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The impact of hydrogen peroxide production in OECTs for *in vitro* applications

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Supplementary information

Figure S1. Transfer curves of PEDOT:PSS and p(gPyDPP-MeOT2) OECTs

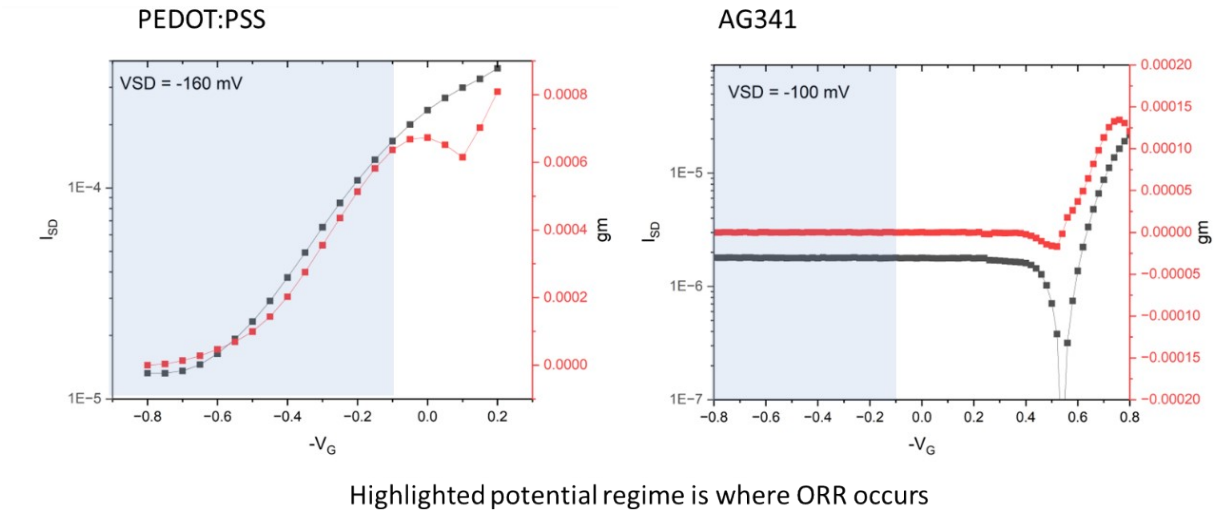
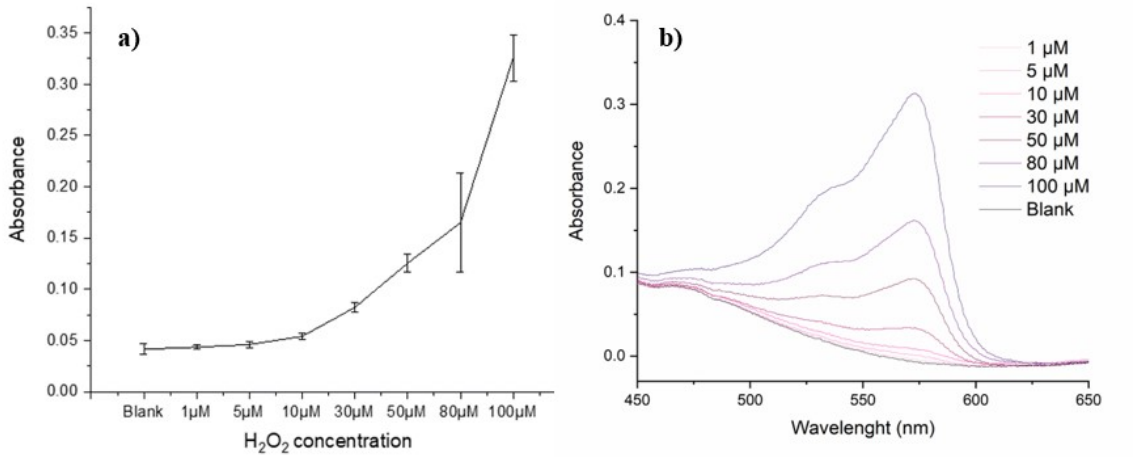


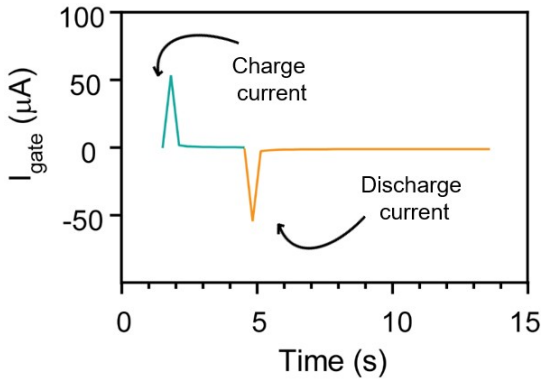
Figure S2. H_2O_2 calibration curve.

a) Colorimetric Standard Curve obtained by mixing the proper amount of H_2O_2 Substrate with the Assay Buffer and the Fluorescent Peroxidase Substrate. The Reaction Mix has been incubated at room temperature for 5 minutes before measuring the absorbance at 570 nm. Different buffer solutions containing concentrations of H_2O_2 ranging from 1 µM to 100 µM have been tested. b) UV-vis spectra of the Reaction Mix solution at different H_2O_2 concentrations. The standard curve has been obtained following the procedure reported in the Peroxidase Activity Assay Kit (Merck Life Science S.r.l., Italy). The absorbance values was measured spectrophotometrically using Dynatech MR580 Microelisa reader.



H_2O_2 Conc (µM)	0	1	5	10	30	50	80	100
Abs at 570 nm	0,045	0,045	0,048	0,052	0,079	0,132	0,199	0,342

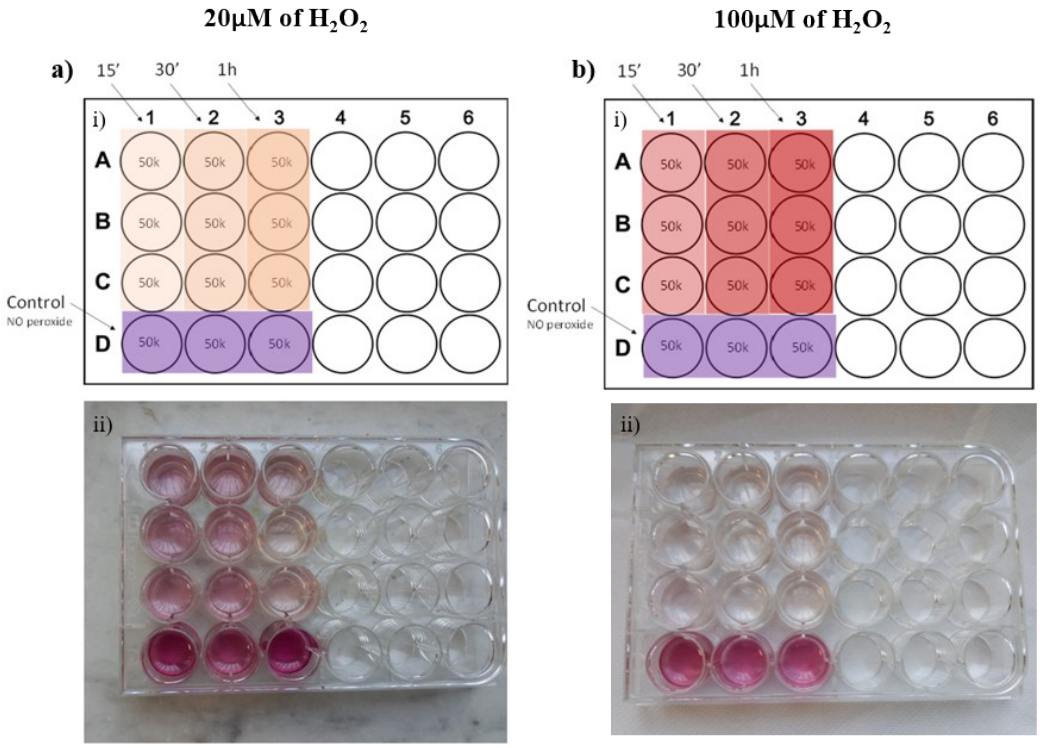
Figure S3. Correlation gate charges with H_2O_2 production. Schematic graph displaying gate current measured during the application of gate voltage pulses: the light blue line corresponds to the current measured during the ON phase of the pulse (charge current), while the orange line is the current measured during the OFF phase of the pulse (discharge current). The number of charges injected from the gate towards the polymeric channel was obtained by the integration of the charge current.



Pulse duration (mins)	Charge injected (□C)		$[H_2O_2]$ (□M)	
1	1023.3 ± 214.7	917.19 ± 84.01	48.85 ± 10.25	29.19 ± 2.67
5	6083.22 ± 2.94	3879.15 ± 540.3	102.51 ± 0.05	68.48 ± 9.54
Large channel				
Small channel				

Figure S4. Test of H_2O_2 produced by the transistor operation on the HT22 cell line. a) 60.000 cells/cm^2 of HT22 have been plated on a 24-well plate. After 24 hours, the cell medium was replaced with a cell media containing a 20 µM solution of H_2O_2 (the same amount produced by the small channel area-transistor and short pulse duration experiment). Different time points have been tested (15', 30', and 60') to evaluate the effect of the H_2O_2 produced by the transistor operation on HT22 cells (i). The H_2O_2-rich medium was then replaced with a fresh medium and then the MTT assay test was carried out to evaluate the metabolic activity of cells (ii). b) 60.000 cells/cm^2 of HT22 have been plated on a 24-well plate. After 24h the cell medium was replaced with a cell media containing a 100 µM solution of H_2O_2 (the same amount produced by the large

channel area-transistor long pulse duration) to evaluate the effect of the H_2O_2 produced by the transistor operation on HT22 cells. (i). The H_2O_2-rich medium was then replaced with a fresh medium and then the MTT assay test was carried out to evaluate the metabolic activity of cells (ii).

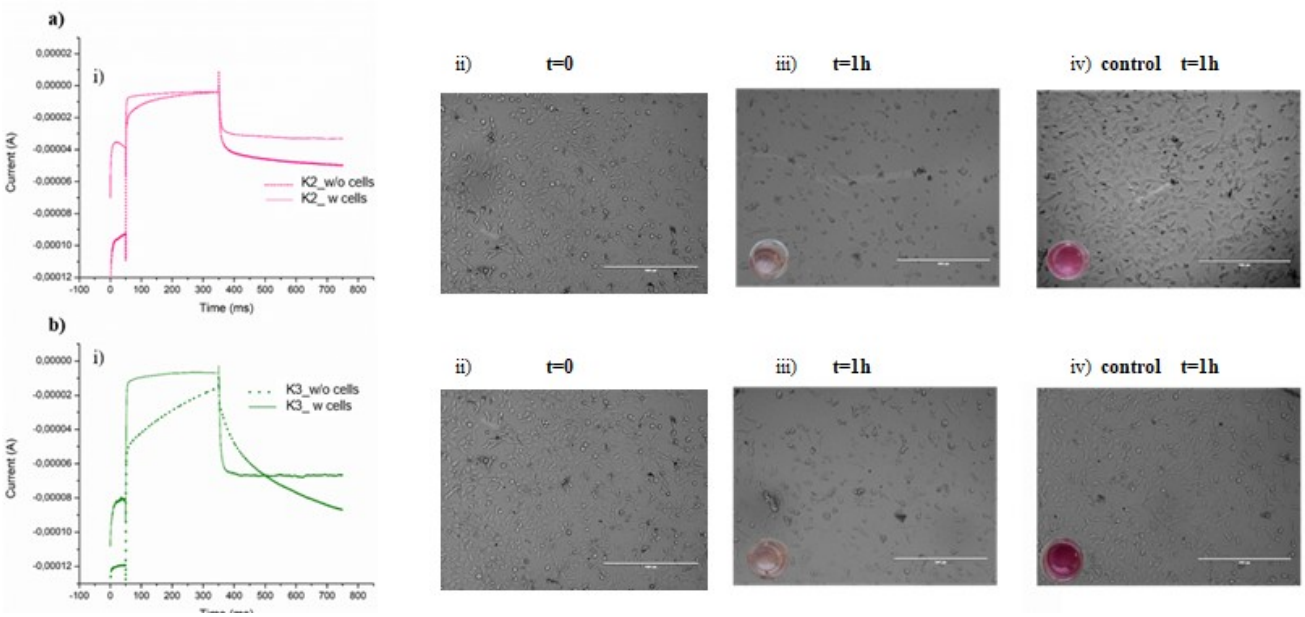


20 µM conc H_2O_2	Abs at 570 nm	100 µM conc H_2O_2	Abs at 570 nm
15'	0,203 (+-0,037)	**15'**	0,075 (+-0,008)
30'	0,194 (+-0,014)	**30'**	0,072 (+-0,007)
1h	0,138 (+-0,007)	**1h**	0,076 (+-0,005)

Figure S5. Effect of H_2O_2 produced by OECT operation on HT22 directly seeded on the device channel area. HT22 (60.000 cell/cm^2) have been seeded on PEDOT:PSS channel area and a 5 minutes square voltage pulse of 0.7 V has been applied at the gate electrode. No significant difference was found in the amount of H_2O_2 produced before and after cell plating in both devices (a-i and b-i).

The MTT assay carried out after 1h of incubation, indicated a drastic decrease in the metabolic activity of the HT22 compared with a PEDOT:PSS sample control (a-iv, b-iv). Such result was confirmed by the brightfield images of the HT22 cells, which exhibited stretched shape at t=0 (a-

ii, b-ii) and round-shaped morphology and cells cluster formation after 1h of exposure at H_2O_2 (a-iii, b-iii).



Peroxide assay

Device K2	**Abs at 570 nm**	**Device K3**	**Abs at 570 nm**
w/ cells	0,113	**w/ cells**	0,100
w/o cells	0,122	**w/o cells**	0,95

MTT assay

	Device K2	**Device K3**
t=1h	0,138	0,185
t=1h control	0,346	0,318