



A unified antifouling and electroactive platform from laser-pyrolyzed PDMS

Wanqing Dai¹, Le Cheng², Jing Sun^{1,3}, Qiang Guo¹, Xinyu Wu², Fred Wang-Fat Lee^{1,4}, Nora Fung-Yee Tam^{1,2}, Yung-Kang Peng² , Ruquan Ye^{2,4,*} , Jian Lin Chen^{1,3,4,*} 

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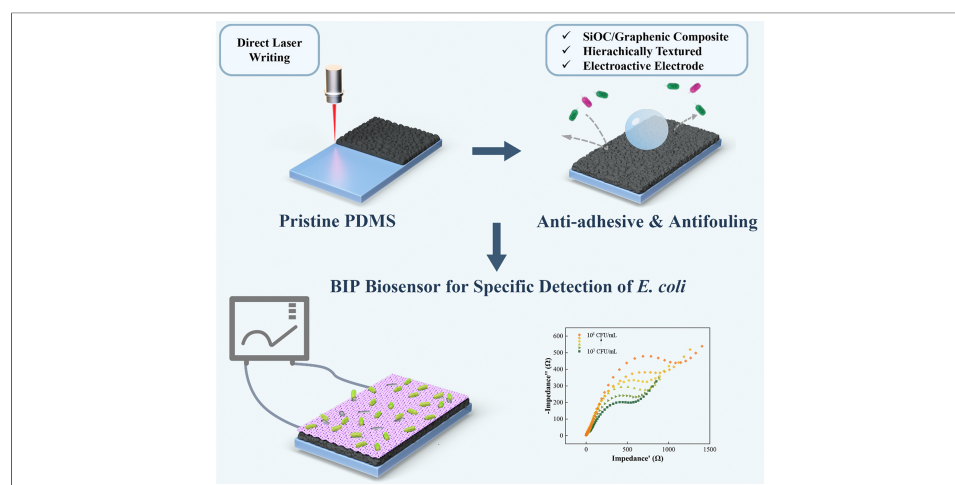
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Abstract

Polydimethylsiloxane (PDMS) is a foundational elastomer for flexible microdevices. Yet, a long-standing materials dichotomy fundamentally constrains its utility: surfaces engineered to resist biofouling typically lack the electrical conductivity and wettability required for electrochemical sensing. Here, we resolve this paradox through a one-step, ambient-air direct laser writing (DLW) strategy that monolithically transforms pristine PDMS into a superhydrophobic hierarchical silicon oxycarbide (SiOC)/graphenic composite (water contact angle = 157°, sliding angle ≈ 3°). Guided by the Young-Dupré equation, the tailored apparent work of adhesion of this architecture provides a thermodynamic barrier to biofouling, achieving a 94.47% reduction in *Escherichia coli* (*E. coli*) attachment under high-load conditions (10⁸ CFU/mL). However, under applied electrochemical potentials, the hierarchical domains undergo localized electrowetting, enabling rapid interfacial charge transfer ($k^0 = 0.00708$ cm/s) without compromising macroscopic antifouling properties. Leveraging this switchable interface, we demonstrate the *in-situ* fabrication of a bacterial-imprinted polymer (BIP) biosensor directly onto the antifouling substrate. Achieving a limit



¹Department of Applied Science, School of Science & Technology, Hong Kong Metropolitan University, Hong Kong, China.

²Department of Chemistry, City University of Hong Kong, Hong Kong, China.

³Shenzhen Research Institute of Hong Kong Metropolitan University, Shenzhen 518000, Guangdong, China.

⁴State Key Laboratory of Marine Environmental Health, City University of Hong Kong, Hong Kong, China.

*Correspondence to: Prof. Jian Lin Chen, Department of Applied Science, School of Science & Technology, Hong Kong Metropolitan University, Hong Kong, China. E-mail: jchen@hkmu.edu.hk; Prof. Ruquan Ye, Department of Chemistry, City University of Hong Kong, Hong Kong, China. E-mail: ruquanye@cityu.edu.hk

of detection of 348 CFU/mL, this work offers a feasible paradigm for monolithic, self-cleaning bioelectronics, eliminating the need for complex multi-material integration.

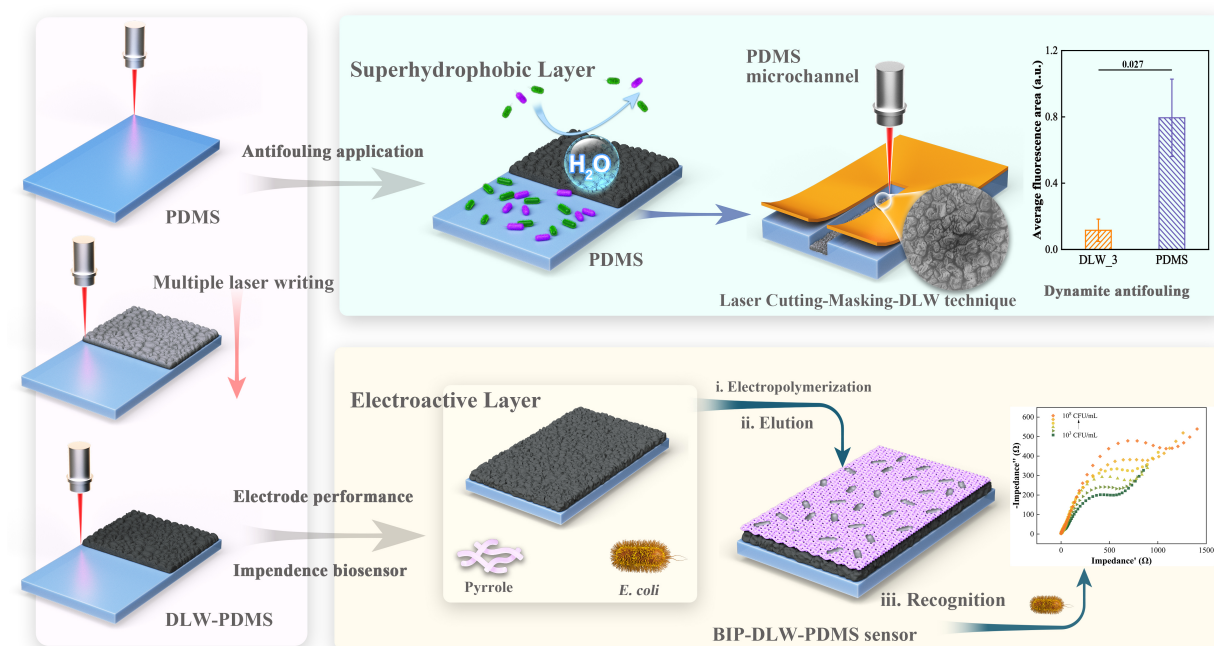
INTRODUCTION

Polydimethylsiloxane (PDMS) remains the dominant material in flexible microdevices, valued for its optical transparency, biocompatibility, and facile fabrication^[1]. While the inherent insulating nature and susceptibility to hydrophobic biofouling of PDMS limit its utility in complex bioelectronics, researchers have historically managed this tradeoff through the intelligent engineering of composite materials. Strategies incorporating polyurethane copolymers^[2], amphiphilic ionic polymers^[3], metallic nanomaterials^[4,5], and graphene-based materials^[6] have successfully demonstrated dual electroactive and antifouling capabilities. However, translating these heterogeneous solutions to PDMS platforms introduces structural and operational compromises.

Surface-grafted hydrophilic polymers and drop-casted conductive inks suffer from thermodynamic incompatibility with the low-surface-energy PDMS substrate, inevitably leading to delamination under sustained microfluidic shear or electrochemical stress. Alternatively, incorporating metallic biocides leads to finite leaching and unintended cytotoxicity, while bulk doping with carbonaceous networks severely embrittles the elastomer. Consequently, a critical gap remains: achieving stable, high-performance electrochemical and antifouling properties in PDMS without relying on unstable heterogeneous coatings or mechanically destructive bulk additives.

While laser-based surface engineering presents a compelling pathway for spatially resolved material modification, its direct application to PDMS is fundamentally limited by the elastomer's low intrinsic carbon yield. Common laser photothermal treatment fails to directly convert pristine PDMS into high-quality laser-induced graphene (LIG), despite the excellent electrochemical properties that render LIG highly desirable for electrochemical biosensing^[7,8]. To circumvent this limitation, current methodologies rely on heterogeneous workarounds, such as doping the matrix with external carbon precursors^[9] or employing cumbersome transfer protocols. Specifically, LIG is pre-fabricated on substrates such as polyimide (PI) and subsequently transferred onto PDMS surfaces^[10]. Exogenous carbon doping alters the inherent physicochemical and mechanical properties of PDMS, whereas LIG transfer methods are hampered by procedural complexity and limited interfacial affinity. Also, ambient-fabricated LIG is inherently hydrophilic, which accelerates bacterial adhesion and biofouling, failing to meet rigorous antifouling and durability demands for long-term *in-situ* microbial monitoring.

Fabricating monolithic conductive PDMS requires harsh laser protocols, such as femtosecond-laser irradiation or oxygen-free pyrolysis, to yield conductive SiC^[11,12]. Ambient CO₂ laser treatment produces more insulating SiO₂ that is primarily engineered to create superhydrophobic micro/nanostructures for wettability tuning^[13-15]; a few reports functionalize them with Au nanoparticles for heavy-metal surface-enhanced Raman spectroscopy (SERS) detection^[16], yet studies on conductive SiC-carbon phases remain largely confined to mechanical strain sensing^[17]. Relevant literature rarely includes systematic characterizations of their electrochemical properties or evaluations of their electrochemical biosensing potential, and synergistic integration of inner conductive networks and outer antifouling textures for pathogen detection remains underexplored. The widespread belief that the outer superhydrophobic silica film blocks solid-liquid contact and hinders Faradaic charge transfer further explains why such materials have been largely overlooked as all-in-one biosensing platforms^[18,19].



Scheme 1. Fabrication of a superhydrophobic and electroactive layer on PDMS via CO₂ laser-based DLW and its bi-applications in antifouling and as functional electrodes and biosensors for BIP technology. PDMS: Polydimethylsiloxane; DLW: direct laser writing; BIP: bacterial-imprinted polymer; *E. coli*: *Escherichia coli*.

This study resolves this limitation by introducing a monolithic material transformation. Rather than applying an external functional layer, we utilize a one-step, ambient-air direct laser writing (DLW) strategy to photothermally transmute pristine PDMS into a hierarchical silicon oxycarbide (SiOC)/graphenic composite. Critically, we demonstrate that repeated laser passes are essential to allow thermal relaxation while building the hierarchical roughness necessary for both superhydrophobicity and enhanced electroactive surface area. The central innovation lies in the synergistic integration of robust antifouling properties and high-performance electrochemistry onto a single monolithic substrate. By systematically investigating the physicochemical and electrochemical properties of the DLW-modified domains, we demonstrate the *in-situ* fabrication of a bacterial-imprinted polymer (BIP) sensor directly onto the electroactive surface. The resulting platform [Scheme 1] presents a highly selective *Escherichia coli* (*E. coli*) biosensor that maintains mechanical and operational stability, advancing the rational design of multifunctional bio-analytical microdevices.

EXPERIMENTAL

Materials

The details of the chemicals and instruments used are provided in the [Supplementary Text 1](#).

DLW method

The PDMS slices were positioned on a metal table and treated with a commercial 10.6 μm CO₂ laser (MSDB-FM60, Minsheng Laser, China, 60 W) in ambient air. The parameters were set at a speed of 1,000 mm/s, a power of 1.8 W, a frequency of 10 kHz, and a pulse density of 5 pulses per dot, henceforth referred to as DLW_1. DLW_*n* denotes *n* repetitions of a laser scan under the DLW_1 condition. After treatment, the samples were sectioned into 1 cm × 1 cm pieces for further experiments.

Characterization

The chemical bonding in DLW-PDMS was investigated using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (Bruker, Germany) over the range 500–3,200 cm^{-1} . A Raman microscope (inVia, Renishaw, UK) equipped with a 532 nm excitation was also employed. The exposure conditions were set to 10% power for 10 s under a 20 $\times$ lens. The X-ray diffraction (XRD) pattern was obtained using a Rigaku instrument (SmartLab, Japan) with Cu K α radiation. Each sample was scanned within the 10°–60° range at a rate of 2°/min. X-ray photoelectron spectroscopy (XPS) spectra were collected using a Thermo Scientific K-Alpha spectrometer (USA) operated at an accelerating voltage of 12 kV. Monochromatic Al K α radiation was employed as the X-ray source. The surface structure of DLW-PDMS was visualized using a Hitachi Regulus 8100 scanning electron microscopy (SEM, Japan). Hydrophobicity was quantified using a DataPhysics OCA 15EC instrument (Germany). A 5 μL deionized-water droplet was deposited onto the sample surface to measure the water contact angle (WCA) and sliding angle (SA). For the SA measurement, the stage was conducted at a tilt speed of 1.0 °/s. Data represent average values from three independent samples per group, with three spots measured on each sample.

Antifouling capabilities assessment

E. coli (ATCC 25922) was prepared in LB broth, resuspended in 1 $\times$ phosphate-buffered saline (PBS) solution, and adjusted to 10⁸ colony-forming units per mL (CFU/mL). The DLW-PDMS samples were submerged in the bacterial solution at 37 °C for 24 h. The slices were then removed, rinsed gently with 1 $\times$ PBS, and transferred to centrifuge tubes containing 5 mL of buffer for a sequential vortex-sonicate-vortex treatment (2-4-2 min). The bacteria were then counted using the plate counting method.

Bacterial viability was evaluated using a live/dead kit (Thermo Fisher, USA) and a microreader (SpectraMax iD3, Molecular Devices, USA). The image was captured with a 40 $\times$ confocal fluorescence microscope (CLSM, Nikon, Japan). For long-term antifouling assessments, a working solution of *E. coli* expressing green fluorescent protein (*E. coli*-GFP, ATCC 25922-GFP) was cultured with the DLW-PDMS sheets at 37 °C for 7 days. Following rinsing, surface biofilm formation was captured using CLSM. The excitation wavelength was 488 nm, with a Z-stacking interval of 2.5 μm . The 3D visualizations were generated using the 3D viewer plugin in FIJI^[20].

DLW-PDMS microchannel preparation and dynamic antifouling performance evaluation

The preparation of the DLW-PDMS microchannel is detailed in [Supplementary Text 3](#). The treated chips underwent a triple ethanol-deionized (DI) water cleaning before evaluation. The chips were positioned on a heating table at 37 °C. A 50 mL syringe containing a bacterial solution of *E. coli*-GFP at 10⁸ CFU/mL was circulated through the DLW-PDMS chip at 5 $\mu\text{L}/\text{min}$, with the solution renewed every 48 h to preserve bacterial activity. Following a week-long circulation of the bacterial solution, unattached bacteria were cleared with a PBS rinse, and the chips were then observed at 20 $\times$ magnification using CLSM.

Electrochemical properties

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) experiments were utilized to examine the electrochemical properties of DLW-PDMS as a working electrode. EIS experiments were conducted on a Zahner electrochemical workstation (Zennium XC, Germany) using a 100 mM solution of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl. The experimental conditions were set to an amplitude of 10 mV and a frequency range of 0.1–100,000 Hz. The CV experiments were performed in a 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. Routine CV characterization was conducted at a scan rate of 50 mV/s over the range of -0.6 to 1.2 V. For parameter-specific measurements, CV at 30 mV/s from -0.1 to 0.5 V was used to calculate the electron transfer rate constant (k^0) and CVs at varied scan rates were recorded to determine electrochemically active surface area (A). For double-layer capacitance (C_{dl}), CVs at varied scan rates were acquired in PBS from 0.2

to 0.4 V. Independent scans from -1.2 to 2.0 V were used to evaluate the electrochemical window (ECW). Detailed calculations are available in the [Supplementary Materials](#).

Electropolymerization fabrication procedure for bacterial (non)-imprinted polymer sensors with DLW-PDMS

The electrochemical polymerization was carried out in a 10 mL solution containing 0.1 M KCl, 0.05 M pyrrole, and *E. coli* at 10^8 CFU/mL. The process was performed within an ECW of -0.3 to 0.7 V at a scan rate of 50 mV/s. After eight electrochemical cycles, the sample was transferred to an elution solution containing 5% w/v sodium dodecyl sulfate (SDS) in 5% v/v HAc solution (pH = 2.47) and immersed for 1 h to remove the bacteria. Following the elution step, the electrode was gently rinsed with sterilized DI water. The resulting electrode was identified as the BIP-DLW-PDMS sensor. A non-imprinted polymer (NIP) electrode was prepared using the same steps without adding bacteria during electropolymerization and served as a control. SEM was employed to study the morphology of each step in the process, while *E. coli*-GFP was used for *in-situ* confocal fluorescence imaging (60× lens).

Impedimetric detection of *E. coli* based on BIP-DLW-PDMS

The prepared BIP-DLW-PDMS was incubated with various concentrations of *E. coli* from 10^3 to 10^8 CFU/mL for 2.5 h. EIS measurement was conducted in a 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl over a frequency range of 0.1 to 100,000 Hz. The impedimetric changes were processed using: $\Delta R_{ct} = |R_{ct1} - R_{ct0}|$, where R_{ct1} is the value of the R_{ct} of the BIP-DLW-PDMS sensor with bacteria, R_{ct0} is the value without bacteria.

Performance evaluation of BIP-DLW-PDMS sensor

S. aureus (ATCC 25923), *S. enterica* (ATCC 13076), *P. aeruginosa* (ATCC 27853), and *E. faecalis* (ATCC 6569) were selected as interferents [[Supplementary Table 1](#)]. Individual measurements for all tested bacteria, including the target *E. coli*, were performed at 10^8 CFU/mL. For mixed-culture competitive selectivity tests, *E. coli* (10^7 CFU/mL) was mixed with pooled non-target bacteria (total 10^8 CFU/mL), corresponding to a target-to-nontarget concentration ratio of 1:10.

The real samples, including tap water, seawater, and milk, were collected from local sources. Milk samples were pretreated by a two-fold dilution before measurement. The real samples were spiked with 10^6 CFU/mL *E. coli* and tested in triplicate.

RESULTS AND DISCUSSION

Formation and structural evolution of the SiOC composite

Initial single-pass DLW treatments under varied parameters [[Supplementary Table 2](#) and [Supplementary Movie 1](#)] produced surfaces with minor visual differences [[Supplementary Figure 1](#)]. Still, they induced significant compositional changes in the Si-O, Si-C, and carbon [[Supplementary Figure 2](#)]. Owing to PDMS's substantial infrared absorption at 10.6 μm , a CO_2 laser at this wavelength enables efficient photothermal modification under ambient-air conditions^[21]. Notably, increased carbon content and reduced SiO_2 correlated with improved antifouling efficacy [[Supplementary Figure 3](#)]. This observation prompted a strategy of increased laser-overlap exposures, hypothesized to promote a more ordered, graphitic carbon structure^[22], which is known to exhibit superior interfacial properties compared to amorphous carbon^[23,24].

To investigate this hypothesis, the PDMS substrate was subjected to multiple laser scans under the optimal antifouling parameter condition identified by principal component analysis (PCA), denoted F10k [[Supplementary Figure 4](#)]. This repetitive process induced a distinct visual transformation from white to black [[Supplementary Figure 5](#)], indicative of substantial photothermal modification. FTIR spectra revealed a

surface chemistry dominated by Si–O and Si–C bonds [Figure 1A]. A progressive narrowing of the Si–O peak [Supplementary Table 3] suggests the selective ablation of the amorphous SiO₂ layer with repeated laser exposure^[25]. Concurrently, Raman spectroscopy demonstrated a pronounced increase in the D and G band intensities [Figure 1B]. The intensity ratio (I_D/I_G) decreased with successive scans, reaching 1.13 for the DLW_3 condition (DLW_*n* denotes *n* laser-scan repetitions), consistent with the formation of an ordered, pyrolyzed carbon structure^[26]. Extended scanning further weakened both carbon-related bands and raised the I_D/I_G ratio to 1.23, reflecting structural evolution and partial degradation of the carbon phase.

XRD analysis provided further insight into the crystalline evolution of the surface [Figure 1C]. The untreated PDMS exhibited broad amorphous peaks ($2\theta = 12^\circ$ and 22.6°). However, the laser-treated surfaces initially showed one broad peak near 23° , characteristic of amorphous SiO₂ or SiOC^[27,28]. With increasing numbers of laser repetitions, distinct diffraction peaks corresponding to crystalline SiC and graphitic carbon emerged^[29,30]. These signals imply phase segregation during the formation of crystalline domains, consistent with previously reported mechanistic analyses^[31,32]. Notably, the absence of characteristic SiC diffraction peaks in DLW_4 agrees with the trends observed in Raman measurements and with earlier reports that excessive thermal exposure drives carbon vaporization and suppresses SiC formation^[28].

To elucidate the precise surface chemistry, XPS was performed on the DLW samples [Figure 1D and Supplementary Figure 6]. Deconvolution of the high-resolution C 1s spectrum resolved peaks at sp²-C (284 eV), Si-C (284.7 eV), sp³-C (286 eV), and C=O (288.3 eV), while the Si 2p region indicated the presence of Si-C (101.5 eV) and a combined Si-O/Si-OC phase (102.9 eV)^[33,34]. The measured elemental composition for the DLW_3 surface (Si = 26.81 at%, O = 34.44 at%, C = 38.75 at%; Supplementary Table 4) is in close agreement with values reported for pure SiOC^[34]. The reduction in carbon content for DLW_4 (33.71 at%, Supplementary Table 4) coincides with structural changes detected by Raman and XRD measurements, suggesting carbon depletion under excessive laser irradiation. These results revealed that repetitive laser pyrolysis in ambient air transforms the PDMS surface into a composite material comprising an amorphous SiOC matrix embedded with crystalline SiC and graphene-like carbon nanoparticles^[17,35].

The laser-induced physicochemical transformation culminated in a hierarchical surface morphology, characterized by a wrinkled, multi-scale structure, as observed by SEM [Figure 1E and Supplementary Figure 7]. The resulting micro- and nanoscale topography, combined with the altered surface chemistry, drove a transition to superhydrophobicity. The WCA increased from 112° on pristine PDMS to 157° for the DLW_3 condition [Figure 1F], accompanied by a dramatic reduction in the SA to 3° [Figure 1G and Supplementary Figure 8], indicating a robust Cassie-Baxter wetting state consistent with a previous report^[15]. Further increases in the number of laser scans produced heavily wrinkled surface topography for DLW_4. These morphological alterations, together with the compositional degradation identified above by Raman, XPS, and XRD, coincided with degraded wetting performance: the WCA decreased to 139° , whereas the SA increased to 11° . The combined set of observations points to partial volatilization of surface-enriched SiOC/C and disruption of the beneficial hierarchical micro-nanotexture, counteracting the advantages achieved under moderate repeated laser exposure.

Thermodynamic antifouling performance

The antifouling efficacy of the DLW-PDMS surfaces was systematically evaluated via CFU assays. The DLW_3 surface exhibited optimal performance, achieving a 94.47% reduction in *E. coli* attachment after 24 h compared to the untreated PDMS control ($P = 0.0019$, Figure 2A). Consistently, confocal images revealed synchronous reductions in both live and dead bacteria [Supplementary Figure 9]. This efficiency is highly competitive with state-of-the-art LIG filtration membranes loaded with 0.5 mg/cm² graphene oxide (GO, 95%)^[36] and PDMS doped with gallium-based liquid metal nanodroplets (90%)^[4].

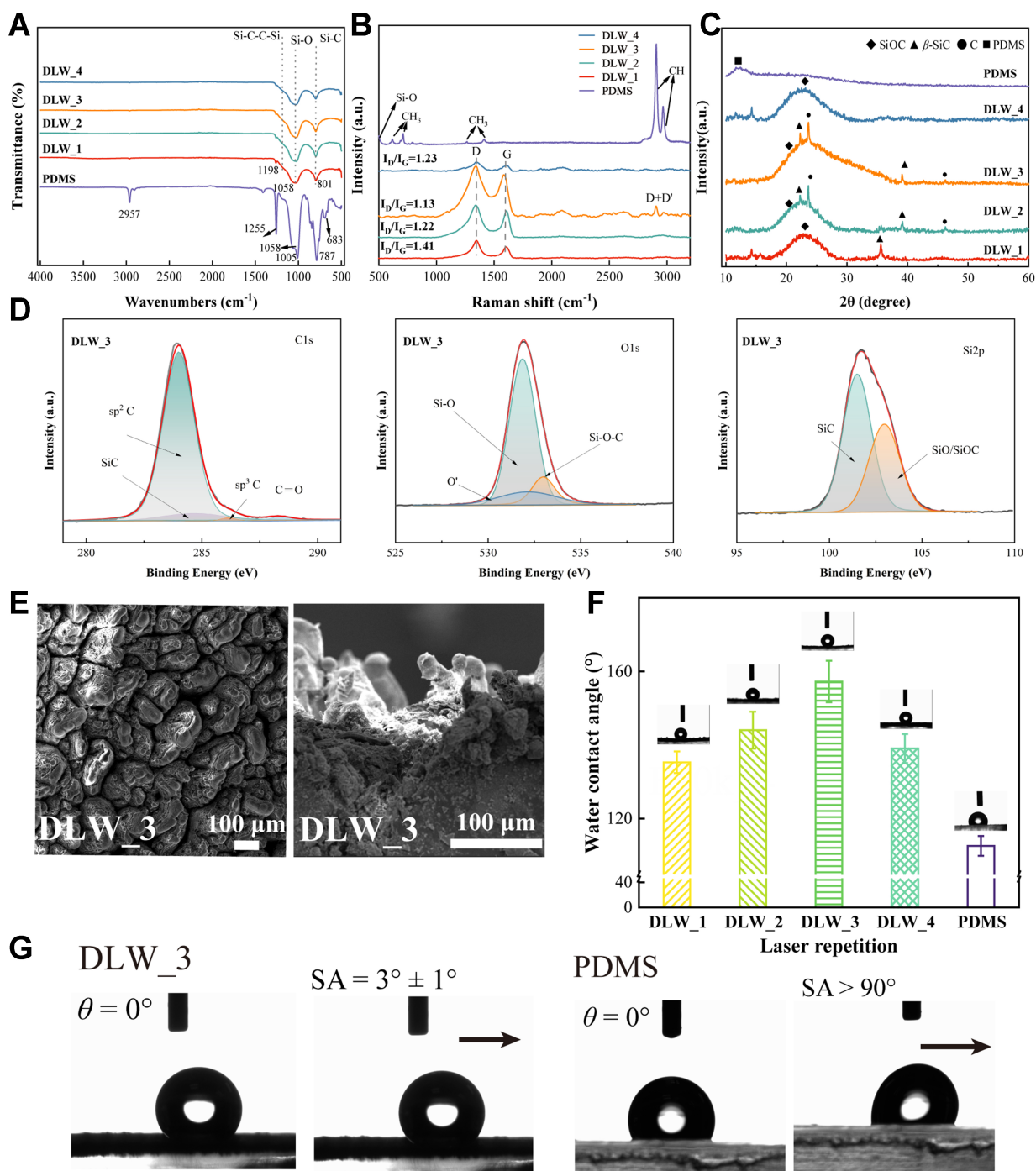


Figure 1. Characterization of the DLW-PDMS under different laser repetitions. DLW_ n denotes n repetitions of a laser scan (1,000 mm/s speed, 1.8 W power, 10 kHz frequency, 5 pulses/dot). PDMS refers to the untreated substrate. (A) ATR-FTIR spectra; (B) Raman spectra showing the evolution of D and G bands; the I_D/I_G ratio is compared; (C) XRD patterns illustrating the emergence of crystalline phases with increased repetitions; (D) XPS spectra of the C 1s, O 1s, and Si 2p regions for the DLW_3 surface. The XPS spectra from other conditions are presented in [Supplementary Figure 6](#); (E) SEM images of the hierarchical morphology of the DLW_3 surface; the scale bar is 100 μm . Other conditions are displayed in [Supplementary Figure 7](#); (F) WCA measurements for untreated PDMS and DLW-treated surfaces; (G) SA measurements of DLW_3 and PDMS. The SA measurements from other conditions are presented in [Supplementary Figure 8](#). Data for WCA and SA are presented as mean $\pm$ SD ($n = 3$, three independent samples per group). DLW: Direct laser writing; PDMS: polydimethylsiloxane; ATR-FTIR: attenuated total reflectance-Fourier transform infrared spectroscopy; XRD: X-ray diffraction; XPS: X-ray photoelectron spectroscopy; SEM: scanning electron microscopy; WCA: water contact angle; SA: sliding angle; SD: standard deviation; SiOC: silicon oxycarbide.

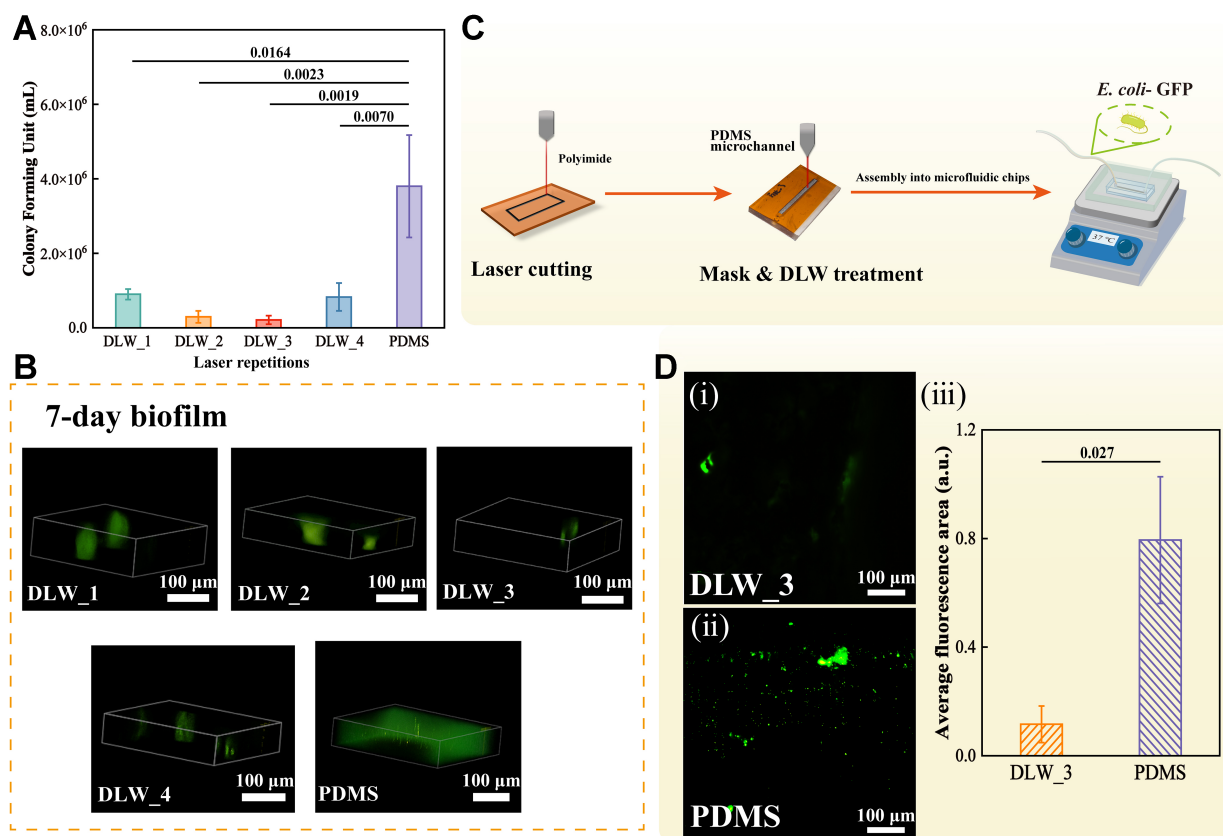


Figure 2. Antifouling performance of DLW-PDMS surfaces. DLW_n denotes *n* repetitions of the laser treatment, and PDMS refers to the untreated substrate. (A) Quantification of bacterial (*E. coli*) adhesion via CFU counts after 24 h of incubation. Data are presented as mean ± SD (*n* = 3, independent substrates per group). Statistical significance was determined using ANOVA with Tukey's test, with pure PDMS as the control group; (B) Representative 3D CLSM reconstructions of *E. coli*-GFP biofilm formation after 7 days of static culture on DLW_n surface and an untreated PDMS control. Scale bars: 100 μm; (C) Schematic of the fabrication process for integrating DLW-modified surfaces into a PDMS microfluidic device; (D) CLSM images showing bacterial adhesion in a DLW₃-treated channel (i) and an untreated PDMS control channel (ii) after 7 days of dynamic flow. Scale bars: 100 μm. (iii) The quantification of the fluorescent area was analyzed using an unpaired *t*-test. Each group replicates (*n* = 3), with 5 sites per sample as technical replicates, at 20× magnification. Data are presented as mean ± SD. DLW: Direct laser writing; PDMS: polydimethylsiloxane; *E. coli*: *Escherichia coli*; CFU: colony-forming unit; SD: standard deviation; ANOVA: analysis of variance; CLSM: confocal fluorescence microscope; GFP: green fluorescent protein.

When moving to the DLW₄ condition, antifouling performance declined. Despite observing a higher ratio of dead attached bacteria on DLW₄ [Supplementary Figure 9F], concurrent increases in both live and dead bacterial and CFU counts do not support a bactericidal effect. The elevated dead-cell proportion likely arises from prolonged residence of bacteria trapped in surface crevices after adhesion, rather than from direct membrane rupture induced by surface topography^[37].

Long-term stability assessments under static culture revealed extensive biofilm formation on the pristine PDMS surface. In contrast, the DLW₃ surface supported only sparse, vertically oriented microcolonies [Figure 2B]. This profound resistance to biofilm development is driven by the hierarchical topography, which stabilizes an interfacial air layer, thereby minimizing the solid-liquid contact area available for bacterial anchoring^[38].

Recent research highlights laser-engineered topographical patterning as an effective strategy for constructing cell-repellent biointerfaces for implantable bioelectronics, in which surface structuring suppresses cell adhesion without relying on biocidal components^[39]. Moreover, prior literature indicates that SiOC particles exhibit favorable biocompatibility and exert no inhibitory effect on bacterial proliferation^[40–42]. These findings

support our inference of a predominantly anti-adhesive mechanism.

To demonstrate the practical utility of this technology for mitigating biofouling, a critical challenge in PDMS-based microfluidics, the DLW process was applied to the interior of microscale channels [Figure 2C]. The modified channels were assembled into microfluidic devices and subjected to 7 days of continuous circulation with a high-load bacterial suspension (7 days with the wall shear stress only at 0.25 Pa, Supplementary Text 3). Post-experiment analysis revealed substantial bacterial accumulation and biofilm formation within the untreated PDMS control chip, particularly along the channel edges [Figure 2D]. Conversely, the DLW_3-treated channel exhibited only minimal bacterial adhesion, representing a statistically significant reduction in surface fouling ($P = 0.027$). These results validate the mechanical robustness and operational stability of the superhydrophobic SiOC layer under dynamic flow conditions.

Due to the hierarchical, superhydrophobic nature of the DLW_3 composite ($WCA = 157^\circ$), the thermodynamic barrier to biofouling was evaluated via the apparent work of adhesion (W_a). Applying the Young-Dupré equation: $W_a = \gamma_w(1 + \cos\theta_w)$, the W_a of the optimized SiOC matrix was calculated to be exceptionally low (≈ 5.8 mN/m). This thermodynamic barrier to aqueous wetting disfavors the stable anchoring of *E. coli* extracellular polymeric substances, which could somewhat explain the observed reduction in bacterial adhesion. Nevertheless, it should be noted that the adhesion derived from WCA measurements serves only as an effective surface-wetting descriptor. Bacterial adhesion is governed by multiple additional factors beyond surface wettability, including hydration state, extracellular substances, and surface charge^[37].

Interfacial electrochemical kinetics

The formation of graphene-like carbon structures within the SiOC matrix^[43], combined with the established biocompatibility and chemical stability of polymer-derived SiOC ceramics as electrode materials^[44], motivated a rigorous investigation of the platform's electrochemical properties. As shown in Figure 3A, the EIS plot revealed a dramatic decrease in R_{ct} , from 44.4 Ω for a single pass (DLW_1) to < 1 Ω (DLW_3). This value significantly outperforms comparable SiOC/C materials ($R_{ct} = 82.78$ Ω)^[45]. These findings, which position the DLW_3 surface as a highly conductive electrode, were corroborated by CV measurements [Figure 3B]. The CV response and redox currents rose with laser repetitions, peaking at DLW_3 and decreasing at DLW_4. This trend aligns with our materials characterization, which indicates that three repetitions yield an optimal balance of high graphitic carbon content and low structural defects [Figure 1 and Supplementary Table 4]. In contrast, excessive exposure may lead to the degradation or vaporization of the conductive carbon phase^[46]. This observation further supports the conclusion that DLW-PDMS forms a composite material consisting of SiOC/C. While pure SiO_2 is intrinsically insulating, the generated interconnected carbon network provides pathways for efficient electron transport across the substrate^[19,47].

Under time-varying electric fields, transient Maxwell electrostatic stress precludes instantaneous steady-state force balance, as illustrated by AC-electrowetting observations where droplet inertia hinders cycle-resolved interfacial relaxation^[48]. In the CV measurements, a continuously swept potential generates a transient Maxwell stress concentrated at the liquid-air-solid three-phase contact lines. This stress may locally overcome the Laplace capillary barrier, inducing spatially confined plastron collapse without a global Cassie-to-Wenzel transition or degradation of hierarchical micro/nanostructures^[49,50]. Unlike static-DC electrowetting, no steady-state Maxwell-Laplace equilibrium is established during potential scanning. Benefiting from a hierarchical microstructure, wetting is confined to isolated domains, and macroscopic superhydrophobicity is preserved^[51].

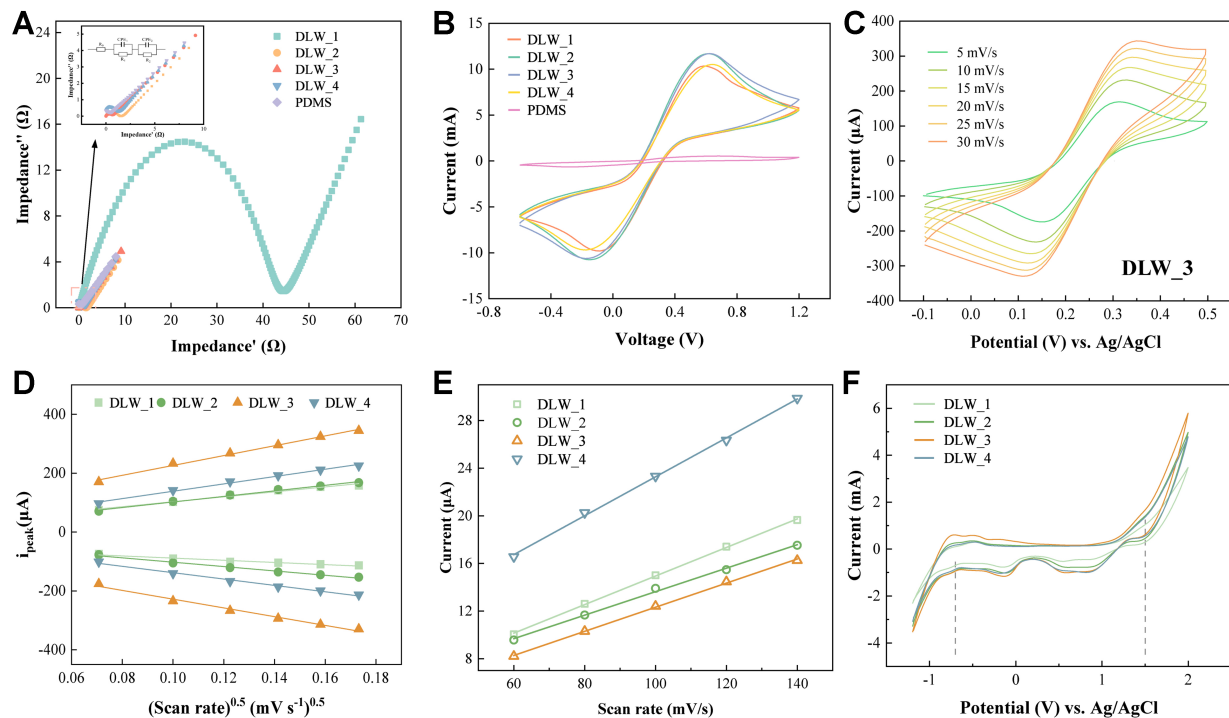


Figure 3. Electrochemical properties of DLW-PDMS electrodes. DLW_*n* denotes *n* repetitions of the laser treatment, and PDMS refers to the untreated substrate. (A) Nyquist plots from EIS analysis in 100 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ for surfaces with different laser repetitions. Inset: equivalent circuit model used for fitting; (B) Cyclic voltammograms in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at 50 mV/s; (C) CV curves for DLW_3 with a redox probe and varying scan rates; (D) Randles-Sevcik plots showing the linear relationship between peak current (i_{peak}) and the square root of the scan rate ($v^{0.5}$) for electrodes with different laser repetitions; (E) Estimation of double-layer capacitance (C_{dl}) of DLW-PDMS electrodes; (F) Comparison of ECWs of DLW-PDMS electrodes. DLW: Direct laser writing; PDMS: polydimethylsiloxane; EIS: electrochemical impedance spectroscopy; CV: cyclic voltammetry; ECWs: electrochemical windows.

Table 1. Comparison of calculated electrochemical parameters based on CV

Parameters for electrochemical performance evaluation	DLW_1	DLW_2	DLW_3	DLW_4
k^0 (cm/s)	0.00268	0.00416	0.00708	0.00533
A (cm ²)	0.841	0.943	1.674	1.247
C_{dl} (μF/cm ²)	142.66	104.62	60.50	131.05

DLW_*n* denotes *n* repetitions of the laser treatment. Key electrochemical parameters were derived from CV analyses to compare the performance of electrodes fabricated with a varying number of laser repetitions. k^0 was obtained based on the ΔE_p obtained at a 30 mV/s sweep rate in Figure 3C and Supplementary Figure 10. A was obtained by plotting and calculating in Figure 3D. The calculation of C_{dl} was obtained in Supplementary Figure 11 and plotted in Figure 3E. CV: Cyclic voltammetry; DLW: direct laser writing.

Quantitative analysis of the CV data [Table 1] revealed a heterogeneous electron transfer rate constant (k^0) of 0.00708 cm/s for DLW_3, rivaling standard electrode materials such as glassy carbon ($k^0 = 0.00782$ cm/s) and pyrolytic carbon ($k^0 = 0.0054$ cm/s)^[52,53]. A good linear relationship between peak current and the square root of sweep rate demonstrates that the electrochemical reaction is governed by semi-infinite diffusion of redox-active species toward the electrode surface [Figure 3C and Supplementary Figure 10]. As shown in Figure 3D, the electrochemically active surface area (A) reached its maximum of 1.674 cm² for DLW_3, which is attributed to the laser-pyrolyzed hierarchical roughness.

Analysis of the non-Faradaic potential regions revealed quasi-rectangular CV curves, indicative of double-layer capacitive behavior [Supplementary Figure 11]. The DLW_3 electrode maintained this ideal rectangular shape even at high scan rates, suggesting excellent charge propagation and electrochemical stability. DLW_3 electrode's double-layer capacitance (C_{dl}) reached a minimum of 60.50 μF/cm² [Figure 3E and Table 1],

minimizing non-Faradaic background currents, thereby improving the signal-to-noise ratio. This value is comparable to conventional noble metal electrodes (platinum: 64 $\mu\text{F}/\text{cm}^2$, silver: 53 $\mu\text{F}/\text{cm}^2$)^[52], and superior to other laser-modified materials, such as carbon black-infused polylactic acid (PLA, 96.2 $\mu\text{F}/\text{cm}^2$)^[54]. The stable ECW of the DLW-PDMS electrode spanned -0.7 to +1.5 V [Figure 3F], a wide operational range similar to that of standard graphite (-0.5 to +1.6 V) and pyrolytic carbon (-0.9 to +1.6 V) electrodes^[52], confirming the platform's suitability for diverse electroanalytical applications.

A defining feature of the DLW_3 platform is its ability to reconcile superhydrophobicity with high-performance electrochemistry. Traditional electrochemical theory dictates that a Cassie-Baxter state (WCA = 157°) should severely impede Faradaic processes due to the insulating air plastron. However, the DLW_3 electrode exhibits interfacial electron transfer kinetics ($k^0 = 0.00708 \text{ cm/s}$). We attribute this unique capability to a potential-driven electrowetting transition. While the hierarchical SiOC matrix thermodynamically repels bacterial suspensions under open-circuit conditions, preventing the initial formation of focal adhesion complexes, applying sweeping potentials during CV locally reduces the liquid-solid interfacial tension. This transient, potential-induced wetting allows the aqueous electrolyte to penetrate the graphitic nano-domains, dynamically facilitating rapid charge transfer. Crucially, upon removal of the applied potential, the robust hierarchical topography prevents total Wenzel wetting, allowing the macroscopic antifouling properties to persist under dynamic microfluidic flow. This dynamic interfacial behavior distinguishes the DLW_3 composite from conventional LIG electrodes, which become rapidly fouled, and standard superhydrophobic polymers, which remain electrochemically inert. The reproducibility of this favorable electrochemical performance was further verified by impedance measurements, which revealed negligible intra-batch variation and excellent batch-to-batch consistency for DLW_3 [Supplementary Table 5].

Repeated laser scanning produces a suite of competing effects that dictate final surface properties: progressive laser-driven carbonization generates electroactive graphitic domains embedded within the SiOC matrix; evolving multiscale surface roughness builds the hierarchical micro-nano topography essential for Cassie-Baxter superhydrophobic antifouling; cumulative laser input induces heat accumulation, thermal stress, and solid-state phase transformations originating from thermal-relaxation processes; and excessive laser fluence causes material ablation. These effects respond differently as the number of repetitions increases. Moderate repeated scanning from DLW_1 to DLW_3 yields favorable carbon enrichment and well-defined hierarchical morphology without severe ablation or thermal damage, delivering balanced antifouling and electrochemical performance. Further increasing scan passes to DLW_4 breaks this optimal trade-off: carbon content decreases, and electrochemical activity weakens owing to ablation-driven loss of graphitic carbon; meanwhile, excess heat generates thermal-stress-mediated structural defects, and ablation degrades the Cassie-Baxter-supporting hierarchical topography. These combined factors explain the superior overall performance observed for the DLW_3 condition.

***In-situ* fabrication and evaluation of the BIP biosensor**

While microfluidic devices offer an attractive platform for rapid bacterial diagnostics^[55,56], integrating biorecognition elements onto PDMS surfaces remains a significant bottleneck, often requiring complex, time-consuming protocols^[57,58]. To address this, we integrated BIP technology, a robust approach for creating synthetic receptors using whole cells as templates^[59], directly onto the DLW_3 electrode.

The *in-situ* electropolymerization of pyrrole in the presence of *E. coli* templates [Figure 4A] resulted in a high-impedance state ($R_{ct} = 1.5 \text{ k}\Omega$ in Figure 4B) due to bacterial encapsulation [Figure 4B-D and Supplementary Figure 12]. SEM and confocal images showed the uniform entrapment of intact bacterial cells within the reticulated 3D Ppy network [Figure 4C(I) and D(I)]. Subsequent template elution generated highly specific, three-dimensional recognition cavities, as evidenced by a sharp decrease in impedance to

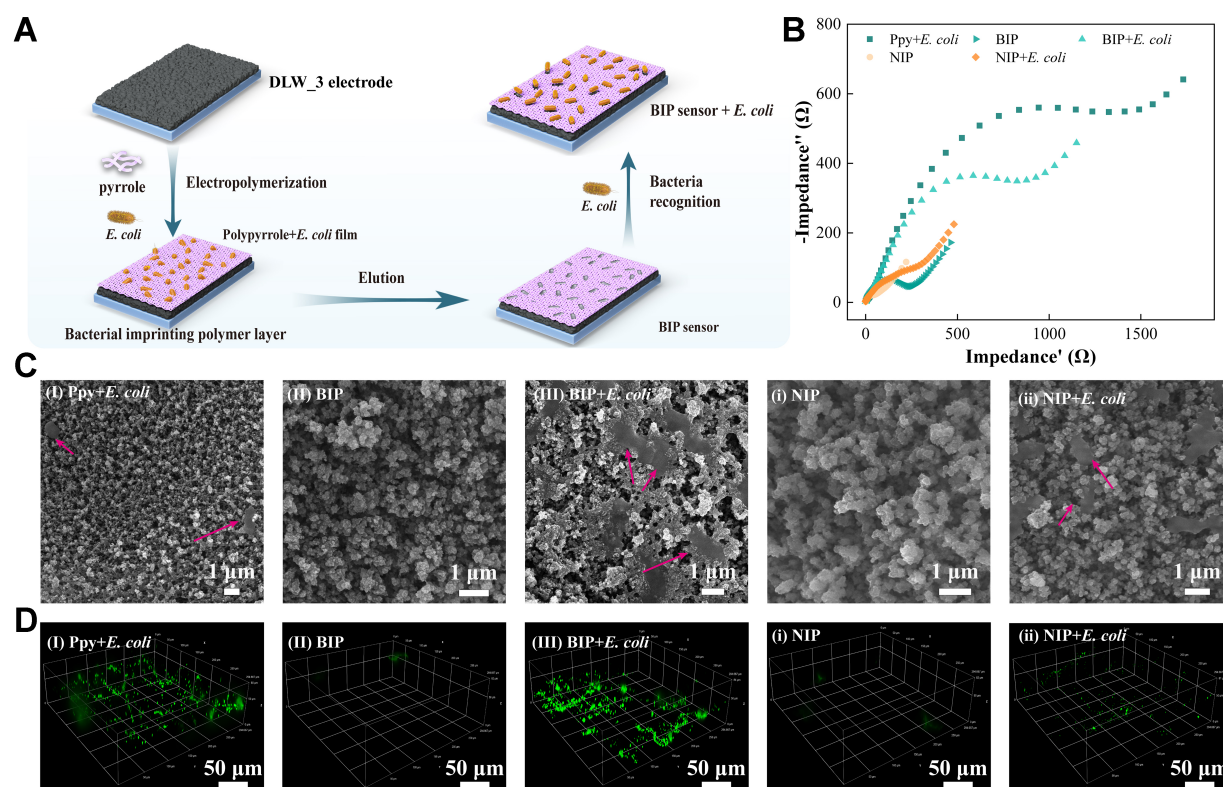


Figure 4. Fabrication and characterization of a BIP sensor on a DLW-PDMS electrode. The DLW_3 condition was used as the base electrode material. (A) Schematic illustrating the fabrication of the BIP sensor via electropolymerization of pyrrole around *E. coli* templates, followed by template removal and specific rebinding; (B) Nyquist plots from EIS analysis tracking the impedance changes at each stage of sensor fabrication and operation; (C) SEM micrographs and (D) corresponding *in-situ* confocal fluorescence maps (60× lens) of the electrode surface after: (I) initial polymerization of the polypyrrole layer with entrapped *E. coli* (Ppy + *E. coli*); (II) template elution to form the BIP; and (III) rebinding of target bacteria (BIP + *E. coli*). Control experiments with a (i) NIP for (ii) recognition of *E. coli* are shown for comparison. The scale bars for SEM and confocal are 1 and 50 μm , respectively. BIP: Bacterial-imprinted polymer; DLW: direct laser writing; PDMS: polydimethylsiloxane; *E. coli*: *Escherichia coli*; EIS: electrochemical impedance spectroscopy; SEM: scanning electron microscopy; NIP: non-imprinted polymer.

234 Ω [Figure 4B], with the formation of these imprinted voids within the polymer film directly visualized in Figure 4C(II) and D(II). Upon re-exposure of this BIP-DLW_3 sensor to the target *E. coli*, a significant increase in impedance was observed (Δ symbol in Figure 4B), indicative of the specific rebinding of bacteria within the complementary cavities, which obstructs charge transfer at the electrode interface. This successful capture event was directly visualized by SEM and confocal imaging, showing bacteria docked within the imprinted sites [Figure 4C(III) and D(III)]. Crucially, a NIP, prepared without the bacterial template as a control, exhibited negligible impedance change and minimal bacterial adhesion upon exposure to *E. coli*. This result demonstrates that the observed sensing response is due to the high-selectivity recognition conferred by the molecular imprinting process, rather than to nonspecific adsorption.

Under the optimal condition [Supplementary Figure 13], the BIP-DLW_3 sensor demonstrated a wide dynamic range for *E. coli* (10^3 to 10^8 CFU/mL), with an increased R_{ct} in a concentration-dependent manner [Figure 5A], yielding a limit of detection (LOD) of 348 CFU/mL (adjusted $R^2 = 0.9882$, Figure 5B). The fabrication protocol proved highly reproducible, with negligible response variation observed across five independent batches (3 replicate sensors per batch) when challenged by 10^8 CFU/mL *E. coli* [Figure 5C].

While the BIP-DLW_3 sensor was exposed to four different non-target bacterial species at a high concentration (10^8 CFU/mL), the impedance response was minimal. In contrast, the response to the target *E. coli* was 2.6- to 3.5-fold greater ($P \leq 0.0001$), confirming the sensor's high specificity [Figure 5D]. This

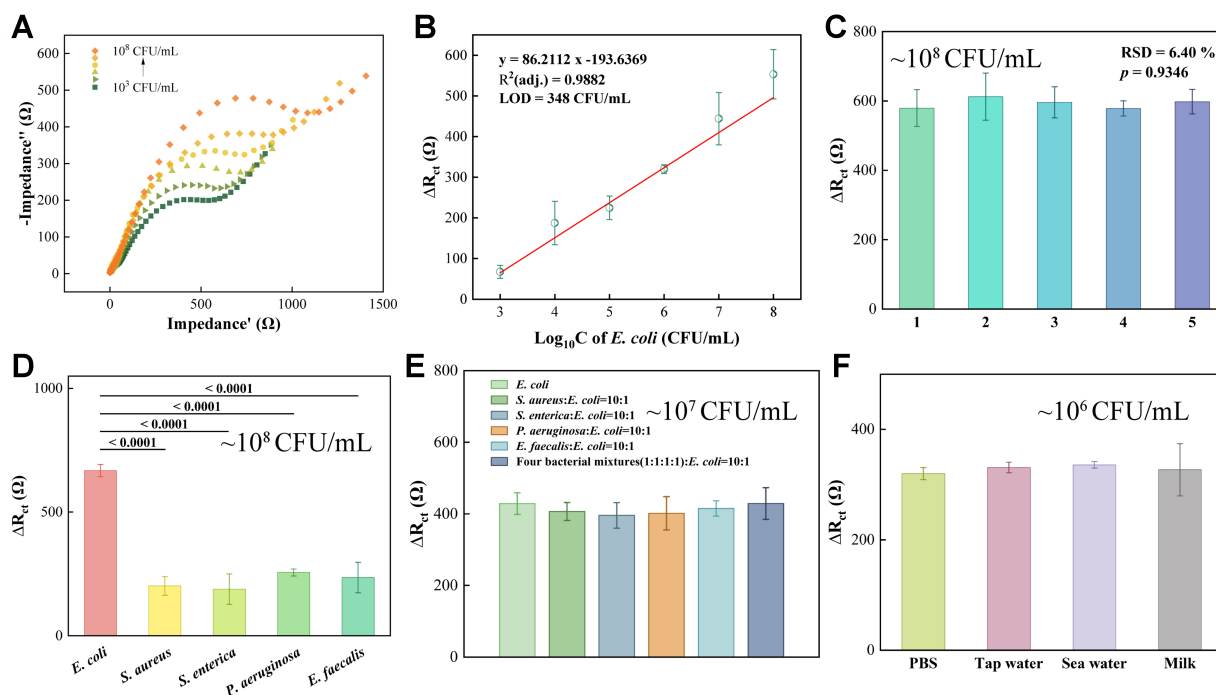


Figure 5. Quantitative and performance evaluations of BIP-DLW-PDMS sensors for *E. coli* detection. The DLW_3 electrode was used for all experiments. (A) Nyquist plots showing the sensor's response to *E. coli* concentrations from 10^3 to 10^8 CFU/mL; (B) Calibration curve plotting the change in charge transfer resistance (ΔR_{ct}) against the logarithm of bacterial concentration. The linear regression equation is $\Delta R_{ct} = 86.2112 \log_{10} C_{E.coli} - 193.6369$; (C) Reproducibility assessment across five independent batches, with three technical replicate sensors ($n = 3$) per batch for detection of *E. coli* at 10^8 CFU/mL; (D) Selectivity evaluation against four non-target bacterial species and the target *E. coli* (all at 10^8 CFU/mL); (E) Competitive selectivity test in a mixed solution containing target *E. coli* (10^7 CFU/mL) and non-target bacteria (10^8 CFU/mL), with a target *E. coli* to non-target bacteria ratio of 1:10; (F) Sensor response to *E. coli* (10^6 CFU/mL) spiked into real-world samples (tap water, seawater, milk). All experiments were performed in triplicate ($n = 3$). Data are presented as mean $\pm$ SD. Statistical significance was determined using ANOVA with Tukey's test. BIP: Bacterial-imprinted polymer; DLW: direct laser writing; PDMS: polydimethylsiloxane; *E. coli*: *Escherichia coli*; CFU: colony-forming unit; SD: standard deviation; ANOVA: analysis of variance; LOD: limit of detection; RSD: relative standard deviation; PBS: phosphate-buffered saline.

selectivity was further validated in a competitive assay, where the sensor preferentially detected target *E. coli* that constituted approximately 9.1% of a mixed bacterial population (Figure 5E, concentration of target *E. coli* is 10^7 CFU/mL). This high degree of selectivity is directly attributed to the specific recognition cavities, complementary in size, shape, and chemical functionality to the *E. coli* template, engineered into the polymer matrix during the imprinting process^[60].

Practical utility was validated in complex matrices (tap water, seawater, milk). The sensor's response to *E. coli* spiked into tap water and seawater (final concentration = 10^6 CFU/mL) closely matched the response observed in the clean PBS buffer control. While greater response variability was observed in minimally diluted milk samples, the results demonstrate the sensor's robust performance and potential for direct application to real-world samples with minimal pretreatment [Figure 5F and Supplementary Table 6].

Compared to conventional bacterial methods such as agar plating and enzyme-linked immunosorbent assay (ELISA)^[61,62], this unified DLW-PDMS platform enables rapid (< 4 h), highly sensitive, and label-free bacterial identification. Notably, our BIP-functionalized DLW-PDMS electrode, without additional nanomaterials pre-modification, achieves a LOD comparable to that of gold and carbon electrodes that employ such nanostructured pre-treatment to enhance sensing performance when functionalized with diverse recognition moieties [Table 2].

Table 2. Comparison of bacterial detection methods

Bacteria	Modified materials and electrode	Detection methods	Concentration range (CFU/mL)	LOD (CFU/mL)	Ref.
<i>E. coli</i> OP50	MIP-stainless steel microwires	EIS	10^2 – 10^7	2×10^2	[63]
<i>E. coli</i> O157:H7	Y6NPs@Au@antibody	Colorimetry and photothermometry	2.5×10^2 – 5×10^6	CL: 1,725 PT: 311	[64]
<i>E. coli</i> K12	Lectin/SWCNT/gold electrode	LSV	2×10^3 – 2×10^8	4.7×10^3	[65]
<i>E. coli</i> O157:H7	Ag@PD-GCE	Amperometry and non-faradaic EIS	-	10^3	[66]
<i>E. coli</i>	Laser-treated GO/antibody	Amperometry	917 – 2.1×10^7	283	[67]
<i>E. coli</i>	ZnO/CdS/Ag ₂ CO ₃ /graphite	CV	10^2 – 10^7	1×10^2	[68]
<i>E. coli</i>	Ppy/DLW-PDMS	EIS	10^3 – 10^8	348	This work

CFU: Colony-forming unit; LOD: limit of detection; *E. coli*: *Escherichia coli*; MIP: molecularly imprinted polymer; EIS: electrochemical impedance spectroscopy; CL: colorimetry; PT: photothermometry; SWCNT: single-walled carbon nanotube; LSV: linear sweep voltammetry; PD-GCE: polymer-derived glassy carbon electrode; GO: graphene oxide; CV: cyclic voltammetry; DLW: direct laser writing; PDMS: polydimethylsiloxane.

Taking advantage of the template-replaceable nature of molecularly imprinted technology, our BIP sensor shows promise for extending detection from single-target detection of *E. coli* to a broader spectrum of analytes, including other bacterial pathogens as well as small-molecule targets such as antibiotics and glucose^[69]. This platform shows great promise for on-site rapid screening and long-term *in-situ* monitoring applications.

CONCLUSION

In this study, we have demonstrated a monolithic materials platform that successfully bridges the gap between thermodynamic bio-repulsion and rapid electrochemical kinetics. By utilizing a single-step DLW process to convert native PDMS into a hierarchical SiOC/carbon composite, we bypass the complex integration protocols that have bottlenecked microfluidic biosensor development. The uniqueness of this system lies in its dynamic interfacial behavior: it acts as a robust, Cassie-Baxter antifouling shield at rest while permitting localized electrowetting for high-sensitivity *in-situ* BIP biosensing under applied potentials. This conceptual advancement not only provides a highly selective diagnostic tool for *E. coli* but also establishes a foundational design principle for the next generation of continuous-monitoring, self-cleaning bioelectronics.

Several limitations of the present work remain to be addressed. Long-term cycling operational stability has not been validated within fully assembled microfluidic chips. Biosensing performance was assessed only against *E. coli* as a single target. Future work will expand this strategy by fabricating multiplexed biosensors integrated into one microfluidic chip that can detect various bacterial targets. Additionally, BIP electrode-interface properties will be optimized to enhance sensing performance. Ultimately, this work provides a rational design route for high-performance microdevices that operate with high fidelity within complex environments.

DECLARATIONS

Authors' contributions

Conceptualization, data curation, methodology, investigation, visualization, validation, formal analysis, software, project administration, writing - original draft, writing - review and editing: Dai, W.

Conceptualization, resources, supervision, project administration, funding acquisition, writing - original draft, writing - review and editing: Chen, J. L.

Resources, supervision, writing - review, and editing: Ye, R.

Methodology, visualization, validation, software: Cheng, L.

Methodology, investigation, visualization, validation, formal analysis, software: Sun, J.

Methodology, investigation, formal analysis: Guo, Q.
Investigation, visualization, formal analysis, software: Wu, X.
Resources, supervision, funding acquisition: Lee, F. W. F.; Tam N. F. Y.
Resources: Peng, Y. K.

Availability of data and materials

All data needed to evaluate the conclusions in the paper are present in the paper and/or the [Supplementary Materials](#).

AI and AI-assisted tools statement

Generative AI tools, ChatGPT (GPT-5.5, released 2026-04-23; OpenAI) and Gemini (Gemini 3.5-Flash, released 2026-05-20; Google DeepMind), were used for language editing and polishing. They were not used to generate the original robot image or any scientific data. These AI-assisted tools did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All AI-assisted content was carefully reviewed and verified by the authors.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Supplementary Materials

[Supplementary Materials](#)

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