Electronic Supporting Information

Electrochemical-Digital Immunosensor with Enhanced Sensitivity and Specificity Detecting Human Salivary Glucocorticoid Hormone

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Electrochemical Equivalent Circuit:

According to the Randles model (Fig. S1B), the equivalent circuit includes ohmic resistance (R_s) of saliva sample, Warburg impedance (Z_w) resulted by the diffusion of ions from sample to micro-Au electrodes and electron-transfer resistance (R_{ct}) at low frequency (< 5Hz). There is an additional distribution of ions during EIS experiments which reflect the non-ideal behavior of a system and is commonly expressed as a C_{dl}. R_s and Z_w represent the features of saliva sample solution diffusion at the probe, while C_{dl} and R_{ct} depend on the insulating and dielectric properties at the interface of electrolyte and electrodes and are affected by the property change occurring at the electrode interface. R_{ct}, the electron transfer resistance is a parameter that can be observed at higher frequencies corresponding to the electron-transfer-limited process and can be measured as the diameter of the semicircle portion in the Nyquist plot as shown in Fig. S1C. Data presented in Fig. 3 shows the Nyquist plot run at 1 Hz to 10 kHz and the effect of ion diffusion can be seen at low frequency data points (<5Hz). The tail at the lower frequencies indicated the presence of diffusion limited electrochemical processes, represented using Z_w. At high frequency, the Z_w becomes negligible compared with R_{ct}. The intercept of the semicircle with the Z_{real} or the real axis at high frequency is equal to R_s.

Au electrode surface modification:

The decrease in impedance from post DTSP functionalization is observed due to the increase in charge conducting molecules bound to the electrode surface forming an electrically charged double layer. After anti-CAb immobilization, the impedance is increased which refers the binding of anti-CAb onto the DTSP SAM attached to Au electrodes. The increase in R_{ct} can be attributed to the non-conducting nature of anti-CAb that resists the charge transfer from solution to the electrode.

Finally, after treating BSA, the impedance is decreased which reflects the removal of unbounded anti-CAb molecules.

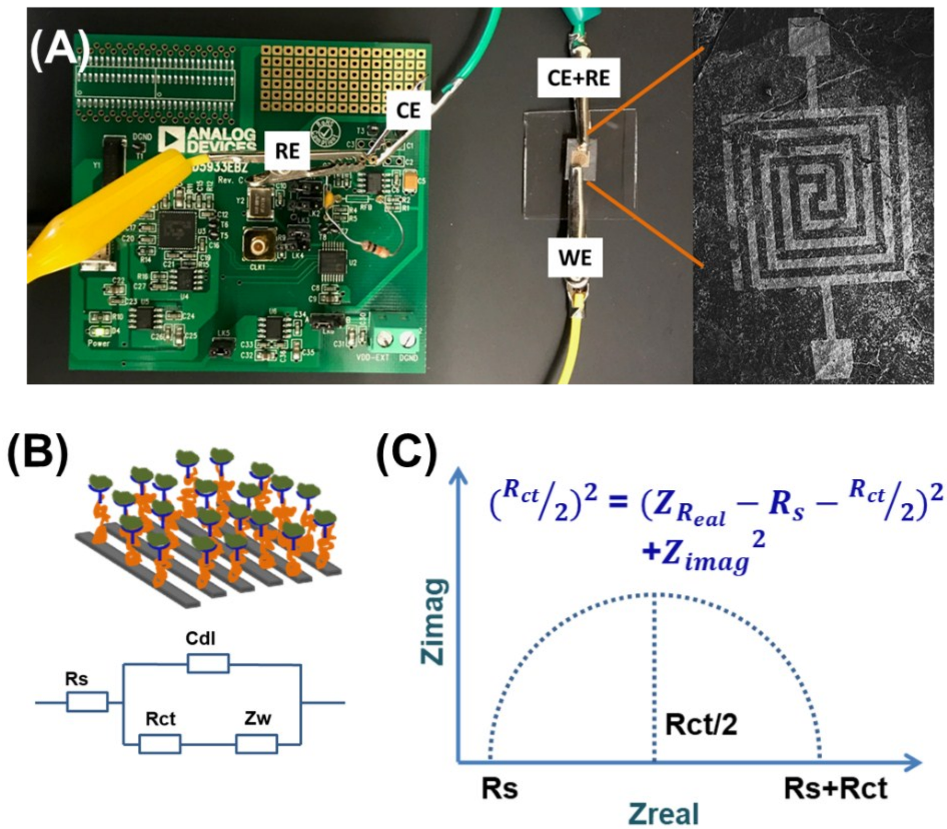

Figure S1. MSP Evaluation board interfaced with biosensor Chip. (A) Optical image of programmed AD5933 instrument purchased from Analog Devices Inc. connected with biosensor (left). SEM image of a tested biosensor chip (right). (B) Electrical equivalent circuit of immobilized anti-CAB on Au electrodes and cortisol antigen specifically linked with anti-CAB. (C) A typical Nyquist plot representing EIS.

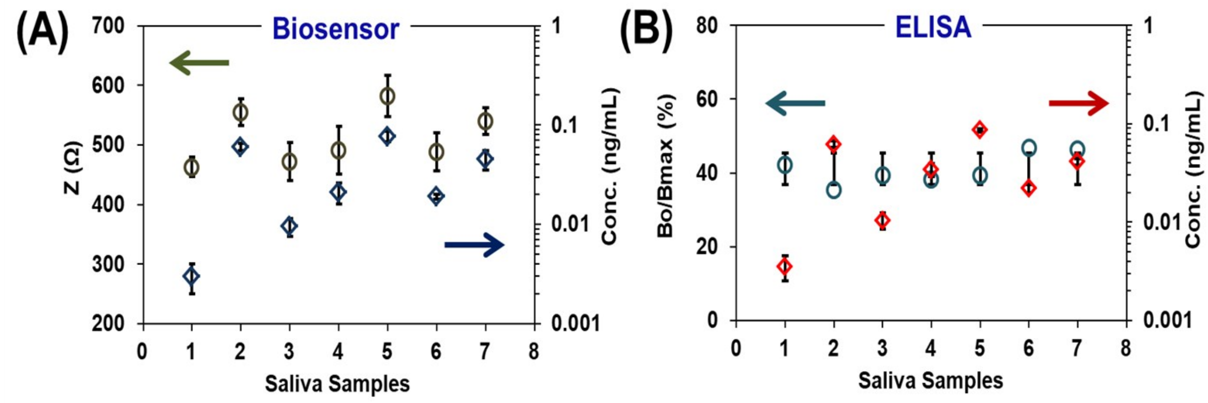

Fig. S2. (A) The net impedance corresponds to cortisol concentration 0.0031, 0.059, 0.0094, 0.0212, 0.0774, 0.0192 and 0.0450 ng/mL in respective samples. (B) ELISA tests to estimate the cortisol concentration (0.0036, 0.0622, 0.0104, 0.0349, 0.0860, 0.0221 and 0.0414 ng/mL) in the same human saliva samples.

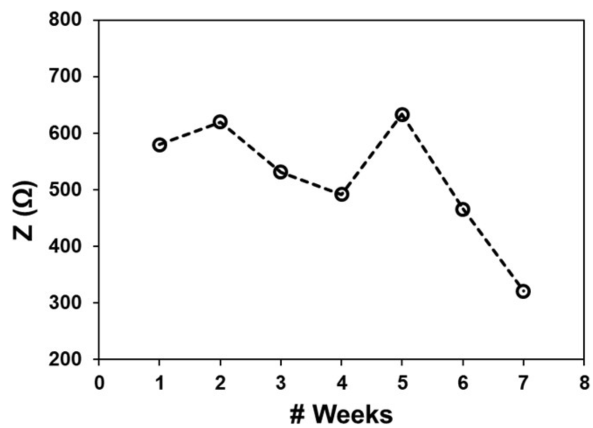

Fig. S3. Reproducibility tests over the period of six weeks. Cortisol aqueous solution 0.1 ng/mL was used and evaluated using an integrated biosensor device via handheld EIS instrument under same conditions.