Supplementary information

Label-Free Impedimetric Immunosensing of Pathogenic *E. coli O157:H7* using Amine Functionalized Carbon

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Electrochemical characterization of Bioelectrode

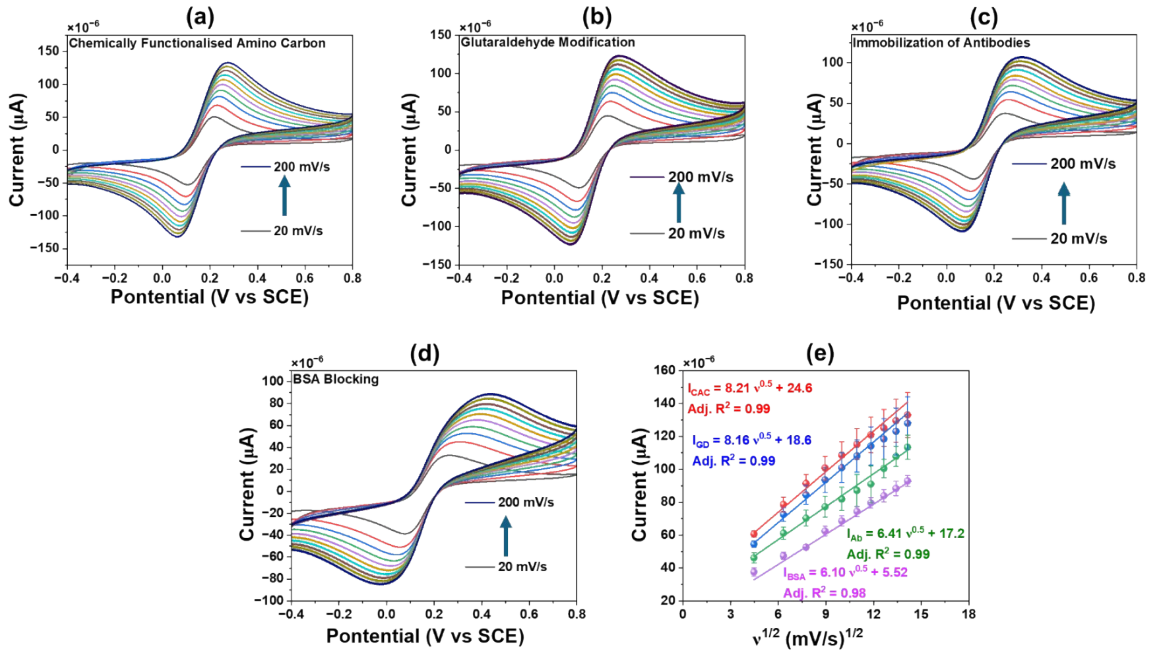


Figure. S1 CV analysis of bioelectrode fabrication at various scan rates ranging from 20 to 200 mv/s **(a)** chemically functionalised amino carbon, **(b)** Glutaraldehyde activation, **(c)** Immobilization of the antibodies on bioelectrodes, **(d)** BSA blocking of bioelectrodes, and **(e)** calibration plot of the square root of scan rate and current responses during bioelectrode fabrication step.

Determination of Electrochemical Active area

The electrochemical active area of the bioelectrodes was obtained with the slope of the intensity of the anodic peak current and square root of the scan rate from the Randle - Sevcik equation (**Equation S1**) for the electron-electron transfer process.

$$\mathbf{I_{pa} = (2.69 \times 10^5)\, n^{3/2}\, D^{1/2}\, C_o\, A_e\, \nu^{1/2}} \quad \text{... (Equation S1)}$$

Where I_{pa} = anodic current response

n = Number of electrons involved in the redox process (n=1)

D = Diffusion coefficient of the electroactive species = 6.7 x 10^{-6} cm^2/s

C_o = Concentration of the electroactive species = 10 mM = 10^{-5} mol/cm^2

A_e = Electrochemically active area

ν = Scan rate.

So, equation S1 can be modified as

$$\mathbf{Slope\ (\mu A/mV^{1/2}) = (2.69 \times 10^5)\, n^{3/2}\, D^{1/2}\, C_o\, A_e}$$

The slope of the anodic sweep from the calibration curve was obtained as 8.21 $\mu A/mV^{1/2}$.

Slope = 8.21 $\mu A/mV^{1/2}$ = 8.21 x 10^{-6} A /$(10^{-3}$ V$)^{1/2}$ = 2.59 x 10^{-4} $A/V^{1/2}$

Thus, the electrochemical active area (A_e) = Slope/(2.69 x 10^5) $n^{3/2}$ $D^{1/2}$ C_o = **0.03756 cm^2 = 3.756 mm^2**.

Table S1: Electrochemical active area calculation using Randles–Sevcik equation.

Electrochemical Active Area Calculation

CFAC	**3.756 mm^2**
CFAC/2.5% GD	**3.733 mm^2**
CFAC/2.5% GD/Ab-E.coli O157	**2.933 mm^2**
CFAC/2.5% GD/Ab-E.coli O157/BSA	**2.791 mm^2**

Determination of Limit of Detection (LOD) and Sensitivity

The limit of detection (LOD) of the biosensor was calculated using the statistical method, using the formula 3σ/slope.

Here, σ = Standard deviation of the blank samples (PBS)

To determine the standard deviation of the blank samples, the responses of ten individual electrodes were measured by incubation with the PBS buffer, as tabulated in the **Table. S2**, and found to be σ = 0.14 (ΔΩ/Ω).

The slope of the calibration plot = 0.31 (ΔΩ/Ω). $(CFU/mL)^{-1}$ …[Figure 4 (b)]

LOD = 3σ/slope

LOD = (3 x 0.14)(ΔΩ/Ω) / 0.31 (ΔΩ/Ω). $(CFU/mL)^{-1}$

LOD = 1.36 CFU/mL

The Sensitivity of the bioelectrodes was calculated using the formula,

Sensitivity = Slope/Area

Here, Slope = 0.31 (ΔΩ/Ω). $(CFU/mL)^{-1}$

Area = 0.0707 cm^2 (geometrical area of the Glassy carbon electrode, d = 0.3 cm)

Sensitivity = Slope/Area = 0.31 (ΔΩ/Ω). $(CFU/mL)^{-1}$) / 0.0707 cm^2

Sensitivity = 4.38 ((ΔΩ/Ω)/(CFU/mL))/cm^2

Table S2: The Blank sample measurement of the bioelectrodes

	R_{ct} (Bioelectrode)	R_{ct} (Blank)	ΔR_{ct}	$\Delta R_{ct}/ R_{ct}$
Bioelectrode-1	1656	1681	25	0.02
Bioelectrode-2	1438	1456	18	0.01
Bioelectrode-3	1992	2056	64	0.03
Bioelectrode-4	1873	2389	516	0.28
Bioelectrode-5	1483	1587	104	0.07
Bioelectrode-6	1423	1932	449	0.3
Bioelectrode-7	1772	2294	522	0.29
Bioelectrode-8	1724	1765	41	0.02
Bioelectrode-9	1540	2021	481	0.31
Bioelectrode-10	1324	1764	440	0.33
			Average (ΔΩ/Ω)	**0.17**
			Standard Deviation	**0.14**

Concentration (CFU/mL)	**Bioelectrode-1**	**Bioelectrode-2**	**Bioelectrode-3**	**Mean (ΔΩ/Ω)**	**Standard Deviation**
1.5	0.38	0.33	0.34	**0.35**	**0.02**
15	0.64	0.64	0.67	**0.65**	**0.01**
150	1.06	0.99	0.99	**1.01**	**0.03**
1500	1.45	1.19	1.21	**1.28**	**0.10**
15000	1.71	1.93	1.46	**1.70**	**0.16**
150000	1.98	2.15	1.71	**1.95**	**0.16**
1500000	2.15	2.37	2.03	**2.18**	**0.13**

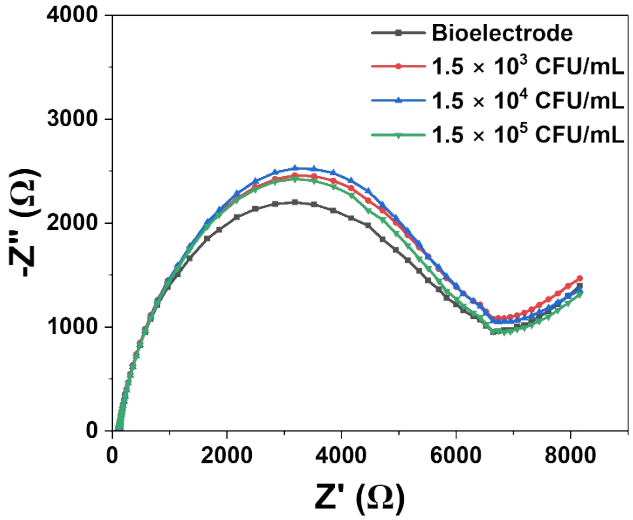
Table S3. Data points of the bioelectrodes for electrochemical sensing of *E. coli O157:H7*

The sensor-to-sensor variation in the form of LOD and sensitivity was calculated using the datapoints of **Table S3**.

Table S4. Sensor-to-sensor variation in the LOD and Sensitivity

ELECTRODE	SLOPE (ΔΩ/Ω). (CFU/ML)$^{-1}$	LOD (CFU/mL)	SENSITIVITY ((ΔΩ/Ω)/(CFU/mL))/cm²
Bioelectrode- 1	0.31	1.36	4.36
Bioelectrode- 2	0.36	1.17	5.09
Bioelectrode- 3	0.27	1.56	3.82
	Mean	**1.36**	**4.43**
	Standard Deviation	**0.16**	**0.52**

The biosensor LOD was found to be 1.36 ± 0.16 CFU/mL and the sensitivity was found to be 4.43 ± 0.52 ((ΔΩ/Ω)/(CFU/mL))/cm².



Comparative study

Figure S2. EIS Nyquist plot of electrochemical immunosensing of *E. coli O157:H7* using non-functionalized graphitic powder.

Real-Sample Analysis

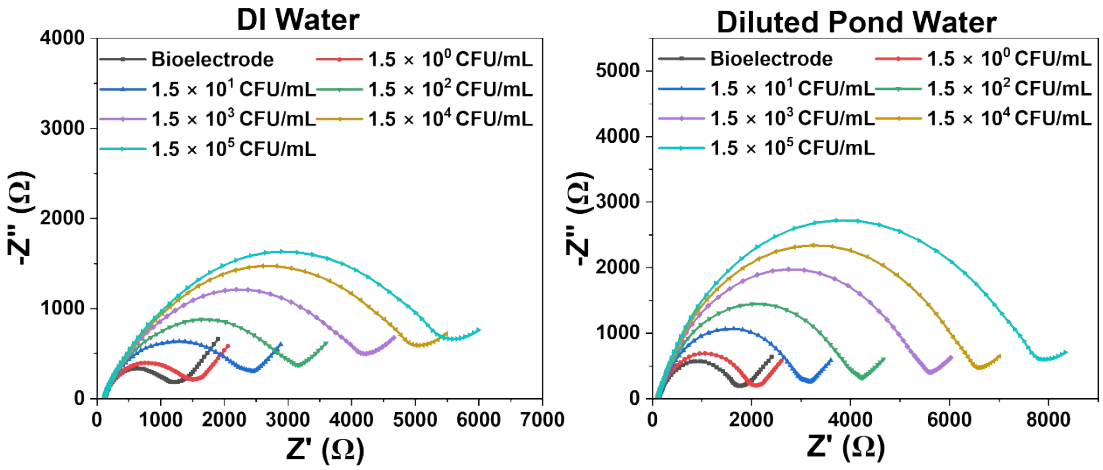


Figure S3. Nyquist plots representing the real sample analysis of *E. coli O157:H7* in DI water (left) and in the diluted pond water (right).

Table S5. Stability study of the bioelectrodes over time.

DAY	ΔRct/Rct	Change In Response (%)	Standard Deviation (n =3)
DAY-3	1.066	6.6	0.032
DAY-6	1.070	7	0.027
DAY-9	1.079	7.9	0.033
DAY-15	1.116	11.6	0.064