2)	0.066	26.1
Delta 1	0.136	14.0
Delta 2	0.131	16.0
Delta 3	0.134	16.2
Delta 4	0.120	19.6
Delta 5	0.115	20.2
Delta 6	0.106	21.3
Delta 7	0.112	21.3
Delta 8	0.106	23.2
Delta 9	0.100	25.5
Delta 10	0.081	27.3

Table S3: Comparative analysis of 50 NP/OP clinical samples using our biosensor (ΔE) and RT-PCR (Copies µL^{-1}). A total of 50 samples were tested; 25 SARS-CoV-2 positive samples consisting of 12 types of SARS-CoV-2 variants, and 25 negative NP/OP samples. The heat-inactivated SARS-CoV-2 variant samples and Ct values were obtained from individuals at the Hospital of the University of Pennsylvania under IRB protocol 823392. The heat-inactivated 25 negative SARS-CoV-2 NP/OP samples were obtained from the Hospital of the University of Pennsylvania under the IRB protocol 844145 and were described in our previous paper [M. D. T. Torres, et al., Detection of SARS-CoV-2 with RAPID: A prospective cohort study. iScience 25, 104055 (2022)].

	ID sample	Lineage	ΔE (V)	Copies µL^{-1}
Positive Samples	228	B.1.350	0.083	2.04 x10^{3}
	263	B.1.350	0.083	2.47 x10^{3}
	266	B.1	0.112	1.53 x10^{6}
	269	B.1	0.066	5.97 x10^{1}
	272	B.1	0.097	1.43 x10^{4}
	346	B.1	0.098	5.52 x10^{4}
	373	B.1	0.106	1.36 x10^{5}
	290	B.1.291	0.083	5.58 x10^{3}
	328	B.1.369	0.064	1.80 x10^{1}
	369	B.1.369	0.082	1.04 x10^{4}
	334	B.1.340	0.075	1.01 x10^{3}
	348	B.1.240	0.083	5.04 x10^{4}
	380	B.1.243	0.068	1.12 x10^{2}
	408	B.1.243	0.072	6.43 x10^{3}
	423	B.1.243	0.072	2.03 x10^{3}
	428	B.1.243	0.051	1.67 x10^{1}
	444	B.1.243	0.077	2.39 x10^{3}
	452	B.1.243	0.061	1.20 x10^{2}
	381	B.1.311	0.062	4.33 x10^{2}
	385	B.1.1.304	0.052	1.61 x10^{2}
	391	B.1.1.317	0.059	3.18 x10^{3}
	406	B.1.2	0.091	4.74 x10^{5}
	455	B.1.2	0.047	4.89 x10^{1}
	459	B.1.1.7	0.093	1.70 x10^{3}
	460	B.1.1.7	0.112	5.20 x10^{4}
Negative	57	-	0.014	0

	58	-	0.009	0
	59	-	0.015	0
	60	-	0.011	0
	61	-	0.014	0
	62	-	0.021	0
	63	-	0.017	0
	64	-	0.011	0
	65	-	0.017	0
	66	-	0.013	0
	67	-	0.013	0
	68	-	0.013	0
	69	-	0.014	0
	70	-	0.016	0
	71	-	0.013	0
	72	-	0.011	0
	73	-	0.016	0
	74	-	0.018	0
	75	-	0.014	0
	76	-	0.018	0
	77	-	0.014	0
	78	-	0.012	0
	79	-	0.019	0
	80	-	0.019	0
	81	-	0.012	0