

Oxygen plasma-engineered resin–carbon interfaces in 3D-printed hollow microneedle electrodes for IL-6 detection

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Abstract

A stereolithographically fabricated hollow microneedle carbon electrode (HMNE) was developed as an integrated platform for interfacial electrochemistry and impedimetric biosensing. The device consisted of hollow resin microneedles with internal channels filled with carbon paste, providing a continuous conductive pathway while preserving the microneedle architecture and structural integrity during insertion. Oxygen plasma treatment markedly modified the resin–carbon interface, decreasing the water contact angle from 79.4° to 15.7° and the apparent charge-transfer resistance from ~120 to 32 kΩ. The heterogeneous electron-transfer rate constant increased from 1.26×10^{-3} to $2.62 \times 10^{-3} \text{ cm s}^{-1}$, while voltammetric and Raman measurements indicated improved electrolyte accessibility and modification of the local interfacial environment without extensive structural transformation. After anti-IL-6 functionalization, the HMNE enabled label-free impedimetric detection of IL-6, producing an approximately fourfold higher response than for TNF- α , IFN- γ , and IL-17. The immunosensor showed a concentration-dependent response from 0.5 to 7.5 ng mL⁻¹, with a limit of detection of 0.178 ng mL⁻¹. The immunosensor also retained its response in simulated interstitial fluid and diluted human plasma, preserving approximately 91% of its initial signal after 40 days of storage at 4 °C. These results demonstrate the potential of oxygen-plasma-engineered resin–carbon hollow microneedles as integrated three-dimensional interfaces for minimally invasive cytokine biosensing.

1. INTRODUCTION

Minimally invasive access to physiologically relevant biomarkers is essential for developing decentralized diagnostics, wearable devices, and personalized health-monitoring technologies.^{1,2} Dermal interstitial fluid has attracted particular attention because it surrounds tissue cells and contains metabolites, electrolytes, proteins, and signaling molecules that can provide information complementary to conventional blood analysis.^{1,3} Compared with blood, interstitial fluid contains no blood cells and generally has a lower protein content, which may reduce matrix-related limitations in biosensing.^{3,4} However, its collection remains challenging because only small volumes are locally available and conventional sampling procedures are not readily compatible with continuous or point-of-care measurements.^{3,5}

Microneedle-based systems provide a promising strategy to overcome these limitations. Their micrometer-scale dimensions allow penetration through the stratum corneum while limiting interaction with deeper, vascularized, and highly innervated regions.^{6,7} Depending on their composition and architecture, microneedles can act as solid biomarker-capture surfaces, porous or swellable sampling structures, hollow conduits for fluid transport, or direct electrochemical sensing interfaces.^{8,9} Recent studies have demonstrated their use for monitoring electrolytes, glucose, lactate, urea, drugs, proteins, and inflammatory biomarkers in interstitial fluid,^{10,11} Nevertheless, most reported systems focus on relatively abundant small molecules, while affinity-based detection of low-abundance proteins still requires highly accessible, stable, and reproducible electrode interfaces.¹²

Hollow microneedles are particularly attractive because their internal channels can establish a direct pathway between the tissue environment and a sensing region. Researchers have integrated these devices with external electrochemical cells, microfluidic components, optical systems, and planar electrodes to sample or monitor biomarkers in interstitial fluid.^{9,13} Recent advances have also demonstrated resin-based three-dimensional printing of hollow microneedles for electrochemical glucose sensing, highlighting the design freedom, rapid prototyping capability, and relatively low fabrication cost offered by additive manufacturing.^{14,15} However, in many hollow-microneedle systems, the microneedles function primarily as passive conduits, while the electrochemical transducer remains spatially separated from the needle structure. This configuration can increase device complexity, require additional fluid transport, and introduce dead volume between sampling and detection.^{6,16}

Integrating the conductive phase directly into the hollow microneedle architecture offers an alternative route in which the needle array serves as both a mechanically functional structure and a three-dimensional electrode interface.¹⁷ Carbon-based materials are attractive for this purpose because of their chemical stability, broad potential window, low cost, and compatibility with different surface functionalization strategies. Carbon paste is particularly useful for filling predefined channels because it conforms to complex geometries and establishes conductive pathways without high-temperature processing or metal deposition.^{17,18} Nevertheless, incorporating carbon into photopolymerized structures creates a heterogeneous interface in which the electrochemical response depends not only on the intrinsic conductivity of the carbon phase but also on carbon loading, electrical continuity, exposure of electroactive domains, channel geometry, and electrolyte wetting.

Three-dimensional printing has substantially expanded the geometries available for electrochemical devices, but polymer-rich or resin-supported electrodes often exhibit limited electrochemical accessibility in their untreated state.^{19,20} In conductive thermoplastic electrodes, insulating polymer can cover the conductive filler and restrict electron transfer, making surface activation an essential fabrication step. Mechanical polishing, solvent treatment, electrochemical activation, laser processing, and plasma exposure have therefore been investigated to expose conductive domains and improve electrode performance.^{20,21} For example, CO₂ laser treatment of carbon black/poly(lactic acid) electrodes partially removes the insulating polymer, generates a porous, carbon-rich surface, increases the electroactive area, and markedly decreases charge-transfer resistance.²² Although effective, treatments that rely on substantial polymer removal may not suit delicate microneedle structures, where preserving tip geometry, channel patency, and mechanical integrity is essential.^{9,23,24}

Oxygen plasma provides a less aggressive approach for tuning polymer–carbon interfaces.^{25,26} Plasma exposure can increase surface energy, introduce oxygen-containing functionalities, and improve the wettability of carbon and polymeric surfaces without liquid reagents or extensive bulk material removal.²⁷ Low-power oxygen plasma has been shown to activate screen-printed carbon electrodes by increasing their electrochemically accessible area, although excessive treatment can also reduce performance, emphasizing the need to consider the relationship among plasma conditions, electrode

composition, and interfacial structure.²⁸ In a hollow resin–carbon architecture, plasma treatment may be particularly beneficial because electrolyte access is restricted to carbon domains exposed within or near the microneedle channel openings. Enhanced wettability may therefore increase the fraction of the conductive phase participating in the electrochemical response.

However, plasma-induced changes in electrode performance may involve not only improved wettability but also modifications in interfacial charge, surface chemistry, and electron-transfer behavior. Therefore, a combination of electrochemical probes with distinct interfacial characteristics is needed to better understand the plasma-modified resin–carbon interface.²⁹

Beyond small-molecule electrochemistry, a major opportunity for microneedle electrodes lies in detecting inflammatory proteins. Interleukin-6 is a widely studied cytokine involved in immune and inflammatory signaling and has been investigated as a target in microneedle-based and electrochemical sensing platforms.^{11,30} Molecularly imprinted microneedle arrays, antibody-functionalized microneedles, and integrated affinity sensors have demonstrated the feasibility of detecting IL-6 and other cytokines using impedance, optical amplification, or related transduction strategies.^{11,31} However, integrating a mechanically robust hollow microneedle structure, an internal carbon electrode, controllable interfacial activation, and label-free impedimetric cytokine recognition remains comparatively underexplored.

In this study, we report a stereolithographically fabricated hollow microneedle electrode in which carbon paste is confined within internal channels extending from the electrical contact region to the microneedle array. The printed resin functions as a load-bearing scaffold, while the internal carbon phase establishes an integrated three-dimensional electrochemical interface. The effects of carbon filling and oxygen plasma treatment were investigated through morphological, mechanical, spectroscopic, and electrochemical analyses, with particular attention to changes in interfacial accessibility and charge-transfer behavior. The plasma-treated platform was subsequently functionalized with anti-IL-6 antibodies and evaluated for label-free impedimetric detection of IL-6. The results show that oxygen plasma improves the electrochemical accessibility of the resin–carbon interface and supports its use as an affinity-based biosensing platform.

2. EXPERIMENTAL METHODS

General Information. All reagents used were of analytical grade. For developing the 3D-printed models, we used Clear V4 and Clear V4.1 photopolymer resins (Formlabs, Massachusetts, USA), compatible with SLA printers from the Form 3 series. Graphite powder, potassium ferricyanide, potassium ferrocyanide, hexaammineruthenium (II) chloride, dopamine hydrochloride, ascorbic acid, and isopropyl alcohol were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Ag/AgCl reference electrode ink was obtained from NanoView Nanotecnologia (Belo Horizonte, Brazil). Acetone, dibasic sodium phosphate heptahydrate, anhydrous monobasic potassium phosphate, and isopropyl alcohol were obtained from Labsynth (Diadema, Brazil). Potassium chloride and sodium chloride were obtained from Dinâmica (São Paulo, Brazil), and colorless nail polish from Mundial (São Paulo, Brazil). The biomolecules used in this study

were polyclonal goat anti-human IL-6 antibody (PeproTech, USA; catalog no. 500-P26G), recombinant human soluble interleukin-6 receptor alpha (rh sIL-6R α) (PeproTech, USA; catalog no. 200-06RC), recombinant human interleukin-17A (rhIL-17A) (PeproTech, USA; catalog no. 200 – 17), human IFN-Alpha Multi-Subtype ELISA Kit (Catalog No. 41110-1)

CAD model preparation and 3D printing of the HMNE models. We developed the CAD model using Fusion 360 (Autodesk, San Francisco, USA) and Tinkercad. The 3D electrode model incorporated an array of hollow microneedles integrated into a structure containing screen-printed electrode-type connector pads. At this stage, we considered combining the microneedle structure with a planar platform, commonly used in electrochemical sensor fabrication.

The model consisted of conical microneedles with a height of 1500 μm , a base diameter of 1000 μm , and a microchannel diameter of 500 μm . The device comprised 14 microneedles, arranged according to the configuration of the working, counter, and reference electrodes, following an arrangement analogous to that of a conventional commercial planar electrode. The microneedle region had dimensions of 1 $\times$ 1 cm, whereas the region corresponding to the conductive tracks had dimensions of 2 $\times$ 1 cm.

The microchannels within the microneedles were directly connected to a three-contact conductive track for the working, counter, and reference electrodes. We printed the models using a Formlabs Form 3 + stereolithography (SLA) 3D printer with Clear V4 and Clear V4.1 photopolymer resins. The models were oriented at 60°, with support generated using PreForm software (Formlabs). After printing, the models were washed in isopropyl alcohol and UV-cured according to the manufacturer's recommended post-processing protocol.

Carbon Paste Preparation and Microchannel Filling. Carbon paste was prepared with 0.7 g of colorless nail polish as a polymeric matrix and binding agent, dissolved in 2.0 mL of acetone to reduce viscosity and facilitate dispersion. Subsequently, 1.0 g of graphite powder was added to the mixture and manually stirred until a homogeneous dispersion was obtained. The carbon paste was deposited into the microchannels designated for the working electrode, counter electrode, and conductive connection tracks.

To prepare the device reference electrode, a commercial silver/silver chloride (Ag/AgCl) ink was modified because its high viscosity hindered penetration into the electrode microchannels. Therefore, 500 μL of acetone was added to 0.5 g of the paste to increase its fluidity and facilitate application. The Ag/AgCl ink was applied to the region designated for the reference electrode.

Optical Microscopy and Scanning Electron Microscopy. We obtained optical microscopy images using a Digilab DI-152T microscope (Santo Andre, SP, Brazil). Optical microscopy was used to validate device dimensions and assess surface quality and structural resolution.

The morphological analysis of the 3D-printed HMNE was performed by scanning electron microscopy (SEM) using an FEI Inspect S50 microscope (São Carlos, Brazil). The micrographs were acquired using

an accelerating voltage of 20 kV and a magnification of 200×.

Mechanical characterization. The mechanical properties of the microneedles with and without carbon paste were evaluated using an Anton Paar MCR 502 rheometer (Santo Andre, Brazil), operated in compression mode with different load capacities and equipped with a 25 mm parallel-plate geometry. To determine the compression force of the microneedles, the HMNE array was fixed to the testing platform, and the geometry was programmed to move vertically toward the microneedles at a speed of 0.035 mm/s until 50N or failure occurred.

Insertion studies. To evaluate the force required for skin penetration, an insertion test was performed using Parafilm M® (Amcor, Neenah, USA) as a skin-mimicking substrate. A previously validated model was employed, consisting of eight layers of Parafilm M® stacked together to form a multilayer substrate.^{32,33} The HMNE were inserted into the Parafilm substrate by applying a compressive force of 50 N using the rheometer.

Optical coherence tomography (OCT). Optical coherence tomography was used to visualize microneedle insertion into a simulated substrate (Parafilm M®) using a 930 nm Callisto OCT imaging system in B-scan mode (Thorlabs, Newton, USA). The acquired images were analyzed using ImageJ (National Institutes of Health, USA) to determine the number of Parafilm M® layers penetrated and the morphometric characteristics of the microchannels.

Plasma treatment and contact angle. The HMNE surface treatment was performed using an HPT-100 plasma cleaner. This equipment is a microprocessor-controlled oxygen plasma treatment system. The plasma treatment was performed at 30% power for 10 min. The contact angle measurements were performed using an SEO Phoenix 300 contact angle analyzer equipped with a camera module connected to a computer. Surfware 9 software was used to analyze the surface wettability of the HMNE before and after oxygen plasma treatment to evaluate potential improvements in their hydrophilicity.

Raman spectroscopy. The spectroscopic characterization of the samples was performed by Raman spectroscopy using a LabRAM Soleil Raman Microscope (HORIBA, France). The analyses were conducted using a 785 nm excitation laser directed onto the sample surfaces via the instrument's microscope system. The acquired Raman spectra were used to characterize the materials' vibrational bands and evaluate their chemical and structural characteristics.

Electrochemical Measurements. Electrochemical measurements were carried out using a μ Autolab Type III potentiostat/galvanostat (Metrohm) operated with NOVA 2.1 software. A Dropsense interface coupled to the μ Autolab ensured a secure connection between the electrode connectors and the instrument. To conduct the tests, approximately 130 μ L of electrolyte solution was added onto the electrodes for the electrochemical measurements.

Two redox probes with distinct electron-transfer mechanisms were employed. Experiments were conducted in a solution containing 5 mmol·L⁻¹ K₄[Fe(CN)₆]/K₃[Fe(CN)₆] in 0.1 mol·L⁻¹ KCl (pH 7.3).

Cyclic voltammetry curves were recorded over a potential range of -0.6 V to + 1.0 V (vs. Ag/AgCl) at scan rates of 25, 50, 100, 125, 150, 175, and 200 $\text{mV}\cdot\text{s}^{-1}$, with 100 $\text{mV}\cdot\text{s}^{-1}$ selected for comparative curves. For the $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$ system, experiments were performed in a solution containing 5 $\text{mmol}\cdot\text{L}^{-1}$ $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3/[\text{Ru}(\text{NH}_3)_6]\text{Cl}_2$ in 0.1 $\text{mol}\cdot\text{L}^{-1}$ KCl (pH 7.3), using the same scan rates applied to the ferri/ferrocyanide system (10–200 $\text{mV}\cdot\text{s}^{-1}$).

To evaluate the device's analytical performance regarding analytes of biological interest, electrochemical experiments were conducted using ascorbic acid and dopamine. For ascorbic acid, experiments were conducted in 0.1 $\text{mol}\cdot\text{L}^{-1}$ phosphate buffer solution (PBS, pH 7.4) containing ascorbic acid at concentrations ranging from 1 to 10 $\text{mmol}\cdot\text{L}^{-1}$. Cyclic voltammetry curves were recorded over a potential range of -0.3 V to + 1.5 V (vs. Ag/AgCl) at a scan rate of 100 $\text{mV}\cdot\text{s}^{-1}$. The anodic peak current (I_{pa}), corresponding to ascorbic acid oxidation, was monitored as a function of analyte concentration to construct calibration curves. As a control, voltammograms were recorded in 0.1 $\text{mol}\cdot\text{L}^{-1}$ PBS (pH 7.4) in the absence of the analyte to verify the absence of interfering signals. For dopamine, experiments were also performed in 0.1 $\text{mol}\cdot\text{L}^{-1}$ phosphate buffer solution (PBS, pH 7.4) containing dopamine at concentrations ranging from 10 to 100 $\mu\text{mol}\cdot\text{L}^{-1}$. Cyclic voltammetry curves were recorded over a potential range of -0.8 V to + 1.4 V (vs. Ag/AgCl) at a scan rate of 100 $\text{mV}\cdot\text{s}^{-1}$. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents, corresponding to the reversible redox process of dopamine (oxidation to dopamine-o-quinone and reversible reduction), were monitored as a function of analyte concentration. The ratio of anodic to cathodic peak currents ($I_{\text{pa}}/I_{\text{pc}}$) and the peak potential separation (ΔE_{p}) were used as indicators of electrochemical reversibility at the electrode surface.

The electrochemical performance of the immunoassays was evaluated using a Metrohm Autolab PGSTAT 302N system equipped with an FRA2 impedance module, and the data were analyzed using NOVA 2.1.3 software. All measurements were performed in a 0.1 $\text{mol}\cdot\text{L}^{-1}$ KCl solution containing 5 $\text{mmol}\cdot\text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe. Electrochemical impedance spectroscopy (EIS) was performed at the half-wave potential (approximately 240 mV), as determined by cyclic voltammetry, over a frequency range of 0.1 Hz to 30 kHz and with a 10 mV RMS sinusoidal perturbation.

Immunosensor fabrication. Electrochemical immunosensors were constructed using previously prepared HMNEs. Initially, 6 μL of a solution containing anti-IL-6 antibodies (6 $\mu\text{g}\cdot\text{L}^{-1}$) was deposited onto the electrode surface, then incubated at room temperature for 60 min to immobilize the antibodies on the sensor surface. After incubation, the modified electrodes were dried and incubated for 30 min with solutions containing the IL-6 antigen at concentrations ranging from 0.5 to 7.5 $\text{ng}\cdot\text{mL}^{-1}$. After each modification and incubation step, the electrodes were rinsed thoroughly with phosphate buffer for 10 s to remove species not bound to the surface and minimize non-specific interactions with the medium. After rinsing, the immunosensors were stored under controlled conditions until electrochemical analysis.

Interference studies. To evaluate the biosensor's selectivity, HMNE electrodes functionalized with anti-IL-6 monoclonal antibodies were incubated separately with the interferents TNF- α , IFN- γ , and IL-17 (all at a concentration of $2.5 \text{ ng} \cdot \text{mL}^{-1}$). To assess potential interference from the physiological matrix, tests were conducted using synthetic interstitial fluid composed of HEPES buffer (pH 7.4) containing NaCl, KCl, NaH_2PO_4 , sucrose, CaCl_2 , and MgSO_4 , spiked with a known amount of the antigen.

Furthermore, the analytical performance of the biosensor was evaluated in a complex biological matrix via recovery assays using human plasma samples from healthy donors, previously diluted 1:100. Different concentrations of IL-6 were added to the samples, and the device's performance was assessed based on charge-transfer resistance (R_{ct}) values, recovery percentage, and relative standard deviation (RSD). All samples were obtained in accordance with ethical procedures approved by the Research Ethics Committee (CAAE: 43139921.2.0000.5594).

Stability studies. The stability of the immunosensors was evaluated over a 40-day period to investigate the maintenance of their analytical performance after storage. After fabrication and functionalization, the immunosensors were stored at a controlled temperature (4°C) until analysis. At predetermined intervals, the electrodes underwent electrochemical measurements using the same IL-6 concentration. We monitored device performance by comparing the charge-transfer resistance (R_{ct}) values at each time point with those recorded on the first day of the experiment. Stability was expressed as the percentage of initial signal retention, calculated as the ratio of the signal obtained after the storage period to the signal recorded on the first day.

3. RESULTS AND DISCUSSION

Fabrication, morphology, and structural assessment of the HMNE. The HMNE was designed as an integrated three-dimensional electrochemical platform combining a hollow microneedle array with planar electrical contacts (Fig. 1A). The device was fabricated by stereolithography and subsequently filled with carbon paste through internal microchannels extending from the contact region to the microneedle array. Each microneedle had a nominal height of 1.5 mm, an outer diameter of 1.0 mm, and an internal channel diameter of 0.50 mm, corresponding to a nominal wall thickness of 0.25 mm. This configuration was designed to provide sufficient structural support while accommodating the conductive phase within the hollow architecture.

After printing and post-processing, optical microscopy and SEM were used to assess how closely the fabricated structures matched the CAD design. The overall electrode geometry was preserved, including the microneedle-containing active region, the planar tracks, and the contact area, with no evident macroscopic collapse or severe distortion (Fig. 1B). Some deviation from the idealized CAD geometry was observed, particularly at the microneedle tips and channel openings, where the contours were smoother and more rounded. Such deviations are consistent with light scattering, lateral photopolymerization, and overcuring commonly observed in SLA-fabricated microstructures.^{34,35} Nevertheless, the hollow architecture and microneedle arrangement were maintained.

SEM analysis provided further evidence of structural integrity. The unfilled microneedles retained an open internal cavity after printing, washing, and post-curing (Fig. 1C), indicating that the approximately 500 μm internal channel remained patent despite the dimensional limitations associated with photopolymerization. Layer-wise surface features characteristic of SLA fabrication were also visible, but no major fractures or discontinuities were observed. Preservation of the channel opening is particularly important because partial obstruction could limit carbon filling and introduce variability in the electroactive area.

Carbon paste filling converted the printed resin scaffold into a functional HMNE while preserving the external microneedle geometry. Optical and SEM images showed that the conductive phase extended through the patterned pathways and occupied the internal microneedle channels without completely sealing the tip openings (Figs. 1B and 1D). This configuration creates an electrochemical interface involving the internal carbon phase, the exposed channel opening, and the surrounding resin surface.

The insertion behavior and structural integrity of the HMNE were evaluated using eight stacked Parafilm layers as a deformable skin-mimicking substrate (Fig. 2-A). Both carbon-filled and unfilled HMNEs exhibited a progressive increase in force with displacement, without an abrupt force drop indicative of catastrophic structural failure within the investigated range. However, the carbon-filled HMNE developed a consistently higher force at a given displacement, indicating greater resistance to deformation during penetration. The apparent insertion stiffness, determined from the approximately linear region between 0.90 and 1.40 mm, increased from $41.5 \pm 1.8 \text{ N mm}^{-1}$ for the unfilled HMNE to $45.9 \pm 1.3 \text{ N mm}^{-1}$ after carbon filling ($n = 2$). Similarly, the mechanical work calculated from the area under the force–displacement curve up to 1.60 mm increased from 13.5 ± 0.3 to $16.7 \pm 1.7 \text{ mJ}$. These results indicate that carbon filling modifies the mechanical response of the hollow structure and increases its resistance to deformation during insertion.

Both configurations withstood an applied load of approximately 50 N without evidence of catastrophic failure. Because the HMNE array contains 14 microneedles, this maximum load corresponds to a nominal value of approximately 3.6 N per microneedle, assuming uniform load distribution across the array. This value is the same order of magnitude as insertion forces reported for individual microneedles in skin, which depend strongly on needle geometry and tip interfacial area. Davis et al., for example, reported insertion forces ranging from approximately 0.08 to 3.04 N for individual microneedles of different geometries.³⁶ The comparison should be considered qualitative because the present measurements were performed using a multilayer Parafilm model and the applied load may not be distributed uniformly among all microneedles. Representative OCT images confirmed penetration through all eight Parafilm layers, further showing that the HMNE maintained its structural integrity during the insertion test (Fig. 2-B).

Interfacial electrochemistry and the effect of oxygen plasma treatment. Once the structural viability of the HMNE platform had been established, oxygen plasma treatment was investigated as a strategy to modulate the electrode/electrolyte interface. In this architecture, the electrochemical response depends

not only on the conductivity of the carbon paste but also on how well the aqueous electrolyte wets the resin–carbon interface and accesses the conductive phase confined within the hollow microneedle channels. Oxygen plasma was therefore employed to modify the surface chemistry and evaluate how these changes affected charge transfer and the response toward redox species with distinct interfacial characteristics.

The effect of plasma treatment on surface wettability was evaluated using static water contact angle measurements (Figs. 3A and 3B). The contact angle decreased from 79.4° for untreated HMNE to 15.7° after oxygen plasma exposure, an approximately 80% reduction. This pronounced decrease indicates a substantial increase in surface hydrophilicity and is consistent with the formation of oxygen-containing functionalities at the resin–carbon interfacial region. In this three-dimensional architecture, improved wettability is particularly relevant because it facilitates electrolyte spreading over the microneedle array and promotes access to the conductive carbon phase exposed at the channel openings. Consequently, a larger fraction of the electrode interface becomes effectively accessible during electrochemical measurements.

Figure 3. (A,B) Representative water-contact-angle measurements for untreated and plasma-treated hollow microneedle carbon electrodes, respectively, showing a decrease from 79.4° to 15.7° after oxygen plasma treatment. (C) Nyquist plots recorded before and after plasma treatment. (D) Cyclic voltammograms obtained with the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple in 0.1 M KCl. (E) Cyclic voltammograms obtained with the $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$ redox couple in 0.1 M KCl. (F) Voltammetric response toward dopamine in PBS at pH 7.4. (G) Voltammetric response toward ascorbic acid in PBS at pH 7.2. Untreated and plasma-treated electrodes were compared under the same experimental conditions.

Electrochemical impedance spectroscopy provided direct evidence that oxygen plasma treatment modified the interfacial electrochemical response (Fig. 3C). The untreated HMNE exhibited a broad semicircular profile with an apparent charge-transfer resistance of approximately 120 k Ω , whereas the plasma-treated HMNE showed a markedly smaller semicircle, corresponding to an R_{ct} of approximately 32 k Ω . Thus, plasma treatment reduced the apparent interfacial resistance by approximately 73%. Because R_{ct} depends on both the heterogeneous electron-transfer rate and the effectively accessible electroactive area, this decrease likely reflects the combined effects of improved electrolyte wetting, greater exposure of conductive carbon domains, and changes in interfacial electron-transfer kinetics.^{37,38} The depressed semicircular profiles are consistent with a heterogeneous interface, as expected for a composite electrode comprising carbon paste confined within a photopolymerized resin structure.

The voltammetric response was first examined using the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple (Fig. 3D). The plasma-treated HMNE showed higher peak currents than the untreated electrode. The anodic peak current increased from approximately 3.0 to 6.7 μA , while the magnitude of the cathodic peak current rose from 3.2 to 6.9 μA . For untreated HMNE, the anodic and cathodic peak potentials were

approximately 0.31 and 0.16 V vs. Ag/AgCl, respectively, giving a peak separation (ΔE_p) of approximately 150 mV. After plasma treatment, the peaks shifted to approximately 0.36 V (anodic process) and 0.21 V (cathodic process), while ΔE_p remained close to 150 mV. Additionally, the anodic-to-cathodic peak current ratio (I_{pa}/I_{pc}) increased from 0.93 to 0.97, approaching unity and indicating a better balance between oxidation and reduction.

The maintenance of ΔE_p , combined with increased peak currents, suggests that plasma treatment did not significantly alter the charge-transfer kinetics of the redox couple. Thus, the improved voltammetric response appears to stem from enhanced accessibility of the electroactive surface to the electrolyte. This behavior is consistent with increased surface wettability from plasma treatment, which facilitates contact between the electrolyte solution (containing the redox probe) and a larger fraction of the HMNE's electroactive domains. Consequently, the results indicate that the primary effect of the surface treatment was not necessarily accelerated electron transfer, but rather an increase in the electroactive area accessible to the electrolyte, contributing to a more intense and well-defined electrochemical response.

The influence of oxygen plasma treatment on heterogeneous electron-transfer kinetics was further investigated by cyclic voltammetry at scan rates ranging from 25 to 200 mV s⁻¹. For both untreated and plasma-treated HMNEs, the peak currents increased linearly with the square root of the scan rate, indicating that mass transport of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple was predominantly diffusion-controlled (Figure S1). The heterogeneous standard electron-transfer rate constant, k^0 , was estimated using the Nicholson approach from the scan-rate-dependent peak-to-peak separations.³⁹ The untreated HMNE exhibited a k^0 of $1.26 \cdot 10^{-3} \text{ cm s}^{-1}$, whereas oxygen plasma treatment increased this value to $2.62 \cdot 10^{-3} \text{ cm s}^{-1}$, corresponding to an approximately twofold increase in the heterogeneous electron-transfer rate. Despite this improvement, both electrodes remained within the quasi-reversible regime, indicating that plasma treatment enhanced, rather than fundamentally changed, the electron-transfer behavior of the resin-carbon interface. The k^0 obtained for the plasma-treated HMNE is also comparable to values reported for established carbon-based electrodes, including carbon paper and pyrolyzed paper electrodes,^{39,40,41,42} despite the conductive area of the present device being confined to the carbon-filled microneedle channels (Table S1). These results indicate that the reduction in R_{ct} observed after plasma treatment is accompanied by a measurable increase in heterogeneous electron-transfer kinetics, in addition to the previously demonstrated improvement in electrolyte accessibility.

The ferri/ferrocyanide response also depends on its sensitivity to surface chemistry and local charge. Oxygen-containing groups generated by plasma treatment may render the exposed interface more negatively charged and could, in principle, hinder the approach of the anionic $[\text{Fe}(\text{CN})_6]^{3-/4-}$ species. Nevertheless, the pronounced increase in peak current after plasma treatment indicates that, under the present conditions, improved wettability and greater access to electroactive carbon domains outweighed any unfavorable electrostatic contribution. Thus, the enhanced ferri/ferrocyanide response results from

increased interfacial accessibility, a larger effectively wetted electroactive area, and changes in the local chemical environment.²⁰

The $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$ couple was subsequently employed as an outer-sphere redox probe that is less dependent on specific adsorption and surface functional groups (Fig. 3E).^{43,44} Plasma treatment produced a more moderate, although still evident, enhancement of the voltammetric response. The anodic peak current increased from approximately 1.8 μA to 2.5–2.7 μA , whereas the cathodic peak currents remained closer in magnitude, at approximately – 3.3 to – 3.6 μA . The moderate enhancement observed for the ruthenium probe is consistent with improved wetting and increased access to the carbon phase. In contrast, the more pronounced response observed for ferri/ferrocyanide indicates that oxygen plasma also modified probe-sensitive interfacial properties, including the local charge distribution and surface functionalization. The different behavior of the two probes therefore confirms that plasma treatment not only increases the electrode's bulk conductivity but also modifies the physicochemical environment at the electrode/electrolyte interface.

The influence of plasma treatment was further evaluated using dopamine and ascorbic acid, which undergo proton-coupled oxidation but interact differently with oxygenated surfaces. Dopamine produced a broad oxidation response in PBS at pH 7.4, and the plasma-treated HMNE reached a current of approximately 4.3 μA , compared with approximately 2.8–3.0 μA for the untreated electrode (Fig. 3F). The oxidation wave also became more clearly defined after plasma treatment. This enhancement is consistent with improved wetting and more effective access of dopamine to electroactive carbon sites. At near-neutral pH, dopamine is predominantly protonated and may also interact favorably with negatively charged oxygen-containing functionalities, further contributing to the increased response.

In contrast, ascorbic acid displayed a substantially higher oxidation current at the untreated HMNE in PBS at pH 7.2 (Fig. 3G). The current reached approximately 5.4 μA for the untreated HMNE, whereas the plasma-treated HMNE exhibited a limiting current of only approximately 2.1–2.2 μA . At this pH, ascorbic acid is predominantly present as negatively charged ascorbate. The lower response after plasma treatment is therefore consistent with unfavorable electrostatic interactions between ascorbate and the negatively charged oxygenated groups introduced at the resin–carbon interface. These results show that plasma treatment does not nonspecifically enhance all electrochemical processes. Instead, it alters the balance among wettability, electrostatic interactions, mass transport, accessible electroactive area, and electron-transfer kinetics in an analyte-dependent manner.

Figure 3 shows that oxygen plasma treatment markedly changes HMNE interfacial behavior. The treatment increases surface hydrophilicity, decreases the apparent charge-transfer resistance, and enhances the response toward selected redox probes and dopamine, while suppressing the oxidation response of negatively charged ascorbate. The contrasting responses of the different electroactive species indicate that these changes cannot be explained solely by increased geometric area or bulk conductivity. Rather, oxygen plasma affects both the accessibility and the chemical environment of the resin–carbon interface.

Raman spectroscopy was further used to examine whether carbon incorporation and oxygen plasma treatment produced detectable changes in the chemical environment at the microneedle tip (Fig. 4). The untreated resin exhibited a complex spectral profile, with prominent contributions around 850–900, 1200–1400, and 1450 cm^{-1} . After carbon paste incorporation, the overall spectral profile remained similar, although the relative intensity of several components changed, particularly between approximately 1200 and 1600 cm^{-1} (Fig. 4B). This region includes contributions commonly associated with the D and G bands of carbonaceous materials;²⁰ however, these signals overlap with vibrational modes of the photopolymer resin.³⁸ The intense feature near 1450 cm^{-1} , which is also present in the resin spectrum, therefore cannot be assigned exclusively to the carbon phase.

Oxygen plasma treatment produced additional changes in the relative intensities and widths of the Raman components for both the resin and the carbon-filled HMNE (Figs. 4C and 4D), without introducing new dominant bands. In the carbon-filled electrode, the most noticeable differences again appeared in the 1200–1600 cm^{-1} region. The preserved overall spectral profile indicates that plasma exposure did not induce extensive structural modification of either the resin or the carbon phase. Instead, the observed spectral changes are consistent with modification of the exposed resin–carbon interface, where plasma treatment can alter the local chemical environment of the resin and accessible carbon domains near the channel opening.

These Raman results are consistent with the pronounced change in wettability and the electrochemical response observed after plasma treatment. Together, the contact-angle, impedance, voltammetric, and Raman measurements indicate that oxygen plasma primarily modifies the exposed resin–carbon interface, improving electrolyte accessibility while also altering its local chemical environment. This combination of effects is reflected in the reduction of R_{ct} , the approximately twofold increase in k^0 , and the probe-dependent electrochemical responses observed for the HMNE.

Electrochemical immunosensing performance toward IL-6. Following oxygen plasma treatment, the HMNE was functionalized with anti-IL-6 antibodies and evaluated as an impedimetric immunosensing platform. IL-6 was selected as an inflammatory biomarker to demonstrate the applicability of the three-dimensional microneedle architecture to affinity-based electrochemical detection.

The stepwise assembly of the immunosensor was first monitored by cyclic voltammetry using the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe (Fig. 5A). The non-functionalized HMNE exhibited the highest anodic and cathodic peak currents, consistent with greater accessibility of the electroactive surface. After anti-IL-6 immobilization, both peak currents decreased, and the voltammetric profile became less pronounced, indicating that the biomolecular layer partially restricted the redox probe's access to the electrode surface. A further decrease in the Faradaic response was observed after IL-6 incubation. This progressive attenuation is consistent with antibody–antigen complex formation, which increases the interfacial barrier and further limits redox-probe access to the electroactive surface.^{30,31,45}

EIS measurements corroborated the changes observed by cyclic voltammetry (Fig. 5B). The non-functionalized HMNE exhibited the smallest semicircle in the Nyquist plot, corresponding to the lowest charge-transfer resistance (R_{ct}). Antibody immobilization increased the semicircle diameter, consistent with the formation of a less conductive biomolecular layer at the electrode/electrolyte interface. Subsequent incubation with IL-6 further increased R_{ct} , reflecting the additional barrier created by the formation of the anti-IL-6/IL-6 complex. Agreement between the CV and EIS responses confirms progressive modification of the electrode interface during immunosensor assembly and antigen recognition.

The selectivity of the immunosensor was evaluated by comparing the change in charge-transfer resistance (ΔR_{ct}) obtained for IL-6 with the responses to other inflammatory proteins, including TNF- α , IFN- γ , and IL-17 (Fig. 5C). IL-6 produced a ΔR_{ct} of approximately 22 k Ω , whereas the anti-IL-6-functionalized HMNE in the absence of analyte and the electrodes exposed to the interfering proteins showed responses of approximately 5–6 k Ω . Thus, the response to IL-6 was approximately fourfold higher than those obtained for the blank and the evaluated interferents. The comparatively small responses observed for TNF- α , IFN- γ , and IL-17 indicate that nonspecific interactions contributed only modestly to the impedimetric signal under the conditions investigated.

The analytical response was evaluated by relating ΔR_{ct} to IL-6 concentration (Fig. 5D). The signal increased progressively from approximately 6 to 50 k Ω over the concentration range from 0.5 to 7.5 ng mL $^{-1}$. A linear relationship was observed within this interval, showing that increasing IL-6 concentration produced a proportional increase in the interfacial resistance associated with antibody–antigen complex formation. The immunosensor exhibited a limit of detection of 0.178 ng mL $^{-1}$.

Although circulating IL-6 concentrations in healthy individuals are generally found in the pg mL $^{-1}$ range, substantially higher levels have been reported under severe systemic inflammatory conditions. Patients with sepsis and septic shock have been reported to have concentrations on the order of 1.7 and 8.5 ng mL $^{-1}$, respectively. The analytical range obtained here therefore encompasses IL-6 concentrations reported for severe inflammatory states, while the LOD is below the lower end of this clinically elevated range.⁴⁶ These comparisons should not be interpreted as diagnostic thresholds, since circulating IL-6 concentrations vary with disease severity, patient population, and analytical method, but they indicate that the HMNE sensitivity is compatible with detecting markedly elevated IL-6 levels.

The performance of the immunosensor was further examined in simulated interstitial fluid (ISF) to evaluate the influence of a more complex matrix (Fig. 5E).^{47,48} The ISF was spiked with IL-6 at 0.5, 1.0, and 2.5 ng mL $^{-1}$, and R_{ct} values were obtained by fitting the Nyquist plots to the Randles equivalent circuit. To account for the baseline response of the functionalized electrode, the results were expressed as ΔR_{ct} . The responses obtained in simulated ISF were comparable to those observed in the calibration medium, and ΔR_{ct} increased with IL-6 concentration under both conditions. These results indicate that

the constituents of the simulated ISF did not markedly impair IL-6 recognition or impedimetric transduction within the evaluated concentration range.

Preservation of the concentration-dependent response in simulated ISF is relevant to the intended application because the matrix contains ionic and molecular components absent from the simplified calibration solution. The comparable ΔR_{ct} responses therefore suggest that the immunosensor retains its recognition capability under conditions that more closely reproduce the physicochemical environment of interstitial fluid.

Storage stability was evaluated over 40 days by monitoring the impedimetric response of the immunosensor (Fig. 5F). After this period, the response decreased by approximately 9%, corresponding to retention of approximately 91% of the initial signal. The relatively small variation indicates that the electrode interface and recognition layer remained sufficiently stable during storage at 4°C to preserve most of the initial impedimetric response.

The immunosensor performance was further evaluated in diluted human plasma to assess its response in a biological matrix. Plasma samples from healthy donors were diluted 1:100 and spiked with IL-6 at 0.5, 1.0, and 2.5 ng mL⁻¹. The impedimetric responses obtained in plasma were compared with those measured in buffer under the same conditions (Table S2). At 0.5 and 1.0 ng mL⁻¹, relative signal recoveries of 95.84% and 92.32% were obtained, respectively, showing that the R_{ct} response was largely preserved in diluted plasma at these concentrations. At 2.5 ng mL⁻¹, the relative signal recovery decreased to 76.75%, indicating a more pronounced matrix effect under this condition.

The corresponding RSD values were 14.95%, 9.19%, and 3.96% at 0.5, 1.0, and 2.5 ng mL⁻¹, respectively (n = 3). The greater variability observed at the lowest concentration is consistent with the larger relative contribution of experimental variability near the lower end of the analytical range. Despite the reduced response at 2.5 ng mL⁻¹, the immunosensor retained a concentration-dependent impedimetric response in diluted human plasma. These results show that the HMNE can transduce IL-6 recognition in a diluted biological matrix, while also indicating that matrix effects should be considered when moving from buffered media to more complex samples.

Recent microneedle-based sensing platforms have demonstrated the feasibility of detecting IL-6 and other inflammatory cytokines using electrochemical and affinity-based approaches (Table S3).^{11,49,50} Several of these systems employ highly engineered interfaces, including microfabricated metal electrodes, carbon-nanomaterial-modified surfaces, hydrogel microneedles, molecularly imprinted polymers, or integrated microfluidic architectures. Some of these platforms have achieved substantially lower detection limits than the present HMNE and, in some cases, have progressed to in vivo measurements.^{49,50} However, these analytical gains generally require additional microfabrication, nanomaterial deposition, or more elaborate surface-functionalization steps.

The main distinction of the present platform lies in the integration of the electrochemical interface directly within a stereolithographically printed hollow microneedle structure. Carbon paste confined within the internal channels establishes a continuous conductive pathway from the electrical contact region to the microneedle array, while preserving the hollow architecture of the device. Oxygen plasma treatment provides an additional way to tune the exposed resin–carbon interface, increasing wettability and improving electron-transfer behavior without metal deposition or extensive modification of the printed structure. Although the detection limit of 0.178 ng mL^{-1} is higher than those reported for some highly engineered microneedle cytokine sensors, the HMNE combines a comparatively simple fabrication route with structural integrity, selective IL-6 recognition, and impedimetric operation in simulated ISF and diluted human plasma.

This comparison indicates that the principal contribution of the HMNE lies in the combination of structural integration, fabrication simplicity, and interfacial tunability within a single hollow microneedle architecture. Further optimization of the electroactive area, bioreceptor immobilization, and antifouling properties may improve analytical sensitivity while preserving the manufacturing advantages of the resin–carbon platform.

4. CONCLUSION

A stereolithographically fabricated hollow microneedle electrode was developed by integrating carbon paste within the internal microchannels of a resin-based microneedle architecture. The resulting HMNE preserved the hollow geometry and maintained its structural integrity during insertion while providing a continuous conductive pathway through the microneedle array. Oxygen plasma treatment effectively modified the resin–carbon interface, decreasing the water contact angle from 79.4° to 15.7° and reducing the apparent charge-transfer resistance from approximately 120 to 32 k Ω . The heterogeneous electron-transfer rate constant increased from 1.26×10^{-3} to $2.62 \times 10^{-3} \text{ cm s}^{-1}$, while Raman spectroscopy indicated that plasma treatment modified the local interfacial environment without extensive structural transformation of the resin–carbon composite.

Following anti-IL-6 functionalization, the plasma-treated HMNE enabled label-free impedimetric recognition of IL-6. The response to IL-6 was approximately fourfold higher than those obtained for TNF- α , IFN- γ , and IL-17, and a concentration-dependent response was observed from 0.5 to 7.5 ng mL^{-1} , with a limit of detection of 0.178 ng mL^{-1} . The immunosensor maintained a concentration-dependent response in simulated interstitial fluid and diluted human plasma, although the lower relative signal recovery at 2.5 ng mL^{-1} in plasma indicated matrix effects under this condition. In addition, the system retained approximately 91% of its initial impedimetric response after 40 days of storage at 4°C .

These findings demonstrate that a hollow microneedle structure fabricated by stereolithography can be converted into an integrated three-dimensional electrochemical interface by incorporating a conductive carbon phase within its internal channels. The combination of hollow microneedle architecture, internal carbon integration, oxygen-plasma interfacial engineering, and impedimetric transduction provides a

comparatively simple and adaptable strategy for affinity-based biosensing. Further optimization of the electroactive interface and bioreceptor functionalization may extend the analytical performance of this platform toward minimally invasive detection of inflammatory biomarkers.

Declarations

Credit authorship contribution statement

Rafael C. Otoni: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Leandro T. Kochi:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Ana Carola D. L. V. Reis:** Methodology, Formal analysis, Writing – review & editing. **Fabíola L. de Castro:** Resources, Investigation, Writing – review & editing. **Ana Alice Zaneli:** Methodology, Formal analysis, Writing – review & editing. **Ana Cristina H. Castro-Kochi:** Methodology, Formal analysis, Writing – review & editing. **Wendel A. Alves:** Conceptualization, Supervision, Project administration, Funding acquisition, Data curation, Writing – review & editing. All authors have read and approved the final version of the manuscript.

Declaration of Competing Interests

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Figures

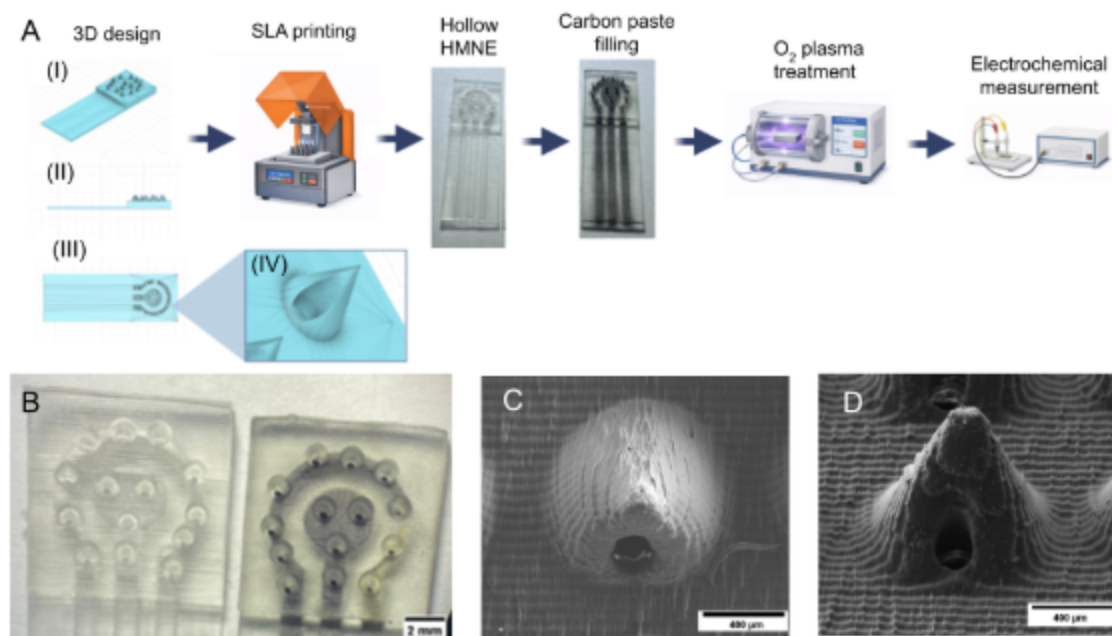


Figure 1

(A) Fabrication workflow comprising CAD design, stereolithographic printing, recovery of the hollow microneedle platform, and filling of the internal channels with carbon paste. Insets I–IV show the isometric, lateral, top, and enlarged single-microneedle CAD views, respectively. (B) Optical images of the platform before and after carbon paste filling. (C) SEM image of an unfilled microneedle showing the open internal channel. (D) SEM image after carbon paste filling, showing preservation of the microneedle geometry and channel opening.

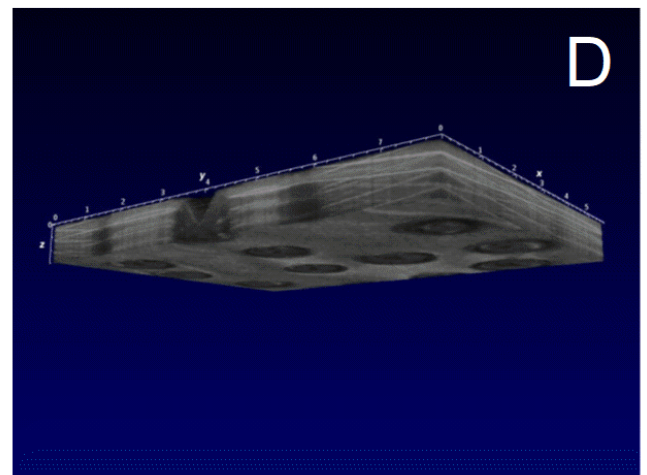
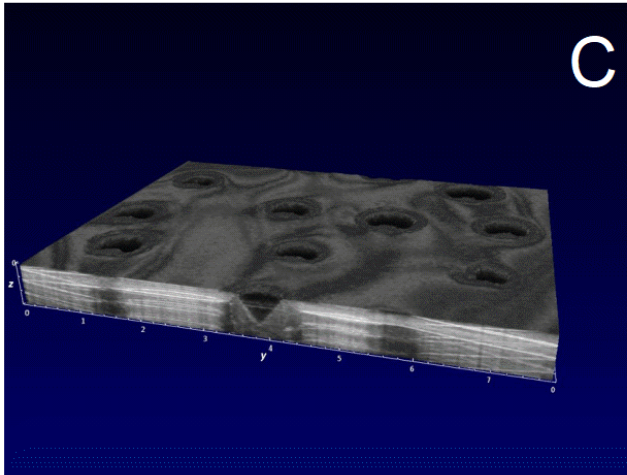
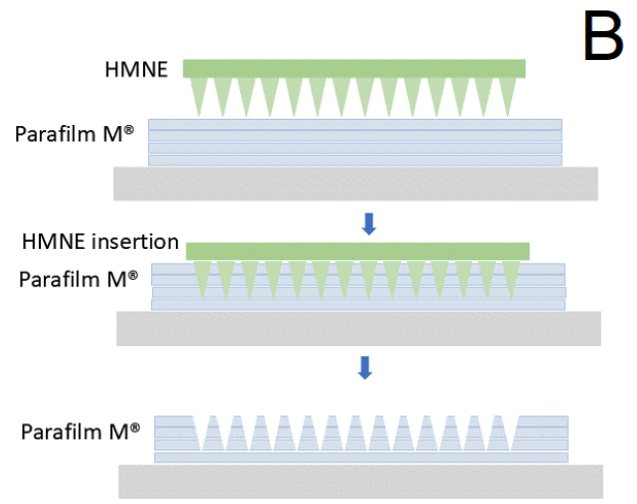
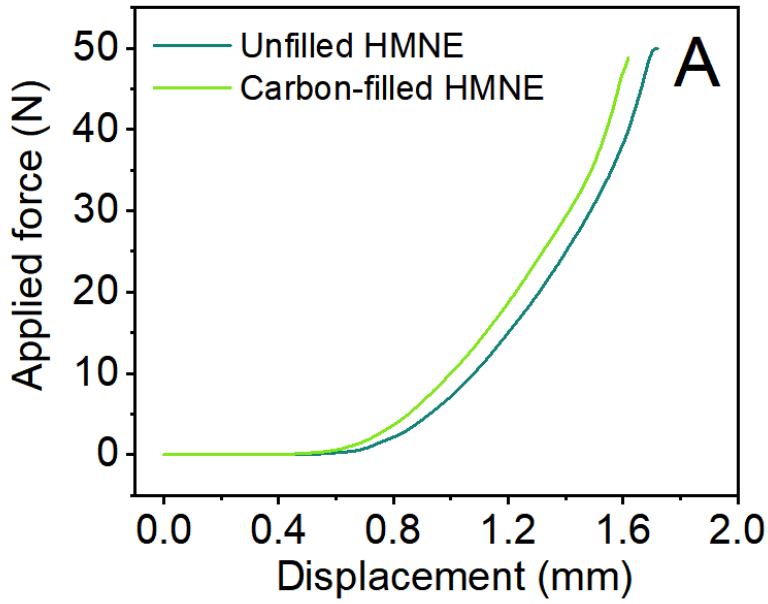


Figure 2

(A) Force–displacement curves obtained during insertion of hollow microneedle electrodes with and without carbon paste filling into eight stacked Parafilm M® layers used as a skin-mimicking substrate. (B) Schematic representation of the microneedle insertion through the multilayer Parafilm structure. (C) Top-view OCT image showing the perforations produced by the microneedles. (D) Bottom-view OCT image showing the corresponding perforations on the opposite side of the substrate, confirming complete penetration through all eight Parafilm M® layers.

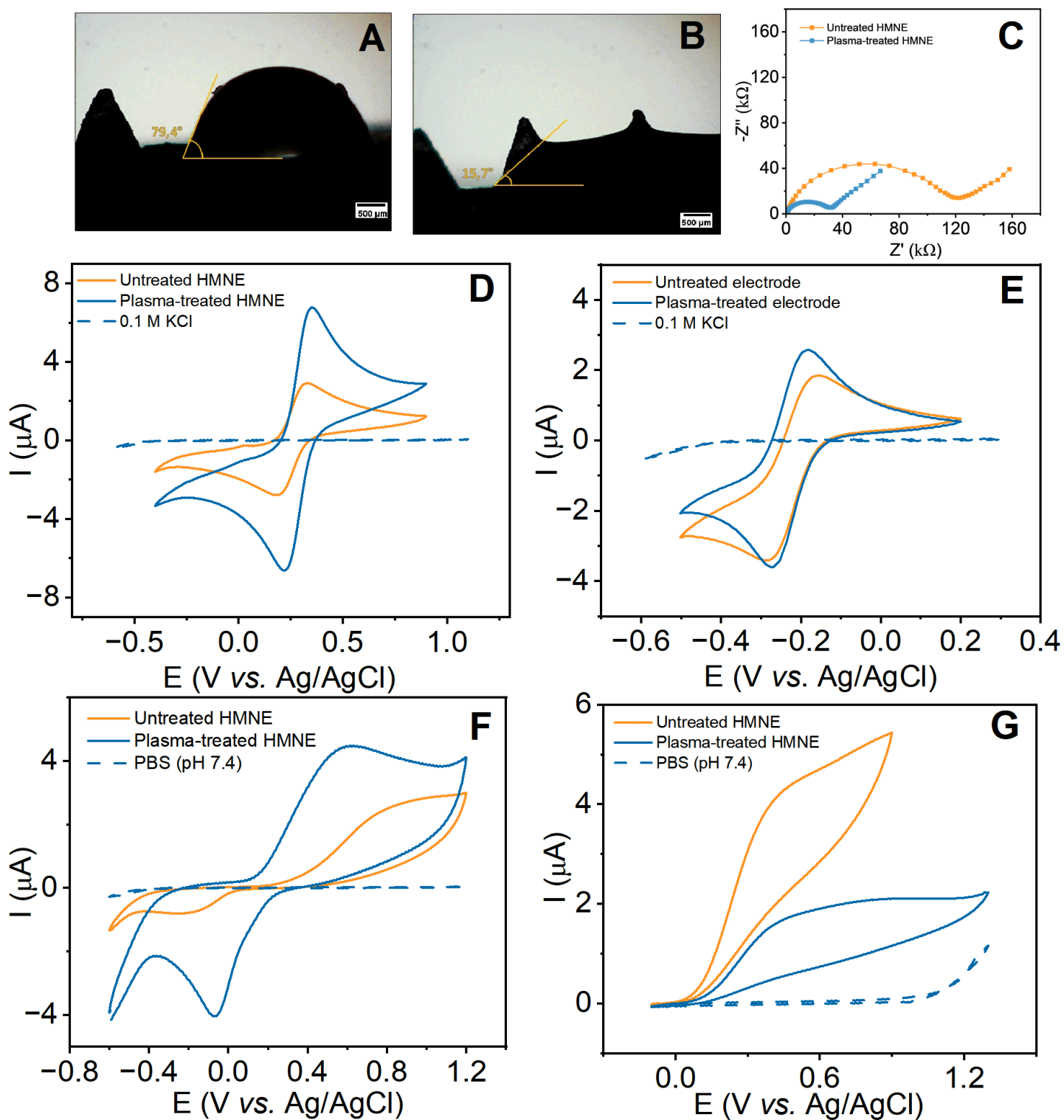


Figure 3

(A,B) Representative water-contact-angle measurements for untreated and plasma-treated hollow microneedle carbon electrodes, respectively, showing a decrease from 79.4° to 15.7° after oxygen plasma treatment. (C) Nyquist plots recorded before and after plasma treatment. (D) Cyclic voltammograms obtained with the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple in 0.1 M KCl. (E) Cyclic voltammograms

obtained with the $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$ redox couple in 0.1 M KCl. (F) Voltammetric response toward dopamine in PBS at pH 7.4. (G) Voltammetric response toward ascorbic acid in PBS at pH 7.2. Untreated and plasma-treated electrodes were compared under the same experimental conditions.

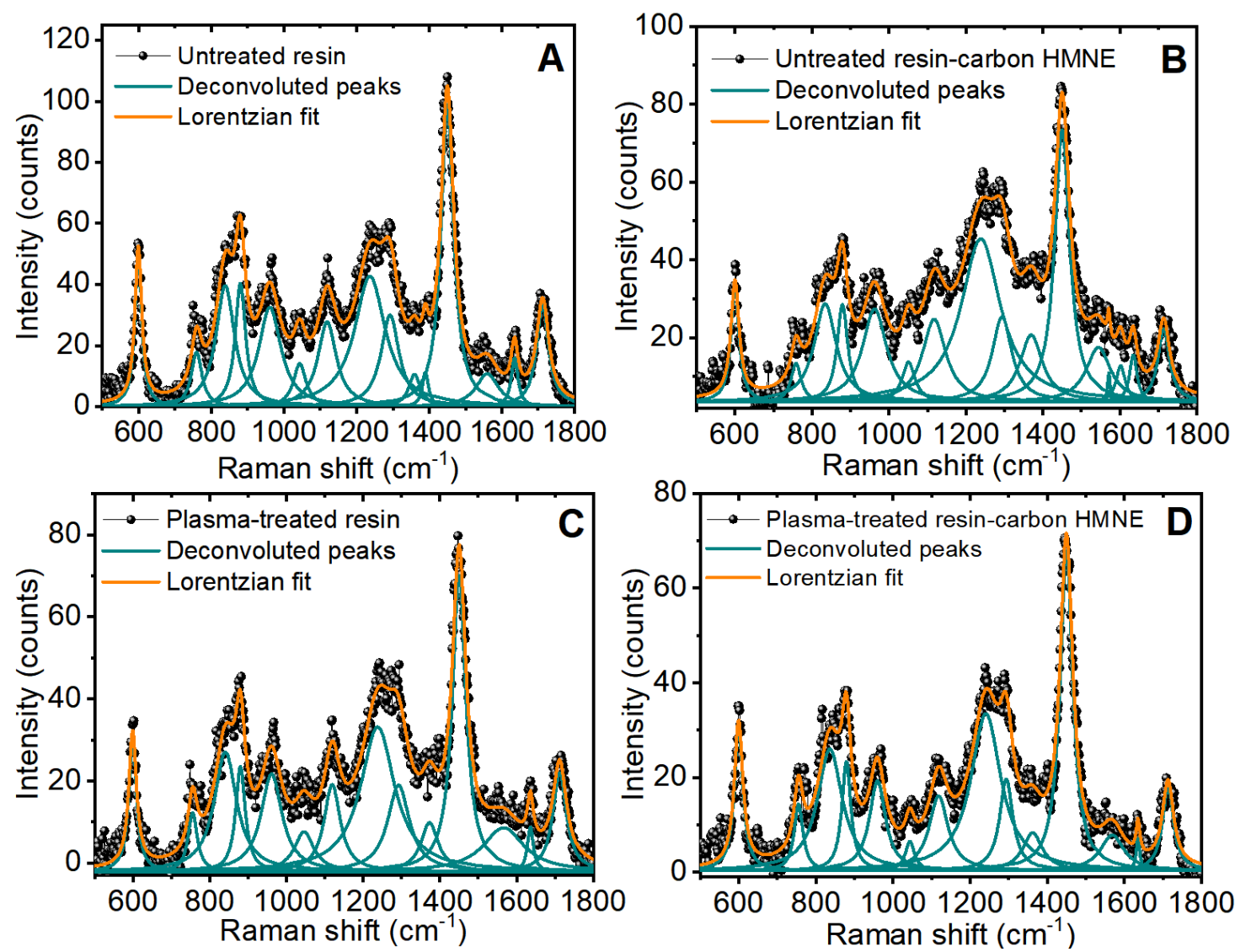


Figure 4

Raman spectra and Lorentzian deconvolution obtained at the microneedle tip for (A) untreated resin, (B) untreated resin-carbon HMNE, (C) plasma-treated resin, and (D) plasma-treated resin-carbon HMNE. Experimental spectra, Lorentzian fits, and individual deconvoluted components are shown for each condition.

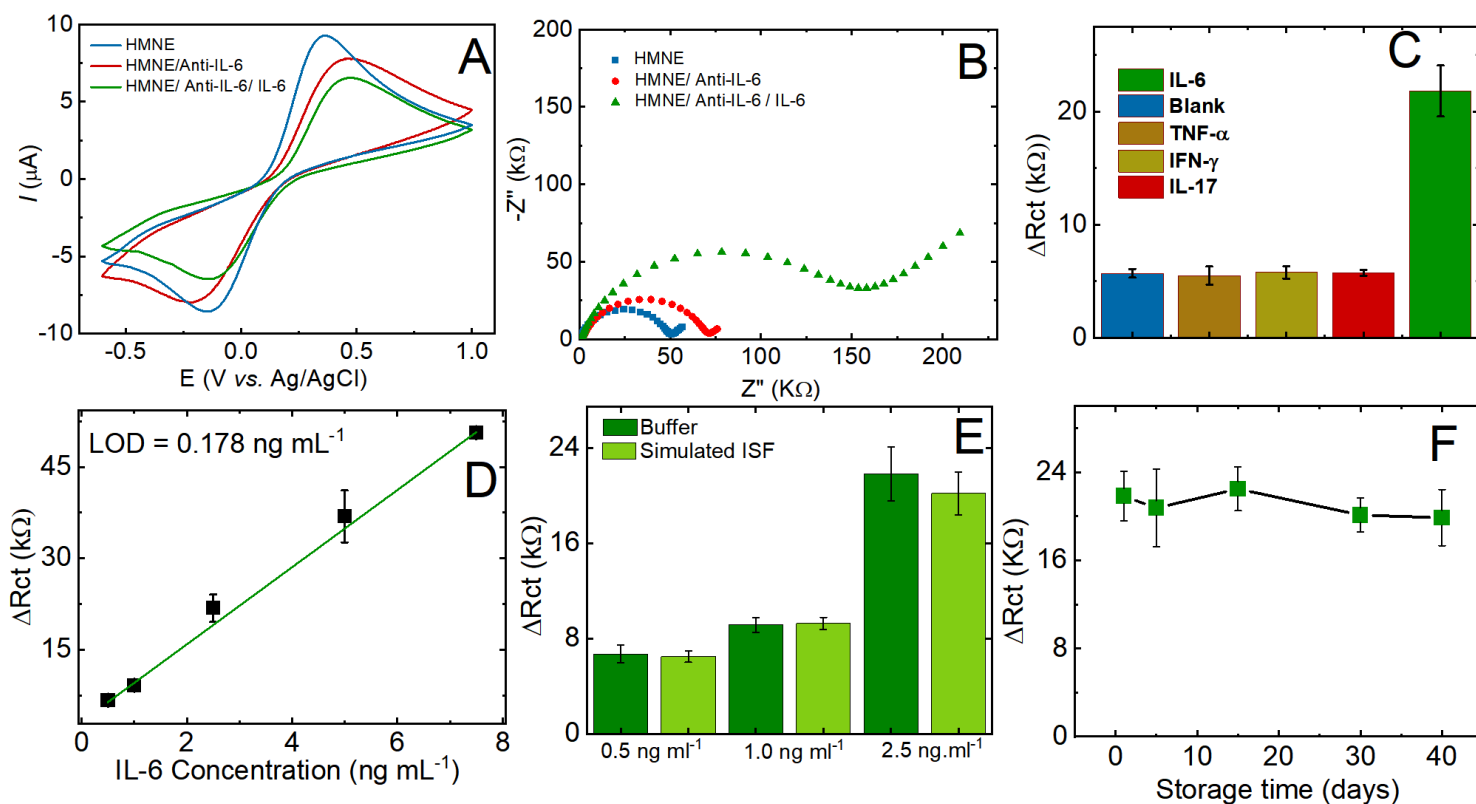


Figure 5

(A) Cyclic voltammograms of the HMNE before functionalization, after anti-IL-6 immobilization, and after incubation with IL-6. (B) Corresponding Nyquist plots showing the progressive increase in charge-transfer resistance (R_{ct}) during antibody immobilization and IL-6 recognition. (C) Selectivity evaluated from changes in charge-transfer resistance (ΔR_{ct}) after exposure to IL-6, TNF- α , IFN- γ , and IL-17. (D) Calibration curve relating ΔR_{ct} to IL-6 concentration. Data are presented as mean $\pm$ SD, and the solid line represents the linear regression fit. The limit of detection was 0.178 ng mL^{-1} . (E) Impedimetric response toward IL-6 in simulated interstitial fluid. (F) Storage stability of the IL-6 immunosensor over 40 days at 4°C .

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