

A Suspended Graphene Field-Effect Transistor for Ultra-Sensitive and Label-Free Detection of Cancer Biomarker miR-21

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Abstract

The sensitive detection of microRNA-21 (miR-21), a key biomarker for various cancers, is crucial for early diagnosis, yet conventional methods often face limitations in sensitivity and operational complexity. Here, we report a label-free biosensor based on a suspended graphene field-effect transistor (GFET) for the direct electrical detection of miR-21. The suspended architecture isolates the graphene channel from substrate-induced interference, resulting in enhanced carrier mobility and reduced electrical noise. After surface functionalization with a specific probe, the GFET demonstrated a clear concentration-dependent response to target miR-21. The binding events were transduced into a monotonic increase in relative resistance ($\Delta R/R_0$) and a positive shift of the Dirac point (V_{Dirac}), achieving a detection limit in the femtomolar (fM) range. These results establish the suspended GFET as a highly sensitive and robust platform for quantifying nucleic acid biomarkers, holding significant potential for biomedical research and point-of-care diagnostics.

Keywords: suspended graphene; field-effect transistor (FET) biosensor; micro RNA; label-free detection; cancer biomarker

1. Introduction

The development of rapid, sensitive, and label-free methods for quantifying the oncogenic biomarker microRNA-21 (miR-21) is critical for early cancer diagnostics and therapeutic monitoring [1–3]. Current standards, such as quantitative Polymerase Chain Reaction (qPCR) and Enzyme-Linked Immunosorbent Assay (ELISA), rely on multi-step sample preparation and enzymatic amplification, processes that present challenges for point-of-care analysis [4–6]. This motivates the investigation of alternative platforms that combine high sensitivity with operational simplicity for real-time detection. This pursuit has led to the exploration of novel materials, such as perovskite derivatives for photodetection, and innovative device architectures, like bipolar junction transistors for protein sensing [7,8].

The performance of conventional substrate-supported graphene field-effect transistors (GFETs), a platform for direct electrical biosensing [9,10], is often limited by the graphene-substrate interface. This interface can introduce charge impurities and scattering sites that decrease carrier mobility and increase electrical noise, constraining detection sensitivity for low-abundance targets like miRNAs [11,12].



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A suspended graphene architecture offers a strategy to mitigate this limitation by physically decoupling the graphene channel from the substrate. This configuration minimizes substrate-induced scattering and preserves the material's intrinsic electronic properties, offering the potential for lower noise and enhanced signal transduction [13,14]. The fabrication of stable suspended structures for liquid-phase sensing remains technically demanding [15,16]. Their successful integration, however, is expected to enhance biosensor performance.

In this work, we report a suspended GFET platform for the sensitive, label-free detection of miR-21, as shown in Figure 1. GFET devices were fabricated by transferring a graphene membrane over pre-etched micro-cavities. A distinctive feature of our design is the exposure of both surfaces of the graphene channel to the electrolyte, which enables dual-sided functionalization. The surface is modified with Peptide Morpholino Oligomer (PMO) probes for specific capture of miR-21. This architecture enhances signal modulation and lowers the limit of detection, offering a technological path for advancing early cancer diagnostics.

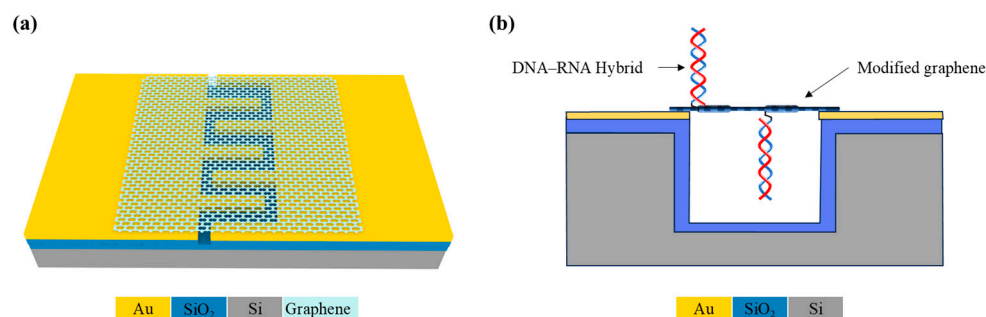


Figure 1. (a) Schematic of the suspended GFET device. The graphene channel is positioned over a micro-cavity to reduce substrate-induced electronic noise. (b) Dual-side functionalization for miR-21 detection. The PMO probes on the top surface capture the target. The accessible bottom surface allows for a higher effective probe density, enhancing the electrical signal from hybridization.

2. Materials and Methods

2.1. Fabrication of Suspended GFET

Monolayer graphene was synthesized via chemical vapor deposition (CVD) on copper foils. The foils were pre-cleaned by sequential immersion in 0.1 M ammonium persulfate, deionized (DI) water, and ethanol, followed by nitrogen drying. To minimize contamination, the foils were folded into enclosed pockets before being loaded into a quartz tube furnace. Growth was initiated at 1050 °C by introducing CH₄ (30 sccm) and H₂ (50 sccm) into an Ar flow (200 sccm). After 30–40 min, the furnace was rapidly cooled under the same gas mixture. Concurrently, microcavities were etched into silicon wafers using standard photolithography and inductively coupled plasma (ICP) etching with a C₄F₈/SF₆ gas mixture. Following resist stripping, a 50 nm gold film was deposited via electron-beam evaporation or sputtering (2×10^{-4} Pa) and patterned into source-drain electrodes using a lift-off process in *N*-methyl-2-pyrrolidone (NMP). Finally, the CVD-grown graphene was transferred onto the patterned substrates using a PMMA-assisted wet-transfer method, positioning the graphene layer to bridge the microcavities. The PMMA support layer was removed with acetone and ethanol. The completed devices were annealed at 150–180 °C in an Ar/H₂ environment to remove transfer residues.

2.2. Surface Functionalization and miR-21 Detection

The overall procedure for surface functionalization and miR-21 detection is schematically illustrated in Figure 2. The graphene surface was first functionalized by incubating

devices in a 7.8 mM solution of 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) in anhydrous dimethylformamide (DMF) for 1 h at room temperature. After rinsing with DMF, isopropanol (IPA), and ultrapure water, the PBASE-modified devices were immediately incubated with a 1–2 μM solution of amine-terminated DNA probes (sequence detailed in Table 1) in PBS (pH 7.8) for 40 min in the dark to facilitate covalent attachment [17]. The probe sequence, complementary to miR-21, included a 5'-terminal FAM label for fluorescence verification. Unbound probes were removed by rinsing with PBS and ultrapure water. Synthetic miR-21 stock solutions (10 μM in RNase-free water) were stored at $-80\text{ }^{\circ}\text{C}$. For hybridization experiments, working solutions (10 fM to 100 nM) were freshly prepared in a buffer (1 $\times$ SSC or 1 $\times$ PBS, pH 7.4) containing 0.01–0.05% Tween-20 and 1 U/mL RNase inhibitor. Detection was performed by incubating the functionalized GFETs with the miR-21 solutions, followed by a buffer rinse before electrical measurement.

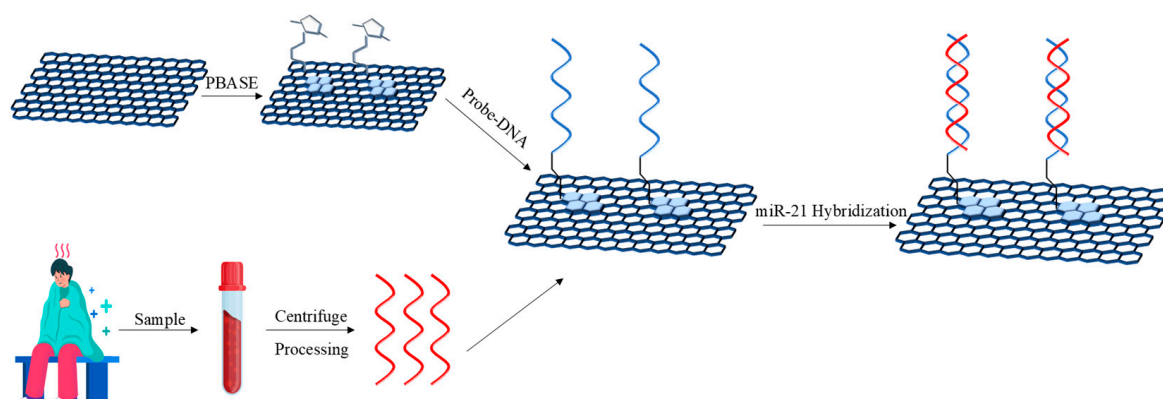


Figure 2. Schematic of the surface functionalization and detection process. (i) PBASE is non-covalently attached to the graphene via π - π stacking. (ii) Amine-terminated DNA probes are covalently immobilized onto the PBASE layer. (iii) Target miR-21 from a sample hybridizes with the complementary probe, inducing an electrical signal change in the underlying GFET.

Table 1. The sequences of oligonucleotides used in the experiments.

Oligonucleotides	Sequence (5'-3')
DNA probe	$\text{NH}_2\text{-C}_6\text{-TTTTTCAACATCAGTCTGATAAGCTA}$
FAM-DNA probe	$\text{NH}_2\text{-C}_6\text{-TTTTTCAACATCAGTCTGATAAGCTA-FAM}$
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-21-1MM	UAGCUUAUCAGACUGAUGUAGA
miR-21-2MM	UAGCUUAUCAGUCUGAUGUAGA

2.3. Characterization and Electrical Measurements

Device morphology and graphene integrity were examined using field-emission scanning electron microscopy (FE-SEM). The successful immobilization and spatial distribution of the FAM-labeled probes were confirmed with laser confocal microscopy. All electrical measurements were performed using a Keithley 4200A-SCS semiconductor parameter analyzer. Transfer characteristics (I_{DS} vs. V_{G}) were recorded by sweeping V_{G} from -2 V to $+2\text{ V}$ (0.05 V step) at a fixed V_{DS} of 50 mV. These sweeps were conducted after each fabrication and functionalization step to monitor the Dirac point and device stability. For real-time sensing, the device resistance was monitored at a fixed $V_{\text{DS}} = 100\text{ mV}$ and $V_{\text{G}} = 50\text{ mV}$. miR-21 solutions of increasing concentrations (5 fM, 50 fM, 500 fM, 5 pM, and 50 pM) were sequentially introduced to the sensor surface. After each addition, the system was allowed to stabilize for 5 min while continuously recording the current. This duration was empirically determined to be sufficient for the signal to reach a stable plateau, which

we defined as a signal drift of less than 0.5% over a 60-s interval. The sensor response was quantified as the relative resistance change ($\Delta R/R_0$), calculated from the real-time current data.

3. Results and Discussion

3.1. Device Fabrication and Characterization

Microscopic analyses confirmed the structural integrity of the fabricated device. The ICP-etched microcavities exhibited uniform depth and well-defined edges (Figure 3a,b), over which gold electrodes were precisely aligned (Figure 3c). Raman spectroscopy was used to evaluate the quality of the transferred graphene channel (Figure 3d). Compared to the substrate-supported area, the suspended graphene exhibited downshifts in the G ($\sim 4 \text{ cm}^{-1}$) and 2D ($\sim 8 \text{ cm}^{-1}$) bands, a higher I_{2D}/I_G ratio, and narrower peak widths. These spectral features indicate strain relaxation and reduced substrate-induced doping, confirming the high crystalline quality of the suspended channel [18]. Successful DNA probe immobilization was verified using fluorescence microscopy. The suspended graphene channel displayed significantly higher fluorescence intensity from FAM-labeled probes than the non-suspended regions (Figure 3e). This result suggests enhanced molecular accessibility to the graphene surface. Figure 3f presents the fully packaged device, configured with an electrolyte reservoir for liquid-gate measurements. These characterizations validate the successful fabrication of a high-quality suspended GFET, establishing a robust platform for high-sensitivity biosensing [19,20].

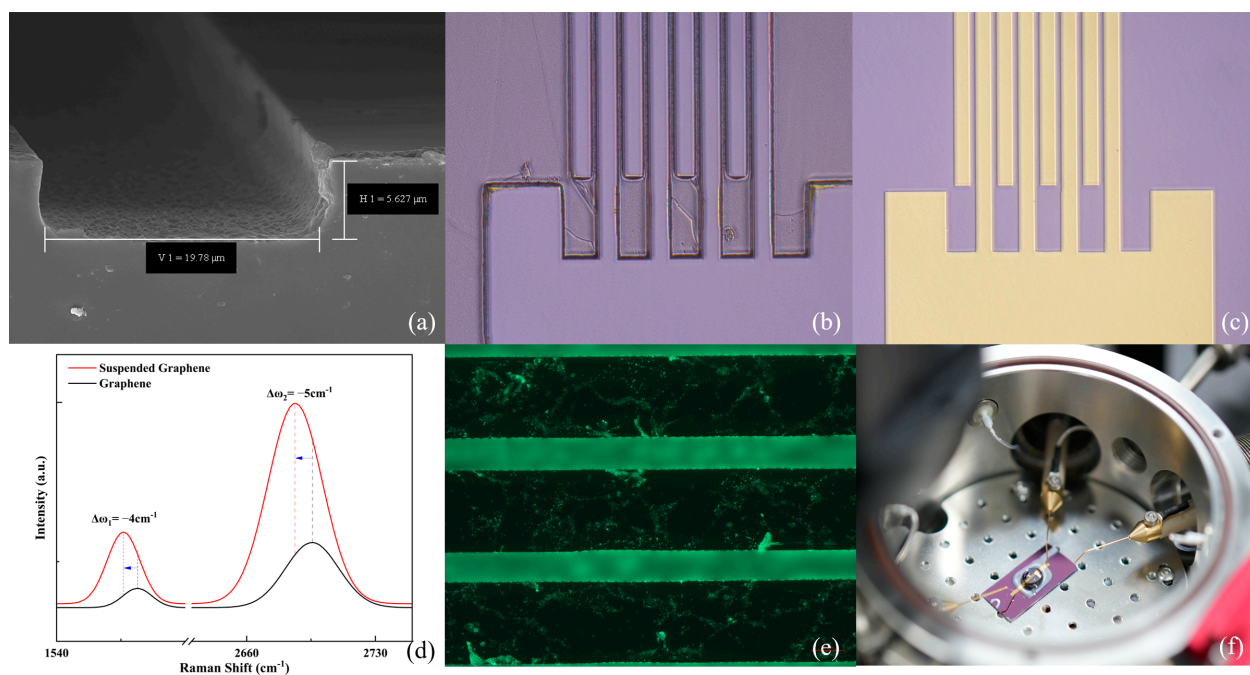


Figure 3. Fabrication and characterization of the suspended GFET device. (a) SEM image of an ICP-etched microcavity. (b) Optical microscopy image of the etched microcavities. (c) Optical image of gold electrodes aligned over the microcavities. (d) Raman spectra of graphene on suspended and non-suspended regions, showing the characteristic G and 2D bands. (e) Fluorescence image of the channel functionalized with FAM-labeled DNA probes, comparing suspended and substrate-supported regions. (f) Photograph of a packaged device during electrical measurement.

3.2. Sensing Mechanism and Performance Characterization

The device's response to surface functionalization and target binding was investigated using both spectroscopic and electrical methods to elucidate the sensing mechanism.

Raman spectroscopy was used to track the stepwise functionalization of suspended and supported graphene devices (Figure 4a,b). In their pristine state, the suspended graphene showed a downshifted G peak (at $\sim 1579\text{ cm}^{-1}$) and a higher I_{2D}/I_G ratio compared to the supported device ($\sim 1585\text{ cm}^{-1}$), indicating reduced initial strain and doping. Subsequent functionalization with PBASE and probe DNA induced a G peak upshift in both devices, consistent with p-type doping. Notably, the total upshift of the G peak for the suspended device was approximately twice as large ($\sim 16\text{ cm}^{-1}$) as that for the supported one ($\sim 8\text{ cm}^{-1}$). Throughout the process, the D peak intensity ($\sim 1350\text{ cm}^{-1}$) remained low, confirming the modification was non-destructive. This significantly larger spectral shift, resulting from the absence of substrate screening, directly demonstrates the superior sensitivity of suspended graphene to surface charge modulation.

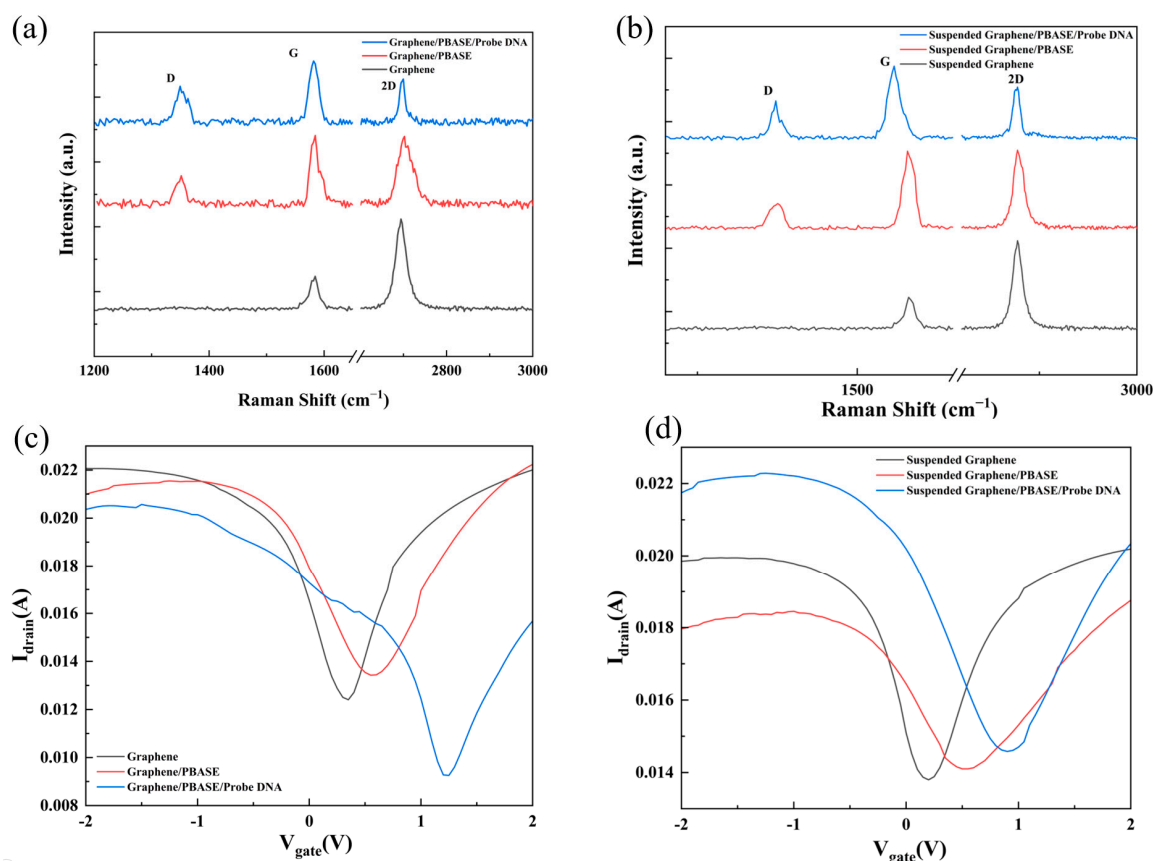


Figure 4. Sensing mechanism and performance characterization. (a,b) Raman spectra of supported and suspended graphene, respectively, at sequential functionalization steps (pristine, PBASE-modified, probe-DNA-functionalized, and RNA-hybridized). (c) Transfer curves of the supported device during functionalization. (d) Comparison of Dirac point shifts (ΔV_{Dirac}) for both devices throughout the functionalization process.

The electrical response to functionalization was tracked by measuring the transfer curves and the corresponding Dirac point voltage (V_{Dirac}) [21]. The pristine suspended device showed a V_{Dirac} near 0 V, whereas the supported device exhibited a slight initial p-doping (Figure 4c). Each subsequent functionalization step induced a stepwise positive shift in V_{Dirac} for both devices, consistent with the expected charge transfer from the attached molecules. Crucially, the magnitude of the Dirac point shift (ΔV_{Dirac}) was consistently larger for the suspended device throughout the process (Figure 4d), confirming that its architecture more effectively transduces surface charge variations into electrical signals [22,23].

3.3. Quantitative Performance and Specificity

To provide quantitative analyses, we characterized the dose–response for both device types using five independent devices (Figure 5a). The target miR-21 concentration ranged from 5 fM to 50 pM. The sensor response, quantified by the relative resistance change ($\Delta R/R_0 = (R - R_0)/R_0$), was measured to construct calibration curves (Figure 5b), which showed high linearity ($R^2 > 0.996$). Linear calibration curves yielded $y = 0.0072 \lg(x) + 0.0015$ for suspended devices and $y = 0.0037 \lg(x) + 0.0013$ for conventional devices. The sensitivity (slope) of the suspended GFET was nearly double that of the conventional device. Blank measurements in PBS yielded $\sigma_{\text{blank}} = 5.2 \times 10^{-6}$ (suspended) and 9.3×10^{-5} (conventional). Furthermore, based on the $3\sigma/\text{slope}$ method, the limit of detection (LOD) was determined to be 7 fM for the suspended device, a tenfold improvement over the 76 fM LOD of the conventional one. This enhanced performance is attributed to the suspended architecture providing both higher transduction efficiency and lower background noise. The relative standard deviation (RSD) at each concentration point was calculated to be no more than 3%, confirming the good device-to-device reproducibility achieved through our standardized fabrication and functionalization protocol.

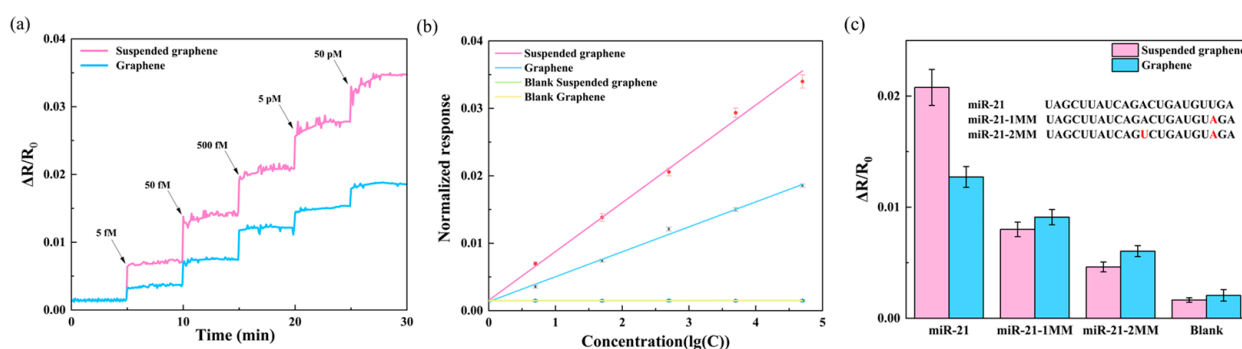


Figure 5. Quantitative Performance and Specificity. (a) Relative resistance change ($\Delta R/R_0 = (R - R_0)/R_0$, where R_0 is the baseline resistance in buffer) of graphene-based devices measured at different target analyte concentrations ranging from 5 fM to 50 pM. (b) Linear fitting of the sensor responses as a function of analyte concentration for suspended graphene devices, supported graphene devices, and their corresponding blank controls. Each data point is the average of measurements from five ($N = 5$) independent devices, and the error bars represent the standard deviation. (c) Specificity test of suspended and supported graphene devices. The relative resistance change ($\Delta R/R_0$) was measured for the target analyte and several non-target analytes, demonstrating selective detection behavior.

To evaluate the sequence specificity of the biosensor, we tested its response against the target miR-21, a single-base mismatch sequence (miR-21-1MM), and a double-base mismatch sequence (miR-21-2MM). As shown in Figure 5c, the electrical response was highest for the complementary miR-21 and decreased progressively for miR-21-1MM and miR-21-2MM, respectively. This suggests the sensor's ability to discriminate single-nucleotide variations. Moreover, the suspended graphene architecture enhances this sequence discrimination. This effect could be attributed to improved electrostatic coupling for specific hybridization, combined with the suppression of non-specific binding in the absence of a substrate. The suspended structure thus improves specificity by increasing the target signal while minimizing the response from mismatched sequences.

These spectroscopic and electrical characterizations consistently demonstrate the enhanced sensitivity of the suspended GFET architecture. By decoupling the graphene channel from the substrate, its response to surface binding events—from linker immobilization to target hybridization—is significantly amplified. This establishes the suspended platform as a more effective transducer for high-sensitivity, label-free biosensing [24,25].

4. Conclusions

In this study, we developed a suspended graphene field-effect transistor (GFET) for the label-free electrical detection of miR-21. The suspended architecture is designed to minimize substrate-induced scattering, which results in improved carrier mobility and lower electrical noise when compared to supported devices. Raman spectroscopy analysis confirmed that the graphene maintained its structural integrity following surface functionalization. The device's performance as a biosensor was systematically evaluated. Concentration-dependent measurements for miR-21 showed a monotonic increase in relative resistance ($\Delta R/R_0$) and a corresponding positive shift in the Dirac point (V_{Dirac}) as target concentration increased, demonstrating a detection limit in the femtomolar range. These results establish that the suspended GFET platform effectively transduces molecular binding events into measurable electrical signals. This work demonstrates that a suspended GFET, combined with a stable surface functionalization strategy, can serve as a sensitive platform for nucleic acid detection. The ability to quantify low-abundance miRNA biomarkers suggests its potential for applications in biomedical research and diagnostics. Furthermore, while the fabrication process utilizes scalable microfabrication techniques, achieving high-yield, large-scale manufacturing will require optimization of the large-area graphene transfer process. Addressing these aspects will be essential for translating this promising technology into practical point-of-care diagnostic tools.

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