Supplementary Information

1. Reagents

1 mg/mL solution of cortisol in methanol and rabbit derived anti-cortisol antibody (Ab) were procured form Sigma-Aldrich (USA). Working solutions of cortisol with concentrations varying in the range from 1 fg/mL to 1 μg/mL were prepared in a pH 7.5 and 50 mM PBS (Phosphate buffer saline) consisting of 0.9% NaCl. 50mM Tris buffer (Tris(hydroxymethyl) Aminomethane) comprising of 150 mM NaCl and pH adjusted to 7.5 was used for further dilution of the Ab. Tris buffer, monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) used in preparation of buffer solutions were all procured from SRL Pvt. Ltd. (India). PBS of varying pH values: 6, 6.5, 7, 7.5 and 8 was prepared by varying the ratio of NaH_2PO_4 and Na_2HPO_4. APTES ((3-Aminopropyl) triethoxysilane) and EDC-NHS (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, N-Hydroxysuccinimide) were purchased from Alfa-Aesar (UK) and Sigma-Aldrich (USA) respectively. The working solutions of cortisol and all the buffer solutions when not in use were stored at 4 °C. The prepolymer and the curing agent for polydimethylsiloxane (PDMS) were acquired from Dow Corning (Sylgard 184). Photoresists S1813 and SU8-2010 along with SU8 developer were purchased from Microchem, USA.

2. Device design

The process flow for the fabrication of EGFET has been depicted in Figure S1. The standard photolithography process was employed for constructing the two electrodes using the mask I. The adhesion of Pt over the glass substrate was improved by depositing in-situ a buffer layer of Titanium (Ti) beneath it. NiO thin film was deposited covering the channel of length 1 mm and 2 mm over the drain and source electrodes using the shadow mask II by employing rf sputtering technique and varying the oxygen content in the deposition gas ambient as 50%, 75% and 100%. A 100 nm thick SiO_2 passivation layer was deposited (through shadow mask III) using plasma enhanced chemical vapor deposition (PECVD)

technique over drain and source electrodes to avoid leakage current due to direct contact between them through the electrolyte.

3. Fabrication of Microfluidic system

A master mold of silicon (Si) was first prepared for the channel design having length of 1 cm, width 300 μm and height 40 μm. SU8 photoresist was spin coated on a thoroughly cleaned and dried silicon wafer. The wafer was exposed to UV light through the 1 channel mask shown in Figure 1 (d) after soft baking for 30 minutes at 90 °C. The pattern was then developed using SU8 developer. Rinsing with iso-propyl alcohol yields the desired mold. Next, PDMS was prepared by mixing the prepolymer with the curing agent in the ratio of 10:1 (Eteshola and Leckband, 2001). The mix was degassed in a vacuum desiccator and poured over the mold. It was solidified by curing at 80 °C for 1 hour. After cooling down to room temperature, the solidified PDMS was removed from the mold and holes were punched for the inlet and outlet. The PDMS was bonded with the previously prepared FET structure by Van der Waals forces. The combinations of two channels and three channels were also prepared following the same procedure (Figure 1 (d)).

4. Cortisol sensing Measurements

The anti-cortisol antibody (Ab) was immobilized over the NiO channel via covalent linkage using EDC-NHS binding agents (Dhull et al., 2019). Prior to immobilization, the device was methodically washed in an ultrasonic bath using trichloroethylene, acetone and isopropyl alcohol successively to remove all pollutants from the surface. The NiO surface was hydroxylated for 30 min at 80 °C using equal portions of ammonia solution and H_2O_2 diluted in de-ionized (DI) water. The NiO channel was then cleaned with DI water and dried using nitrogen gun. Then silanization was carried out overnight at room temperature using 1 % solution of APTES in toluene. The unbound silanes from the NiO surface were removed by rinsing with toluene. This was followed by functionalization of the channel using 0.4 M EDC and 0.1 M

NHS solution. 10 µg/mL concentration of the Ab (volume: 10 µL) was then dropped over NiO and provided an incubation of 3 h at 27 °C. Afterwards, the device was washed meticulously using PBS to remove any loosely bound or unbound antibody molecules. For cortisol measurement, 20 µL of each concentration in PBS was dropped over the Ab/NiO surface and a sufficient time of 2 h incubation at 27 °C was given for interaction of cortisol with Ab molecules. The device was stored at 4 °C when not in use.

5. Structural characterization

The XRD patterns of NiO thin film, as depicted in Figure S3, reveals well defined diffraction peaks at 2θ values of 38.28°, 44.48°, 64.89° and 78.04° corresponding to the (111), (200), (220) and (222) crystalline planes, respectively (JCPDS file #00-001-1239). It is noteworthy that for the NiO film deposited at 50% O_2 gas ambient, the (200) plane is dominant. As the O_2 content is increased to 100%, the (111) plane becomes dominant. Thus, the XRD spectra confirms the growth of polycrystalline NiO thin film in cubic phase.

6. Morphological characterization

The AFM images for bare NiO thin film deposited over glass substrate in 100% oxygen gas ambient, after immobilization of Ab and after binding of cortisol with Ab/NiO/glass FET structure are shown in Figure S4. The images confirm the growth of a uniform nanostructured NiO thin film over glass substrate using rf sputtering technique having a rms surface roughness of 3 nm. AFM image taken after antibody immobilization unambiguously exhibits the adequate and uniform immobilization of the biomolecules on NiO with rms roughness increased to 11 nm. Further, on incubation of the Ab/NiO/glass with cortisol, evenly distributed structures can be clearly seen. The surface roughness decreased to 5 nm due to presence of an overlayer of cortisol over Ab/NiO/glass.

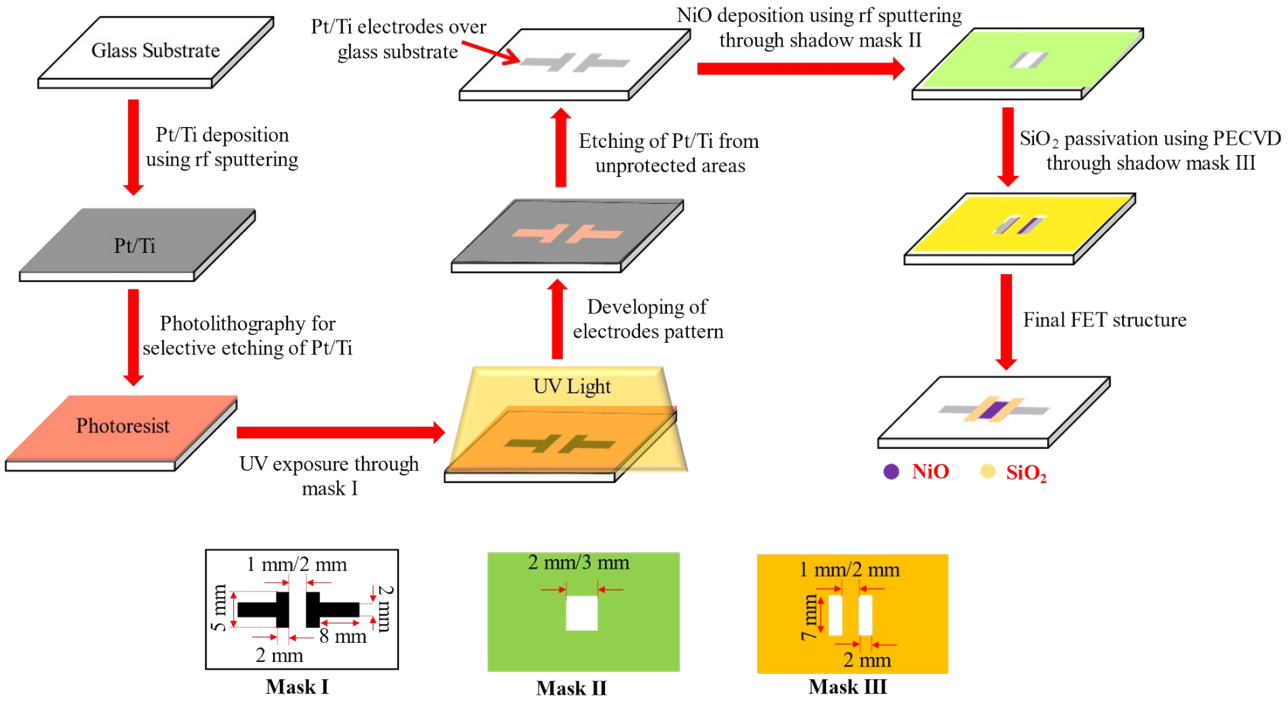
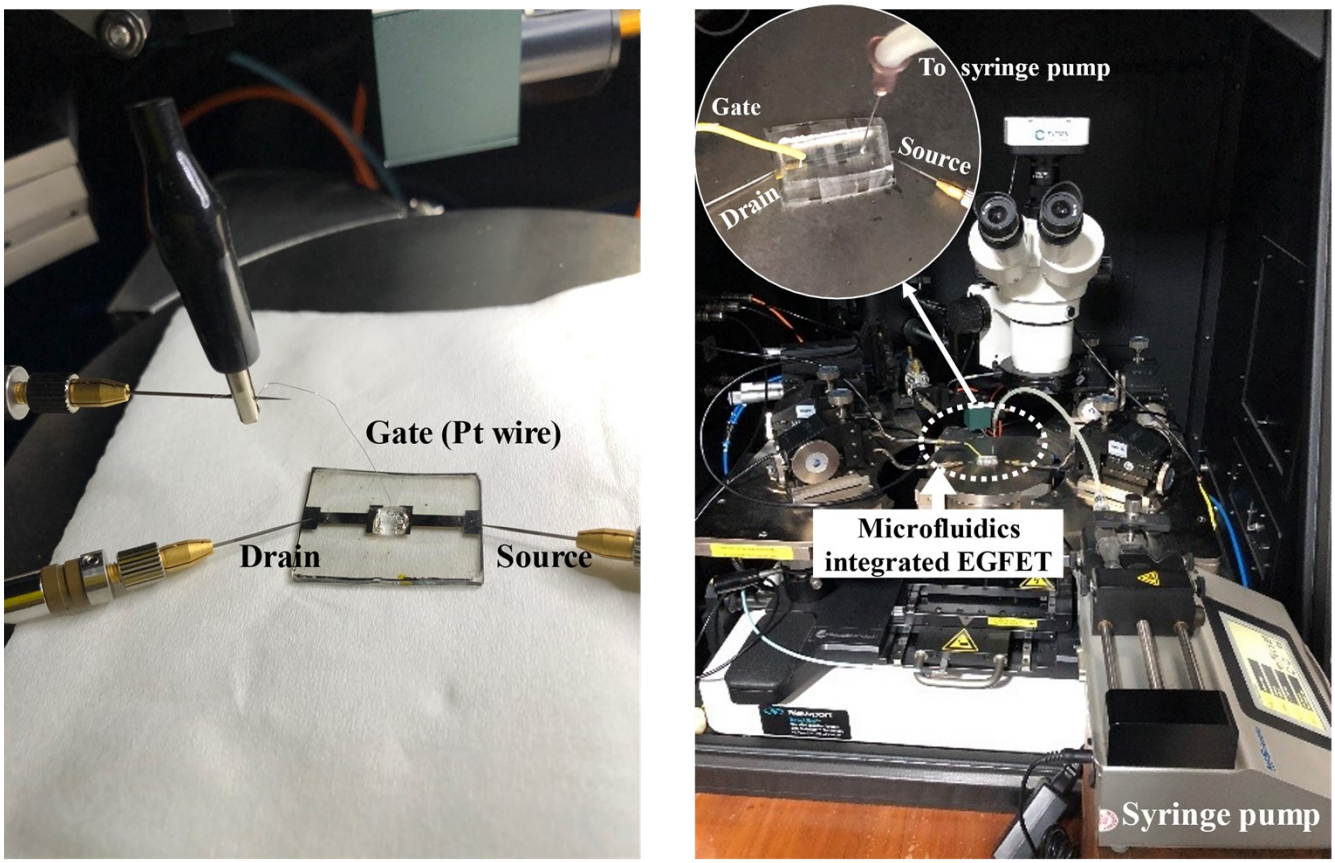


Figure S1: Process flow for the fabrication of FET strucutre.



(a) (b)

Figure S2: Images for the measurement setup of (a) FET device with electrolyte gating by platinum wire, (b) EGFET device integrated with microfluidic system (inset shows the zoomed-in top-view).

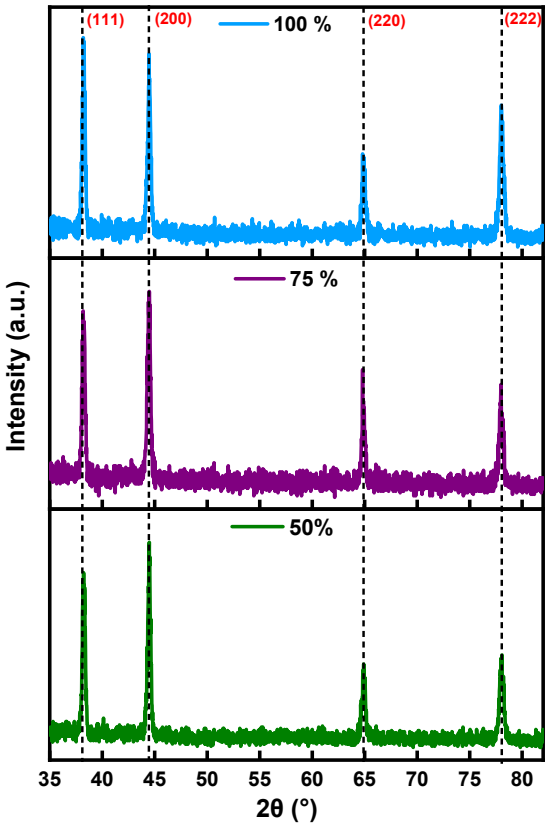
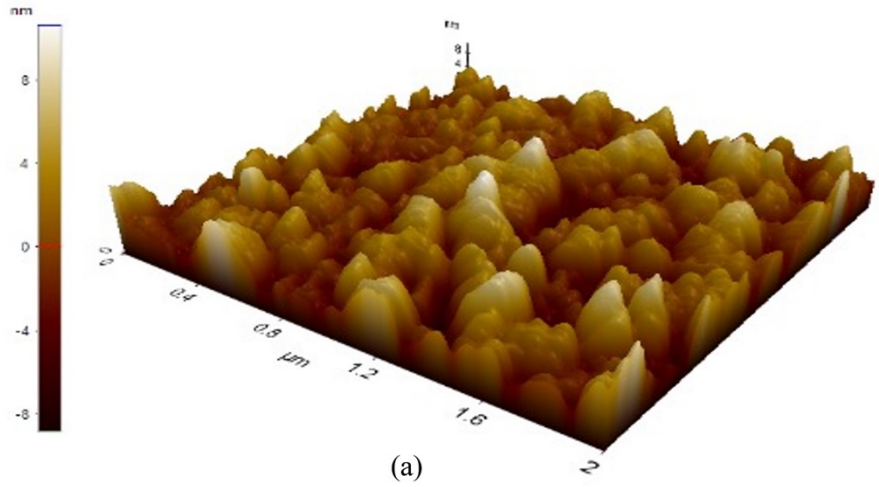
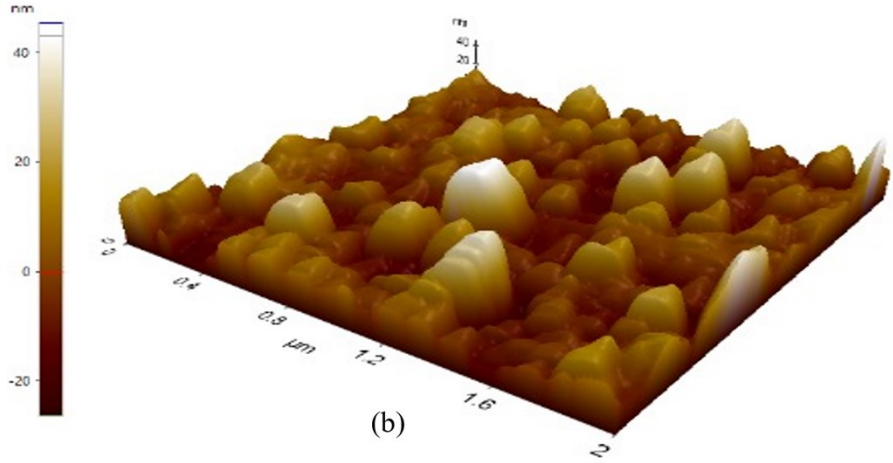


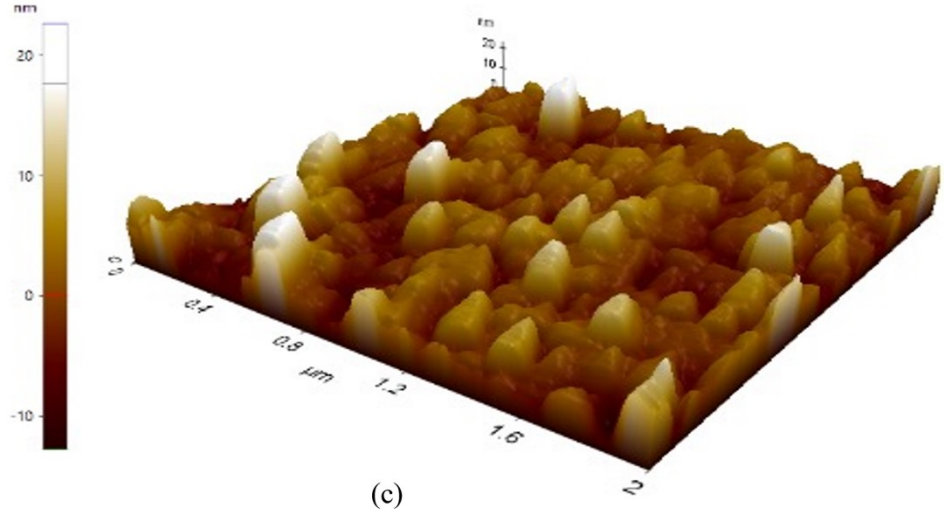
Figure S3: XRD spectra obtained for NiO thin film grown at varying oxygen content (50%, 75% and 100%) in the deposition gas ambient.



(a)



(b)



(c)

Figure S4: AFM images for (a) NiO/ITO, (b) Ab/NiO/ITO and (c) E. coli/Ab/NiO/ITO.

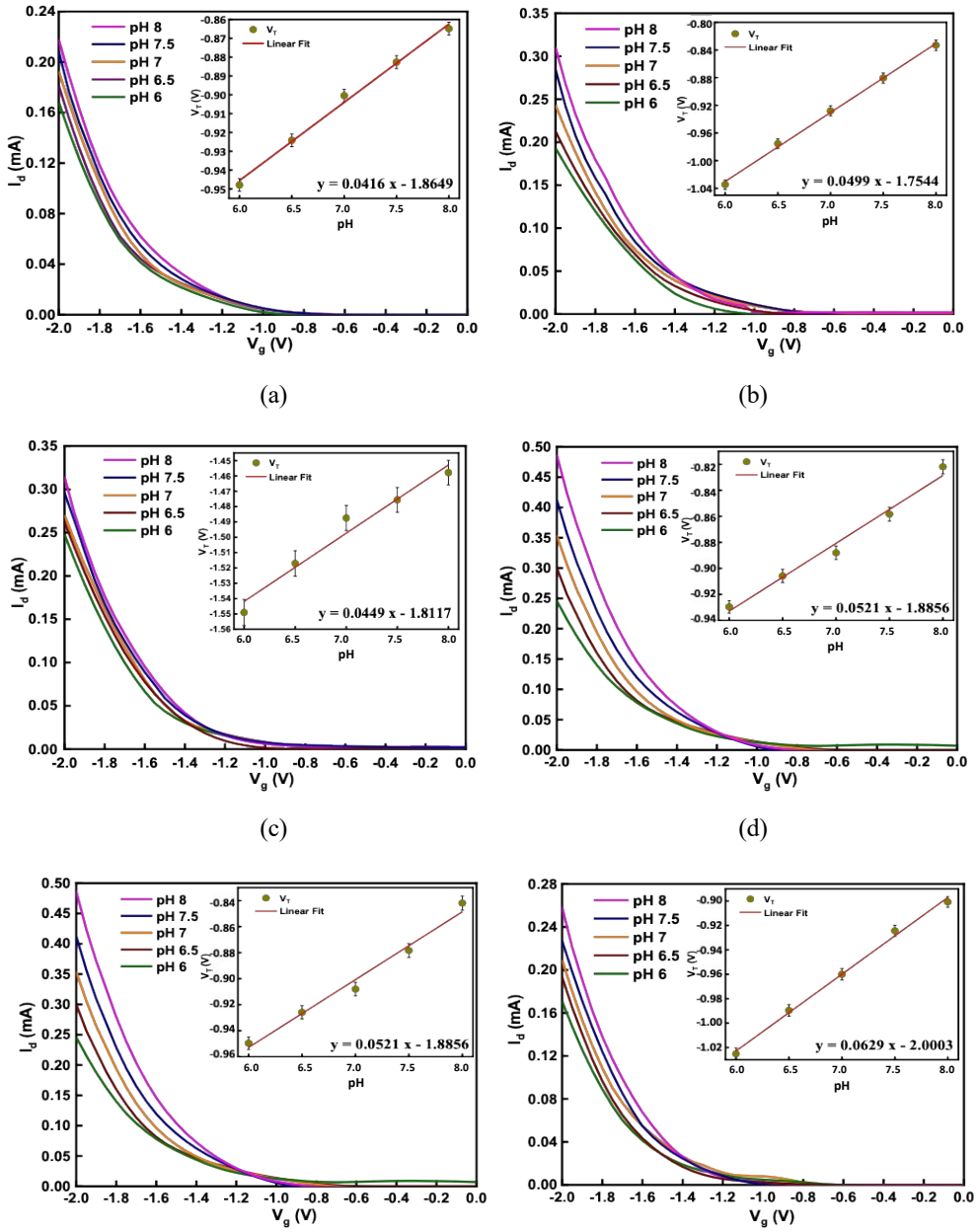
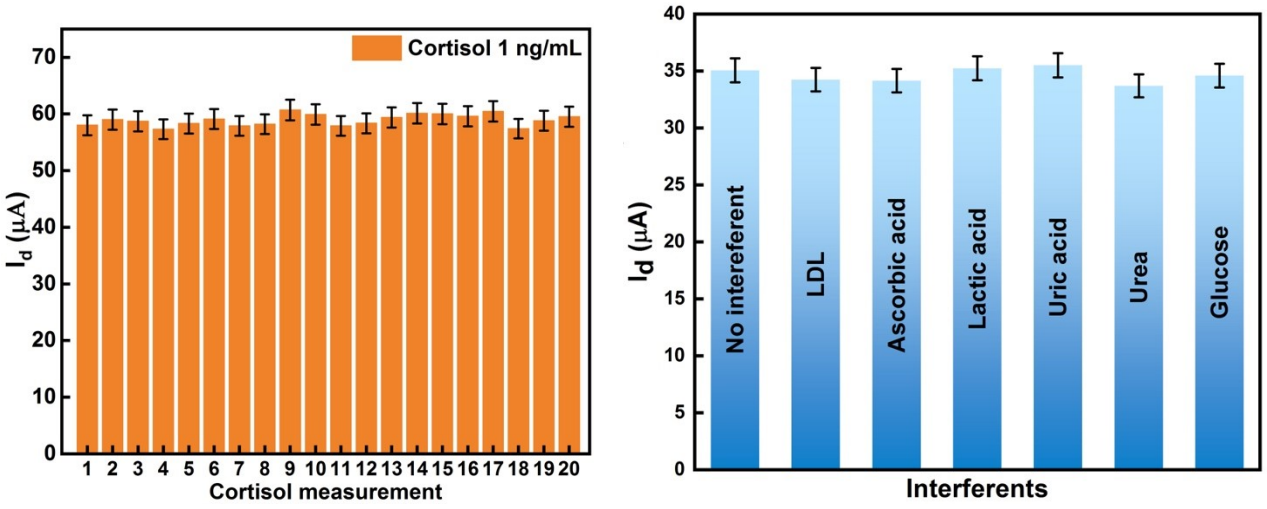


Figure S5: pH sensing response of EGFET devices: (a) 1mm50%O_2, (b) 2mm50%O_2, (c) 1mm75%O_2, (d) 2mm75%O_2, (e) 1mm100%O_2 and (f) 2mm100%O_2.



(a) (b)

Figure S6: Sensing response of the EGFET device (a) for 20 repeated cortisol measurements and (b) in absence of cortisol in the test sample.

Table S1: Deposition parameters for fabrication of the drain-source electrodes and the NiO channel

Deposition Parameter	**Titanium**	**Platinum**	**Nickel Oxide**
Target Size	4" (99.999% pure)	3" (99.999% pure)	2" (99.999% pure)
Base pressure	6×10^{-6} Torr	6×10^{-6} Torr	6×10^{-6} Torr
Deposition Pressure	10 mTorr	10 mTorr	20 mTorr
Substrate Temperature	Room Temperature	Room Temperature	Room Temperature
Gas Ambient	100% Ar	100% Ar	1. 50% Ar + 50% O_2 2. 25% Ar + 75% O_2 3. 100% O_2
Film Thickness	10 nm	100 nm	100 nm

Table S2: Summary of pH sensitivity values for all EGFET devices

O_2 %	pH sensitivity (mV/pH) (Channel length = 1 mm)	pH sensitivity (mV/pH) (Channel length = 2 mm)
50	42	50
75	45	52
100	53	63

Table S3: Analysis of recent literature on detection of cortisol employing various sensing techniques

Sensing platform	Detection Technique	Linear Range	Limit of Detection	Reference
Molecularly imprinted polypyrrole	Electrochemical	1 pM – 10 µM (0.36 pg/mL – 3.6 µg/mL)	1 pM (0.36 pg/mL)	(Manickam et al., 2017)
Colorimetric detection using CMOS photodiode	Optical	0.01 – 20 ng/mL	18 pg/mL	(Pinto et al., 2017)
Graphene-Nanoplatelet-Amphiphilic-diblock-co-Polymer Composite	Electrical	3 pg/mL – 10 µg/mL	3 pg/mL	(Khan et al., 2017)
LMR and ZnO/PPY nanocomposite of MIP	Optical	10^{-12} – 10^{-6} g/mL	25/9 fg/mL	(Usha et al., 2017)
Antibody-embedded	Electrical (FET)	10 fg/mL – 10	1 pg/mL	(Jang et al.,

PSMA		ng/mL		2018)
MTPP and MWCNTs nanocomposite functionalized electrodes	Electrochemical	50 fM – 1 pM (18.11 fg/mL – 0.36 pg/mL) and 1 pM – 100 nM (0.36 pg/mL – 36.23 ng/mL)	50 fM (18.11 fg/mL)	(Manickam et al., 2018)
Cortisol-conjugated BSA based cuvette-type sensor	Optical (LSPR)	1 – 10000 ng/mL	8 ng/mL	(Jeon et al., 2018)
n-doped multidimensional carbon nanofibers	Electrical (FET)	100 aM – 10 nM (36.23 ag/mL – 3.62 ng/mL)	100 aM (36.23 ag/mL)	(Jeong et al., 2019)
Conductive carbon yarn functionalized with Fe_2O_3	Electrochemical	1 fg – 1 ug	0.005 fg/mL	(Sekar et al., 2019)
NiO thin film	Electrochemical	1 pg/mL – 10 µg/mL	0.32 pg/mL	(Dhull et al., 2019)
Graphene functionalized by tris(4-carboxyphenyl) porphyrin and cortisol aptamer	Electrical (FET)	0.01–10^4 nM (3.62 pg/mL – 3.62 µg/mL	-	(Zhang and Jia, 2021)
NiO thin film based EGFET	**Electrical (FET)**	**1 fg/mL to 1 µg/mL (2.76 fM to 2.76 µM)**	**0.02 fg/mL (0.06 fM)**	**Present work**
Microfluidics integrated NiO based EGFET			**0.5 fg/mL (1.38 fM)**	