

Supplementary Materials

A unified antifouling and electroactive platform from laser-pyrolyzed PDMS

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Supplementary Movie 1

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Supplementary Texts

Supplementary Text 1. Chemical and materials

The PDMS kit (Sylgard™ 184) was obtained from Dow Co., Ltd., USA. The preparation of PDMS slices was carried out as follows: A mixture of PDMS and crosslinker in a 10:1 ratio was mixed for 10 minutes, then vacuumed for 10 minutes. Then, 9 grams of the mixture was poured into a petri dish and baked in an oven at 60 °C for two hours to yield 2 mm-thick PDMS slices. All samples were sealed to prevent dust contamination before the laser treatment stage. LB broth and LB agar powder were procured from Sigma, Germany. Tryptone soya broth (TSB) was purchased from OXOID, UK. Brain heart infusion (BHI) broth was from Hopebio, China. The Live/Dead BacLight bacterial viability kit, which quantified bacterial activity, was obtained from Invitrogen, Thermo Fisher, USA.

Potassium chloride (KCl) was purchased from Acros Organics, Belgium. Potassium ferricyanide ($K_3[Fe(CN)_6]$) was procured from Sigma, Germany, while potassium hexacyanoferrate (III) hexacyanoferrate ($K_4[Fe(CN)_6]$) was purchased from VWR, USA. Pyrrole was obtained from Maclin, China. Sodium dodecyl sulfate (SDS) and acetic acid (HAc) were acquired from Sigma-Aldrich, Germany. All solutions were prepared using Milli-Q deionized water. All electrochemical experiments were performed using a three-electrode electrochemical cell. The reference electrode was a silver/silver chloride (Ag/AgCl) electrode, and a platinum sheet served as the counter electrode. The sample was fixed in the electrode holder of the platinum sheet, which acted as the working electrode.

Supplementary Text 2. Bacterial culture

The bacterial stains, namely *E. coli* (ATCC 25922), *E. faecalis* (ATCC 6569), *S. enterica* (ATCC 13076), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), and *S. aureus* (ATCC 25923), were stored at -80°C. The *E. coli* samples were collected in LB broth, and *E. faecalis* was cultured in BHI. *S. enterica*, *P. aeruginosa*, and *S. aureus* were collected in TSB. All bacterial samples were resuspended by centrifugation at 8,000 rpm for 5 minutes in a sterilized PBS buffer solution (pH 7.2) to remove the medium. The density of all bacterial samples was adjusted by OD_{600nm} (OD_{600nm} = 1.0), using the plate counting method and obtaining bacterial counts of about 10⁸ CFU/mL.

Supplementary Text 3. PDMS microchannel fabrication

The PDMS chip was fabricated via soft lithography, with the mold design crafted in *AutoCAD* and produced using a CADworks3D Printer (Ultra 50, Canada) to create the master mold. The main channel, Y-shaped, was 1 mm height, 2 mm wide and 35 mm long. The cross-linked PDMS mixture was cast into the mold and thermally cured at 65 °C for 4 hours. The challenge of laser treatment on concave channels necessitates a focus on the channel surface. This paper introduces a simple laser-cutting, masking, and DLW technique, in which the laser-cut PI sticker is masked onto the PDMS microchannel. After laser fabrication, the DLW-PDMS microchannels and the pure PDMS underwent a 3-minute oxygen plasma treatment (900 mTorr and 18W, PDC-32G-2, Harrick Plasma, USA), and the components were then assembled into the microfluidic chip. A control group with non-laser-treated microchannels was also included. The wall shear stress within the rectangular microchannel was calculated using the laminar-flow formula^[1]:

$$\tau = \frac{6\mu Q}{wh^2} \quad (1)$$

where τ is wall shear stress (Pa), μ is the dynamic viscosity of the PBS-based bacterial suspension, (0.001 Pa·s, approximated as water at 25 °C). At a bacterial concentration of 10^8 CFU/mL, the particle-volume fraction of *E. coli* in suspension is negligible. Under such dilute conditions, suspended microbial cells are not expected to alter the bulk viscosity of the aqueous buffer^[2]. Q is volumetric flow rate (m^3/s), w is channel width (m), and h is channel height (m). This flow rate yielded a target wall shear stress of 0.25 Pa.

Supplementary Text 4. Estimation of electron transfer rate constant (k^0)

The evaluation of the standard heterogeneous electron transfer rate constant (k^0) for the DLW-PDMS was performed in the 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl solution, but within a narrower potential window of -0.1 V to 0.5 V. Scan speed intervals of 5 mV/s were employed to study the relationship between the sweep speed and the peak currents.

k^0 for the quasi-reversible reaction occurring at the DLW-PDMS electrode was estimated by calculating the peak potential difference (ΔE_p) between the cathodic peak potential (E_{cp}) and the anodic peak potential (E_{ap}) for different numbers of laser repetitions (Supplementary Figure 10). This analysis was conducted using the

Nicholson method, as described in Equation 2^[3].

$$\Psi = k^0 \left(\frac{D_O}{D_R} \right)^{\frac{\alpha}{2}} \sqrt{\frac{RT}{\pi n F D_O}} \nu^{-\frac{1}{2}} \quad (2)$$

Where Ψ is a dimensionless parameter obtained from ΔE_p calculations ($\Delta E_p < 220$ mV), and D_O/D_R is the ratio of the diffusion coefficients of the corresponding oxidized and reduced forms in the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system, with $D_O = 7.6 \times 10^{-6}$ cm²/s and $D_R = 7.6 \times 10^{-6}$ cm²/s^[4]. The α represents the electron transfer coefficient, which equals 0.5. And n is the number of electrons transferred in the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system, which takes the value of 1. F is the Faraday constant, R is the gas constant, and T is the absolute temperature in K^[5]. And ν represents the scan rate. All parameters were chosen with reference to the relevant calculations from the carbon material electrodes.

Supplementary Text 5. Estimation of electrochemically active surface area (A)

The electrochemically active surface area of the DLW-PDMS electrodes was calculated using the Randles-Sevcik equation, as shown in Equation 3. This parameter was determined by plotting the anodic peak current (i_{ap}) and cathodic peak current (i_{cp}) as a function of the square root of the scan rate ($\nu^{1/2}$) in the CV plot presented in Supplementary Figure 10^[6].

$$i_p = 2.69 \times 10^5 n^{\frac{3}{2}} A C \sqrt{D} \nu^{\frac{1}{2}} \quad (3)$$

Where i_p is the peak current, and n is the number of electrons transferred, which takes the value of 1 in the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system. While A is the electroactive surface area (cm²), C is the concentration used in the redox system, D is the diffusion coefficient, which was used in this system with $\text{K}_3[\text{Fe}(\text{CN})_6]$, and ν is the scan rate^[7].

Supplementary Text 6. Evaluation of double-layer capacitance (C_{dl})

The double-layer capacitance characterization of the DLW-PDMS was further investigated using the CV method in a PBS buffer solution. The measurements were conducted within a potential window of 0.2 V to 0.4 V at various scanning speeds to investigate the capacitive behavior of the modified PDMS surface.

The CV curves obtained at different sweep rates were analyzed to determine the C_{dl} values of the DLW-PDMS electrodes, as shown in Supplementary Figure 11. The C_{dl} value of the DLW-PDMS electrode was calculated based on the non-faradaic current response at the surface of the DLW-PDMS electrode. This calculation was performed

according to Equation 4, where i represents the current density and C_{dl} is the capacitance^[8].

$$i = C_{dl} \frac{dv}{dt} \quad (4)$$

In comparison, metallic electrodes such as platinum and silver have reported double-layer capacitance values of 64 $\mu\text{F}/\text{cm}^2$ and 53 $\mu\text{F}/\text{cm}^2$, respectively^[8].

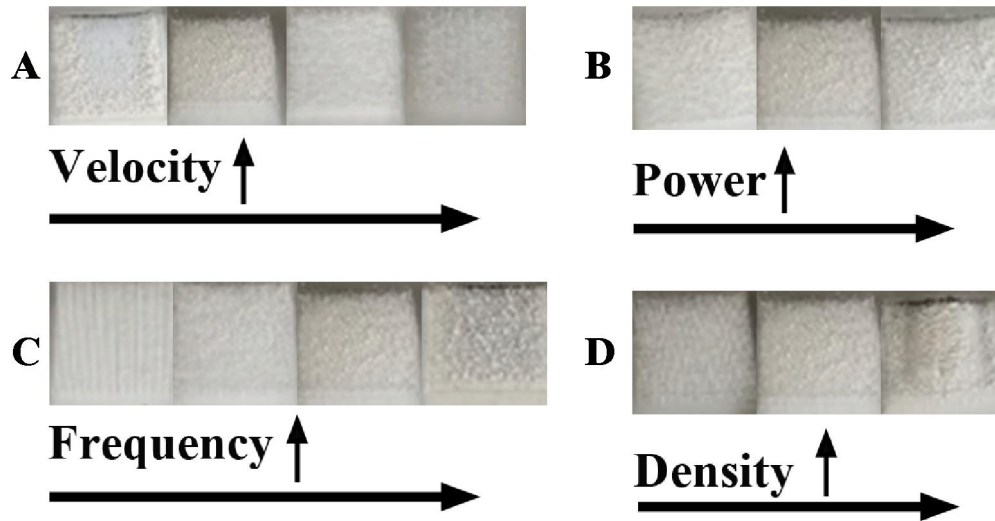
Supplementary Text 7. Electrochemical windows

The electrochemical window of the DLW-PDMS electrode was investigated in PBS solution under varying laser repetition conditions. The condition was conducted under 50 mV/s with a potential step of 10 mV and a voltage range of -1.2 to 2.0 V.

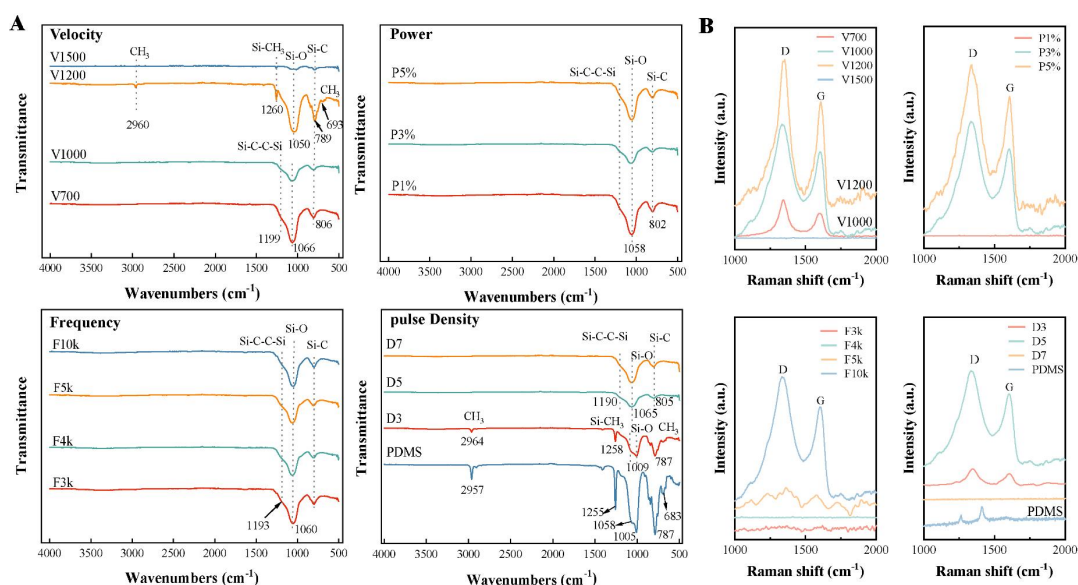
Supplementary Text 8. Optimization of BIP-DLW-PDMS

The optimization of the BIP-DLW_3 sensor was performed by systematically varying key parameters, using EIS for the characterization technique. The experiments were conducted in a 0.1 M KCl solution containing a 5 mM redox probe of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The parameters optimized included the number of electropolymerization cycles, elution time, and recognition time.

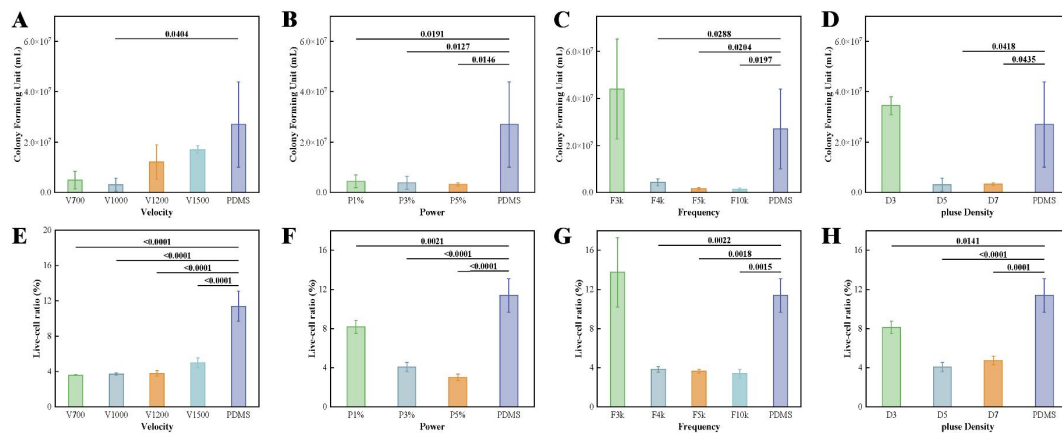
Supplementary Figures



Supplementary Figure 1. Appearance of single DLW-treated PDMS.

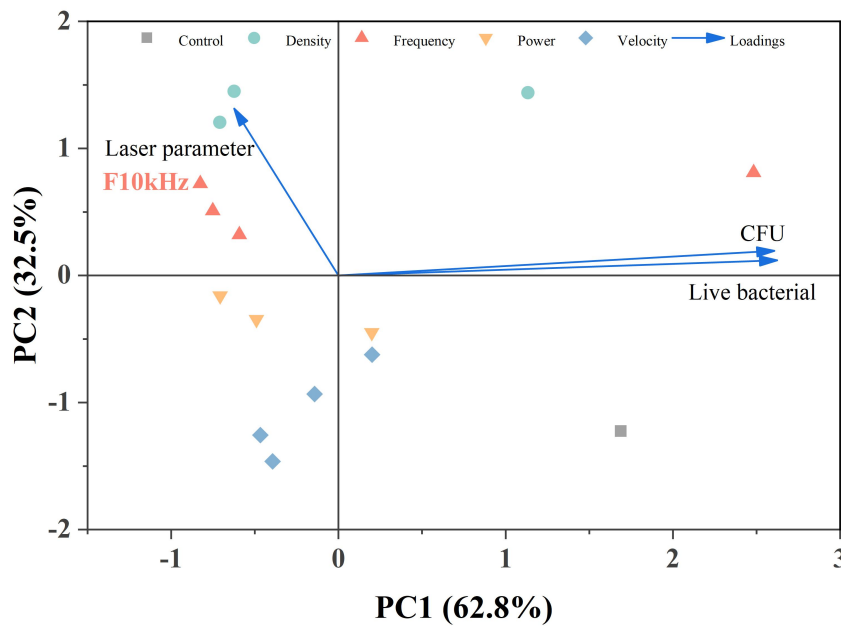


Supplementary Figure 2. Characterization of the single DLW treatment. (A) ATR-FTIR spectra of sample powders obtained under different laser parameters (Supplementary Table 1). Label V represents velocity, with V700 indicating a processing speed of 700 mm/s. Other parameters are listed in Supplementary Table 1. Label P represents power; label F represents frequency, and label D represents pulse density. (B) Raman spectra of samples under different laser parameters showed the appearance of D and G peaks characteristic of carbon material.

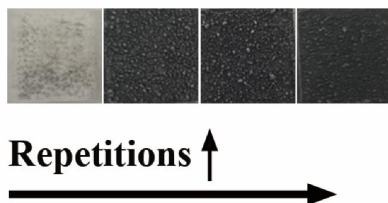


Supplementary Figure 3. Antifouling performance of the single DLW treatment.

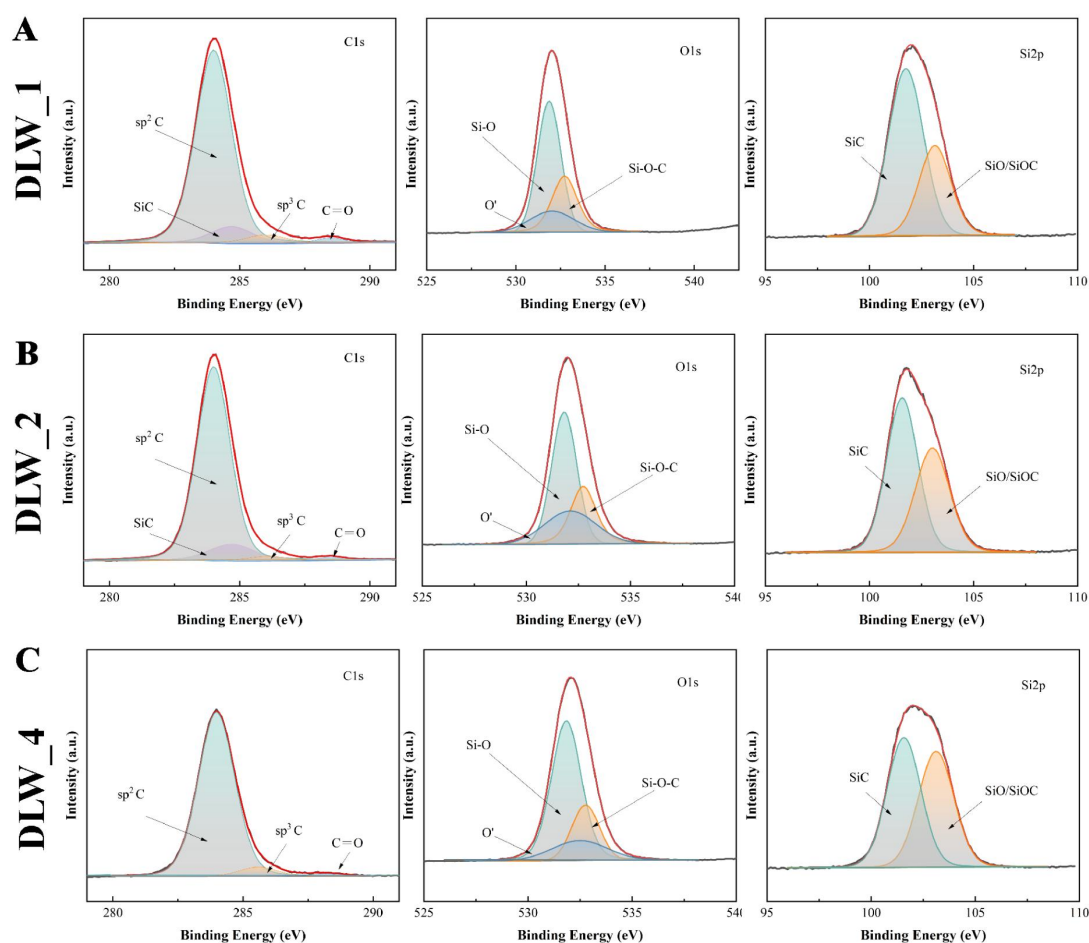
(A-D) The total number of colonies on the sample slices after 24h of incubation was analyzed for a significant difference ($\alpha = 0.05$) relative to the total number of colonies attached to the pure PDMS slices, using the Turkey test ($n=3$). (E-H) Live bacterial ratio of the samples after live and dead bacterial staining. Significant difference analysis was performed using the Tukey test in the pure PDMS group ($n=4$).



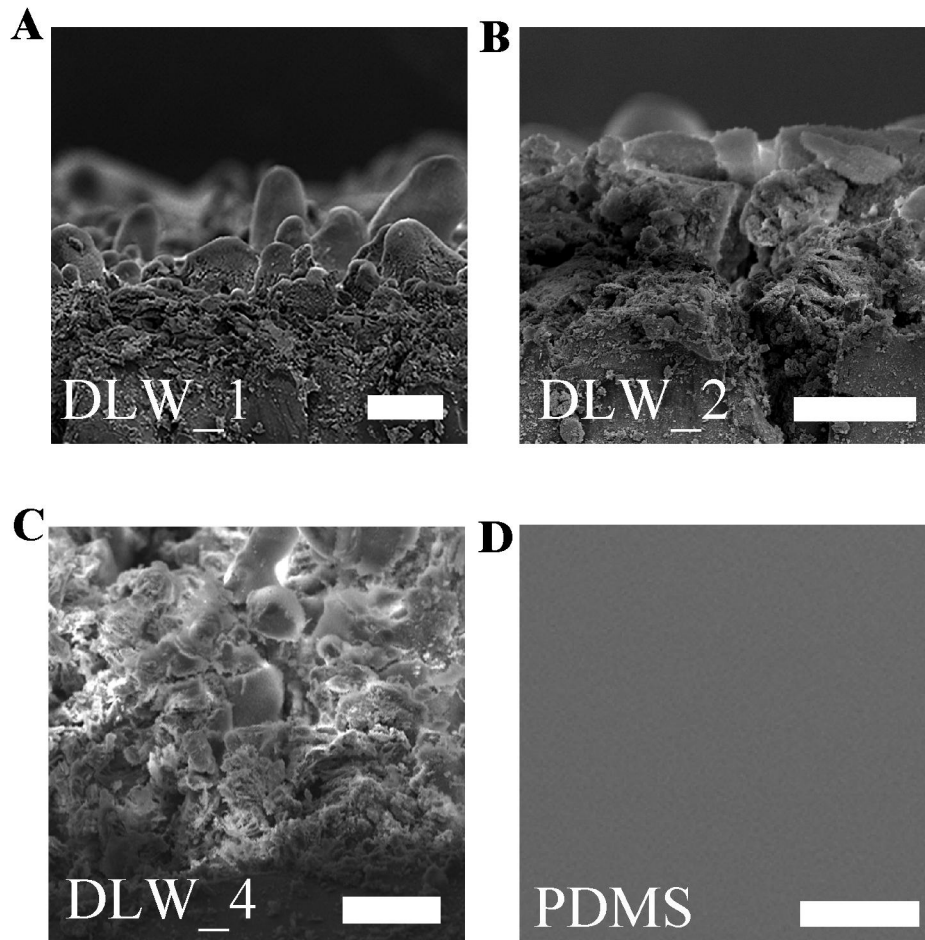
Supplementary Figure 4. Principal component analysis (PCA) biplot for the assessment of antifouling performance of PDMS surfaces treated with different laser parameters, based on bacterial CFU and live bacterial ratio. Different symbols denote distinct laser parameter groups (Power, Velocity, Density, Frequency) and the control group, with the optimal 10 kHz Frequency (F10 kHz) highlighted in the Frequency group. PC1 and PC2 account for 62.8% and 32.5% of the total variance, respectively.



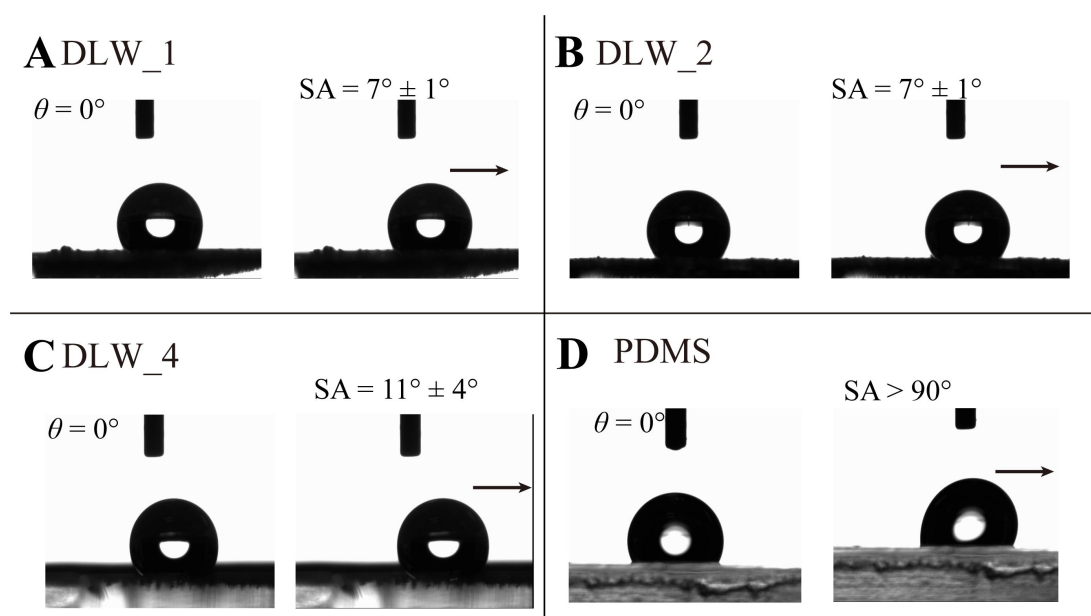
Supplementary Figure 5. Appearance of PDMS with different laser repetitions. From left to right, they are DLW_1, DLW_2, DLW_3, and DLW_4.



Supplementary Figure 6. XPS maps showing the compositional changes of the samples with different laser repetitions. The C1s, O1s, and Si2p regions correspond, from left to right, respectively. (A) shows the elemental composition of DLW_1, which is a single DLW treatment; (B) shows DLW_2, which is two repetitive DLW treatments; and (C) shows DLW_4, which is four repetitive DLW treatments.

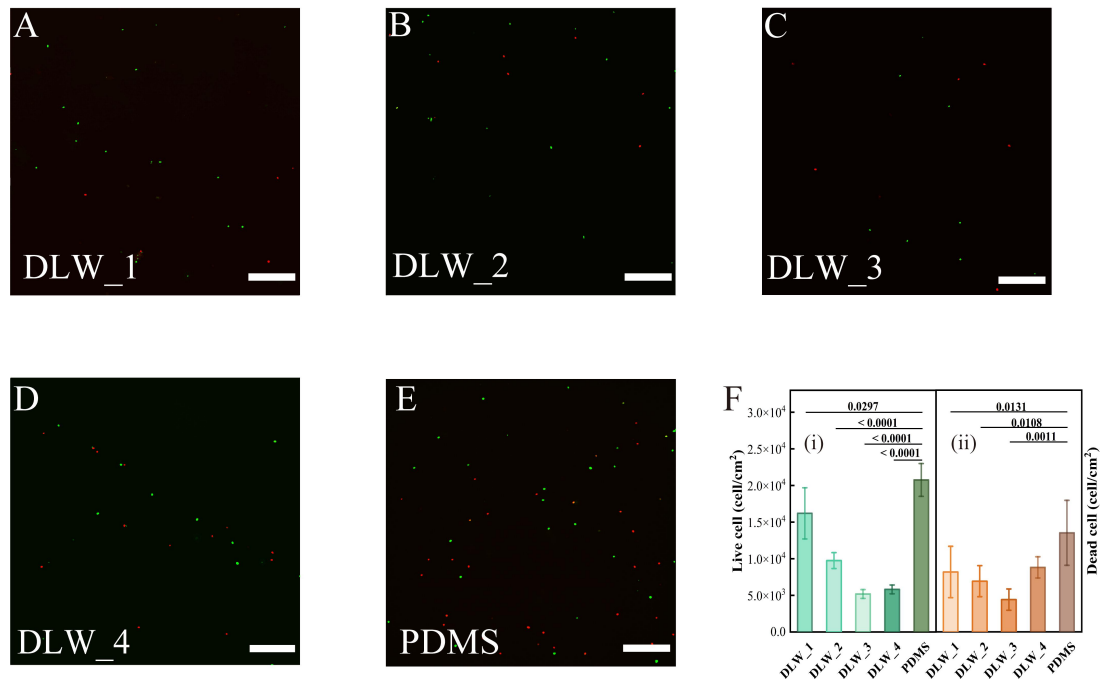


Supplementary Figure 7. SEM image of the different laser repetitions. (A) is a single DLW treatment labelled DLW_1; (B) are two DLW treatments labelled DLW_2; (C) are four DLW treatments labelled DLW_4; and (D) is pure PDMS without DLW treatment labelled PDMS.

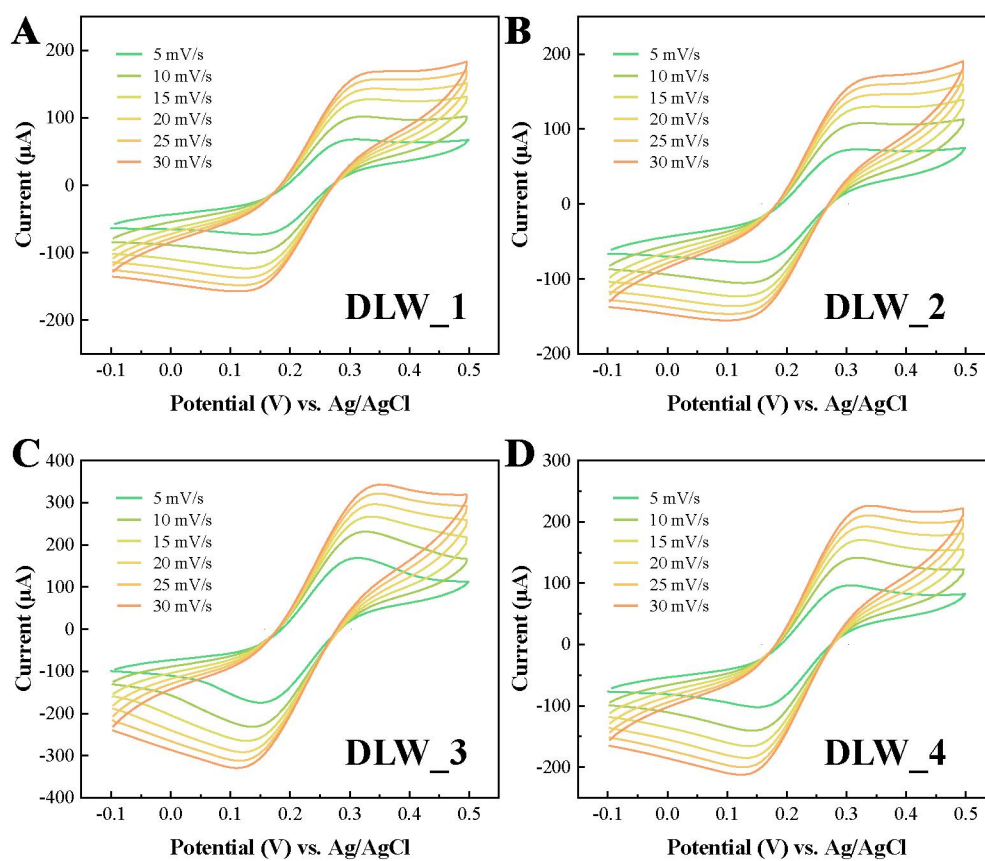


Supplementary Figure 8. Sliding angle (SA) measurements of DLW samples prepared with different laser repetition numbers. (A–C) DLW_ n samples exhibit low-adhesion property, n represents the laser repetition. (D) The PDMS control group shows high-adhesion behavior, with a $SA > 90^\circ$, as the water droplet remains firmly attached to the surface even at near-vertical tilting. All measurements were performed using 5 μ L deionized water via the tilting platform method. Data is presented as mean $\pm$ standard deviation from three independent measurements.

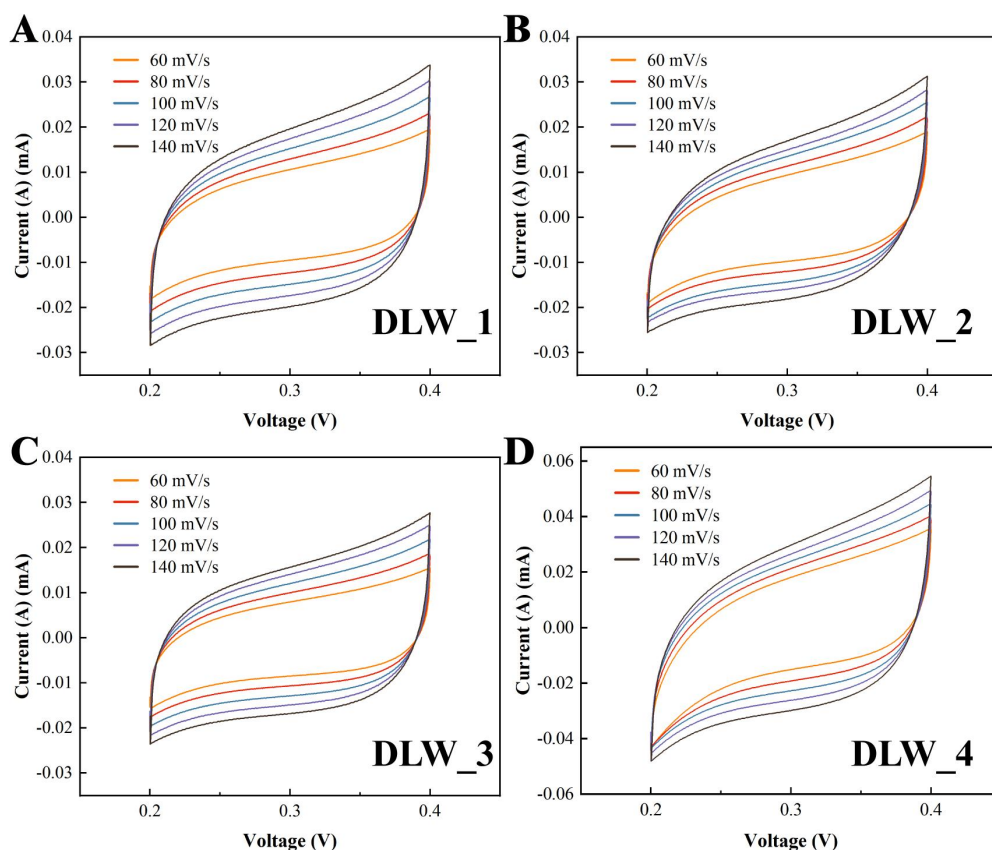
24h antifouling



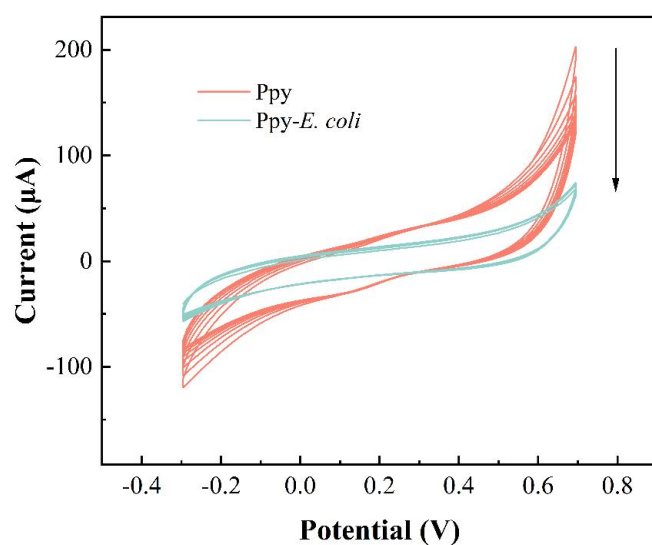
Supplementary Figure 9. Fluorescence images and analysis of live and dead bacteria after different laser repetitions. (A–E) FITC (green) and TRITC (red) channels represent live and dead bacteria, respectively. Scale bars: 100 μm . (F) Bacterial quantification was performed using *FIJI* and converted according to the number of fields under 40 $\times$ lens view. (i) shows live bacterial counts, and (ii) shows dead bacterial counts. Statistical analysis was performed by one-way ANOVA followed by Tukey's test ($n = 3$, 5 sites for each group). Only significant differences between DLW_n and PDMS are shown. In inset (i), DLW_3 is significantly different from DLW_1 and DLW_2, but not from DLW_4.



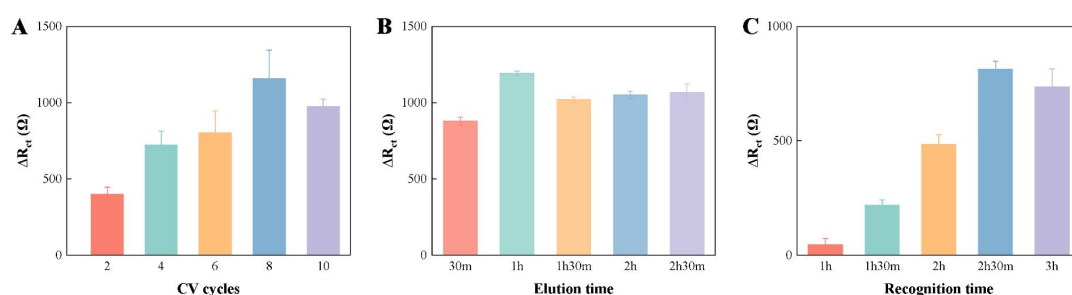
Supplementary Figure 10. CV for different laser repetition rates with a redox probe with varying scan rates. Where (A) represents the CV behavior of DLW_1 at varying scan rates, (B) represents the CV plot with two laser repetitions (DLW_2), (C) is three laser repetitions (DLW_3), and (D) is four laser repetitions (DLW_4).



Supplementary Figure 11. CV results for DLW-PDMS electrodes at different laser repetitions and scan rates. The potential interval was set to 0.2-0.4 V, and the electrolyte was PBS buffer solution. (A) represents the CV behavior of DLW_1 at varying scan rates, (B) represents the CV plot with two laser repetitions (DLW_2), (C) with three laser repetitions (DLW_3), and (D) with four laser repetitions (DLW_4).



Supplementary Figure 12. Electropolymerization processes on DLW_3 electrodes in the absence (pyrrole, Ppy) and presence of *E. coli* (pyrrole-*E. coli*, Ppy-*E. coli*).



Supplementary Figure 13. Impedance response for BIP-DLW-PDMS optimization. (A) is the number of polymerization cycles, (B) is the elution time, and (C) is the recognition time.

Supplementary Tables

Supplementary Table 1. Morphology and size of the target bacteria and four interfering bacteria

Bacteria	Morphology	Size
<i>E. coli</i>	Rod-shaped	1.5 μm (L) $\times$ 0.5 μm (W)
<i>S. aureus</i>	Spherical or Elliptical	0.5 - 1 μm
<i>S. enterica</i>	Rod-shaped	2-5 μm (L) $\times$ 0.7-1.5 μm (W)
<i>P. aeruginosa</i>	Rod-shaped	1-5 μm (L) $\times$ 0.5-1.0 μm (W)
<i>E. faecalis</i>	Spherical or Elliptical	0.5 - 1 μm

Supplementary Table 2. Laser parameter setting

Parameter	Velocity (mm/s)	Power (W)	Frequency (kHz)	Pulse Density (pulse/dot)
V group	700	1.8	5	5
	1000			
	1200			
	1500			
P group	1000	0.6	5	5
		1.8		
		3		
F group	1000	1.8	3	5
			4	
			5	
			10	
D group	1000	1.8	5	3
				5
				7

Note: A parameter label identifies each variable group. For example, velocity change is labeled V, and a velocity of 700 mm/s corresponds to the parameter group labeled V700. Specifically, the parameter labeled DLW in the main text corresponds to the F10k frequency group.

Supplementary Table 3. FWHM of the Si-O peaks in the laser repetition FTIR map obtained from Gauss fitting

Repetitions	FWHM
DLW_1	167.03
DLW_2	159.91
DLW_3	159.42
DLW_4	161.25

Supplementary Table 4. Elemental proportions based on XPS full spectrum

Repetitions	Atomic (at%)		
	C	O	Si
DLW_1	35.51	37.17	27.32
DLW_2	37.66	35.08	27.26
DLW_3	38.75	34.44	26.81
DLW_4	33.71	38.23	28.05

Supplementary Table 5. Batch consistency of DLW_3 in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$

Electrode Batch	Fabrication Date	R_{ct}
Batch 1	Day 1	177 ± 24
Batch 2	Day 14	181 ± 17
Batch 3	Day 30	172 ± 10

Note: three independent sensors per batch

Supplementary Table 6. Impedance detection results of the BIP-DLW-PDMS sensor for *E. coli* in real samples

Sample source	Calculated	Recovery (%)	RSD (%)
	concentration (CFU/mL)		
PBS	1.07×10^6	93	3.39
Tap water	1.20×10^6	105	2.91
Sea water	1.32×10^6	115	1.75
Milk	9.12×10^5	79	14.49

Note: The bacterial concentration used for spiking was confirmed to be 1.15×10^6 CFU/mL using the plate method. Bacterial concentrations were determined using calibration curves. This calculated value was determined by averaging the values. The recovery was calculated from the computed value of bacterial concentration. RSD was calculated from three samples measured simultaneously.

Supplementary Movie 1. Direct laser writing on PDMS.

The laser direct action on pristine PDMS was conducted under ambient conditions. The movie was accelerated 3x.

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