Supplementary Information (SI) for Nanoscale.

Supporting Information

Bio-modified hydrogels in electrolyte-gated organic field-effect transistors for sensing applications

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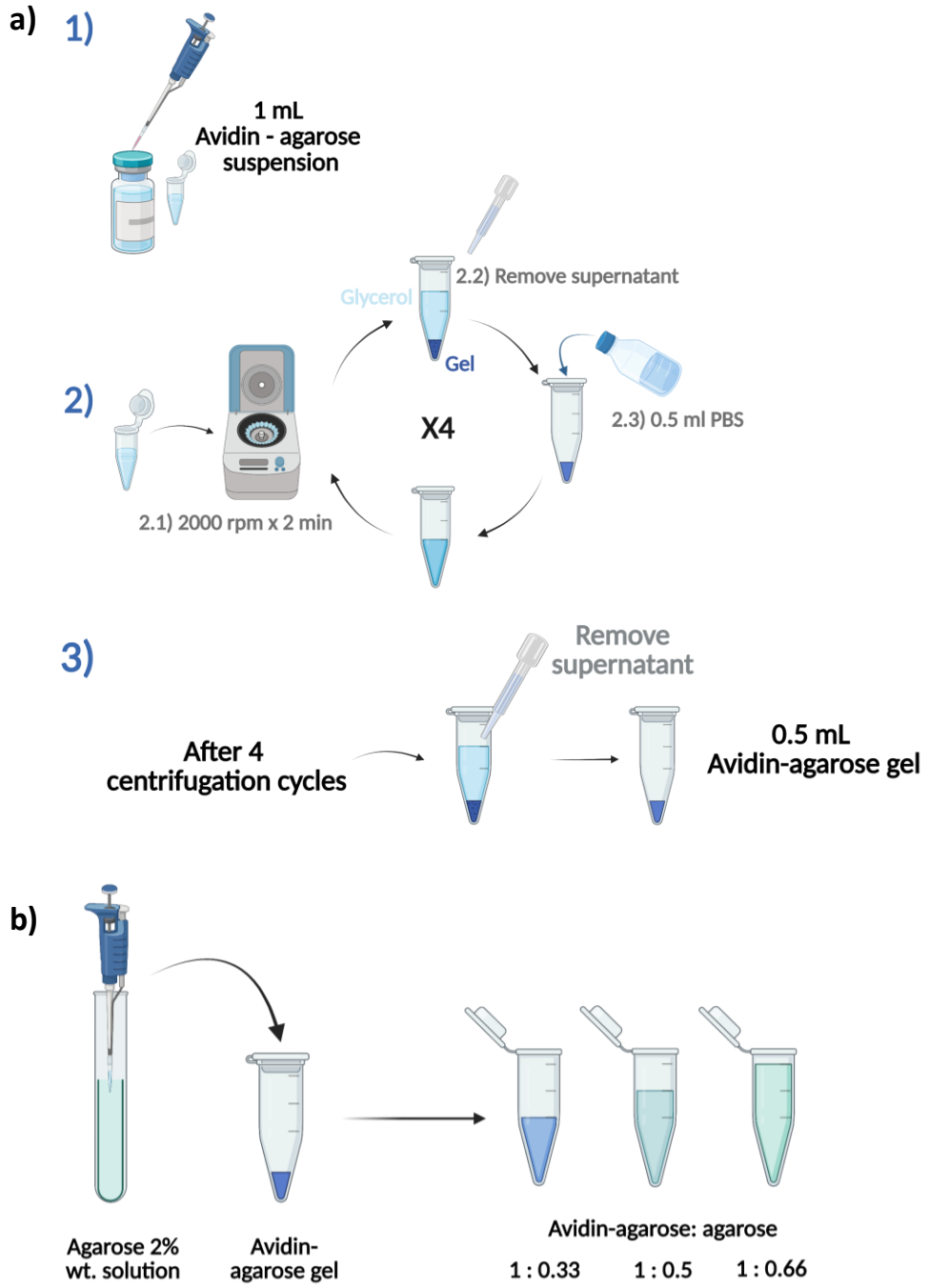
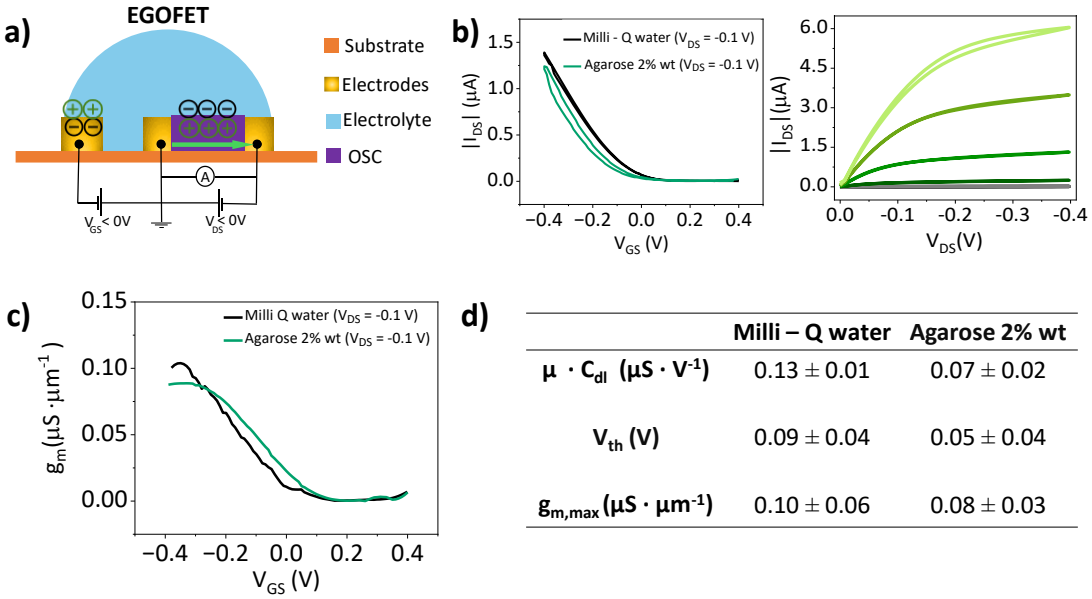


Figure S1. a) Avidin-agarose gel obtaining process. 1 mL of the commercial avidin-agarose suspension was centrifuged. Then, the supernatant was removed and 0.5 mL of a PBS buffer solution were added and the resulting mixture was again centrifuged. This process was repeat three times more, yielding finally 0.5 mL of packed gel after discarding the last supernatant. b) Schematic representation of the avidin-agarose:agarose mixture prepared by adding 1, 1.5 or 2 mL of a 2% wt. of a non-modified agarose solution in water to the previously obtained 0.5 mL of packed avidin-agarose gel, giving weight ratios avidin–agarose:agarose: 1:0.33, 1:0.5 and 1:0.66.



d)

	Milli – Q water	Agarose 2% wt
$\mu \cdot C_{dl}$ ($\mu S \cdot V^{-1}$)	0.13 ± 0.01	0.07 ± 0.02
V_{th} (V)	0.09 ± 0.04	0.05 ± 0.04
$g_{m,max}$ ($\mu S \cdot \mu m^{-1}$)	0.10 ± 0.06	0.08 ± 0.03

Figure S2. a) Schematic representation of an Electrolyte Gated Organic Field Effect Transistor (EGOFET). b) Transfer characteristics (V_{DS} = -0.1 V) of an EGOFET operating in water and an HYGOFET based on agarose as electrolyte (left) and corresponding output characteristics of the HYGOFET device (V_{GS} = 0.1, 0, -0.1, -0.2, -0.3 and -0.4 V) (right). c) Transconductance (g_m) normalized to channel length (L = 50 µm) values as a function of V_{GS} for devices measured using Milli-Q water (black) and 2 wt% agarose (green). d) Comparison of $\mu \cdot C_{dl}$, V_{th} and $g_{m,max}$ extracted from the EGOFET and HYGOFET (N=6).

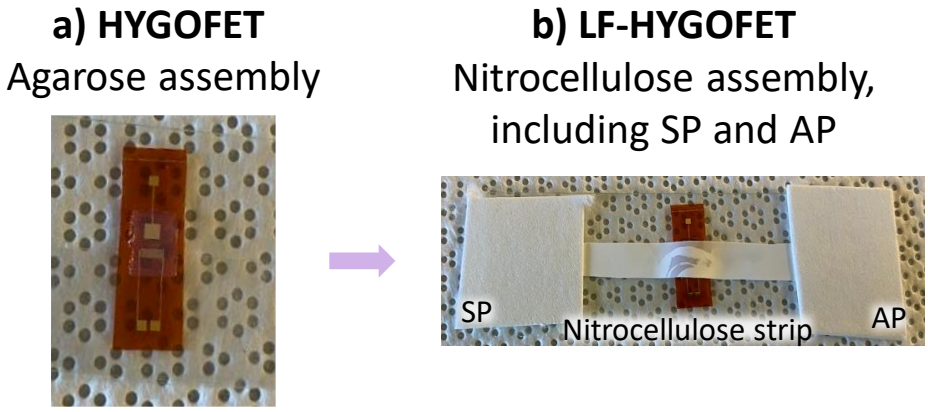


Figure S3. Assembly process of the Hydrogel-Gated Organic Field-Effect Transistor coupled with Lateral Flow (LF-HYGOFET). (a) Assembly of the agarose hydrogel onto the electrodes and organic semiconductor. (b) Integration of the lateral flow components, including the sample pad (SP), nitrocellulose strip and absorbent pad (AP), to enable capillary-driven sample transport.

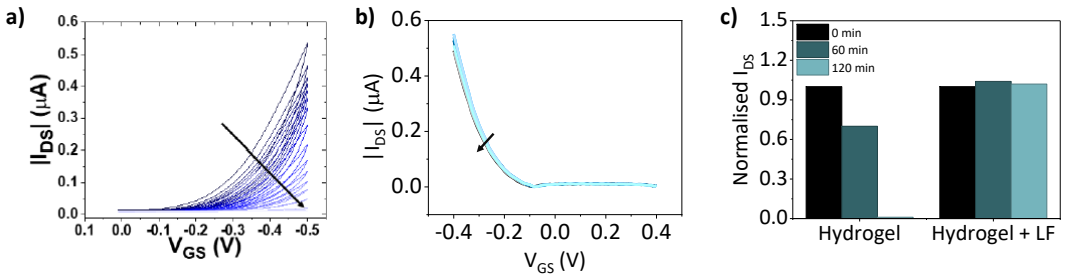


Figure S4. a) Transfer curves for HYGOFET devices measured every 10 minutes for 2 hours, showing the progressive decrease in IDS as the hydrogel dehydrates (V_{DS} = -0.1 V). b) Transfer characteristics for LF-HYGOFET devices measured every 10 minutes after the addition of 200 µL of Milli-Q water to the sample pad. Transfers were acquired for 2 hours. c) Normalised I_{DS} (at V_{GS} = -0.4 V) at 0, 60, and 120 min comparing HYGOFET and LF-HYGOFET devices, highlighting the improved stability when hydrogel dehydration is avoided.

Table S1. Capacitance values (C, µF/cm²) measured at different frequencies for avidin-agarose, and avidin-agarose after exposure to a biotin solution (10^{-14} M) (N = 3). The data illustrate the impact of avidin–biotin interaction on the electrical properties of the sensing surface.

Frequency (Hz)	C (µF/cm^2) Avidin-agarose	C (µF/cm^2) Avidin-agarose + Biotin (10^{-14} M)
10^3	3.79 ± 0.06	1.02 ± 0.03
10^2	5.83 ± 0.09	1.43 ± 0.04
10	8.2 ± 0.2	2.16 ± 0.07
1	11 ± 0.3	3.8 ± 0.1
0.1	16 ± 0.4	9.0 ± 0.3

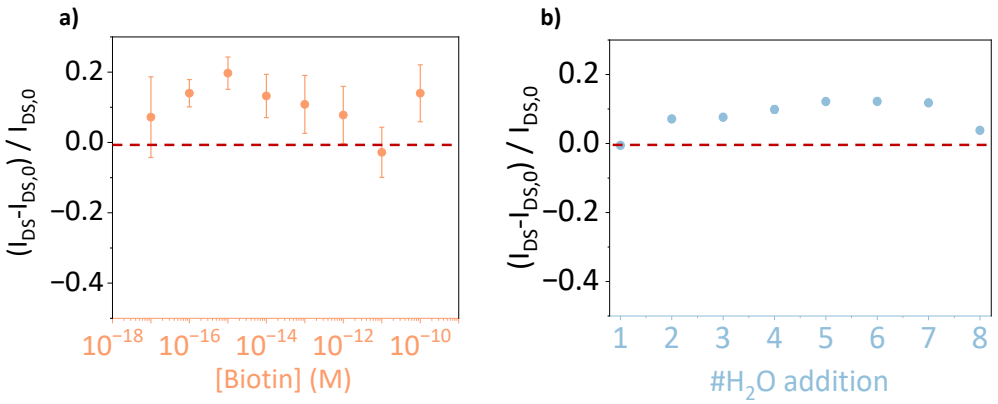


Figure S3. Relative current variation of LF-HYGOFET using agarose 2% wt (0:1 ratio) as a) a function of biotin concentration (N=6) to evaluate the biosensor's specificity across the tested range, and b) as a function of water additions (N=3) simulating sensing experiments to study the sensor's stability.