SUPPLEMENTARY DATA FOR

ElectroELISAProbe: Low-cost and miniaturized cell for on-site electrochemical microtiter plate readout

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Table of Contents

Page 2 Immunoassay protocols

Page 2 **Figure S1**. Initial setup for the ElectroELISA Probe

Page 3 **Figure S2**. Pictures of the components of the final device.

Page 3 **Table S1**. Data of peak current intensities and potentials of the CVs presented in Figure 1F.

Page 4 **Figure S3**. Reproducibility evaluation of devices with carbon-ink WE modified with different nanomaterials.

Page 4 **Table S2**. Data of peak current intensities and potentials of the CVs presented in Figure S3.

Page 5 **Table S3**. Comparison of characteristics and costs of commercially available transduction approaches for immunoassays and the one presented in this work

Page 5 **Figure S4**. Calibration curve for TMB solutions in 0.1 M H_2SO_4

Page 6 **Table S4**. Recovery study for the determination of anti-VCA IgG using the ElectroELISAProbe.

Page 6 **Table S5**. MMP9 concentrations found in saliva samples obtained by the ElectroELISAProbe and by a bench microplate spectrophotometer.

Page 7 **Figure S6**. Details of the design of the 8-cell ElectroELISAProbe.

IMMUNOASSAY PROTOCOLS

Human Epstein-Barr virus capsid antigen (EB-VCA) antibody (IgG) ELISA immunoassay

Human anti-VCA IgG concentrations were quantified using a sandwich ELISA kit (MyBioSource, MBS706901) following the manufacturer's protocol. Briefly, 100 µL of standards or appropriately diluted samples (spiked serum) were added to microplate wells (pre-coated with anti-VCA IgG capture antibody) and incubated for 45 minutes at room temperature. After three washes with the provided wash buffer, 100 µL of HRP-conjugated detection antibody was added and incubated for 30 minutes at room temperature. Wells were washed four times before adding 100 µL of TMB substrate solution (50 µL of each provided substrate solution called A and B). After 10 minutes of incubation in the dark, the reaction was stopped with 100 µL of stop solution. Then, determination was either performed by absorbance measurement at 450 nm using an EonTM plate reader, or electrochemically with the ElectroELISAProbe.

Human MMP9 ELISA immunoassay

Quantification of human MMP9 was performed using the SimpleStep ELISA® Kit (Abcam, ab246539) according to the manufacturer's instructions. Briefly, 50 µL of standards or appropriately diluted samples (saliva) were added to each well of the pre-coated 96-well plate, followed by 50 µL of antibody cocktail containing capture and detector antibodies (SimpleStep Technology®, Abcam). Plates were incubated for 1 hour at room temperature and then washed three times with the provided wash buffer. Provided TMB development solution (100 µL) was added and incubated for 10 minutes in the dark. The reaction was stopped with 100 µL of Stop Solution. Then, determination was either performed by absorbance measurement at 450 nm using an EonTM plate reader or electrochemically with the ElectroELISAProbe.

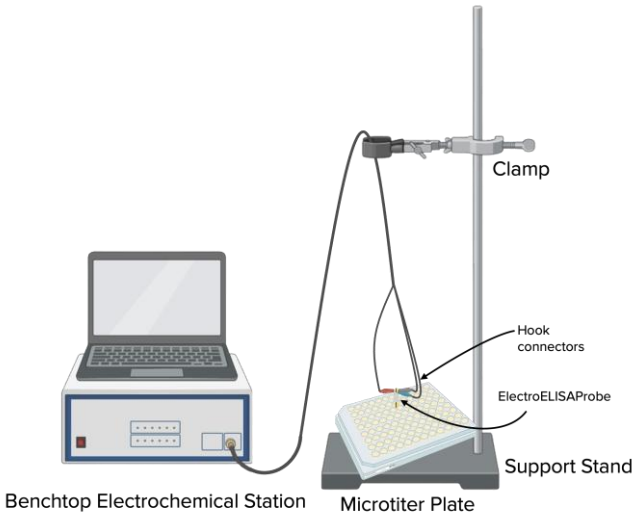


Figure S1. Initial setup for the ElectroELISA Probe

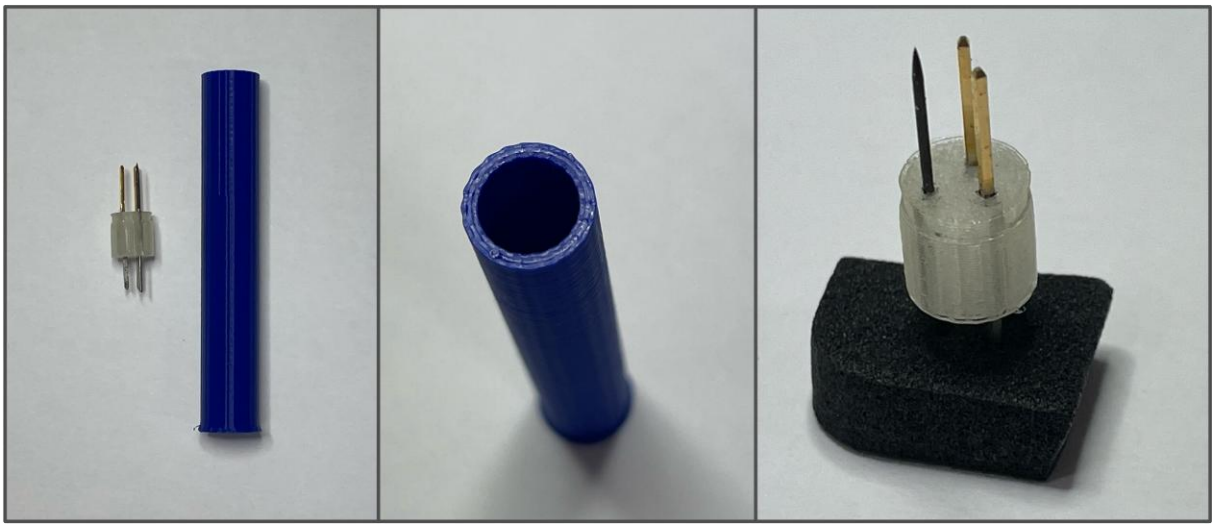


Figure S2. Pictures of the components of the final device. Left: Electrochemical cell (transparent material, that is TPU) with gold connectors as RE and CE and carbon-ink modified stainless-pin as WE, and shaft (blue, made of PLA). Centre: Detail on the hollow inside of the shaft, where the cell is placed. Right: Detail on the electrochemical cell (placed over a black foam substrate for a better viewing).

Table S1. Data of peak current intensities and potentials of the CVs presented in Figure 1F (performed with devices including a carbon-ink WE modified following different procedures).

Modification	i_{pa} (µA)	E_{pa} (V)	i_{pc} (µA)	E_{pc} (V)
1 Layer at 70^0C (15 min)	124	0.46	-154	0.02
3 Layers at 70^0 (15 min)	151	0.38	-164	0.15
2 Layers at 70^0C (15 min) + 1 Layer at 37^0C (12 h)	261	0.24	-291	0.08

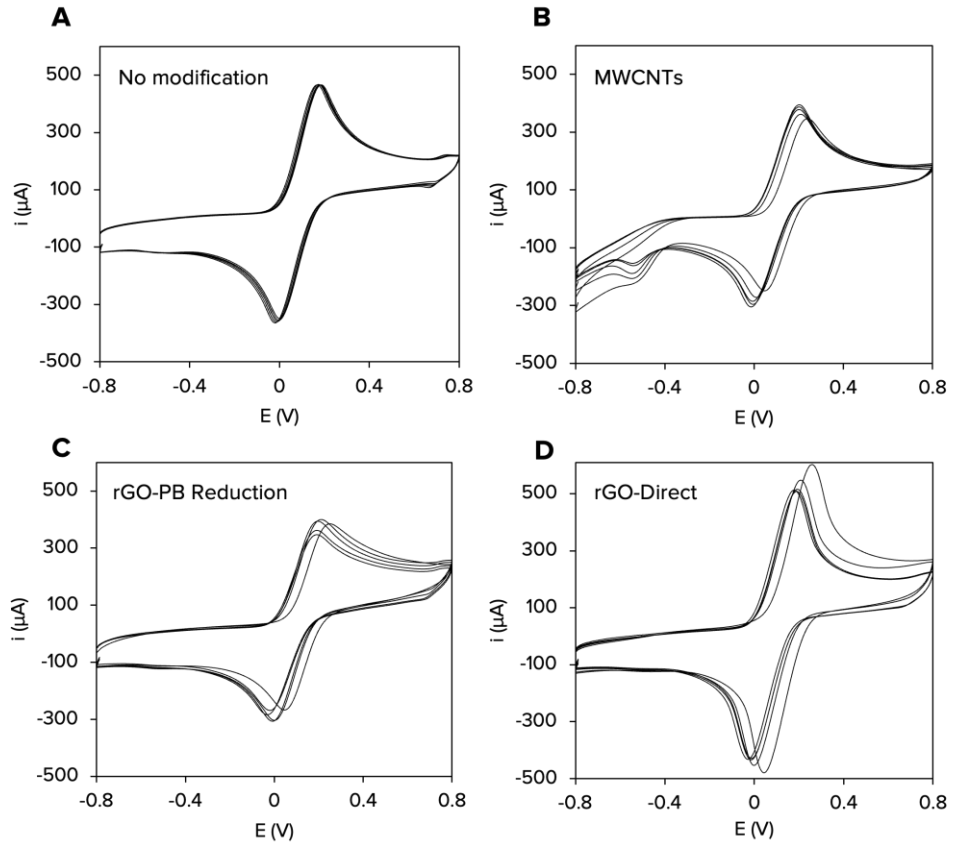


Figure S3. Reproducibility evaluation of devices with carbon-ink WE modified with different nanomaterials. CVs recorded using cells with carbon-ink WE (A) unmodified, (B) modified with MWCNTs, (C) modified with GO subsequently reduced in PB (rGO-PB reduction), and (D) modified with rGO directly reduced (rGO-direct). All CVs were recorded in a 20 mM ferrocyanide solution in 10 mM PB pH 7.0, from -0.80 V to +0.80 V at 0.1 V/s. For all the experiments, n = 5.

Table S2. Data of peak current intensities and potentials of the CVs presented in Figure S3 (performed with devices with carbon-ink WE modified with different nanomaterials). SD (and RSD in brackets) are calculated from measurements performed with 5 different WEs in each case.

Modification	i_{pa} (µA)	E_{pa} (V)	i_{pc} (µA)	E_{pc} (V)
None	385.0 ± 0.6 (0.2%)	0.17 ± 0.01	-383 ± 3 (0.7%)	-0.01 ± 0.01
MWCNTs	309 ± 16 (5.2%)	0.21 ± 0.02	-297 ± 19 (6.5%)	0.01 ± 0.03
rGO-PB reduction	281 ± 17 (6.0%)	0.20 ± 0.03	-307 ± 24 (7.9%)	0.01 ± 0.03
rGO-direct	435 ± 18 (4.2%)	0.20 ± 0.03	-463 ± 18 (4.0%)	0.01 ± 0.03

Table S3. Comparison of characteristics and costs of commercially available transduction approaches for immunoassays and the one presented in this work

Feature	Optical Plate Reader	SPE-based microplate	SPE-based immunoplatforms	ElectroELISAProbe (this work)	
Portability	Medium	Low	High	High	
Costs (EUR) not including ELISA Kit	Microtiter plate: 9 Portable optical reader: 4000	SPE-based microplate: 120 Plate adapter: 5400 8x-potentiostat: 6800	SPE: 2 Portable potentiostat: 700	ElectroELISAProbe: 0.15 Microtiter plate: 9 Portable potentiostat: 700	8x-pot.: 6800
TOTAL (EUR)	4009	12320	702	709.15	6809.15
TOTAL per assay (EUR) (no reagents or readers)	0.1	1.25	2	0.1*/0.25**	
Compatibility with commercial ELISA kits	High	Low	Low	High	
Affected by ambient light/turbidity	Yes	No	No	No	

*Assuming the reusability of the probe/**Assuming a new probe for each measurement.

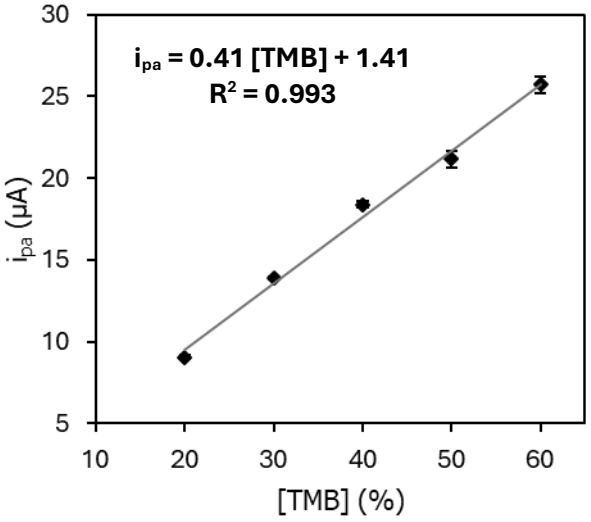


Figure S4. Calibration curve obtained by plotting the anodic peak current intensity obtained recording CVs of TMB solutions in 0.1 M H_2SO_4 at different dilutions (20, 30, 40, 50 and 60%). Error bars represent the standard deviation (SD) of measurements obtained with 3 different devices.

Table S4. Results of the recovery studies for the determination of anti-VCA IgG using the ElectroELISAProbe. SD (and RSD in brackets) are calculated from 3 different measurements.

Spiked [Anti-VCA IgG] (ng/mL)	[Anti-VCA IgG] found (ng/mL) (RSD value)	Recovery (%)
27	26 ± 1 (5%)	96 ± 5
33	36 ± 1 (2%)	110 ± 2
100	107 ± 9 (9%)	106 ± 9
200	210 ± 20 (9%)	106 ± 9

Table S5. MMP9 concentrations found in saliva samples determined by the ELISA immunoassay using the ElectroELISAProbe compared to the results obtained using a bench microplate spectrophotometer. SD (and RSD in brackets) are calculated from 3 different measurements.

[MMP9] (pg/mL) by spectrophotometry (RSD value)	[MMP9] (pg/mL) by ElectroELISAProbe (RSD value)	Recovery (%)
400 ± 83 (20%)	361 ± 180 (50%)	90 ± 45
1258 ± 170 (14%)	1094 ± 69 (6%)	87 ± 6
1934 ± 220 (11%)	1734 ± 240 (13%)	90 ± 12
3920 ± 170 (4%)	3305 ± 240 (15%)	84 ± 13

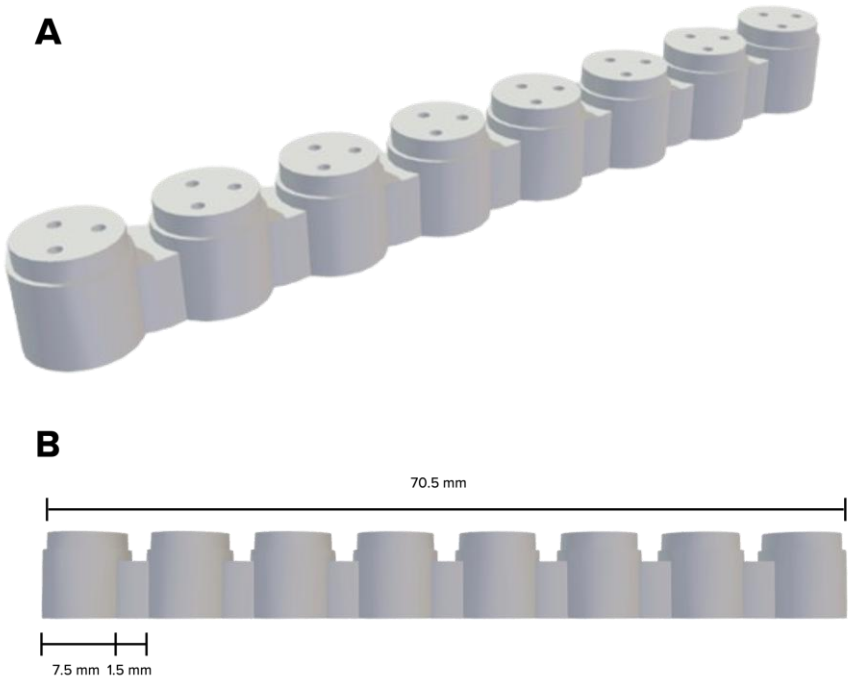


Figure S5. Details of the design of the TPU adapter for the 8-cell ElectroELISAProbe. **A**: 3D render of the design. **B**: Front view of the design, with size details for total width, individual probe width and probe separation. The remaining dimensions are the same as those shown in Figure 1.