Supplementary Information (SI) for Analyst.

Facile and Scalable Fabrication of Molecularly Imprinted Polymer (MIP) Sensors on Poriferous Laser-Engraved Graphene Electrodes for Stress Monitoring

Atul Sharma [a,b], Rachel E. Owyeung [a,b], Ayanna Thomas [c], Sohel Siraj [d], Bishal Kumar Keshari [d], Parikshit Sahatiya [d], and Sameer Sonkusale [a,b*]

[a] Sonkusale Research Lab, Advanced Technology Laboratory, Tufts University, USA

[b] Department of Electrical and Computer Engineering, Tufts University, USA

[c] Department of Psychology, Tufts University, Medford 02155, MA, USA

[d] Department of Electrical and Electronics Engineering, BITS Pilani Hyderabad, Secunderabad, 500078, India

*Correspondence: sameer@ece.tufts.edu

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Figure S1. Fabrication of LEG electrodes

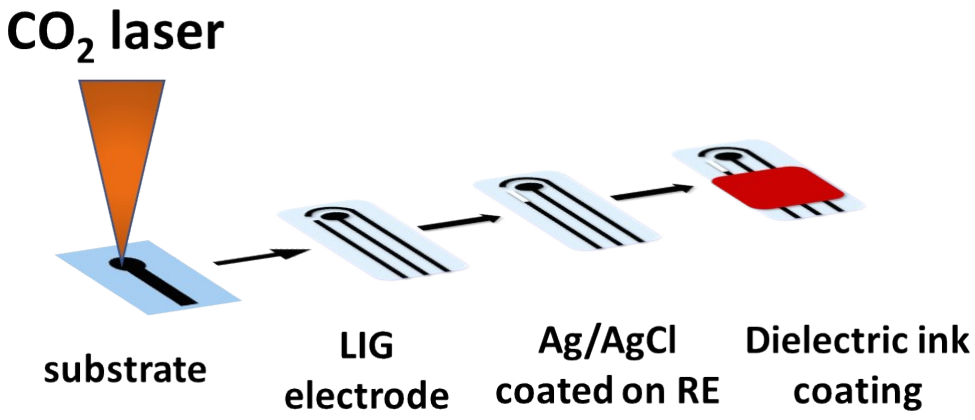

Figure S1: Step-by-step fabrication of laser-induced graphene (LEG) electrode using carbon ablation technique (CO_2 laser-induced graphene electrode). WE: 0.5 mm (diameter of circle) and 0.5 mm width of tail; CE: 0.5 mm width; RE: 0.5 mm width)

Supplementary Table 1 (ST1): A summary of the list of parameters used for carbonization of the Polyimide (PI) sheet for LEG electrode fabrication.

Substrate	**Support**	**Laser**	**Mode cut (Raster)**	**Power (W)**	**Speed (%)**	**No. Passes**	**Defocus (mm)**	**Resistance (ohm/ cm)**
Polyimide (PI)	PET with silicone adhesive	1	Raster	35	5.25	1	0.50	N/A
Polyimide (PI)	PET with silicone adhesive	1	Raster	35	7.25	1	0.50	70
Polyimide (PI)	PET with silicone adhesive	1	Raster	35	7.5	1	0.50	100
Polyimide (PI)	PET with silicone adhesive	1	Raster	30	7.5	1	0.50	80
Polyimide (PI)	PET with silicone adhesive	1	Raster	30	7.5	1	0.25	78
Polyimide (PI)	PET with silicone adhesive	1	Raster	30	5.25	1	0.50	70
Polyimide (PI)	PET with silicone adhesive	1	Raster	25	5.25	1	0.50	55
Polyimide (PI)	**PET with silicone adhesive**	**1**	**Raster**	**22**	**5.25**	**1**	**0.50**	**35**

Note- Nitrogen (N_2) gas flow 50%

Figure S2: XRD Characterization

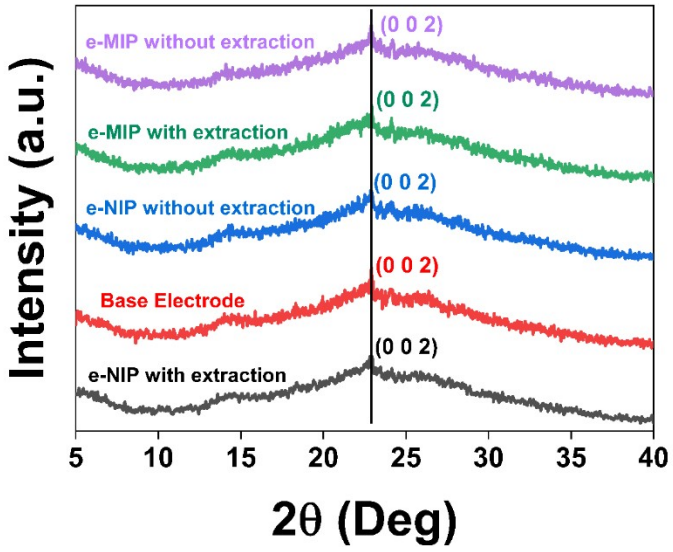

Figure S2. XRD patterns of e-NIP with extraction, base electrode, e-NIP without extraction, e-MIP with extraction and e-NIP without extraction.

Figure S3: XPS- eNIP without template extraction

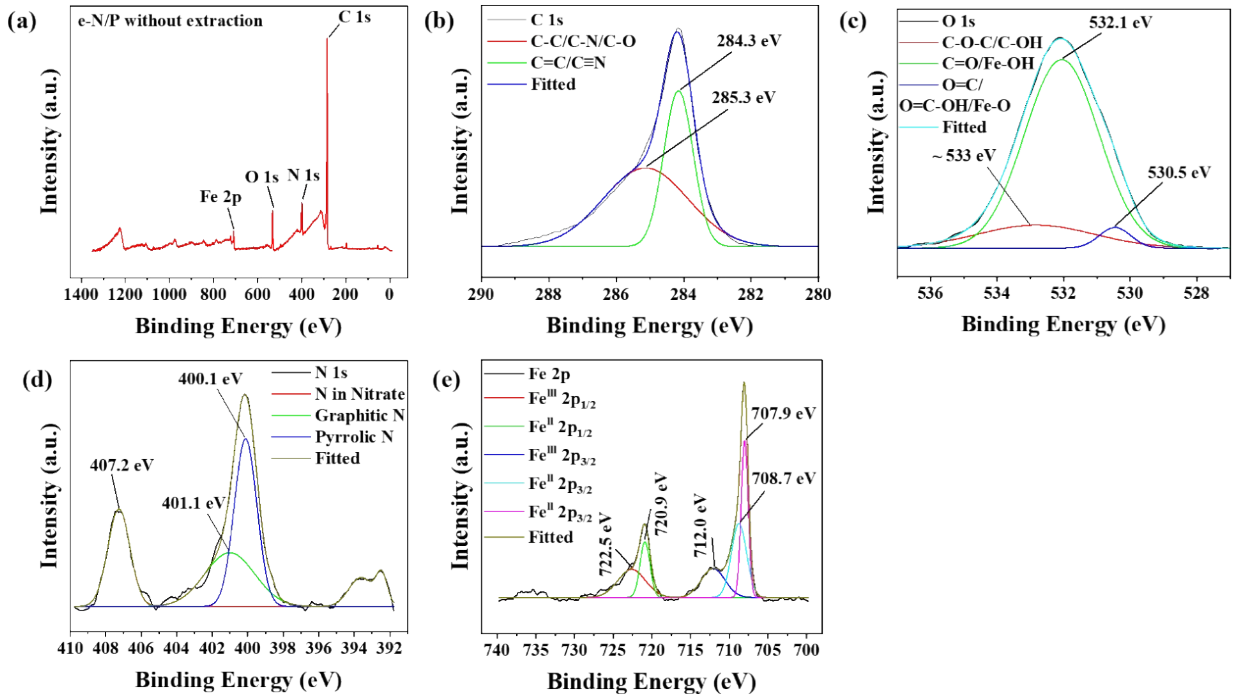

Figure S3. High-resolution XPS survey and deconvoluted spectra for e-NIP without cortisol (template) extraction.

Figure S4: XPS- eMIP without template extraction

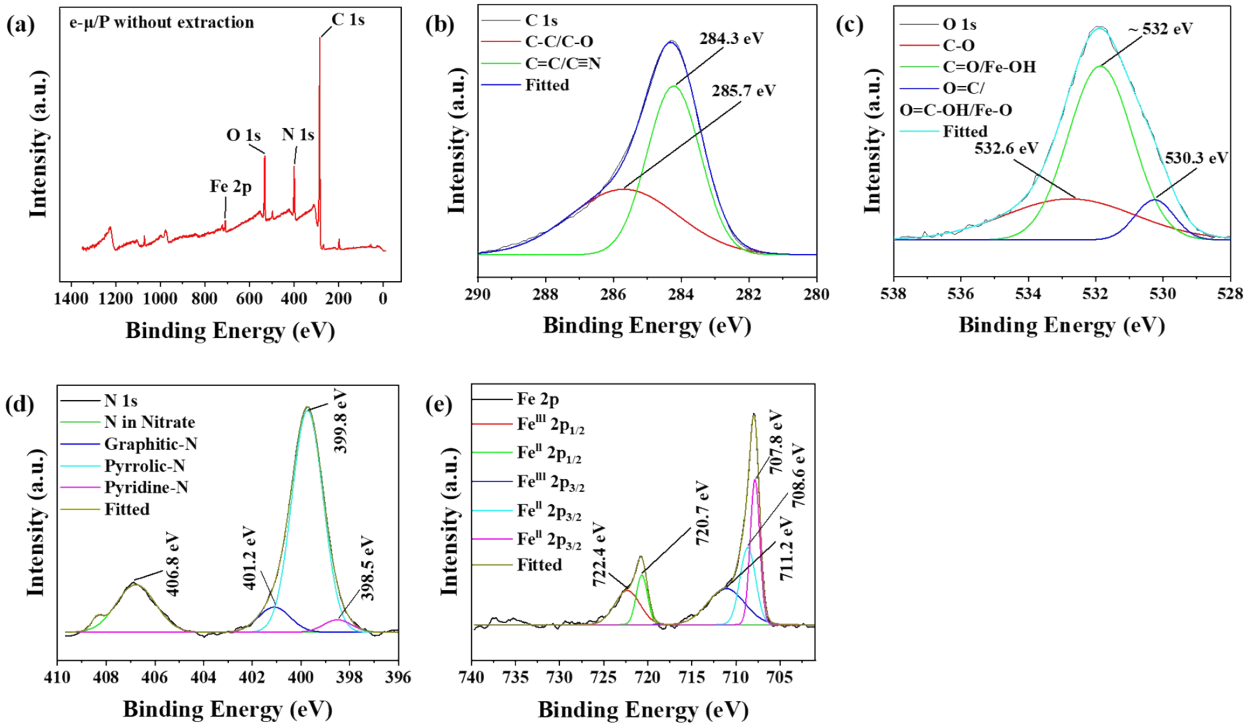

Figure S4. High-resolution XPS survey and deconvoluted spectra for e-MIP sensor without cortisol (template) extraction.

Supplementary Table 2 (ST2): XPS peak binding energy assignment and functional groups percentages on bare, cort-eNIP and cort-eMIP sensors (before and after cortisol extraction) procedure.

Sample 1: Base electrode			
C1S	C-C/C-O/C≡N	285.6 eV	43.91%
	C=C	284.8 eV	56.08%
O1S	C-O-C/C-OH	533.6 ev	16.69%
	C-O	532.8 eV	67.66%
	C=O/Fe-OH	531.9 eV	15.63%
Sample 2: e-NIP without extraction			
C1S	C-C/C-N/C-O	285.3 eV	59.40%
	C=C/C≡N	284.3 eV	40.59%
O1S	C-O-C/C-OH	533 eV	25.40%
	C=O/Fe-OH	532.1 eV	40.83%
	O=C/O=C-OH/FeO	530.5 eV	33.75%
N1S	N in Nitrate	407.2 eV	25.05%
	Graphitic N	401.1 eV	31.11%
	Pyrrolic N	400.1 eV	43.83%
Fe2P	Fe3 2p 1/2	722.5 eV	18.34%
	Fe2 2p 1/2	720.9 eV	13.07%
	Fe3 2p 3/2	712.0 eV	16.23%
	Fe2 2p 3/2	708.7 eV	26.51%

	Fe2 2p 3/2	707.9 eV	25.83%
Sample 3: e-NIP with extraction			
C1S	C-C/C-N/C-O	285.9 eV	13.04%
	C=C	284.7 eV	47.27%
	C=O	286.7 eV	35.91%
	π-π*	291.6 eV	3.76%
O1S	C=O/Fe-OH	531.1 eV	30.43%
	C=C	532.2 eV	37.80%
	C-O-C	533 eV	31.75%
N1S	Pyridine N	397.3 eV	14.78%
	Pyrrolic N	399.4 eV	48.00%
	Graphitic N	400.7 eV	37.21%
Fe2P	Fe3 2p 1/2	724.8 eV	3.87%
	Fe2 2p 1/2	720.9 eV	0.40%
	Fe3 2p 3/2	714.3 eV	2.36%
	Fe3 2p 3/2	711.3 eV	90.33%
	Fe2 2p 3/2	708.3 eV	2.39%
	Fe2 2p 3/2	707.5 eV	0.62%
Sample 4: e-MIP without extraction			
C1S	C-C/C-O	285.7 eV	43.90%
	C=C/C≡N	284.3 eV	56.09%
O1S	C-O	532.8 eV	29.59%
	C=O/Fe-OH	532 eV	61.70%
	O=C/O=C-OH/FeO	530.3 eV	8.70%
N1S	N in Nitrate	406.8 eV	18.75%
	Graphitic N	401.2 eV	8.02%
	Pyrrolic N	399.8 eV	69.74%
	Pyridine N	398.5 eV	3.48%
Fe2P	Fe3 2p 1/2	722.4 eV	16.11%
	Fe2 2p 1/2	720.7 eV	10.35%
	Fe3 2p 3/2	711.2 eV	25.04%
	Fe2 2p 3/2	708.6 eV	22.61%
	Fe2 2p 3/2	707.8 eV	25.87%
Sample 5: e-MIP with extraction			
C1S	C-C/C-N/C-O	285.8 eV	40.73%
	C=C/C≡N	284.4 eV	59.26%
O1S	C-O	532.7 eV	29.86%
	C=O/Fe-OH	532 eV	55.13%
	O=C/O=C-OH/FeO	530.4 eV	14.99%
N1S	Graphitic N	401.5 eV	3.69%
	Pyrrolic N	399.7 eV	51.58%
	Pyridine N	398.8 eV	26.06%

	Fe2(CN)x	397.1 eV	18.66%
Fe2P	Fe2 2p 1/2	720.9 eV	9.41%
	Fe3 2p 3/2	711.6 eV	38.65%
	Fe2 2p 3/2	708.0 eV	25.91%
	Fe3 2p 1/2	723.3 eV	26.02%

Figure 5: AFM topographic studies

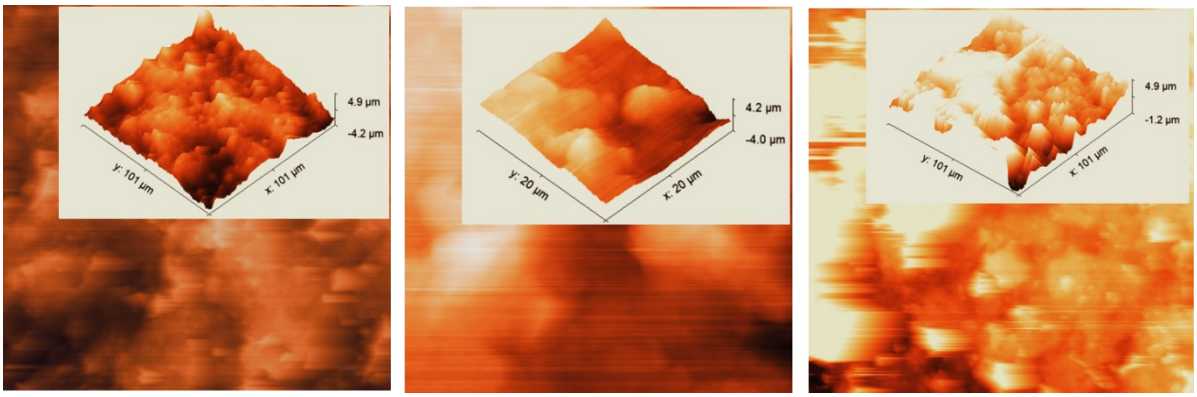

Figure S5. AFM microscopic images of (a) Cort-eNIP, (b) cort-eMIP before template extraction, and (c) cort-eMIP after template extraction. Inset showing the 3D images.

Figure S6: Effect of scan rate

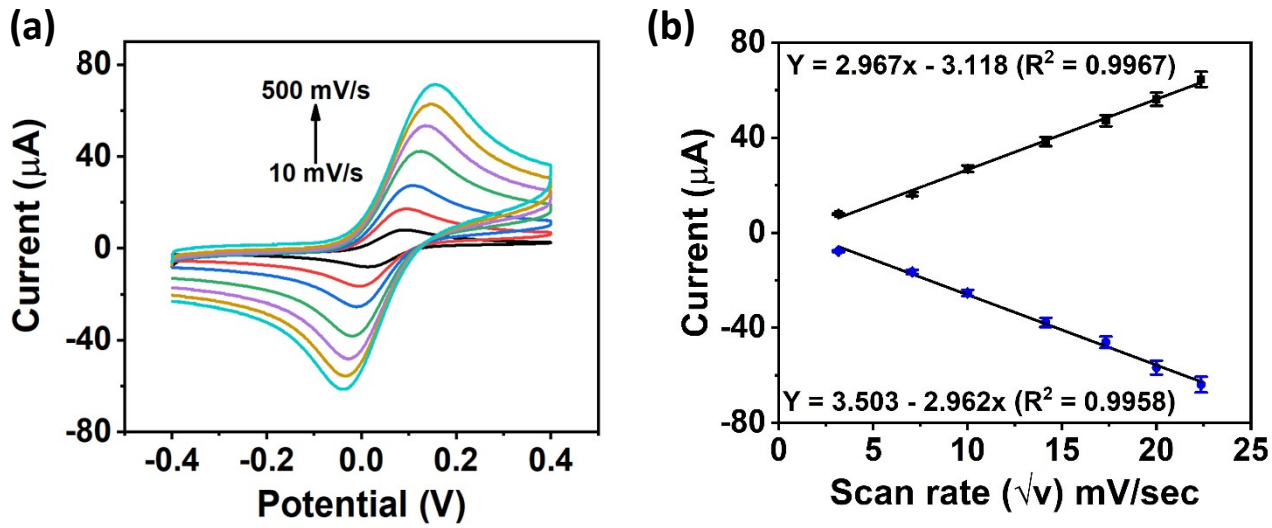

Figure S6: Measured voltammograms at different scan rates (10 mV/sec to 500 mV/sec) in the presence of 1.0 mM ferro/ferri cyanide in PBS (0.10 M, pH 7.4). Each measurement was performed in triplicate.

Figure S7: Effect of incubation time

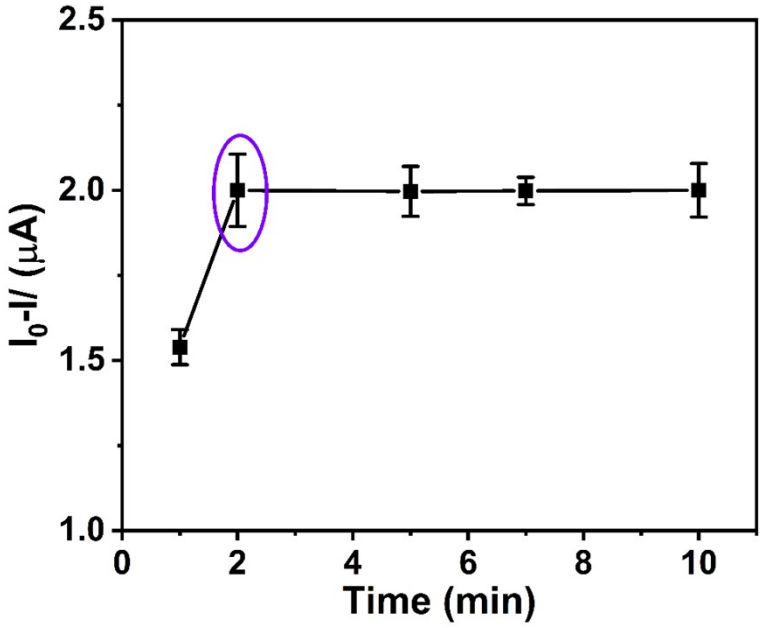

Figure S7: Effect of incubation time on the cort-eMIP/LEG sensor strip for cortisol detection at 1.0 pg mL^{-1} cortisol in 0.10 M phosphate binding buffer (PBS, 0.10 M, pH 7.4). Each measurement was performed in triplicate.

Figure S8: Effect of pH

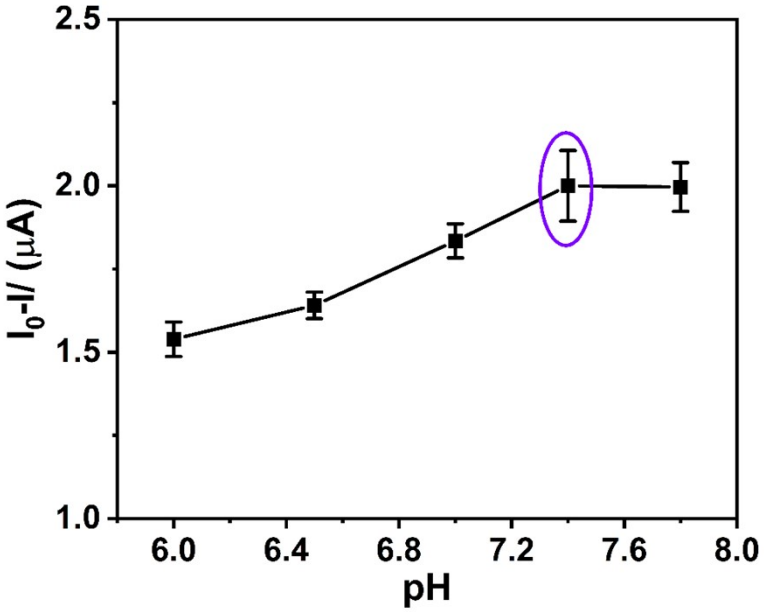

Figure S8: Effect of pH on the cort-eMIP/LEG sensor strip for cortisol detection at 1.0 pg mL^{-1} cortisol in 0.10 M phosphate binding buffer (PBS, 0.10 M, pH 7.4). Each measurement was performed in triplicate.

Figure S9: Effect of monomer concentration

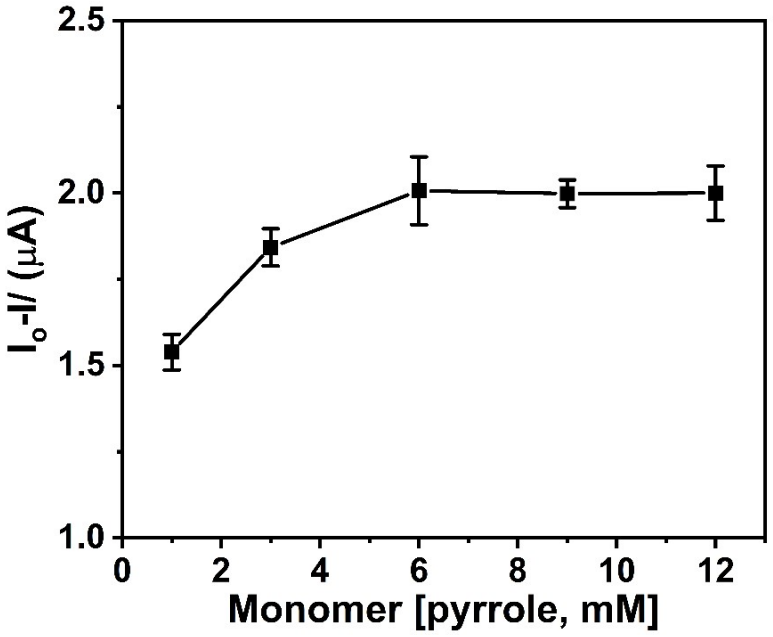

Figure S9: Effect of monomer concentration (pyrrole) on the binding capacity of cort-eMIP/LEG sensor strip for cortisol detection at 1.0 pg mL^{-1} cortisol in 0.10 M phosphate binding buffer (PBS, 0.10 M, pH 7.4). Each measurement was performed in triplicate.

Figure S10: Effect of monomer: template ration

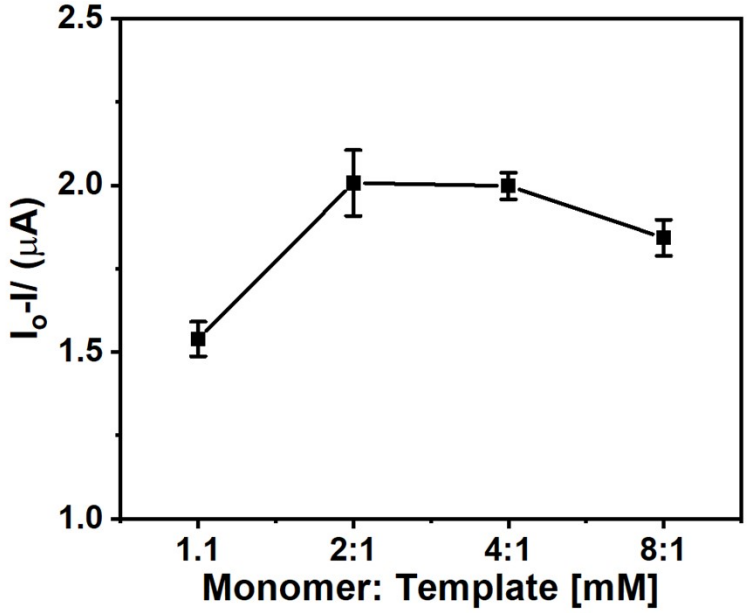

Figure S10: Effect of monomer: template ration (pyrrole: cortisol) concentration on the binding capacity of cort-eMIP/LEG sensor strip for cortisol detection at 1.0 pg mL^{-1} cortisol in 0.10 M phosphate binding buffer (PBS, 0.10 M, pH 7.4). Each measurement was performed in triplicate.

Supplementary Table 3 (ST3). Comparison of analytical performance between our proposed method and other methods for the detection of salivary cortisol

S. No	Method	Material	Linear range	LOD	Sample	Ref.
1.	SWV	1-nalphtyl phosphate (1-NP)/SPE*	0.2−44.6 ng mL^{-1}	0.6 ng mL^{-1}	Saliva	[1]
2.	EIS	d-BSA/ rGO*	0.01–100 nM	–	Saliva	[2]
3.	DPV	Nanostructured NiO film	0.001−10 µg mL^{-1}	0.32 pg mL^{-1}	Saliva	[3]
4.	DPV	Metalloporphyrin /MWCNT	50 fM to 100 nM	–	Saliva	[4]
5.	CV	Graphene-pyrrole	0.5 to 5.0 ng	0.5 ng	Artificial	[5]

			mL^{-1}	mL^{-1}	saliva	
6.	**CA**	**Cort-eMIP-PPy/LEG electrode**	**0.10 to 10,000 pg mL^{-1}**	**0.08 pg mL^{-1}**	**Saliva**	**This work**

Note: SWV- square wave voltammetry; DPV- differential pulse voltammetry; CV- cyclic voltammetry; CA- chronoamperometry; EIS- electrochemical impedance spectroscopy; SPE- screen printed electrode; MWCNT- multiwall carbon nanotubes; MIP- molecularly imprinted polymer.

Supplementary Table 4 (ST4): Validation of developed cort-eMIP sensor strip with ELISA and Salimetric (third party) testing for Human Saliva Samples collected as part of the study.

Sample No	**Stress level (Time point)**	**Cortisol concentration found (μg/dL) (Salimetric* data)**	**Cortisol concentration found (μg/dL) (Cortisol Sensor data)**	**Cortisol concentration found (μg/dL) (Cortisol-ELISA data)**	**% Relative error (Sali to Sensor data)**	**% Relative error (ELISA to Sensor data)**	**% Relative error (ELISA to Sali data)**
SS-1	Baseline	0.207	0.199	0.207	3.86	3.73	-0.15
SS-2	Immediately pre-control	0.206	0.199	0.205	3.64	3.38	-0.27
SS-3	Immediately post-control	0.193	0.195	0.203	-1.04	3.94	4.92
SS-4	20min post-control	0.186	0.179	0.188	3.76	4.79	1.06
SS-5	Baseline	0.212	0.202	0.213	4.72	5.16	0.47
SS-6	Immediately pre-control	0.18	0.171	0.176	5.00	2.82	-2.30

SS-7	Immediately post-control	0.153	0.148	0.154	3.40	4.03	0.65
SS-8	20min post-control	0.124	0.119	0.123	4.03	3.38	-0.68
SS-9	Baseline	0.106	0.106	0.1	0.107	5.66	6.54
SS-10	Immediately Pre-Stress	0.108	0.108	0.0986	0.105	8.70	6.10
SS-11	Immediately Post-Stress	0.096	0.096	0.098	0.10	-2.08	2.00
SS-12	20min post-Stress	0.178	0.178	0.172	0.176	3.37	2.27

*** SS- Saliva sample; Salimetric is a third-party commercial service provider for independent measurement.**

References

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