Supplementary Material

A Nanostructured microfluidic device for plasmonic-assisted electrochemical detection of Hydrogen peroxide.

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Figure S1. A fabrication process of the microfluidic device schematic. A SAM is fabricated on a water interface and later transferred on top of (a) a Si wafer. Followed by the e-beam deposition of (b) a thick layer of ZnO and (c) a thin layer of gold. The (c-i) gold-coated beads are removed through (c-ii) chemical wet-etching. An exfoliated Gr solution is (c-iii) drop-casted on top of the sensing area. The (d) PDMS mold is attached on top of the platform and (e) the tubes are added to complete the microfluidic device. The detection of H_2O_2 is (f) conducted under visible light.



Figure S2. AFM study. AFM image of the electrode surface featuring three NCs. Two NCs are partially covered by graphene nanosheet. Scale bar: $1 \mu m$.

Supplementary note 1: analyte concentration relation to absorbance.

The absorption intensity depends directly on the presence and concentration of the target analyte as well as on the electrode's plasmonic properties.^{3, 4} To confirm this the absorption of our electrode was registered in presence of higher and lower concentrations of H_2O_2 , the target analyte. In **Figure S3** it can be observed how the absorbance peak is decreased or increased for a smaller concentration (10 μ M) and higher concentration (500 μ M), respectively. A spectrum of an optimally dispersed nanocavities gold electrode drop-casted with graphene and one of a bare gold electrode is presented as reference.



Figure S3. UV-Vis dNCs/Gr electrode H_2O_2 absorbance. Three different concentrations (10 μ M, 100 μ M and 500 μ M) of H_2O_2 absorbance spectra are tested on the proposed electrode.

Supplementary note 2: FDTD Simulation.

FDTD Simulation represents the nanocavities as a result of the empty space caused by removing polystyrene beads. The refractive index value of "etch" material is 1, which is equal to the background refractive index, as the representative of the empty space in the layers. In the simulation geometry.

Due to the symmetry in both x and y directions, the simulation is done in a quarter of geometry (the simulation region shown in **Figure S4** to reduce the computational cost. To consider the symmetry in simulation, the boundary conditions in surfaces 1 and 2 (**Figure S4a**) are considered as symmetric and anti-symmetric boundary conditions, respectively. On the other surfaces including surfaces 3-6 (**Figure S4b**), the boundary condition is a perfectly matched layer (PML). In the PML boundary condition, the surfaces fully absorb electromagnetic energy with minimum reflection. We used Total-Field Scattered-Field (RFSF) as the optical source. The optical source applies uniform electromagnetic energy and the electromagnetic scattered from our structures. We made sure that the quality of the mesh is accurate enough, and the results are independent of the mesh size. To measure the absorbance energy in the platform, the transmission power from the line above the platform (Upper line in **Figure S4b**).

To decrease the computational cost, symmetric and anti-symmetric boundary conditions are considered in x and y directions, respectively. Moreover, a perfectly matched layer (PML) boundary condition is considered in other surfaces to implement infinity boundary conditions which fully absorbed electromagnetic energy with minimum reflection. Total-Field Scattered-Field (TFSF) source is used as a plane wave light source considering the scattering from the platform. Mesh accuracy (**Figure S4d**) is set as 5 after getting the independency of results to mesh quality. (The absorbance is measured by subtracting the transmission power before and after the platform. A study of the metal thickness and its relation to the maximum electrical field was performed for both ZnO and Au. Optimized values for the thickness of ZnO and Au (100 nm ZnO and 60 nm Au) were selected for optimal performance of the electrode according to the plots shown in **Figure S4e** and **Figure S4f**.



Figure S4. FDTD Simulation of electromagnetic field prop. Surfaces (a) 1-4 and (b) 6 and 5 in simulated region (c) Magnified image of heighted area in (b) showing material choice for the simulation. (d) Mesh accuracy plot. Study of (e) ZnO and (f) Au thickness in terms of maximum electrical field.



Figure S5. Study of electrochemical sensitivity of the four configurations of nanocavities. The sensitivity of the four configurations of nanocavities (a) without and (b) with graphene towards H_2O_2 .



Figure S6. Long run stability study. Three different electrodes, the proposed dNCs/Gr, dNCs and Gr on Au were studied in 3 mM K3FeCN6 with a fixed potential of 0.3 V vs Ag/AgCl.



Figure S7. Calibration curve in plasma. Inet-H2O2-concentrations responses in plasma.

Supplementary note 3: contact angle study.

To further study the surface properties of the proposed electrode a contact angle study was performed (**Figure S8**). As previously reported, graphene coating modifies the water interactions of bare metal substrates lowering the free energy in the interface [1]. Accordingly, increasing the hydrophobicity of the surface from 54.3° to 65.3° in water. Further increasing to 69.2° in the presence of PBS, media in which the cancer cells are tested, MCF-7) which decreases to 55.8 when H_2O_2 is released and PC3 is reduced from 55° to 47.3°. Non-cancer cells also present the same trend, the hydrophobicity decreases when H_2O_2 is released in CBFE and hVFF cells from 58.6 and 54.1 to 50.3 and 32.2 respectively. The characterization of the contact angle is a significant parameter to understand the interface properties between the proposed sensor and the media. Concluding that the release of H_2O_2 increases the free energy at the surface then increasing the hydrophilicity at a unique level for each cell type.



Figure S8. Wettability of the surface. The contact angle of the different medias was measured. The first measurement to the left was performed in the conventional Au electrode. The rest were run on the dNCs/Gr.

Table S1. Electrochemical-ba	ased detection of H ₂ O ₂
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Electrode	Method	Fabrication method	LOD	Sensitivity	Linear range	Ref
AuNi NDAs		One-step electrodeposition.	2 μΜ	994 μA mM ⁻¹ cm ⁻ 2	0.005-15 mM	[2]
Ag-decorated TiO2 nanowire arrays		Ti H_2O_2 corrosion and photodeposition of Ag.	1.2 mM	8.33 μA mM ⁻ ¹ cm ⁻²	0-22.5 mM	[3]
Ultrathin Ag concave nanosheets	Electrochemical	Galvanic replacement reaction.	0.17 μM	320.3 μA mM ⁻ ¹ cm ⁻²	0.005-6 mM	[4]
a-MnO2/GCE		One-step acidification of flowers -like MnO2.	0.02 μM		0.00008-1.78 mM	[5]
PtNi/CeO2/NCNFs		Heat-treating the electrospun nanofiber	25 nM	345 μA mM ⁻¹ cm ⁻ 2	0.0005-15 mM	[6]

		hybrid of polyvinyl pyrrolidone and inorganic metal salts.				
HRP/GCIS/ITO		Flower-like CIS- graphene hybrid (GCIS) protocol and drop- coat.	0.47 μM	11.2 μA mM ⁻¹	0.0005-0.53 mM	[7]
RGO-Pt NPs/GCE	RGO-Pt NPs/GCE Pt		~0.2 μM	459 mA M ⁻¹ cm ⁻²	0.0005-3.475 mM	[8]
AuNPs-NH2/Cu- MOF/GCE		Cu-MOF ionothermal synthesis, grafted with ammoniated Au NPs drop-cast on GCE.	1.2 μΜ	1.71 μΑ μΜ ⁻¹ cm ⁻²	0.005–0.850 mM	[9]
Au@PtNP/GO		Chemical protocol for Au@PtNP/GO solution packed into ITO.	1.62 μM		0.001-3 mM	[10]
Au-Ag bimetallic NPs on SWCNT		SWCNT photolithographed on glass, Au covered Ag NPs electrochemically deposited on top.	26.8 μM	13.1 μA mM ⁻ ¹ cm ⁻²	0.3-1.8 mM	[11]
Au@C-Co3O4	Plasmonic- Assisted Electrochemical	Fabrication of Au@ZIF- 67 and pyrolysis. Drop- cast onto GCE.	19 nM	7553 μA mM ⁻ ¹ cm ⁻²	0.00005-0.1 mM 0.1-3 mM	[12]
dNCs/Gr		Au deposition on Si. Ps beads fabless patterned. Gr drop- cast on top.	1 pM	1520 μA mM ⁻ ¹ cm ⁻²	0.000000001- 0.010 mM	This work

Table S2. Determination of H_2O_2 released from cells

Electrode	Cell line	I _{max}	H ₂ O ₂ amount/ total	H_2O_2 amount /each cell	Ref
Pt NPs/gold microelectrodes	single MCF-7 cell	~120 nA	N.A.	N.A.	[13]
Graphene QDs/gold electrode	MCF-7 cells	82.5 nA	N.A.	7360 amol	[14]
Graphene-Pt Nanocomposites	PC12 cells	46.9 nA	N.A.	105 amol	[15]
MnO ₂ Nanosheets	SP2/0 cells	65 nA	N.A.	40 amol	[16]
	HeLa	1.1 μA	2.06 μM	2060 fM	
Au@C-Co3O4/GCE	MCF-7	1.2 μA	2.38 μM	2380 fM	[17]
	НКС	0.7 μΑ	1.32 μM	1320 fM	
Gold dNCs/Gr	MCF-7	24.1 μA	0.245 μM	2042 fM	
	PC3	23 μΑ	0.212 μM	1767 fM	This work
	CBFE	18.3 μA	0.056µM	464 fM	
	hVFF	17.7 μA	0.042 μM	350 fM	

Table S3. Determination of H₂O₂ concentration in plasma

Test	H_2O_2 spiked sample (μ M)	Determined by concer (UNA)	Recovery
		Determined by sensor (µivi)	(%) (n=5)
1	0.1	0.12±0.01	120±5
2	1	1.11±0.08	111±6
3	2	1.82±0.11	91±4
4	5	5.15±0.23	103±4
5	10	10.10±0.47	101±3

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