Supplementary Information File

Inflammatory biomarker detection in saliva samples by printed graphene immunosensors

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- ***S.1 IL-6 incubation time***

EIS measurements have been used to investigate the dynamics of IL-6 binding at different analyte concentrations. We fixed the frequency and amplitude of the probing signal at 1 Hz and 10 mV, respectively (equilibration time=5s). Figure S.1 shows the total impedance as a function of time for four IL-6 concentrations (from 1pg/mL to 1000 pg/mL) in PBS. At the beginning, the total impedance Z increases for all the analyzed concentrations (Figure S2 left panel), due to the occurrence of binding events; then, it shows a saturation in time that is concentration dependent. The right panel of Figure S.1 shows the phase related to Z for the lowest concentration of 1 pg/mL (higher concentrations provide a similar trend of phase vs time). It shows a transition from a dynamic diffusive regime at the working electrode (phase angle around 50°), which is a fingerprint of antigen-antibody binding events in action, to a static capacitive one (phase angle around 90°), indicating the completion of binding events. The lowest IL-6 concentration provides a saturation of Z value and a stable phase at 87.7 ° within about 1500 s. Hence, 1h of analyte incubation represents a good compromise to get a reliable response for incubation of IL-6 [1] for concentrations falling in the window comprising both the normal level and the cytokine storm range.

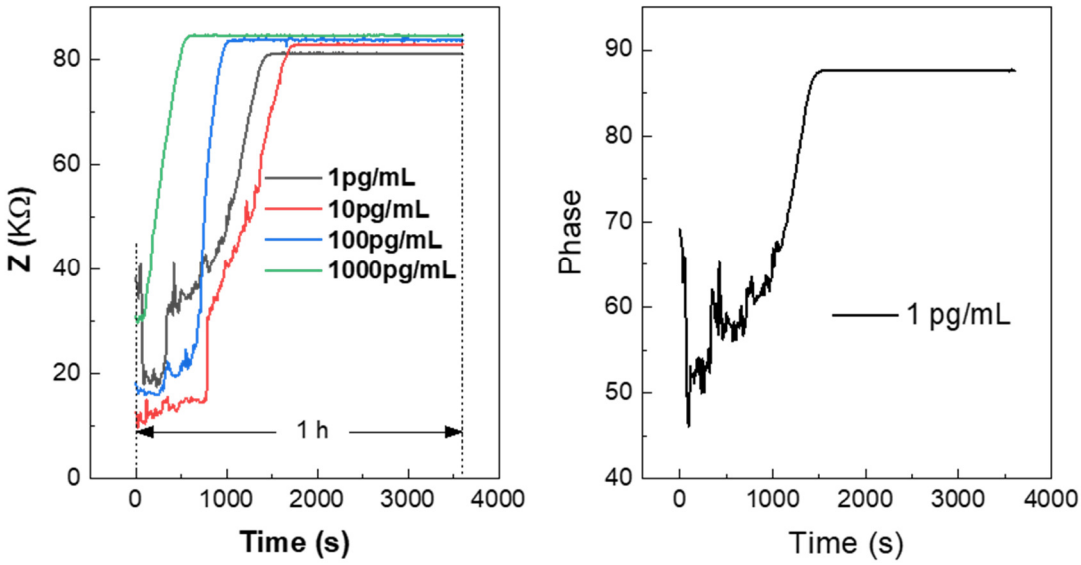

Figure S.1. Total impedance Z vs time for different IL-6 concentrations in PBS solution (left panel); phase vs time for IL-6 nominal concentration of 1 pg/mL.

- ***S.2 Comparison between the SPE biosensors and standard ELISA assay for salivary IL-6 detection***

Our biosensors exhibit a LoD calculated from calibration curves that is far below the pg/mL level and a larger dynamic range than that provided by the ELISA assay based on the same monoclonal antiIL-6 (Human IL-6 ELISA development kit by Vinci Biochem; ELISA standard range from 10 pg/mL to 1000 pg/mL, as indicated by the producer); all this, with a low variability in their individual response, above all for concentrations falling within the cytokine storm interval, where they are highly effective by fact.

To correlate the biosensor and standard ELISA assay responses, using the as-collected saliva (blank) and salivary nominal IL-6 concentrations of 10, 100 and 1000 pg/mL, we have measured the ELISA signals by a Simple Plex Human IL-6 2nd Gen ELISA assay kit (LoD 0.1 pg/mL lower and upper limit of quantification 0.28 and 2652 pg/mL, i.e. wider dynamic range than that of Vinci Biochem Human IL-6 ELISA development kit) (Figure S.2, left panel). The ELISA response (Relative Fluorescence Unit, RFU) has been correlated to the SPE response upon comparing the ΔR_{ch} parameter and RFU for the selected concentrations [2].

The linear fit at the response correlation curve (ΔR_{ch} vs. RFU, Figure S.2 right panel) indicates that the responses of the TEGO-SPE sensor and ELISA assay well correlate with each other (coefficient of determination R^2=0.99636), as also expected because of the lower LoD and the wider linearity over the spun concentration window (dynamic range) of the SPE biosensor.

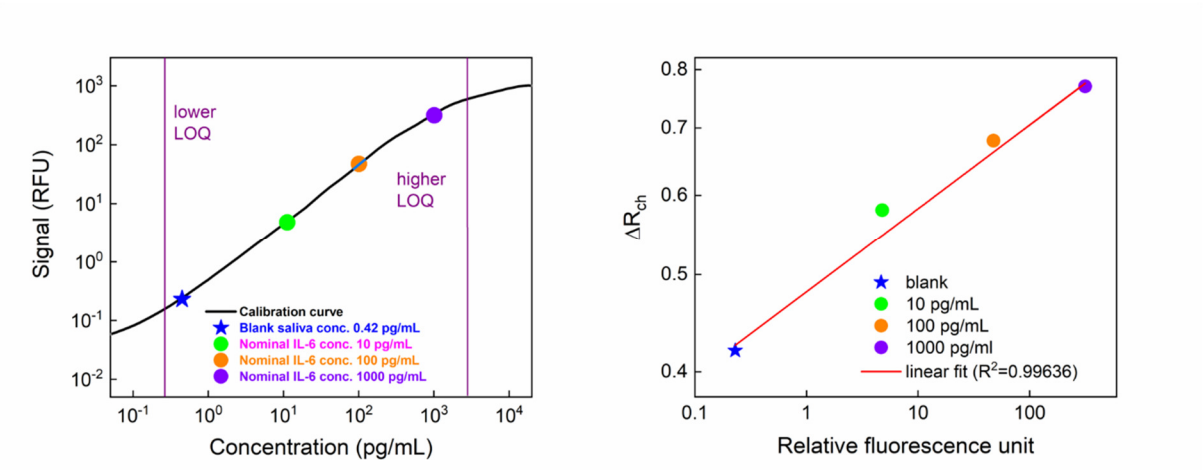

Figure S.2. (left) ELISA assay on saliva samples with blank saliva (concentration of 0.42 pg/mL, blue symbol) and saliva samples with nominal IL-6 concentrations of 10 (green symbol), 100 (orange symbol) and 1000 (violet symbol) pg/mL; (right) correlation plot between the SPE signal (ΔRch) and the ELISA signal (RFU). The red line represents the fitting curve to the response correlation plot.

- ***References***

[1] R. Reverberi, L. Reverberi, Factors affecting the antigen-antibody reaction, Blood Transfus. 5 (2007) 227–240. https://doi.org/10.2450/2007.0047-07.

[2] A. Cruz, M. Vieira, A.R. Mesquita, A. Sampaio, I. Mendes-Pinto, I. Soares, P.P. Freitas, Non-invasive diagnosis and monitoring tool of children's mental health: A point-of-care immunosensor for IL-6 quantification in saliva samples, Front. Neurosci. 16 (2022) 919551. https://doi.org/10.3389/fnins.2022.919551.