

ARTICLE

Electrochemical Monitoring of Cell Death in Lung Cancer-Derived Spheroids Using Lab-Fabricated Sensors

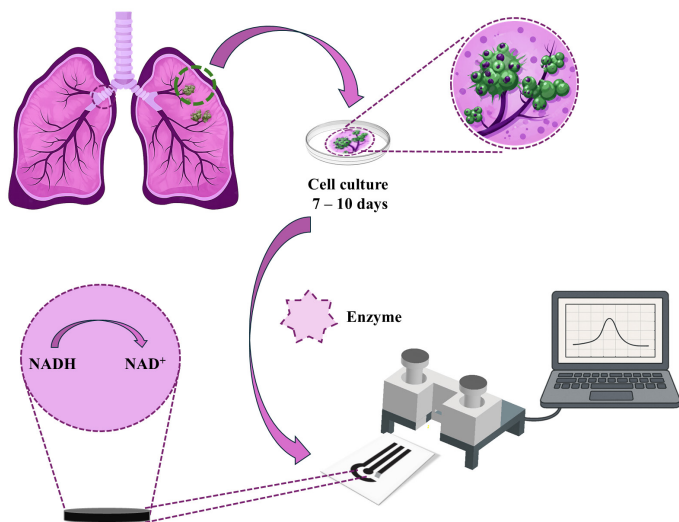
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A low-cost and portable electroanalytical platform was developed for the indirect determination of cell death in three-dimensional (3D) lung cancer models. The system is based on a lab-made graphite-pine rosin composite stencil electrode (GPC-SE) integrated with a 3D-printed support, enabling straightforward coupling with a portable potentiostat. Human lung adenocarcinoma A549 cells were cultured as multicellular tumor spheroids (tumoroids) to provide a physiologically relevant *in vitro* model. The analytical strategy relies on the indirect quantification of extracellular lactate dehydrogenase (LDH) released during tumoroid cell death. LDH activity was evaluated by monitoring the electrochemical oxidation of NADH produced during the enzymatic conversion of lactate to

pyruvate in the presence of NAD⁺. The GPC-SE fabrication was optimized in terms of graphite-to-rosin ratio and electrochemical pretreatment, resulting in improved signal stability and reproducibility. The proposed platform demonstrated a linear response for NADH over 400–900 $\mu\text{mol L}^{-1}$ ($R^2 = 0.98$), with limit of detection of 60 $\mu\text{mol L}^{-1}$ and limit of quantification of 200 $\mu\text{mol L}^{-1}$. Application to A549 tumoroids treated with doxorubicin (DOX) revealed a concentration-dependent increase in electrochemical signal, consistent with drug-induced cytotoxicity and LDH release. The integration of 3D cell culture models with a miniaturized electrochemical

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system provides a simple and effective approach for monitoring therapeutic responses, thus revealing huge potential for bioanalytical and drug-screening applications.

Keywords: Lactate dehydrogenase, cancer models, tumoroids, graphite-pine rosin composite, stencil electrode

INTRODUCTION

In vitro bioassays are essential tools for investigating cellular behavior and physiological responses under controlled conditions. Human lung adenocarcinoma A549 cells are widely employed as a representative model of non-small cell lung cancer and are commonly cultured either as conventional two-dimensional (2D) monolayers or as three-dimensional (3D) systems. Notably, 3D culture platforms provide a more realistic representation of the *in vivo* tumor microenvironment.¹ Under non-adherent conditions, A549 cells self-assemble into multicellular spheroids (tumoroids), typically formed in low-attachment culture plates. These 3D structures exhibit key physiological features absent in 2D systems, including gradients of oxygen, nutrients, metabolites, and therapeutic agents, as well as enhanced cell-cell and cell-matrix interactions.² In addition, spheroids often display increased resistance to drug exposure, better reflecting tumor behavior *in vivo*. As a result, A549-derived tumoroids represent a robust and physiologically relevant model for evaluating drug responses, metabolic activity, and biomarker release.

Among these biomarkers, lactate dehydrogenase (LDH) plays a central role as an indirect marker of cell death.^{3–5} Chemotherapeutic agents such as doxorubicin (DOX) induce cytotoxicity in A549 tumoroids through mechanisms including DNA intercalation and the generation of reactive oxygen species, ultimately compromising cell membrane integrity and leading to LDH release into the extracellular medium.^{5,6} The oxidation of NADH produced during the enzymatic conversion of L-lactate to pyruvate can be employed to monitor LDH activity.⁷ This indirect detection strategy provides a sensitive and physiologically relevant approach for assessing drug efficacy in lung cancer models, bridging the gap between cellular biology and analytical chemistry. Thus, integrating tumoroid-based assays with electrochemical platforms enables quantitative monitoring of therapeutic responses under conditions that closely mimic the tumor microenvironment.^{8,9}

Despite these advances, conventional methods for evaluating cell viability and drug response in 3D models often rely on optical or biochemical assays that require labeling steps, multiple reagents, or costly instrumentation. These limitations restrict their applicability for rapid and portable analysis. In this context, electrochemical biosensing has emerged as promising approach owing to its sensitivity, low cost, and compatibility with portable and miniaturized systems.

Three-dimensional (3D) printing enables the rapid and low-cost fabrication of customized analytical devices, including electrochemical cells, electrode holders, and integrated sensing platforms.¹⁰ This approach allows greater design flexibility, system miniaturization, and improved component integration.¹¹ In addition, 3D-printed supports can incorporate functional elements such as magnets and electrical contacts, ensure proper alignment, and facilitate the use of disposable electrodes in portable platforms.¹² Thus, prototyping via 3D printing is a versatile, low-cost strategy for developing electrochemical devices for analytical applications.^{13,14}

In this work, a lab-made graphite-pine rosin composite stencil electrode (GPC-SE) was employed for the indirect screening of LDH via NADH electrooxidation. The electrode material incorporates pine rosin, a natural and sustainable resin previously reported as an efficient binder in graphite-based conductive inks.^{15,16} The *in vitro* electrochemical monitoring of cell death was performed using immortalized lung cancer-derived spheroids, minimizing the variability commonly associated with primary cell cultures and enabling a more reliable evaluation of the biological response in the culture medium. The proposed sensor was optimized by evaluating different graphite-to-rosin ratios and applied to NADH determination, providing a simple, low-cost, and portable analytical platform. When combined with a 3D-printed electrochemical interface, this approach represents a practical and sustainable strategy for bioanalytical applications.

MATERIALS AND METHODS

Chemicals and materials

All experimental solutions were prepared with ultrapure water (18.2 MΩ cm), supplied by a Millipore Direct-Q®3 purification system (MA, USA). The GPC-SE was fabricated using graphite powder purchased from Sigma-Aldrich (St. Louis, MO, USA), silver ink (ADB Sensors, Brazil), EVA-coated polyester substrate and vinyl adhesive obtained locally.

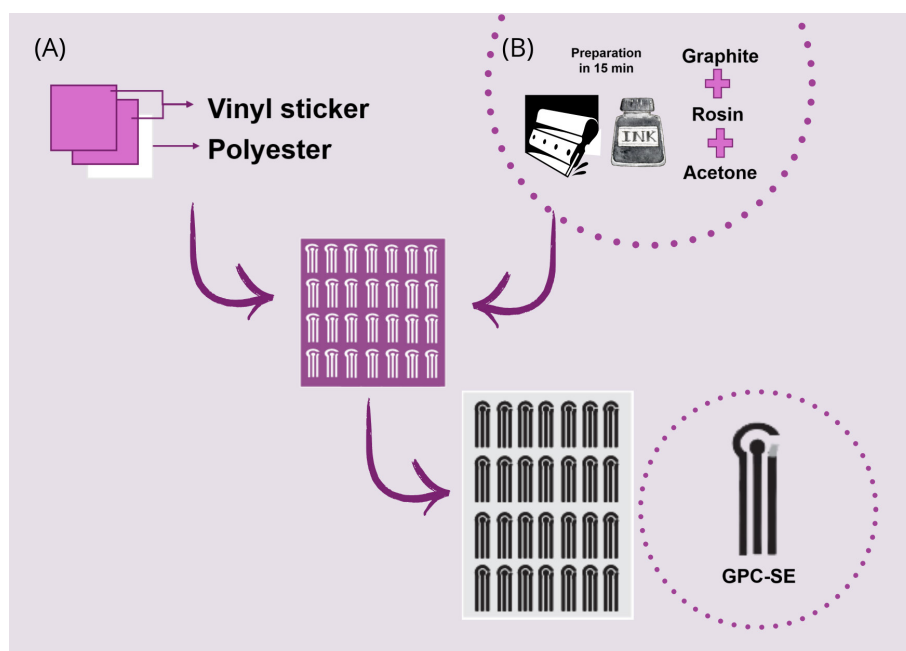
Acetic (CH_3COOH , 5.0 mol L⁻¹), nitric (HNO_3 , 1.0 mol L⁻¹), and sulfuric (H_2SO_4 , 1.0 mol L⁻¹) acids were investigated for the electrochemical cleaning of GPC-SE. Electrochemical characterization was performed using ferro-/ferricyanide ($[\text{Fe}(\text{CN})_6]^{4-/3-}$) and hexaammineruthenium ($[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$) redox probes at 1.0 mmol L⁻¹ in 0.1 mol L⁻¹ KCl.

Lactate dehydrogenase (LDH), doxorubicin (DOX), lactate, and nicotinamide adenine dinucleotide in both reduced (NADH) and oxidized (NAD⁺) forms were purchased from Sigma-Aldrich (St. Louis, MO, USA). The A549 cell was purchased from Banco de Células do Rio de Janeiro.

Electrode fabrication

The device was manufactured following the procedure previously described by Campos et al. (2026) with specific adaptations.¹⁶ Two layers of vinyl adhesive (Vinyl sticker – 8 cm × 11 cm) were applied onto a polyester substrate (80 mm wide × 110 mm long × 250 μm thick). The integrated electrode geometry, composed of a working electrode (WE; $\varnothing = 2.5$ mm), a reference electrode (RE), and an auxiliary electrode (AE), was designed using Silhouette Studio® software. The electrode stencil was subsequently patterned using a Silhouette cutting plotter (Belo Horizonte, MG, Brazil).

The adhesive stencil layers were mechanically detached from the polyester substrate using tweezers, as illustrated in Scheme 1A. The previously prepared carbon ink was then deposited onto the areas corresponding to the working electrode (WE), auxiliary electrode (AE), and electrical connections (Scheme 1B). The ink was evenly spread with a spatula and dried at room temperature for 40 min. Following solvent evaporation and curing, the residual adhesive layers were removed, exposing the final printed electrode pattern (Scheme 1B). Different proportions of the conductive material (graphite) and the binder (rosin) were evaluated and homogenized under magnetic stirring for 15 – 20 min.¹⁶



Scheme 1. (A) Schematic representation of the fabrication steps involved in the preparation of the GPC-SE. (B) Step-by-step preparation of the conductive ink composed of graphite, rosin and acetone.

A hydrophobic barrier was subsequently applied to define the sample deposition region on the electrode surface. A commercial nail polish (Colorama Cremoso Leite de Coco, 8 mL) mainly composed of nitrocellulose in volatile solvents, was employed due to its ability to rapidly form a homogeneous coating. The RE was then fabricated using commercial silver paint.

To facilitate device assembly and improve mechanical robustness, a custom 3D-printed support was employed to connect the electrochemical platform with the potentiostat via a USB connection. The support was designed in Autodesk Inventor 2025 and sliced in Ultimaker Cura 5.11.0. It was printed on a desktop 3D printer (Easythreeed, China) via fused deposition modeling, using polylactic acid (PLA, $\phi = 1.75$ mm). Electrical connection was established using a USB interface. The support is an adaptation of a previous study,¹⁷ and the complete design with all dimensional details is available in Figure S1 of the Supplementary Material.

Surface characterization

Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) analyses were performed using a JSM-6610 microscope (Jeol, Tokyo, Japan) equipped with a Thermo Scientific NSS Spectral Imaging system. Double-sided conductive carbon tape was used to fix the samples, which were coated with a thin gold layer using a Desk V sputter coater (Denton Vacuum LLC, Moorestown, New Jersey, USA).

Spectroscopic analyses were performed by Raman spectroscopy, using a LabRAM HR Evolution high-resolution Raman microscope (Horiba, UV-Vis-NIR spectral range of 220–2200 nm, Villeneuve-d'Ascq, France). All analyses were conducted at the Multi-User High-Resolution Microscopy Laboratory (LabMic) at the Federal University of Goiás (UFG).

3D Cell culture of lung cancer

Cell culture

25 cm² flasks containing 5 mL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin solution were used to culture human lung adenocarcinoma A549 cells (ATCC® CCL-185™). Cell cultures were kept at 37 °C, 86% humidity, and 5% CO₂ in a humidified incubator (Thermo Fisher Scientific). Cells were routinely passaged to maintain a confluency of approximately 80%. To assess potential mycoplasma contamination, cells were cultured for three days in antibiotic-free medium. Subsequently, DNA was extracted, followed by polymerase chain reaction (PCR) amplification and agarose gel electrophoresis to detect mycoplasma contamination.

Tumoroids formation and treatment

Human lung adenocarcinoma cells (A549) were seeded at densities of 1×10^4 and 5×10^3 cells per well in ultra-low attachment 96-well V-bottom plates (untreated surface), in a final volume of 200 μ L of DMEM supplemented with 10% (m/v) FBS. To promote uniform tumoroid formation, plates were centrifuged at $400 \times g$ for 10 min. To allow the formation of multicellular tumor spheroids (MCTS), the cultures were left at 37 °C in a humidified atmosphere (5% CO₂) for 5 days.

After tumoroid establishment, the MCTS were treated with increasing concentrations of DOX (1, 10, and 100 μ mol L⁻¹) prepared in complete culture medium for 48 h. For drug exposure, 100 μ L of culture medium was gently removed and replaced with 100 μ L of DOX solution at twice the intended final concentration, maintaining a constant final volume and minimizing tumoroid disruption.

Following treatment, tumoroids were carefully transferred to flat-bottom plates for morphological assessment using an optical microscope (Lonza) under phase-contrast mode with a 4 $\times$ objective. Subsequently, the conditioned medium (supernatant) was collected and diluted in phosphate-buffered saline (PBS) at a ratio of 1:1.

Electrochemical measurements

A μ Stat 400 bipotentiostat/galvanostat (DropSens, SL, Oviedo, Spain), controlled by DropView 8400 software, was used for all electrochemical characterization measurements. The electrodes were subjected to an electrochemical pretreatment by cyclic voltammetry (CV) before each measurement, employing a scan rate of 100 mV s^{-1} , in a potential window of -1.0 V to $+1.0 \text{ V}$, for 10 scans, in a 1.0 mol L^{-1} sulfuric acid solution. All experiments were performed using only the GPC-SE pseudo-reference electrode (Ag/AgCl). The quasi-reversible Randles-Ševčík equation and CV¹⁸ were used to calculate the electroactive area – using scan rates varying from 5 to 500 mV s^{-1} and in triplicate ($n = 3$).

All electrochemical measurements (application in NADH determination) were carried out using a portable and miniaturized potentiostat, PalmSens4 (PalmSens), controlled through PStace 5.12 software. LDH activity was monitored by measuring the electrochemical response of NADH generated through the LDH-catalyzed conversion of L-lactate to pyruvate in the presence of NAD⁺. To circumvent substrate limitation and ensure maximal enzymatic activity, L-lactate and LDH concentrations were optimized for 3 mmol L^{-1} and 500 U mL^{-1} , respectively. The concentration of NAD⁺ and the incubation time were optimized, resulting in selected conditions of 3 mmol L^{-1} and 2 min. Electrochemical measurements of NADH were conducted in 10 mmol L^{-1} phosphate-buffered saline (PBS, pH 7.4) over a concentration range from 400 to $900 \text{ } \mu\text{mol L}^{-1}$, using the differential pulse voltammetry (DPV) technique. Measurements were conducted within a potential window of 0.25 to 0.90 V under optimized parameters (step potential: 0.01 V, pulse amplitude: 0.2 V, and pulse time: 0.02 s). To minimize electrode fouling and mitigate matrix-related effects, a single-use electrode strategy was adopted, employing one sensor per measurement.

RESULTS AND DISCUSSION

Fabrication of GPC-SE

Graphite-rosin compositions were evaluated in different proportions of 40:60, 45:55, 50:50, 55:45, and 60:40 (w/w), corresponding to 1.20/1.80, 1.35/1.65, 1.50/1.50, 1.65/1.35, and 1.80/1.20 g of graphite/rosin, respectively, prepared with 5 mL of acetone for GPC-SE fabrication. Electrochemical performance was assessed using the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe in 0.1 mol L^{-1} KCl by CV at 50 mV s^{-1} . The results were evaluated in terms of reversibility (ΔE_p and I_{pa}/I_{pc}) and are summarized in Table S1, available in the Supplementary Material. Based on these parameters, the composition containing 60% graphite and 40% rosin was selected for subsequent experiments.

Electrochemical pretreatment and optimization

Acid pretreatment procedures were investigated to improve sensor performance.¹⁶ Sulfuric acid (H_2SO_4 , 1.0 mol L^{-1}), acetic acid (CH_3COOH , 5.0 mol L^{-1}), and nitric acid (HNO_3 , 1.0 mol L^{-1}) were evaluated in triplicate. CVs analyses were performed at a scan rate of 100 mV s^{-1} over a potential window from -1.0 to $+1.0 \text{ V}$ for 10 cycles, and the results are shown in Figure S2.

Among the tested conditions, sulfuric acid pretreatment provided improved peak resolution (Figure S2), characterized by higher peak currents (I_p) and lower peak-to-peak separation (ΔE_p), and it was therefore selected for further studies. In addition, the influence of the final scan potential was evaluated by terminating the CV in the anodic region, cathodic region, and at 0 V (Figure S3). Termination in the cathodic region yielded a more well-defined signal with higher current response and was selected as the optimal condition.

Reproducibility and stability of the GPC-SE

Under optimized conditions, the repeatability and reproducibility of the electrochemical signal after pretreatment were evaluated using the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe. Measurements were conducted using electrodes from the same batch ($n = 5$) and from different batches ($n = 3$), as shown in Figure S4 and Figure 1, respectively.

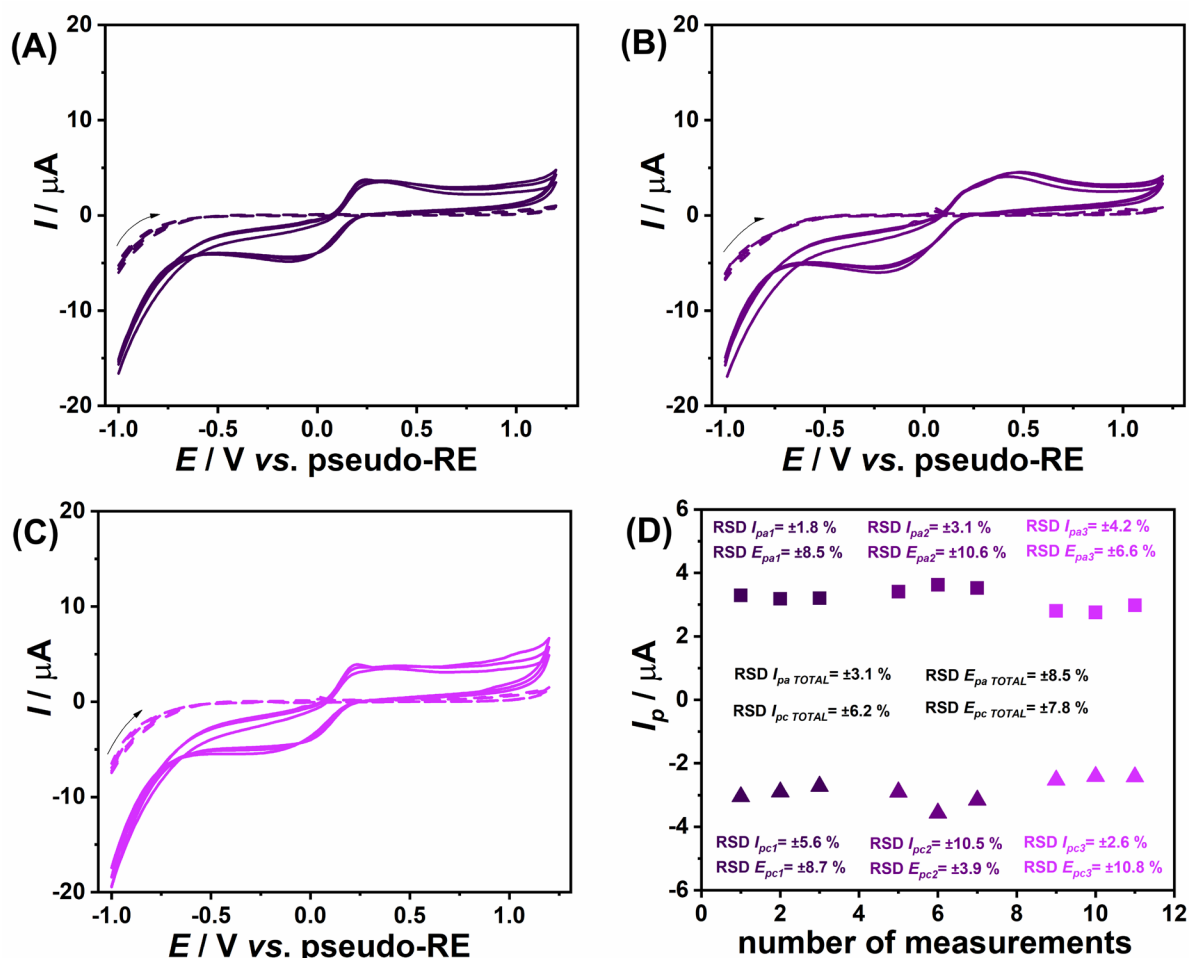


Figure 1. CVs for 0.1 mol L⁻¹ KCl solution in absence (blank: dashed lines) and the presence of 1 mmol L⁻¹ [Fe(CN)₆]^{4-/3-} (solid lines) on GPC-SEs (A – C) after acid pretreatment. Scan rate of 50 mV s⁻¹. (D) Plot of I_p vs. number of measurements ($n = 3$) performed on three different GPC-SEs from different batches.

The proposed pretreatment yielded consistent electrochemical responses across all assays (Figure S4 and Figure 1). The responses showed good stability, with low relative standard deviation (RSD) values for peak potential (E_p , < 8.5%) and current (I_p , < 6.2%), as summarized in Table S2. These results demonstrate good reproducibility and stability of the graphite-rosin stencil-printed electrodes after sulfuric acid pretreatment. Accordingly, GPC-SEs represent a reliable platform for screening applications requiring portability, low sample volume (50 μ L), and operational stability.

Physicochemical and electrochemical characterization of GPC-SE electrodes

SEM and Raman spectroscopy were used in the physicochemical characterization of the GPC-SE electrodes (Figure 2).

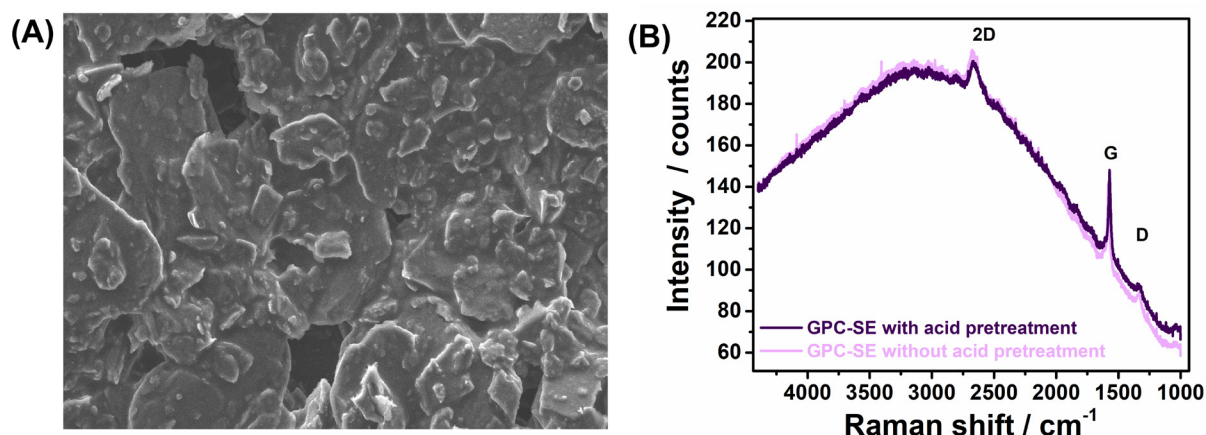


Figure 2. (A) SEM micrographs for the surface of the GPC-SE with pretreatment at 1 k magnification. (B) Raman spectra without and with acid pretreatment of electrode surface.

SEM images (Figure 2A) revealed a typical carbonaceous morphology with increased surface roughness and the presence of porous domains after pretreatment. This behavior is likely associated with partial removal of the binder phase, thereby increasing exposure of the conductive graphite structure.

Characteristic bands of graphitic materials: D ($\sim 1331\text{ cm}^{-1}$), G ($\sim 1571\text{ cm}^{-1}$), and 2D ($\sim 2672\text{ cm}^{-1}$) were observed in the Raman spectra (Figure 2B). I_D/I_G ratios were calculated as 0.85 for the untreated GPC-SE and 0.86 after pretreatment, indicating that the acid activation did not significantly alter the structural disorder of the carbon framework.

These results suggest that pretreatment primarily enhances surface accessibility rather than modifying the bulk structure.¹⁶ This interpretation is supported by EDS analysis (see Figure S5), which showed a decrease in surface oxygen content after treatment, indicating removal of oxidized species or surface contaminants. Such changes are expected to improve electrode-electrolyte interaction and facilitate electron transfer.

Scan rate studies using representative inner- and outer-sphere redox probes $[\text{Fe}(\text{CN})_6]^{4-/3-}$ and $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$, respectively (1.0 mmol L^{-1} in $0.1\text{ mol L}^{-1}\text{ KCl}$), were used in the electrochemical characterization as shown in Figure 3.

To investigate mass transport characteristics and surface sensitivity, two electrochemical probes with distinct electron-transfer pathways were employed: the inner-sphere couple $[\text{Fe}(\text{CN})_6]^{4-/3-}$ and the outer-sphere couple $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ (Figure 3). In the case of $[\text{Fe}(\text{CN})_6]^{4-/3-}$, the anodic and cathodic peak currents exhibited linear dependence on the square root of scan rate ($v^{1/2}$), with correlation coefficients of $R^2 = 0.97$ for both processes. The calculated slopes, 8.05×10^{-6} and $-7.85 \times 10^{-6}\text{ A (V s}^{-1})^{-1/2}$, are consistent with predominantly diffusion-controlled electron transfer accompanied by limited surface-mediated interactions.

Similarly, the $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox couple also exhibited diffusion-controlled behavior, with slopes of 8.42×10^{-5} and $-6.57 \times 10^{-5}\text{ A (V s}^{-1})^{-1/2}$ and high linearity ($R^2 = 0.99$ and 0.98). These results are consistent with faster electron-transfer kinetics and lower sensitivity to surface chemistry, as expected for an outer-sphere probe.¹⁸

The electroactive surface area was determined using the Randles–Ševčík equation for *quasi*-reversible systems, while the heterogeneous electron-transfer rate constant (k_o) was obtained using the Nicholson method based on scan-rate studies ranging from 5 to 500 mV s^{-1} .^{19,20} All values represent the mean $\pm$ standard deviation obtained from three independently fabricated electrodes from each batch. The estimated k_o value was $1.94 (\pm 0.1) \times 10^{-4}\text{ cm s}^{-1}$, and the electroactive area was calculated as $0.07 \pm 0.009\text{ cm}^2$. A comparable value was obtained from double-layer capacitance measurements ($0.08 \pm 0.005\text{ cm}^2$, Figure S6), indicating good agreement between the method employed.

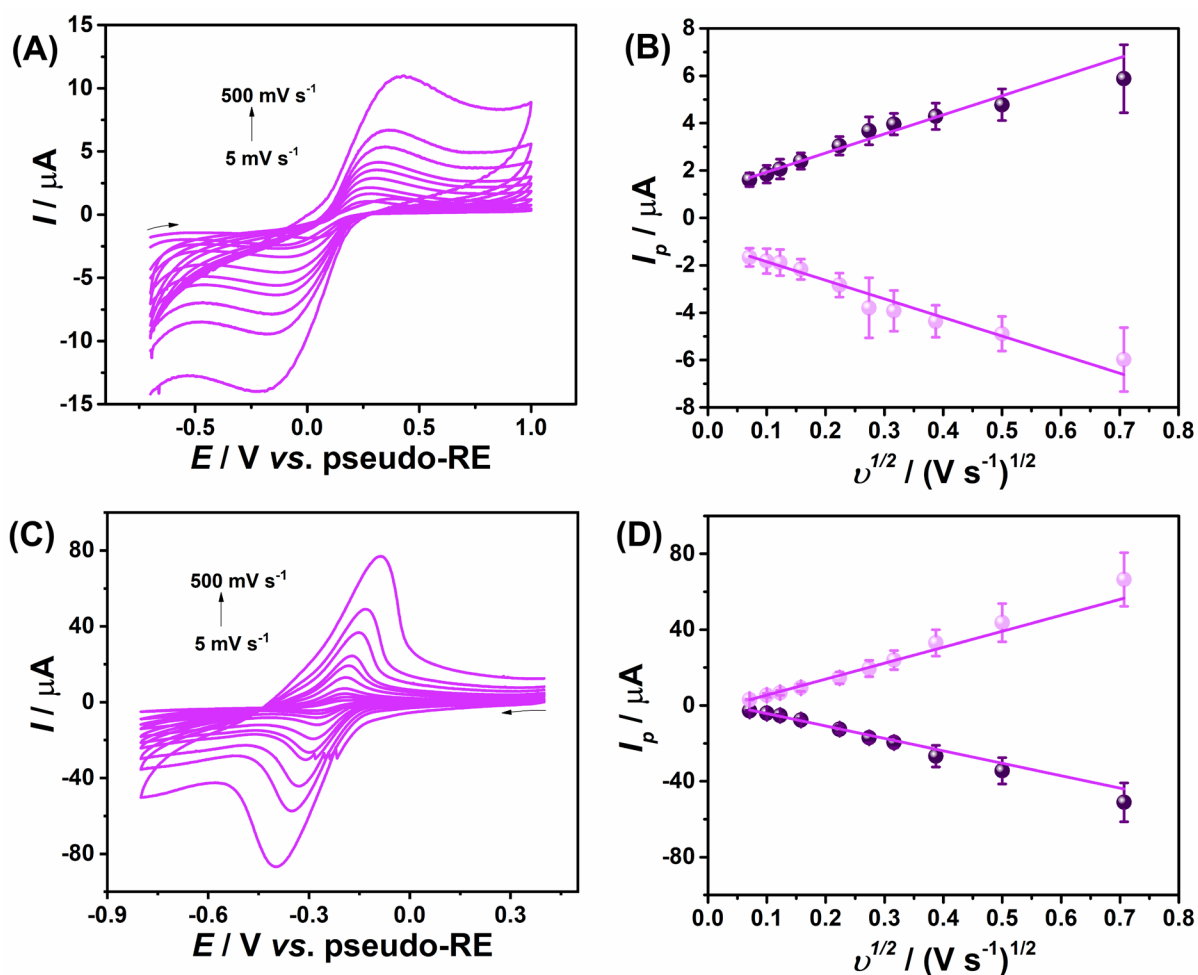


Figure 3. CVs at 5 to 500 mV s^{-1} for (A) $[\text{Fe}(\text{CN})_6]^{4-/3-}$ and (C) $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ (1 mmol L^{-1} in 0.1 mol L^{-1} KCl) performed on GPC-SE. Randles–Ševčík plots (B and D).

Electroanalytical determination of LDH

Three-dimensional (3D) tumor cell models, commonly referred to as tumoroids, have been employed as an *in vitro* platform for cell viability (Figure 4A), as they better replicate the tumor microenvironment than conventional 2D cultures. This enables chemotherapeutic agents such as DOX more physiologically relevant.

DOX primarily exerts its cytotoxic effects through DNA intercalation and the generation of reactive oxygen species (ROS), ultimately activating cell death pathways, including apoptosis and necrosis.^{21,22} As a result, cell membrane integrity is compromised, leading to the release of intracellular components into the extracellular environment, including LDH.²³ Hence, quantification of LDH levels plays an important role in assessing cell death in 3D tumor models. This approach enables a more accurate evaluation of drug efficacy under conditions that better mimic the physiological tumor environment.²⁴

The extracellular release of LDH from tumoroid models enables a direct electrochemical biosensing strategy for monitoring drug response without modifying the working electrode. LDH catalyzes the oxidation of L-lactate to pyruvate while simultaneously reducing NAD^+ to NADH (Figure S7). Its enzymatic activity can be quantitatively monitored by electrochemically oxidizing NADH at the electrode surface.^{25,26} This approach supports the development of sensitive, rapid, and miniaturized analytical platforms, in which the electrochemical signal is directly correlated with LDH concentration and, consequently, with the extent of tumor cell death.

To circumvent potential limitations associated with NADH generation during the enzymatic reaction, key parameters, including L-lactate concentration and reaction time, were systematically optimized. Optimal conditions were established at 3 mmol L⁻¹ for L-lactate and 2 min for reaction time, based on the resulting NADH current response (Figure S8).

Because NADH is susceptible to oxidation, all supernatants were analyzed immediately after collection, protected from light, and processed under identical experimental conditions. The short analysis time and the use of relative comparisons between treated and untreated samples minimize the influence of spontaneous NADH degradation on the analytical response.

The relationship between NADH concentration and its corresponding oxidation peak current was evaluated using DPV profiles, revealing a linear response over the range of 400 to 900 μmol L⁻¹ (Figure 4 B–C), resulting in a correlation coefficient ($R^2 = 0.98$), which is adequate for the proposed method. The limits of detection (LOD) and quantification (LOQ) were determined using IUPAC equation,²⁷ resulting in values of 60 μmol L⁻¹ and 200 μmol L⁻¹, respectively.

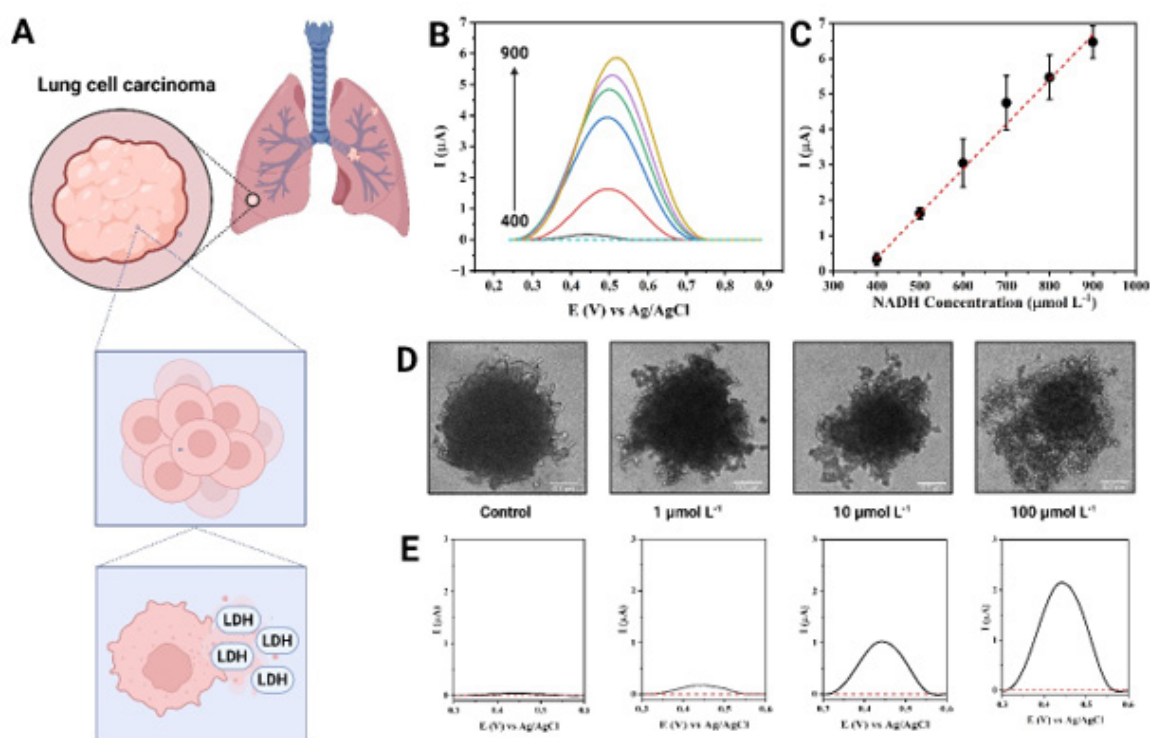


Figure 4. Electrochemical assessment of A549 tumor spheroid response to DOX treatment. (A) Schematic illustration of the 3D tumor model, emphasizing LDH release following cell death. (B) Baseline-corrected DPV profiles recorded at increasing NADH concentrations (500 to 1000 μmol L⁻¹), showing a concentration-dependent rise in current response. (C) Corresponding calibration plot of peak current versus NADH concentration, demonstrating a linear relationship within the evaluated range. (D) Representative optical images of tumoroids under untreated conditions and after exposure to different concentrations of DOX (1, 10, and 100 μmol L⁻¹), highlighting treatment-induced morphological alterations. (E) Baseline-corrected DPV profiles obtained from treated tumoroids, exhibiting a progressive increase in current signal with increasing DOX concentration, expressed as mean (solid line). All experiments were conducted in triplicate ($n = 3$). (Figure 4 was created with the assistance of BioRender.)

The lung tumoroid models displayed a well-defined spherical architecture, consisting of densely aggregated cells that remained suspended in the culture medium. This three-dimensional model was exposed to DOX at concentrations of 1, 10, and 100 $\mu\text{mol L}^{-1}$ for 48 h. Optical microscopy analysis revealed a concentration-dependent disruption of tumor cell composition and spheroid structure, with increasing degrees of fragmentation observed at higher DOX levels, indicating the cytotoxic effect of the drug (Figure 4D). Following DOX treatment, the collected supernatant was supplemented with L-lactate and NAD^+ and subsequently analyzed by DPV (Figure 4E). The electrochemical profiles showed a progressive increase in current intensity proportional to the level of cell death, which was effectively detected using the electrochemical platform.

Although basal intracellular NADH is present in viable cells, its contribution to the electrochemical signal is expected to be minimal because measurements were performed in the culture supernatant. Since all responses were compared with untreated controls collected under identical conditions, the measured signal mainly reflects the increase in extracellular NADH associated with cell damage rather than basal intracellular NADH levels.

This electrochemical approach provides a direct and sensitive method for evaluating chemotherapeutic efficacy in 3D tumor models. Furthermore, it provides a physiologically relevant platform for drug evaluation, supporting the development of more effective therapeutic strategies and contributing to personalized treatment. The proposed platform demonstrated promising performance; however, validation using additional cell models and therapeutic agents, together with improvements in electrode fabrication, could enhance its reproducibility and broaden its applicability. Therefore, appropriate fabrication and quality control procedures are important to ensure consistent analytical performance.

CONCLUSIONS

A portable and cost-effective electrochemical platform based on a lab-made graphite-pine rosin composite stencil electrode was successfully developed in this study for the indirect assessment of cell death in 3D lung cancer models. The fabrication process yielded reproducible, stable electrodes with satisfactory electrochemical performance after acid pretreatment. The use of tumoroid-based assays enabled a more physiologically relevant assessment of drug response compared to conventional 2D systems. The proposed method relies on electrochemical detection of NADH, a product of LDH-catalyzed reactions, enabling quantitative monitoring of cell membrane disruption and biomarker release. The platform demonstrated suitable analytical performance, including linear response, acceptable detection limits, and consistent reproducibility. Application of doxorubicin to doxorubicin-treated A549 tumoroids showed a clear correlation between drug concentration and the electrochemical signal, confirming its ability to monitor cytotoxic effects. The integration of low-cost electrode fabrication, 3D-printed device architecture, and biologically relevant models represents a promising strategy for developing accessible bioanalytical tools. This approach may advance drug screening methodologies and support future applications in personalized and translational cancer research.

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Conflicts of interest

The authors declare no conflict of interest.

Declaration on the use of artificial intelligence

The authors declare that ChatGPT and Grammarly were used exclusively for grammar and language correction during the preparation of this article. BioRender was used to assist in creating Figure 4. The authors take full responsibility for the accuracy, integrity, and originality of the article content.

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SUPPLEMENTARY MATERIAL

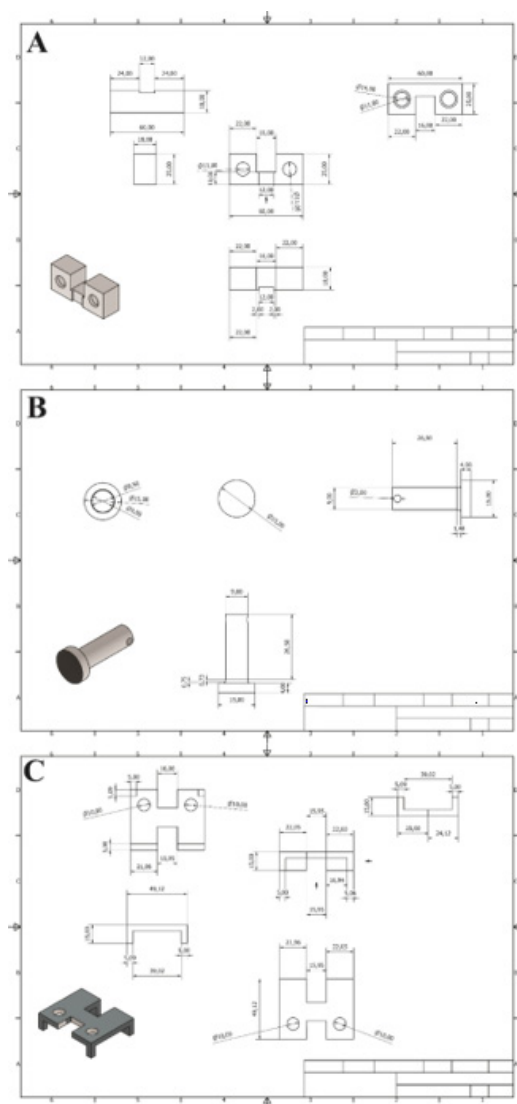


Figure S1. Representative schematic of the components of the 3D-printed support (A) base for fitting the USB cable (B) base for fitting the two springs (C) base for integrating the set of springs and USB cable.

Table S1. Composition of GPC-SE vs. reversibility of the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ electrochemical response

Composition (Graphite:Rosin) / %	$\Delta E_p (E_{pa} - E_{pc}) / V$	$I_{pa} / I_{pc} $
40:60	0.492	1.186
45:55	0.242	1.085
50:50	0.232	1.076
55:45	0.155	1.049
60:40	0.195	1.011

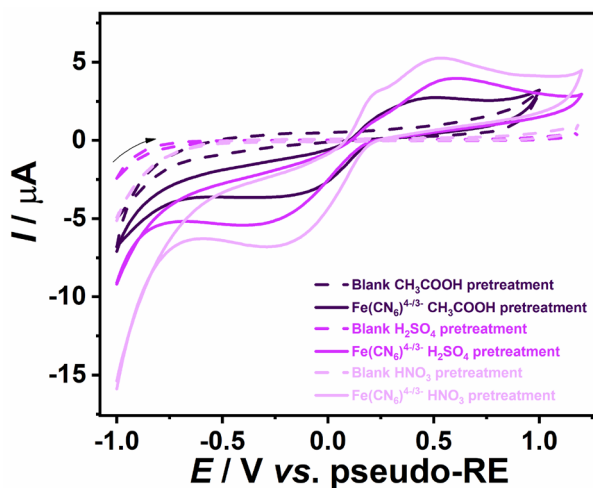


Figure S2. Cyclic voltammograms for 0.1 mol L⁻¹ KCl solution in absence (blank: dashed lines) and the presence of 1 mmol L⁻¹ [Fe(CN)₆]^{4-/3-} (solid lines) on GPC-SE. All potential scans started at -1.0 V, with a scan rate of 50 mV s⁻¹.

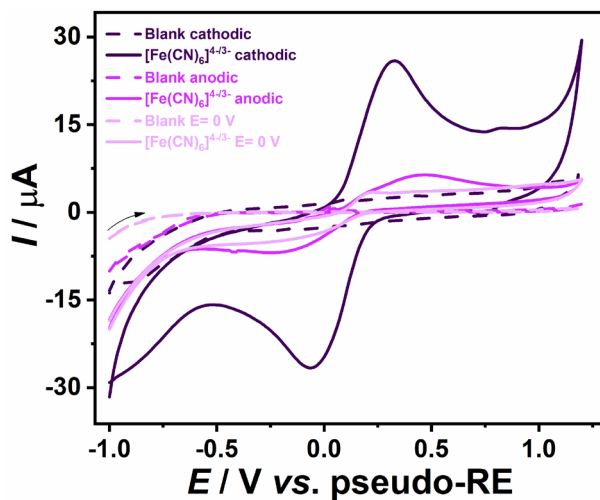


Figure S3. Cyclic voltammograms for 0.1 mol L⁻¹ KCl solution in absence (blank: dashed lines) and the presence of 1 mmol L⁻¹ [Fe(CN)₆]^{4-/3-} (solid lines) on GPC-SE. All potential scans started at -1.0 V, with a scan rate of 50 mV s⁻¹.

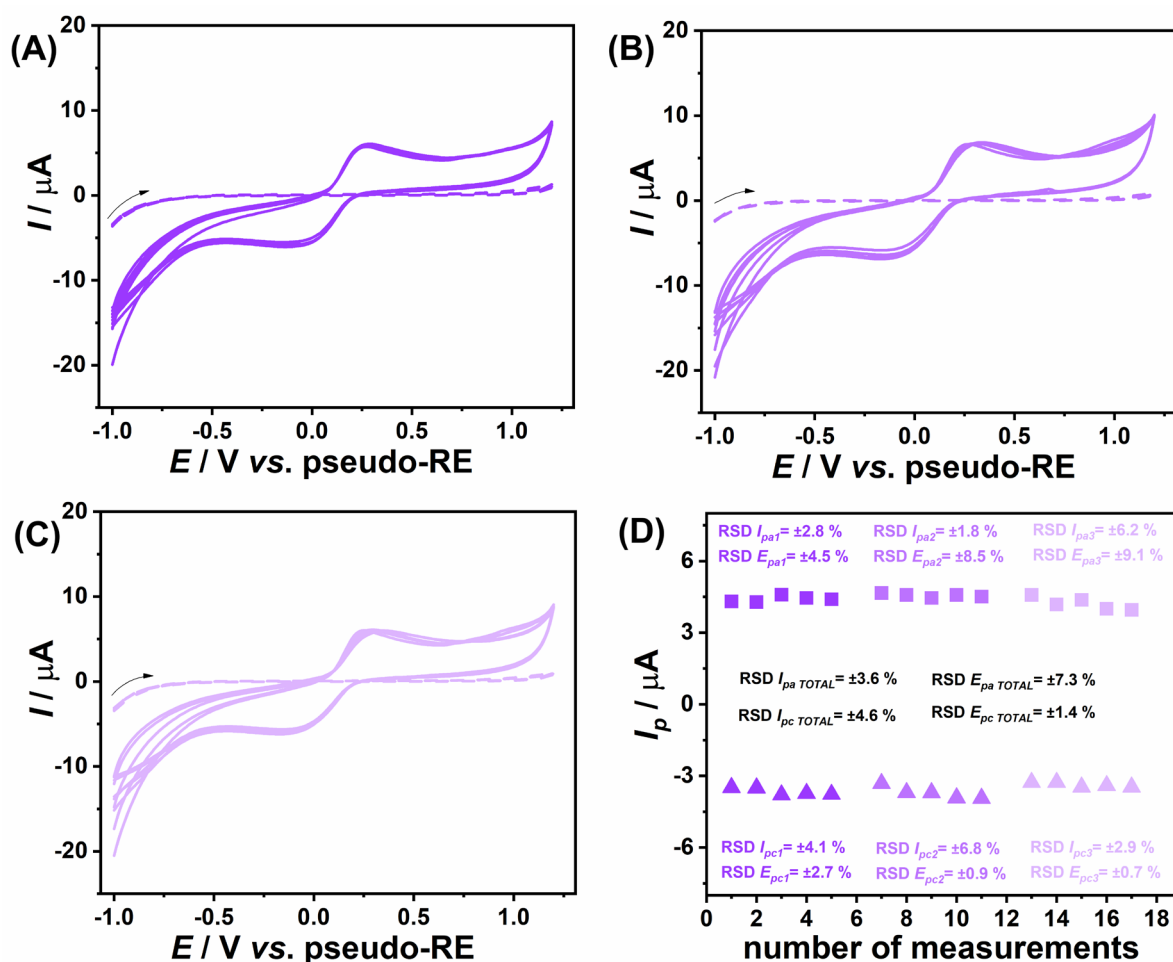


Figure S4. Cyclic voltammograms for 0.1 mol L⁻¹ KCl solution in absence (blank: dashed lines) and the presence of 1 mmol L⁻¹ [Fe(CN)₆]^{4-/3-} (solid lines) on GPC-SEs (A – C) after acid pretreatment. All potential scans started at -1.0 V, with a scan rate of 50 mV s⁻¹. (D) Plot of I_p vs number of measurements ($n = 5$) performed on three different GPC-SEs from same batch.

Table S2. RSD values for E_p and I_p of 1 mmol L⁻¹ [Fe(CN)₆]^{4-/3-} in 0.1 mol L⁻¹ KCl solution

	Electrode	RSD I_{pa} / %	RSD E_{pa} / %	RSD I_{pc} / %	RSD E_{pc} / %
Same batch	1	2.8	4.5	4.1	2.7
	2	1.8	8.5	6.8	0.9
	3	6.2	9.1	2.9	0.7
	Total	3.6	7.3	4.6	1.4
Different batches	1	1.8	8.5	5.6	8.7
	2	3.1	10.6	10.5	3.9
	3	4.2	6.6	2.6	10.8
	Total	3.1	8.5	6.2	7.8

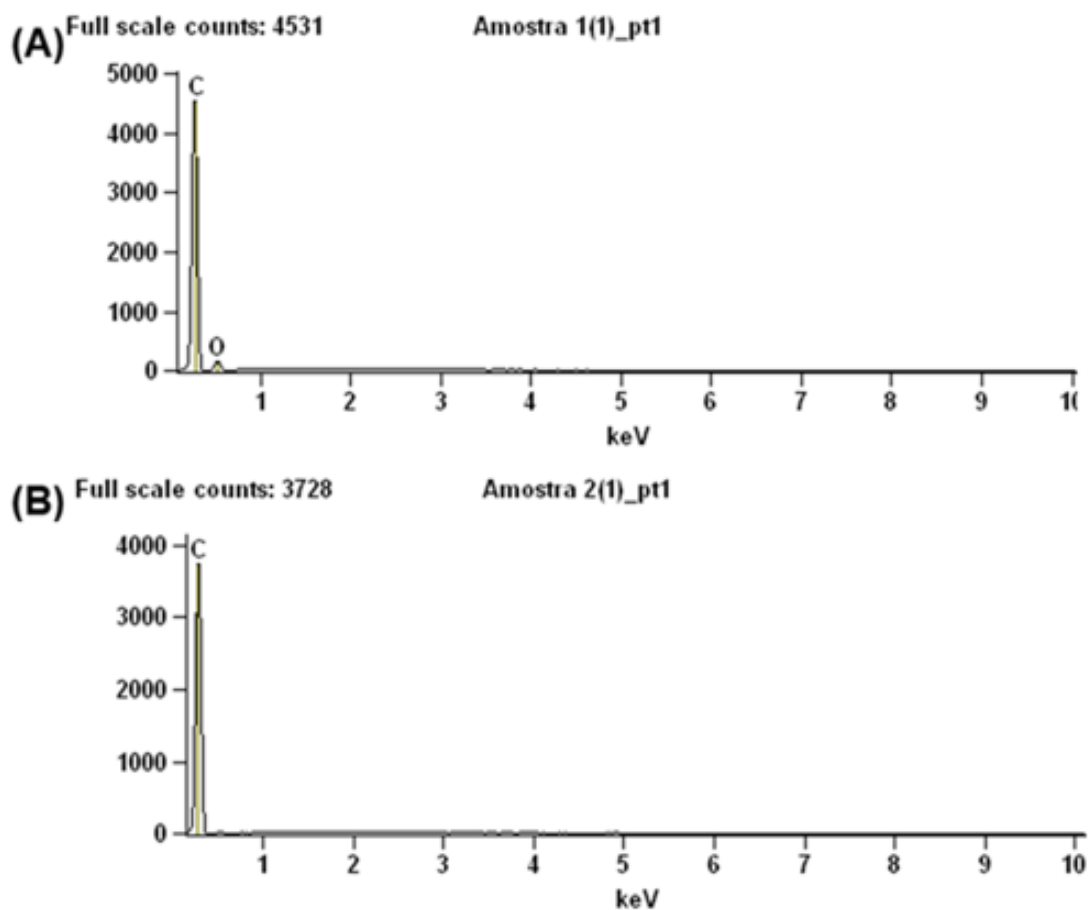


Figure S5. EDS spectra of GPC-SE electrodes before (A) and after (B) acid pretreatment, showing changes in the surface elemental composition.

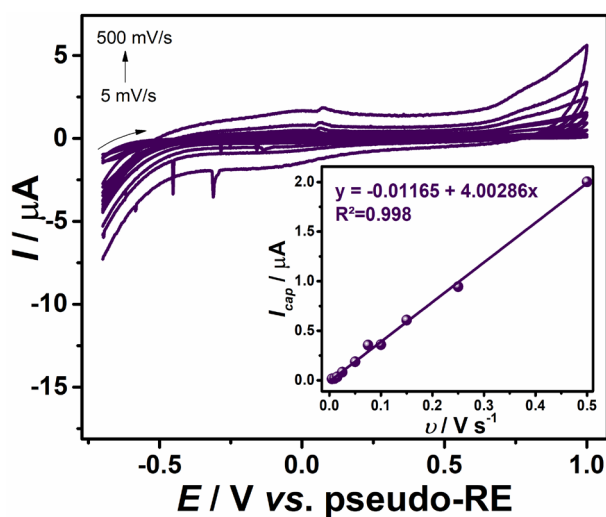


Figure S6. Cyclic voltammograms recorded in blank 0.1 mol L⁻¹ KCl solution at different scan rates (5 to 500 mV s⁻¹), used for double-layer capacitance measurements and estimation of the electroactive surface area of GPC-SE. Inset: the linear regression for capacitance current (I_{cap}) vs scan rate (ν).

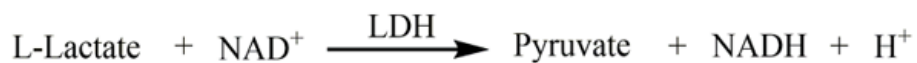


Figure S7. Biochemical reaction of the LDH in the presence of NAD⁺ and L-lactate, generating NADH for electrochemical detection.

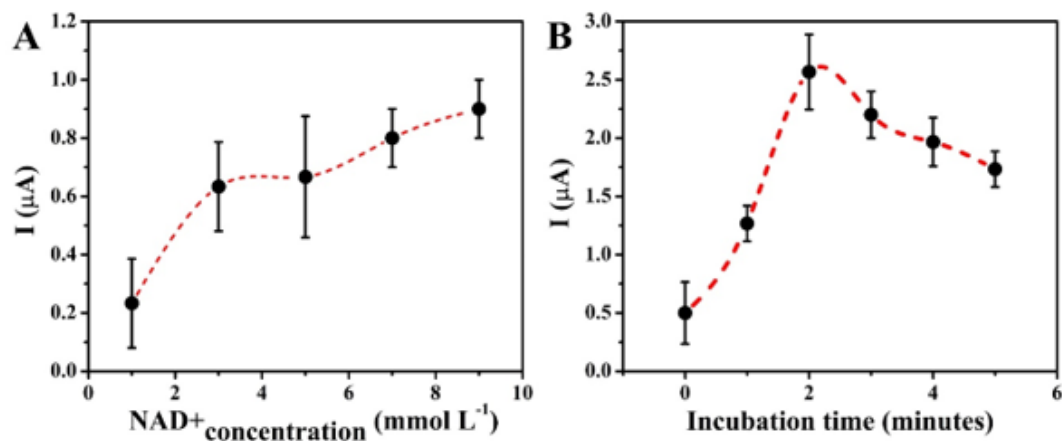


Figure S8. Optimization of electrochemical conditions for LDH detection in 10 mmol L⁻¹ PBS (pH 7.4), using 500 U mL⁻¹ LDH and 3 mmol L⁻¹ L-lactate. (A) Effect of NAD⁺ concentration on the electrochemical response. (B) Influence of incubation time on signal generation.