

Flexible Neural Interfaces based on Graphene–PAN Composite for the Treatment of Constipation via Sacral Nerve Stimulation

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ABSTRACT

Background: Conventional Sacral Nerve Stimulation (SNS) devices are widely used to treat constipation but are often limited by their lack of mechanical flexibility, which can reduce long-term therapeutic efficacy. Developing advanced electrode materials may improve stimulation performance and patient outcomes.

Objective: This experimental study aimed to introduce a proof-of-concept flexible cuff electrode composed of a graphene–polyacrylonitrile (PAN) composite and evaluate its electrochemical properties, their mechanical characteristics, and short-term functional performance in a rabbit SNS model.

Material and Methods: In this experimental study, graphene–PAN composite electrodes were fabricated and characterized for electrochemical properties, including charge injection capacity and impedance, as well as mechanical properties, such as flexibility and tensile strength. *In vivo* testing was conducted on rabbits to evaluate their performance in SNS therapy and their effectiveness in relieving constipation.

Results: The graphene–PAN composite electrodes demonstrated a high charge injection capacity ($1062.58 \pm 73.36 \mu\text{C}/\text{cm}^2$), low impedance ($31.27 \pm 2.76 \Omega$), and favorable mechanical flexibility (modulus: $1.31 \pm 0.45 \text{ MPa}$; strain at break: $27 \pm 2\%$). *In vivo* stimulation progressively improved fecal output over four days, approaching pre-injury levels.

Conclusion: Graphene-based electrodes exhibited suitable electrochemical and mechanical performance, enabling effective neural stimulation and enhanced therapeutic outcomes in constipation management. As an initial proof-of-concept, these flexible graphene-based materials represent promising candidates for future neural stimulation devices.

Keywords

Electrodes; Electric Stimulation Therapy; Constipation

Introduction

In modern medicine, the integration of implantable electronic devices with the complexities of the nervous system has opened a new era of therapeutic possibilities [1]. Among these innovations, Sacral Nerve Stimulation (SNS) has emerged as a promising approach for treating a range of disorders, including chronic constipation, which can

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significantly affect patients' quality of life [2]. By delivering controlled electrical impulses to the sacral spinal nerves (S3 or S4) via implanted electrodes, SNS modulates neural activity, providing relief in cases where conventional treatments are insufficient [3].

Several commercially available SNS systems, such as the InterStim system (Medtronic), Axonics system (Axonics), and BetterStim system, have successfully translated this technology from research to clinical practice, offering clinicians effective tools for managing pelvic floor disorders and related conditions [4-7]. However, despite their clinical efficacy, these devices have limitations.

A major challenge in current SNS therapy is the formation of fibrosis around implanted electrodes. Fibrosis, characterized by scar tissue development, can insulate electrodes from surrounding nervous tissue, reducing stimulation efficiency [8]. This is especially critical for SNS, where the dynamic environment of the sacral nerves requires electrodes with exceptional flexibility and adaptability.

Traditional electrode materials-including gold, titanium, tungsten, platinum, and their alloys-have long been used in neural stimulation applications [9-14]. While effective in many respects, these materials often fall short in addressing the mechanical challenges of neural interfaces. The mismatch between the stiffness of conventional electrodes and the delicate tissues of the nervous system can provoke inflammatory responses, potentially compromising device performance and patient outcomes [15-18].

To overcome these limitations, researchers have explored novel materials for neural interfaces. Flexible and stretchable electronic devices offer a promising strategy to reduce inflammatory responses and improve long-term implant reliability [19-22]. Among these materials, graphene has attracted significant interest due to its high conductivity, biocompatibility, and mechanical flexibility [23-26].

In this study, we investigated a proof-

of-concept graphene-polyacrylonitrile (PAN) composite cuff electrode for SNS applications. This composite was selected for its mechanical softness, promising Charge-Injection Capacity (CIC), and electrochemical stability. While carbon-based electrodes have been explored in various neural applications, graphene-based interfaces remain underexplored in SNS models, particularly for bowel dysfunction following pudendal nerve injury. Here, we describe the fabrication of graphene-PAN electrodes, characterize their electrochemical and mechanical properties, and evaluate their ability to restore bowel function in a rabbit injury model. This work represents an initial step toward assessing flexible graphene-based composites for future neural stimulation technologies.

Material and Methods

Electrode design

This experimental study evaluated the InterStim system electrode (Medtronic, Minneapolis, MN, USA), commonly used for SNS in treating conditions such as overactive bladder and fecal incontinence. Figure 1 compares the commercial InterStim II sacral neuromodulation stimulator, which features four metal contacts, with a schematic of a proposed electrode incorporating graphene-PAN composite contacts embedded in a protective medical-grade silicone elastomer (German-manufactured, locally sourced from the Tehran market).

As shown in Figure 1A, the SNS electrode consists of four cylindrical contacts positioned adjacent to a sacral nerve root, most commonly the third sacral nerve (S3). Each contact measures 3 mm in length and is evenly spaced along a 3-mm lead. The electrode head, located near the sacral nerve, is equipped with tines to prevent displacement during tissue implantation. The metal contacts, typically platinum or platinum-iridium, are located at the electrode tip and connect to a signal generator. The tail end, which interfaces with the signal

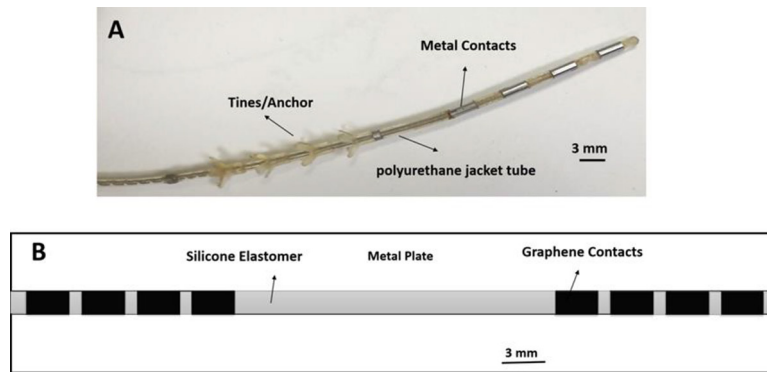


Figure 1: Comparison of SNS (Sacral Nerve Stimulation) electrodes. **(A)** Commercial InterStim II electrode with four platinum-iridium contacts. **(B)** Proposed flexible graphene–PAN (polyacrylonitrile) composite-contact electrode encased in medical-grade silicone elastomer tubing.

generator, does not include tines. The contacts are encased in a polyurethane tube, providing durability and protection. For SNS testing, a single metal contact stimulator electrode was also used.

In this study, the conventional metal contacts were replaced with graphene–PAN composite contacts (Figure 1B). The electrode geometry and lead configuration were as follows: outer diameter 1.27 mm, inner diameter 1 mm, total electrode length 27 mm, contact length 3 mm, and spacing between contacts 3 mm.

The use of a graphene–PAN composite was motivated by the need for materials with mechanical properties closer to soft neural tissue. Conventional metal electrodes, while effective, exhibit a significant mechanical mismatch with neural tissue, which may provoke inflammatory responses and reduce long-term efficacy. Graphene-based composites offer a combination of softness, flexibility, and favorable electrochemical properties, making them promising candidates for neural interfaces. This study investigates whether a graphene–PAN composite can be fabricated into a functional cuff electrode suitable for preliminary SNS testing.

Fabrication

The fabrication of graphene–PAN composite electrodes involves several carefully controlled steps to ensure optimal performance and

reliability.

The first step is the preparation of the graphene–PAN composite solution. Graphene powder (few-layer, technical grade, Nanotech Innovations) and PAN powder (molecular weight 250,000 g/mol (Lianxu, China)) were used. PAN (5 wt%) was dissolved in dimethylformamide (DMF), stirred, and heated on a hot plate for one hour until a uniform solution was achieved. Graphene powder was then added to the solution at a 70:30 ratio of graphene to PAN, based on prior studies indicating that this ratio optimally forms graphitic crystallites [27]. The mixture was further stirred and heated at 70°C for an additional hour (Figure 2A).

Next, a test graphene–PAN composite stimulating electrode was prepared. Stripped wires with 3 mm exposed tips were wrapped with filter paper, immersed in the graphene–PAN solution, and dried in a temperature-controlled environment. Electrical connectivity between the two ends was verified using a digital multimeter (Figure 2B).

The third step involved forming a thin graphene–PAN composite sheet, which served as the contact points for the four stimulating electrodes. The prepared solution was slowly poured onto filter paper placed over a lid and left to settle for several hours. The sheet was then flipped and allowed to sit for additional hours to complete formation (Figure 2C).

Finally, the four-contact graphene–PAN

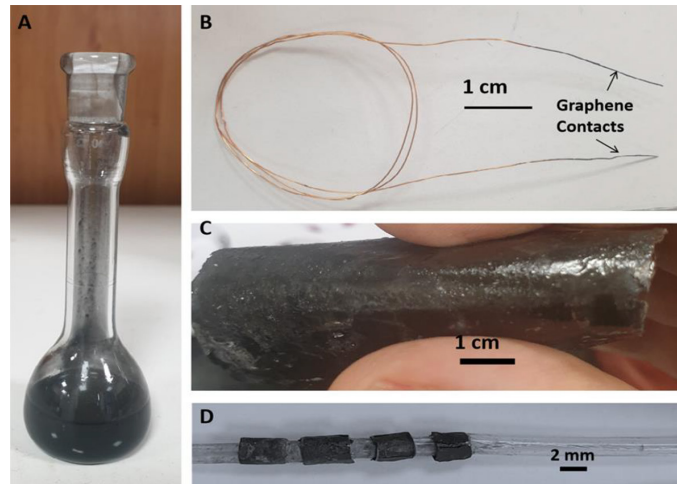


Figure 2: Fabrication and characterization of graphene-PAN (polyacrylonitrile) composite electrode. (A) Graphene-PAN composite solution preparation. (B) Preliminary test electrode with graphene-PAN tips on exposed wire sections. (C) Thin, flexible graphene-PAN composite sheet formed on filter paper. (D) Final four-contact cuff electrode with graphene-PAN composite contacts.

composite cuff electrode was fabricated. A mandrel matching the internal diameter of the contacts was used. The graphene-PAN composite sheet was wrapped around the mandrel, and the edges were heated to form cylindrical contacts. Wires were attached to each contact using silver epoxy, with each wire sequentially passing through all contacts to ensure proper electrical connection. The assembly was placed in a silicone mold, with metal plates shaping a hollow tube at designated locations. All epoxy and wire connections were fully encapsulated, and the wires were routed inside the tube to prevent direct contact with biological tissue. Graphene-PAN composite contacts were positioned at eight locations: four for neuronal contact and four for connection to the signal generator. Silicone elastomer was applied between the contacts at both wire ends. The mold was secured and heated at 120 °C for ten minutes, then cooled to room temperature before electrode removal. The final four-contact cuff electrode is shown in Figure 2D.

Surface Characterization

The morphology of the graphene-PAN

composite sheet was analyzed using Scanning Electron Microscopy (SEM) with a TESCAN-Vega system (HV 20 kV, SE mode). Raman spectra were recorded using a confocal Raman microscope (LabRAM HR, Japan) with a 532 nm excitation laser at room temperature.

Mechanical properties

The mechanical properties of the graphene-PAN composite sheet were evaluated by measuring tensile strength according to the British Standard International Organization for Standardization (BS ISO) 1924-3 standard, with a minimum of 10 successful tests performed to ensure statistical validity. Samples were carefully cut to a width of 15 mm and a length of 150 mm, providing a 100 mm test span as required by the standard. Sample thickness was measured following the ISO 534 standard and recorded as 0.15 mm.

Tensile testing was conducted using a SANTAM-50kN universal testing machine equipped with a 200 kg load cell. Specialized roller grips were employed to secure the samples, preventing slippage and minimizing damage during testing. The tensile tests provided data on the mechanical behavior of the

graphene–PAN composite sheets, including tensile strength, elasticity, and elongation at break.

Electrode characterization

To ensure that the electrode design could safely deliver the required stimulation currents without incurring damage, the electrochemical properties of the graphene–PAN composite electrodes were evaluated using Cyclic Voltammetry (CV), Electrochemical Impedance Spectroscopy (EIS), and CIC measurements. CIC is a critical parameter in neural stimulation, defined as the maximum charge that can be safely injected during a stimulation pulse without exceeding the water window, thereby maintaining electrochemical stability and preventing irreversible electrode reactions [28].

The EIS was performed to assess the electrical impedance of the electrodes across a range of frequencies. All electrochemical experiments employed a three-electrode setup consisting of a working electrode, a reference electrode, and a counter electrode. A potentiostat (OGF500) was used to control the potential of the working electrode relative to the reference electrode. The electrodes were immersed in an electrochemical cell containing platinum counter electrodes and Ag/AgCl reference electrodes. All measurements were conducted in 1× Phosphate-Buffered Saline (PBS) at room temperature without nitrogen gas bubbling. The graphene–PAN composite-stimulating contacts served as the working electrodes.

For EIS measurements, the frequency range was set from 0.1 Hz to 10 kHz, with a small AC voltage amplitude of 10 mV applied. CV scans were performed over a voltage range of -2 to 2 V to evaluate the graphene–PAN composite's electrochemical behavior, including redox reactions, capacitive properties, and any signs of electrolysis.

To assess *in vitro* stability, electrodes underwent 1000 CV cycles. Impedance at 1 kHz

was measured after every 100 cycles. CV cycling was performed within the water window range of -1 to 0 V to ensure safe and stable operation.

In Vivo Experiments

The *in vivo* performance of the graphene–PAN composite electrode for SNS was evaluated through a series of carefully designed experiments, approved by the Iran National Committee for Ethics in Biomedical Research, Shiraz, Iran.

Animal Model Selection: Five healthy male rabbits, each weighing approximately 2 kg, were selected for the study.

Behavioral Observation (Control): Rabbits were housed in standard cages at room temperature with *ad libitum* access to water and solid food. Over four days, fecal output was measured via 24-hour collection and weighing to establish baseline parameters under normal conditions.

Pudendal Nerve Injury: To model pudendal nerve injury, a surgical procedure was performed under anesthesia to ensure animal welfare. The injury was induced by repeated clamping of the pudendal nerve for 60 seconds per application, with 60-second intervals between repetitions, as illustrated in Figure 3A. Daily monitoring of fecal output over four days was conducted to assess the effects of the injury on bowel function.

Electrode Implantation: Prior to surgery, graphene–PAN composite electrodes were sterilized using Ethylene Oxide (ETO). Under anesthesia, the electrodes were carefully positioned near the sacral nerves to enable precise electrical stimulation. Once implanted, the electrodes were connected to a signal generator capable of delivering controlled electrical pulses, allowing adjustment of parameters, such as voltage, frequency, and pulse width (Figure 3B).

Stimulation Parameters: Electrical stimulation was delivered with adjustable settings: voltage 0–10 V, frequency 5–300 Hz, and

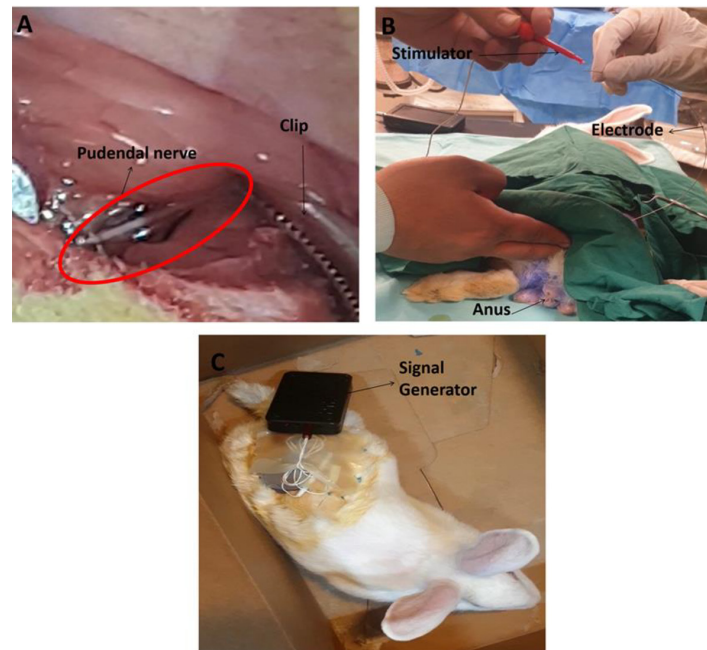


Figure 3: Experimental procedure. (A) Pudendal nerve clamping injury. (B) Electrode implantation and sacral nerve stimulation setup. (C) Daily 90-minute stimulation protocol.

pulse width 30–300 μ s. Remote monitoring allowed real-time adjustment of parameters, while visual observation of motor contractions served as an indicator of stimulation efficacy (Figure 3B).

Efficacy Assessment: Following implantation, rabbits were individually housed in a temperature-controlled environment with access to food and water. The signal generator was activated daily for 90 minutes over a four-day period (Figure 3C). Fecal output was collected and weighed daily to evaluate the effect of SNS on bowel function recovery.

Data Presentation: Experimental results were summarized as mean $\pm$ Standard Deviation (SD), providing a clear and quantitative assessment of the electrode's performance *in vivo*.

Results

Stimulating Electrodes Enhanced by Graphene–PAN composite

Graphene–PAN composite stimulating electrodes were successfully fabricated,

exhibiting electrical resistances between 1 and 5 Ω . Figure 4 summarizes the structural and mechanical characterization of the graphene–PAN composite sheet. Light microscopy images (Figure 4A) show a homogeneous, uniform sheet, while SEM images at various magnifications (Figures 4B–D) reveal a flake-type hybrid structure formed by graphene dispersed within PAN. The flakes measure several micrometers in size. PAN acts as a binder, facilitating microstructural rearrangement, while directional carbonization occurs due to the catalytic effect of graphene. The resulting hybrid exhibits a largely random orientation across the sheet [27].

Raman spectroscopy (Figure 4E) confirmed the composite's structural features. The D peak (~ 1345 cm^{-1}) indicates defects and disorder in the graphene lattice, corresponding to breathing modes of sp^2 carbon atoms in rings [29]. The G peak (~ 1582 cm^{-1}) arises from the E_{2g} mode, reflecting in-plane vibrations of sp^2 carbon atoms. The 2D peak (~ 2701 cm^{-1}) represents a second-order overtone, providing insight into the number of graphene layers.

Peaks at 2326 cm^{-1} and 1621 cm^{-1} correspond to the stretching vibrations of nitrile ($-\text{C}\equiv\text{N}$) groups and amide groups in PAN, respectively [30].

Gaussian peak fitting using OriginLab 2024 was performed to extract Full-Width at Half Maximum (FWHM) values and intensity ratios. The D, G, and 2D bands were fitted, yielding an $I_{\text{D}}/I_{\text{G}}$ ratio of 1.085 and an $I_{2\text{D}}/I_{\text{G}}$ ratio of 0.812. The $I_{\text{D}}/I_{\text{G}}$ ratio greater than 1 indicates a measurable level of lattice defects, edge sites, or functional groups despite overall graphitic ordering [31]. The $I_{2\text{D}}/I_{\text{G}}$ ratio suggests a few-layer to multi-layer graphene structure rather than a monolayer. FWHM values were 73.65 (D band), 53.15 (G band), and 75.79 (2D band), consistent with a heterogeneous, multi-layered, or turbostratic graphene structure. Overall, these results indicate that the material possesses a predominantly sp^2 -bonded network with significant defect density and multi-layer graphene architecture.

Mechanical testing of the graphene–PAN composite sheet was conducted according to the BS ISO 1924-3 standard. Load-

displacement curves (Figure 4F) indicate the sheet exhibits suitable tensile strength, elasticity, and flexibility, consistent with the requirements for neural interface applications.

Table 1 summarizes the calculated mechanical properties of the graphene–PAN composite samples, as referenced in Figure 4F, providing quantitative measurements of tensile strength, elasticity, and elongation at break.

The fabricated graphene–PAN composite sheet demonstrated a modulus of elasticity of $1.31\pm 0.45\text{ MPa}$ and a strain at break of $27\pm 2\%$, indicating good flexibility. Recent literature supports the mechanical suitability of graphene–PAN composites, which typically exhibit a modulus of elasticity ranging from a few MPa to several hundred MPa along with high stretchability [32]. For comparison, Cao et al. [33] reported graphene with a strain at break up to 5%, highlighting that our composite's higher strain at break ($27\pm 2\%$) offers enhanced flexibility for flexible electronic applications.

Conventional electrode materials, such as platinum and platinum-iridium alloys are far

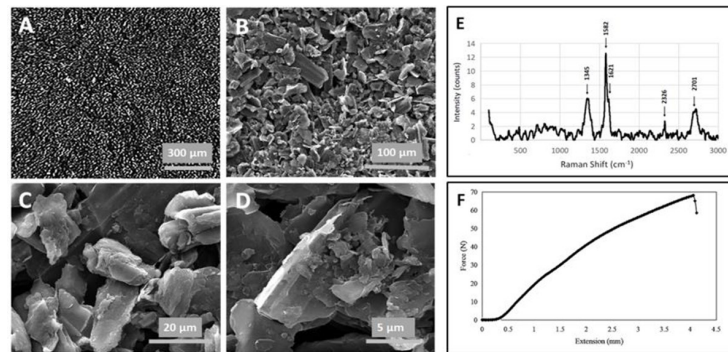


Figure 4: Structural and mechanical characterization of graphene–PAN (polyacrylonitrile) composite sheet. (A) Light microscopy image showing uniform structure. (B–D) Scanning Electron Microscopy (SEM) images at increasing magnifications revealing flake-type hybrid morphology. (E) Raman spectrum indicating defects, graphitic ordering, and multi-layer structure ($I_{\text{D}}/I_{\text{G}} = 1.085$, $I_{2\text{D}}/I_{\text{G}} = 0.812$). (F) Load-displacement curve from tensile testing.

Table 1: Mechanical Properties of Graphene–PAN (polyacrylonitrile) Composite Samples

Tensile strength	Strain at break	Tensile stiffness	Modulus of elasticity
$4.54\pm 0.68\text{ N/mm}^2$	$27\pm 2\%$	$197.38\pm 18.78\text{ N/mm}^2$	$1.31\pm 0.45\text{ MPa}$

stiffer, with moduli of elasticity around 169 GPa and 202 GPa, respectively [34]. Their tensile strengths typically exceed 124 N/mm², and their tensile stiffness values are correspondingly high [35]. In contrast, the graphene–PAN composite contacts in this study exhibited a tensile strength of 4.54 ± 0.68 N/mm² and a tensile stiffness of 197.38 ± 18.78 N/mm², reflecting a favorable combination of mechanical compliance and durability.

The observed flexibility and stretchability of the graphene–PAN composite suggest it may reduce mechanical mismatch with soft neural tissues, supporting its potential for use in sacral nerve stimulation applications.

Electrochemical measurements

Figure 5 provides comprehensive insights into the electrochemical properties of the graphene–PAN composite electrodes: cyclic voltammograms with the water window identified, impedance spectroscopy with equivalent circuit modeling, capacitance estimation from current–scan rate analysis, and

stability testing where impedance at 1 kHz remained consistent during repeated cycling. Figure 5A presents the cyclic voltammogram (CV) of the graphene–PAN composite electrode recorded over a wide potential window from -2 V to $+2$ V versus Ag/AgCl to identify the electrochemical stability limits and determine the safe operating region for neural stimulation applications. The highlighted region between approximately -1 V and 0 V represent the electrode's safe voltage window, where no significant faradaic reactions occur and the electrode behavior is predominantly capacitive. Within this potential range, the electrode surface remains electrochemically stable, minimizing irreversible redox reactions, electrode degradation, and potential tissue damage during stimulation. As the potential is swept toward more negative values, several distinct cathodic peaks emerge at approximately -1.5 V, -1.8 V, and -2.0 V versus Ag/AgCl. These reduction waves are attributed to the electrochemical reduction of oxygen-containing functional groups present

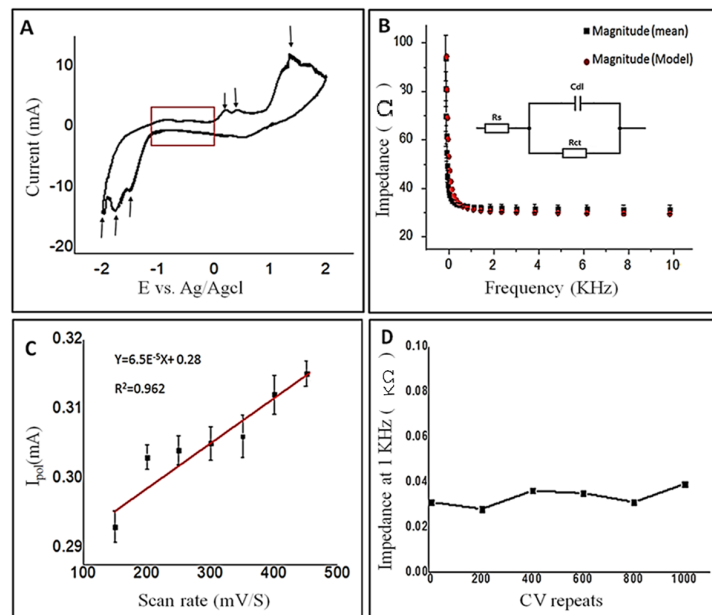


Figure 5: Electrochemical properties of graphene–PAN (polyacrylonitrile) composite electrodes. (A) Cyclic voltammograms showing water window (-1 to 0 V, red rectangle) and redox features. (B) Impedance spectroscopy with equivalent circuit model (inset); impedance at 1 kHz = 31.27 ± 2.76 Ω . (C) Double-layer capacitance estimation from CV (Cyclic Voltammetry) at varying scan rates (slope yields 65.03 ± 4.49 μ F). (D) Impedance stability at 1 kHz over 1000 CV cycles.

on the graphene surface. Graphene-based materials commonly contain a variety of oxygen functionalities introduced during synthesis or processing, including quinone, hydroxyl, carbonyl, aldehyde, epoxy, carboxyl, and peroxide groups. Each functional group possesses a different reduction potential, resulting in multiple cathodic peaks distributed across the negative potential range. The appearance of these cathodic peaks indicates that the graphene–PAN composite contains electrochemically active surface functionalities capable of participating in reversible or quasi-reversible redox reactions [36, 37]. Weak peak at 0.39V might due to a reversible redox reaction related to PAN [38]. The broad peak between +1V and +2V is attributed to the oxidation of graphene–PAN composite.

Figure 5B illustrates the EIS used to estimate parameters like double-layer capacitance (C_{dl}) and electrode impedance. The impedance at 1 kHz is $31.27 \pm 2.76 \Omega$. Following EIS sweeps, a linear equivalent circuit was used for data fitting, extracting capacitance and series resistance values. The simplified Randles cell model consists of a double-layer capacitance (C_{dl}) parallel to charge transfer resistance (R_{CT}) in series with ohmic Resistance (R_{Ω}) [39]. Using EIS and circuit modeling, a C_{dl} value of $48 \pm 1.6 \mu F$ was extracted for graphene–PAN composite electrodes.

Figure 5C shows the calculation of C_{dl} using cyclic voltammetry at different scan rates (dV/dt). For each scan rate, the polarization current (I_{pol}), was calculated as the average of the anodic and cathodic currents ($I_{pol} = (I_a - I_c)/2$), which represents the capacitive contribution of the electrode. These values were then plotted against the scan rate (dV/dt), giving a straight-line relationship. Since the capacitive current is directly proportional to scan rate, the slope of this line equals the double-layer capacitance (C_{dl}) [40]. Using this method, C_{dl} was calculated to be $65.03 \pm 4.49 \mu F$.

The CIC was computed using capacitance extracted from either EIS or sweeps at

different scan rates within the water window between -1 to 0 V (Equation 1):

$$CIC = \frac{C_{dl} \times V_m}{GSA} \quad (1) [21]$$

where Geometric of Surface Area (GSA) is the geometric surface area of the electrode exposed to the solution, V_m is the voltage threshold for electrolysis of water, and C_{dl} is specific electrochemical capacitance.

To estimate the surface area based on the physical dimensions of the graphene material, the graphene–PAN composite contact is considered as a hollow cylinder. Using this GSA of 6.123 mm^2 and extracted C_{dl} from both methods (EIS or repeated CV), the resulting CIC values are $783.91 \pm 26.14 \mu C/cm^2$ and $1062.58 \pm 73.36 \mu C/cm^2$, respectively. Some stimulation materials, along with our electrodes, were included in the Table 2 for direct comparison.

Figure 5D presents the changes in impedance after repetitive cyclic voltammetry, demonstrating the stability of graphene–PAN composite electrodes. The impedance at 1 kHz remains stable, with fluctuations within a few Ω during repeated cyclic voltammetries. This stability indicates that the graphene–PAN composite electrodes do not undergo significant changes that would affect their performance over time. Recent literatures demonstrated that carbon-based electrodes, including graphene, maintain lower impedance and stable performance over extended periods of CV [44], compared to metal-based electrodes [28]. This is largely due to the inherent chemical stability and high surface area of graphene, which resist surface fouling and degradation [45].

In Vivo Sacral Nerve Stimulation (SNS)

During *in vivo* testing, the stimulation parameters were set at a frequency of 60 Hz and a pulse width of $100 \mu s$, with voltage as the only variable. At an initial voltage of 100 mV, no muscle contractions were observed. Muscle

Table 2: Electrode materials commonly employed in neural stimulation applications

Material	Dimensions	CIC ($\mu\text{C}/\text{cm}^2$)	Impedance at 1 KHz ($\text{K}\Omega$)	Water window (V)	Reference
Graphene-PAN	6.123 mm^2	783.91 $\pm$ 26.14 1062.58 $\pm$ 73.36	0.031 $\pm$ 0.002	-1 to 0	This Work
Pt Macroelectrode	>0.001 cm^2	34 to 54	---	-0.6 to 0.9	[41]
PtIr	(1.4-3.8) $\times 10^{-4}$ cm^2	23 $\pm$ 5	--	-0.6 to 0.8	[15, 42]
TiN	2000 μm^2	960 $\pm$ 85	54.3 $\pm$ 0.74 10.2 $\pm$ 0.53	-0.9 to 0.9	[43]

CIC: Charge-Injection Capacity, PAN: Polyacrylonitrile

responses began at 500 mV and increased with higher voltages. At 2.5 V, the stimulus was excessively strong, causing noticeable distress in the animals. To ensure consistency and safety across all subjects, a voltage of 1 V was selected for subsequent experiments.

Comparatively, Thüroff *et al.* [46] investigated ventral sacral root stimulation in dogs, reporting effective bladder contractions and micturition at stimulation voltages ranging from 0.5 to 3 V, depending on electrode placement and frequency. Their findings demonstrate that controlled sacral root activation can reproducibly induce functional responses, providing foundational voltage thresholds for sacral nerve stimulation therapies. The behavioral responses observed in this study, particularly the distress at 2.5 V, highlight the importance of optimizing stimulation parameters to balance therapeutic efficacy with animal welfare.

In Figure 6 under normal conditions, fecal weights ranged from 76.33 $\pm$ 8.50 to 90 $\pm$ 5.56 g. Pudendal nerve injury caused a marked reduction in fecal output, with an average of 14.16 $\pm$ 2.43 g (18.7% of normal), indicating severe constipation. SNS treatment progressively restored fecal output: Day 1, 31.66 $\pm$ 6.50 g (55.3% of normal); Day 2, 50 $\pm$ 3.60 g (71.7%); Day 3, 72.66 $\pm$ 4.04 g (80%); and Day 4, 82.33 $\pm$ 9.86 g, approaching baseline levels.

Repeated-measures one-way ANOVA revealed a significant effect of treatment ($F(2, 8)=384.02$, $P\text{-value}<0.001$). Post-hoc Tukey

tests confirmed significant differences between normal vs. injury ($P\text{-value}<0.001$), injury vs. SNS ($P\text{-value}<0.001$), and normal vs. SNS ($P\text{-value}<0.01$), supporting the efficacy of SNS in reversing constipation induced by pudendal nerve injury.

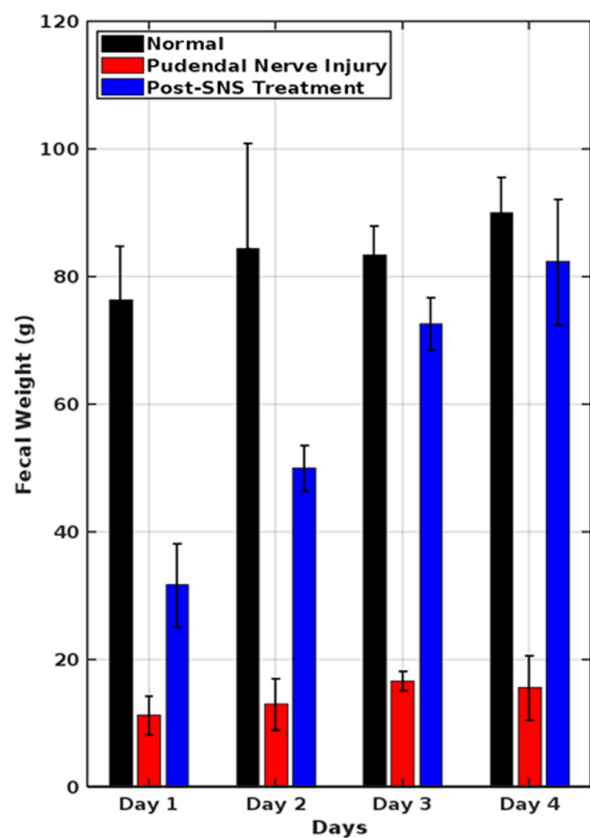


Figure 6: Fecal output in rabbits (mean $\pm$ SD, $n=5$) under control (black), post-pudendal nerve injury (red), and during SNS (Sacral Nerve Stimulation) treatment (blue) over four days.

Discussion

The present study demonstrates that graphene–PAN composite electrodes possess favorable electrochemical, mechanical, and functional characteristics, making them promising candidates for SNS applications.

A key finding is the high CIC of the graphene–PAN composite electrodes ($0.78\text{--}1.06\text{ mC/cm}^2$), which supports the safe delivery of stimulation currents within established electrochemical limits. A high CIC broadens the usable stimulation range and can accommodate variability in electrode-to-nerve positioning, reducing the likelihood of overstimulation under suboptimal placement conditions. Additionally, the low impedance ($\sim 31\ \Omega$ at 1 kHz) facilitates efficient current transfer to target nerve tissue and supports energy-efficient operation—an important consideration for long-term implantable neuromodulation devices.

Mechanically, the graphene–PAN composite exhibits a low elastic modulus ($1.31\pm 0.45\text{ MPa}$) and high strain at break ($27\pm 2\%$), reflecting flexibility and compliance closely aligned with soft neural tissues. Such mechanical softness can minimize tissue–electrode mismatch, potentially reducing fibrotic encapsulation and chronic inflammatory responses that compromise long-term device performance. These results are consistent with prior studies highlighting the benefits of flexible materials in neural interfaces [16–18], particularly in dynamic anatomical regions like the sacral nerve environment.

Functional evaluation *in vivo* further underscores the advantages of graphene–PAN composite electrodes. Rabbits subjected to pudendal nerve injury exhibited severe constipation, with fecal output reduced by $>80\%$. SNS treatment using graphene–PAN composite electrodes progressively restored bowel function over four days, with fecal output approaching baseline levels. Statistical analysis confirmed the significance of this improvement, demonstrating that graphene–PAN composite-based SNS can effectively compensate for nerve

injury and restore motility. In agreement with previous clinical and preclinical studies reporting the efficacy of sacral nerve stimulation in treating constipation and bowel dysfunction [2,46].

Several limitations of the study should be acknowledged. First, experiments were conducted in a small animal model with a limited sample size. Although rabbits provide a useful proof-of-concept platform, physiological differences necessitate careful consideration when extrapolating results to humans. Second, the long-term stability and biocompatibility of graphene–PAN composite electrodes were not fully assessed. While cyclic voltammetry suggests electrochemical durability under repeated use, chronic implantation studies are required to evaluate structural integrity, potential degradation, and tissue response over extended periods. Third, large-scale, reproducible fabrication of medical-grade graphene electrodes remains a manufacturing challenge. Variability in graphene synthesis and processing could influence electrode performance, emphasizing the need for standardized production methods for clinical translation.

Finally, bowel motility was primarily assessed via fecal output. While informative, a more comprehensive evaluation—including gastrointestinal transit time, defecation frequency, anal EMG, and intraluminal pressure—would provide deeper mechanistic insights. These additional endpoints were beyond the scope of this study but should be prioritized in future research to strengthen both biological interpretation and clinical relevance.

Overall, this study provides proof-of-concept evidence that flexible graphene–PAN composite electrodes combine favorable electrochemical performance, mechanical compliance, and functional efficacy, supporting their potential as next-generation materials for SNS and broader neural stimulation applications.

Conclusion

This study demonstrates the potential of

graphene–PAN composite electrodes as a promising platform for SNS therapies for constipation. The composite exhibited a high charge injection capacity ($0.78\text{--}1.06\text{ mC/cm}^2$) and low impedance ($\sim 31\ \Omega$ at 1 kHz), supporting safe and efficient current delivery during stimulation. Mechanically, the graphene–PAN composite showed a low elastic modulus ($1.31\pm 0.45\text{ MPa}$) and high flexibility, indicating favorable integration with soft neural tissues.

The *In vivo* evaluation in rabbits revealed improved bowel function, demonstrating that the material can effectively support functional neuromodulation within a dynamic anatomical environment. This study did not include a direct experimental comparison with Pt/PtIr electrodes; however, it introduces and validates a graphene–PAN composite as a promising alternative for SNS electrode applications. The results indicate that the composite shows favorable electrochemical and functional performance, supporting its potential for future development in neural stimulation technologies.

Future research should expand this proof-of-concept study toward long-term implantation, clinical translation, and broader exploration of graphene–PAN composites in additional SNS applications, including therapies for urinary incontinence.

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Authors' Contribution

M. Hejazi designed the study, collected the data, and drafted the manuscript. MM. Jahangiri assisted with the *in vivo* surgeries. Kh. Gorgi performed the sacral surgeries. O. Koohi-Hosseinabadi, as a veterinarian, prepared the animals and assisted in the surgeries. SA. Seyedi contributed to the development of

the signal generator. E. Mirzaei assisted with the preparation of the graphene–PAN composite solution and performed electrochemical experiments. J. Rouzegar conducted the mechanical experiments and analyzed the mechanical data. AR. Mehdizadeh supervised the project, provided consultation, and revised the manuscript. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

The methodology for conducting *in vivo* experiments to evaluate the performance of a graphene–PAN composite electrode for SNS involved several critical steps, meticulously designed and approved by the Iran National Committee for Ethics in Biomedical Research, Shiraz city (IR.SUMS.AEC.1402.009).

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Conflict of Interest

AR. Mehdizadeh, Editor-in-Chief and Chairperson, was not involved in the peer-review or decision-making processes for this manuscript. The peer review was independently managed by the non-author members of the Editorial Board and external reviewers.

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