Supporting Information for

Non-Invasive Label-Free Detection of Cortisol and Lactate Using Graphene Embedded Screen-Printed Electrode

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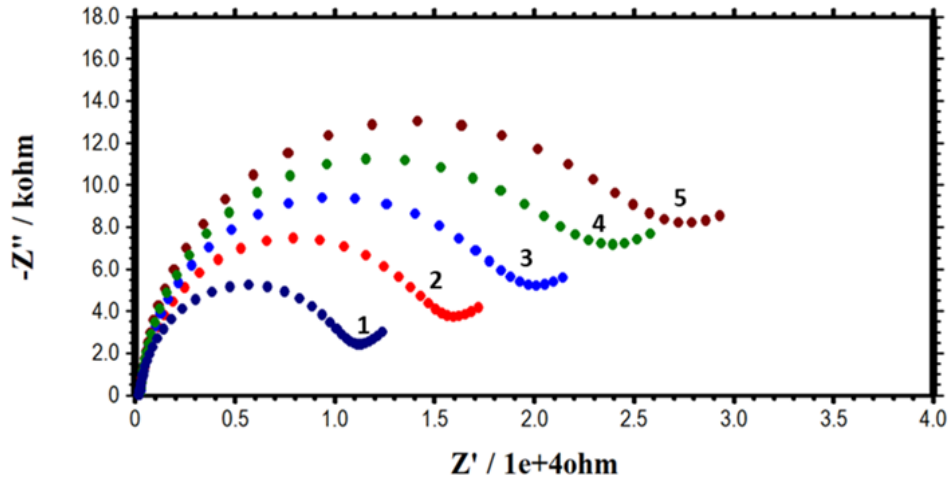
Supplementary Figures and Table



Modified Electrode	R_{ct} (diameter of semicircle)
1. e-RGO	9.52
2. Bare	13.59
3. GO	20.07
4. Ab@e-RGO	23.4
5. Ag+Ab@e-RGO	24.85

Fig. S1 The step by step modification of SPE using electrochemical impedance spectroscopy (EIS)

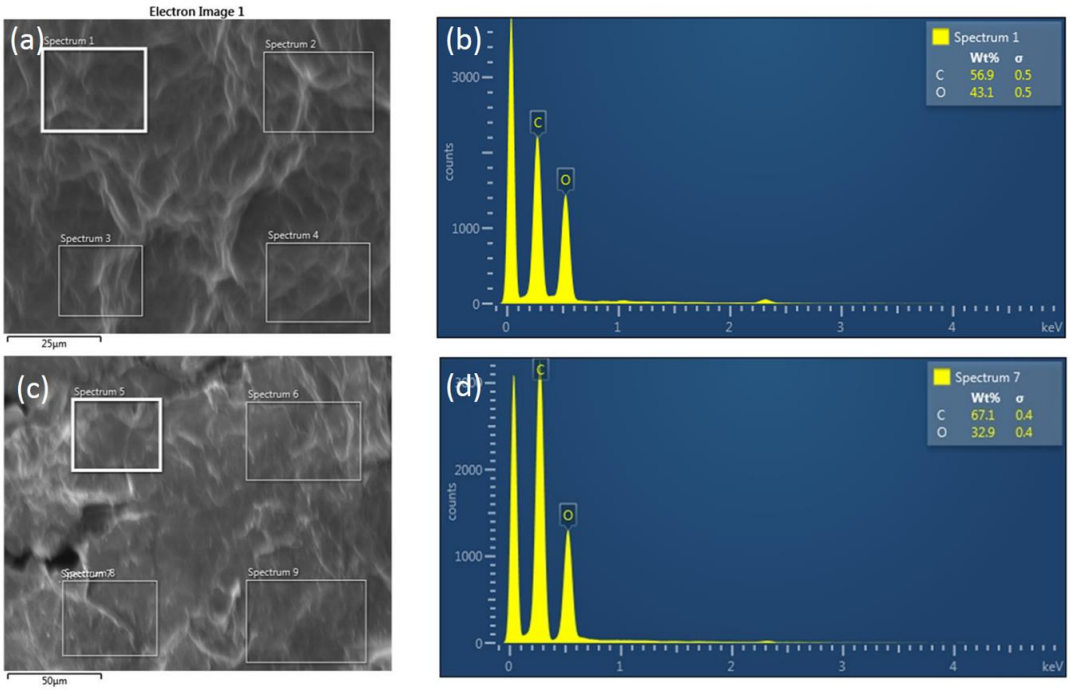


Fig. S2 a, c SEM microscopic images of GO and e-RGO. **b, d** EDS analyses of GO and e-RGO

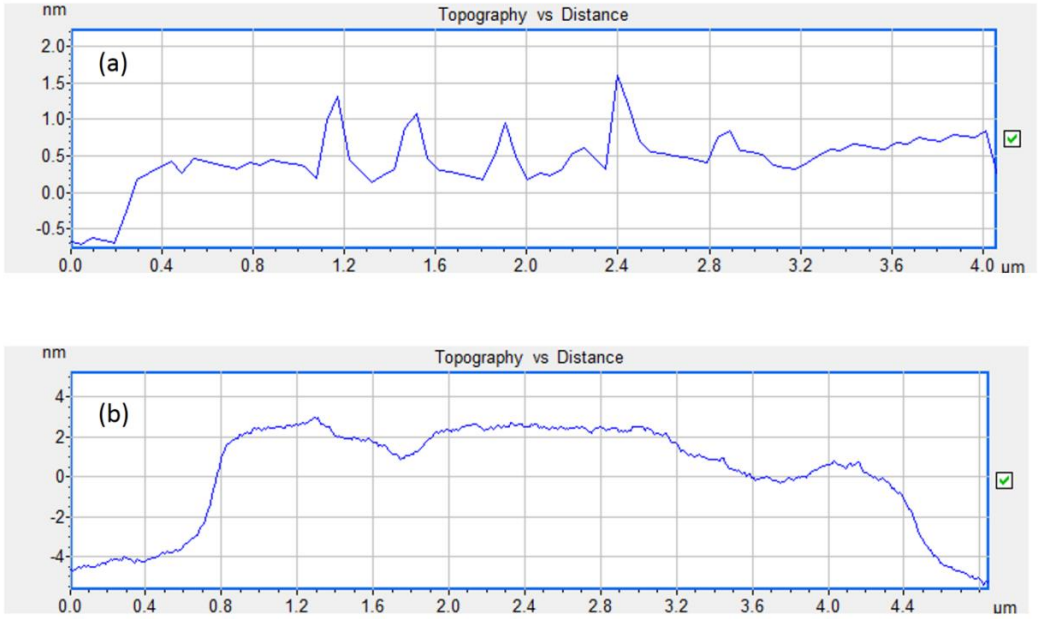


Fig. S3 a, b AFM line profile mapping of e-RGO deposited SPE surface

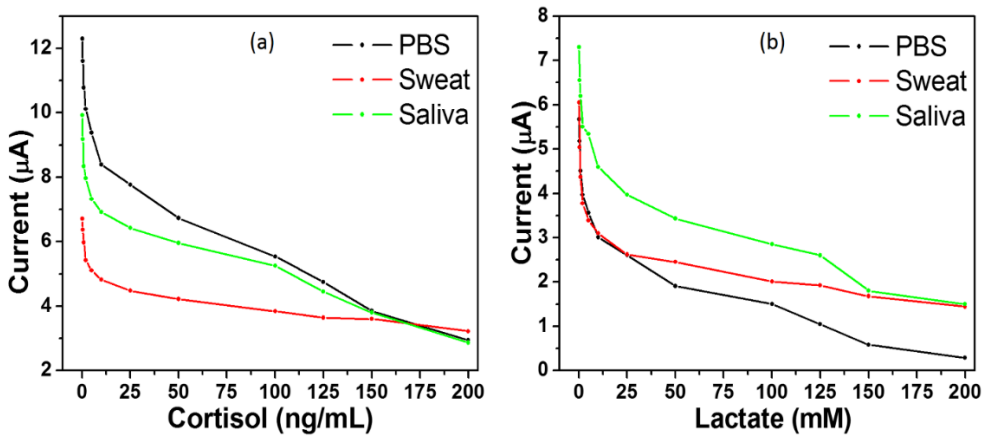


Fig. S4 a The calibration curves obtained from chronoamperometric responses of various cortisol antigen concentrations recorded using anti cortisol Ab@e-RGO SPE in standard PBS buffer, spiked sweat and saliva solution. **b** The calibration curves obtained from chronoamperometric responses of various lactate antigen concentrations recorded using anti lactate Ab@e-RGO SPE in standard PBS buffer, spiked sweat and saliva solution. The curves represent the decreasing relationship between current and antigen concentration generated by the insulating effect of the antigen-antibody complex.

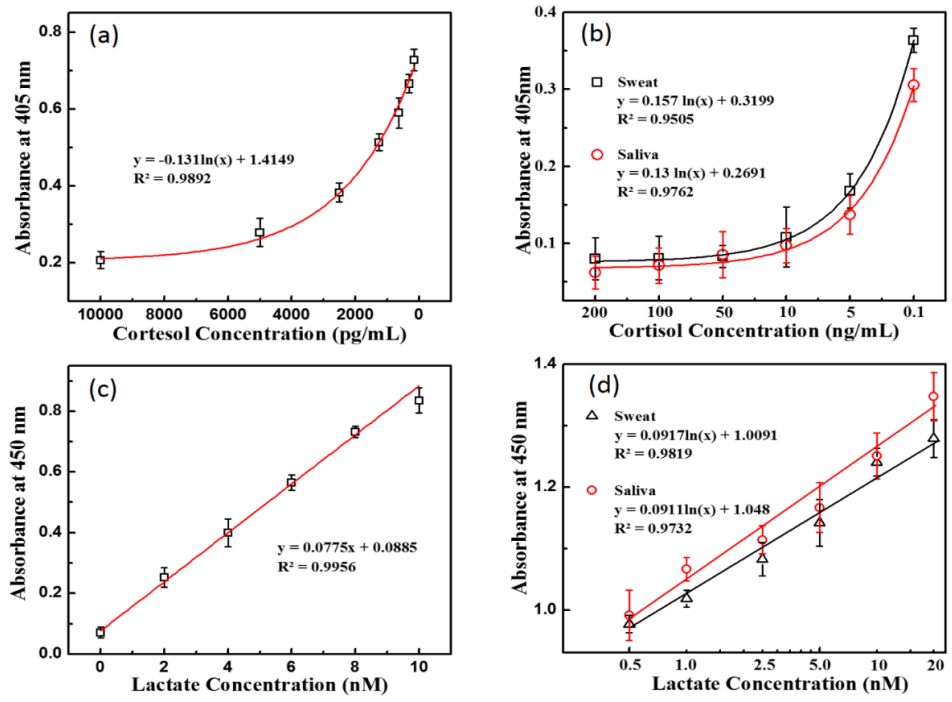


Fig. S5 a Analysis of standard samples with known concentrations of cortisol using color based absorbance (at 450 nm) with a cortisol commercial colorimetric kit. **b** Analysis of cortisol spiked sweat and saliva samples using cortisol commercial colorimetric kit. **c** Analysis of standard samples with known concentrations of lactate using color based absorbance (at 450 nm) with a lactate commercial colorimetric kit and **d** Analysis of lactate spiked sweat and saliva samples using lactate commercial colorimetric kit

Table S1 Comparison with existing reports

Target	Platform	Technique	LOD	Ref.
Cortisol	SPR based anticortisol modified HC 80 on Au disk	Surface plasmon resonance	4 μg mL^{-1}	[1]
Cortisol	glucose oxidase (GOD)-cortisol conjugated labeled sensor	Calorimetric Chromatography	1 ng mL^{-1}	[2]
Cortisol	LFIA with anti-CAB	Chemiluminescence	0.3 ng mL^{-1}	[3]
Cortisol	1D ZnO NRs and 2D ZnO NFs	Electrochemical	0.36 mg mL^{-1}	[4]
Lactate	Chitosan/MWCNTs with LOx	Electrochemical	22.6 μM	[5]
Lactate	TiO_2-NPs, rGO with Lox	Electrochemical	0.6 μM	[6]
Lactate	Prussian	Electrochemical	0.01 mM	[7]
Lactate	HRP with LOx	Amperometric	10 mmol L^{-1}	[8]
Lactate	CNT-FET with LOx	Electrical	1 pM	[9]
Cortisol and Lactate	e-RGO dual working area	Amperometric Detection	0.1 ng mL^{-1} 1 mm	Present work

Validation with Commercial ELISA Kit

The proposed sensing assays were validated using the commercial lactate colorimetric assay kit II (Cat. no. K627-100, BioVision Incorporated, CA, USA) and cortisol ELISA kit (Cat. no. ADI-900-071, Enzo Life Sciences Inc., NY, USA). At first, different concentrated solutions of both lactate and cortisol were prepared using the sample diluent that was received with the commercial ELISA kits and the manufacturer's assay protocol was strictly maintained during the bioassay. Then, the same concentrated solutions of lactate and cortisol were prepared in complex media, i.e., sweat and saliva, and the assay protocol, as mentioned in the booklet, was strictly followed. The yellow color developed with different absorbance intensity related to the lactate and cortisol concentration, which was recorded at 450 nm in the 96-well plates. The sensitivity of the proposed electrochemical sensor was validated with the commercial colorimetric detection kit for both lactate and cortisol. The visual color response of the commercial kit was up to 0.1 ng mL^{-1} and 0.5 nM for cortisol and lactate respectively, indicating that the prototype electrochemical method is sensitive and consistent with the commercial kits. The results are depicted in Fig. S5.

References

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