# SYNTHESIS OF HIGHLY SENSITIVE GRAPHENE NANOCOMPOSITE FOR BIOSENSING GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP)

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## INTRODUCTION

Every year approximately 10 million central nervous system injuries, including 500,000 cases of spinal cord injury (SCI) are reported globally. In Canada, there are over 170,000 cases each year [1]. The majority of SCIs are as a result of car accidents and falls, followed by violence and sport related activities. Apart from physical and psychological disabilities, individuals with SCI experience a deteriorated quality of life and cost the global economy around US$4 billion annually [2, 3].

The pathophysiology of spinal cord injuries can be divided into the primary and secondary injuries. Primary injury consists of the mechanical damage to the spinal cord caused by the impact, while secondary injury is the resulting cellular and molecular events that are initiated by the primary injury [4]. The biochemical processes present in secondary injury include edema, ischemia, hypoxia, cytotoxicity, apoptosis, and hemorrhagic necrosis [5]. It is imperative to diagnose and monitor the course of injury SCI progression accurately in order to administer effective clinical interventions and help treat the SCI.

One way to monitor injury progression is by monitoring the concentration of proteins or biomarkers released after the onset of the secondary injury from the site of injury into the bodily fluids. One of the most prominent and extensively studied biomarkers is glial fibrillary acidic protein (GFAP), an astroglial and ependymal protein [6]. GFAP is found in blood and in cerebrospinal fluid (CSF) and has been quantified to determine if a spinal cord injury has occurred.

Despite the fact that several injury proteins could provide information about the status of the injury, they are not currently used clinically. One of the reasons for this is the absence of a device which has a rapid turnaround time and is easy to use, portable, and inexpensive [7].

Here, we report the development of a highly sensitive immunosensor fabricated using graphene composite. This graphene composite-based immunosensor created on screen-printed electrode would allow detection of GFAP using electrochemical impedance spectroscopy.

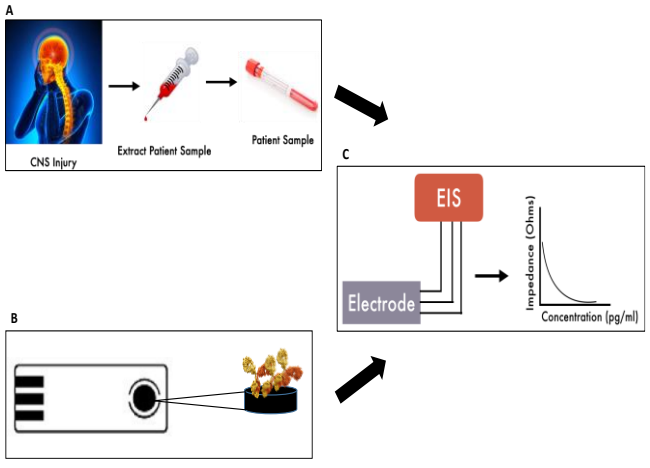

**Figure 1.** The schematic representation of the modification of graphene electrode surface, sample collection, and electrochemical detection of GFAP: **(A)** Samples artificially prepared or collected from blood and cerebrospinal fluid (CSF) of patients (**B)** Modification of surface electrode for binding the GFAP antibody. **(C)** Electrochemical impedance spectroscopy (EIS) biosensing for detecting GFAP biomarker in the CSF and blood.

## EXPERIMENTALS

Standard three pin screen printed electrodes were cleaned with Deionized (DI) water to make the surface hydrophilic and NaOH solution was prepared in DI water. Electrode was dipped in the NaOH solution for 2hrs at 55$^{o}$C. Afterwards, the electrode was again cleaned with DI water to remove the unbounded ions from the surface. To create a surface for the detection of the GFAP, 0.2%, 0.5%, and 1% branched Polyethylenimine solution was prepared in DI. To complete the functionalization process and reduce the surface to form Schiff base, the electrode was washed with DI to remove all the unbounded molecules. Later the process was completed by adding 1% Glutaraldehyde (GA) prepared in DI on the electrode surface. 10$\mu$g/ml GFAP monoclonal antibody was added on the surface of the electrode to make the surface detect the presence of GFAP from the samples introduced.

## RESULTS

After the electrode was functionalized, EIS was performed with each surface modification procedure to validate the efficacy of the electrode. EIS was measured between frequencies of 0.5Hz and 250 KHz and we observed a unique detection signal at 10 KHz. Characterization of the electrode was performed using EIS and the impedance signal was recorded at every stage of surface modification. For the characterization of the immunosensor, logarithmic concentrations of Phosphate buffered saline (PBS) were prepared and tested after every stage of the surface modification.

The blank graphene electrode when tested with logarithmic concentrations of PBS. It detected ions due to PBS in the sample and gave an increasing EIS trend from higher to lower concentrations with the maximum impedance recorded for 0.001% PBS in DI (Figure 2).

In order to select the optimal concentration of PEI for creating the nanocomposite, logarithmic concentrations of PBS were tested on the electrode surfaced modified with 0.2%, 0.5% and 1% PEI. Electrode surface modification was validated for each of these concentrations and EIS spectra was found to decrease with the increase in the concentrations of PEI. However, spectra for 0.5% and 0.1% PEI were nearly the same (Figure 3). Next, GA was deposited on the three concentrations of PEI and the Schiff base was created on the electrode surface.

A sustained and consistent decreasing EIS trend with increasing concentration was observed after modifying the surface for all the concentration of PEI and GA. Impedance was found to be lower than for the blank electrode in all the concentrations after the deposition of PEI and after the deposition of GA. One of the reasons for this decrease is because of the increasing conductivity of the electrode surface due to the formation of an electrically charged nanocomposite with the addition of PEI. Monoclonal GFAP antibody deposited on the surface of the electrode was found to be bound to the surface of the electrode, proving the validity of the graphene nanocomposite and the formation of Schiff base for the detection of GFAP.

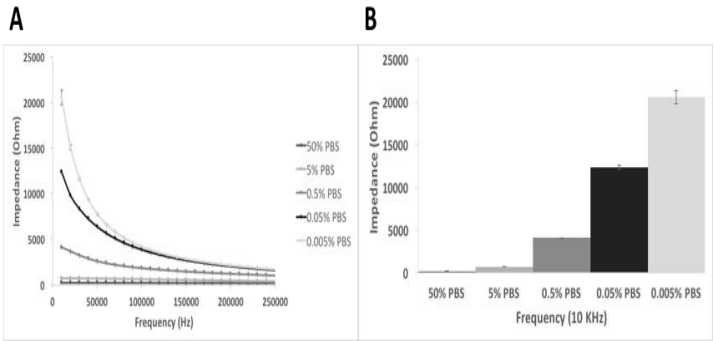


**Figure 2.** Impedance characterization on blank graphene electrode. (A) EIS of logarithmic concentration of PBS diluted in DI on blank electrode. (B) Impedance offered by the blank electrode at 10 KHz

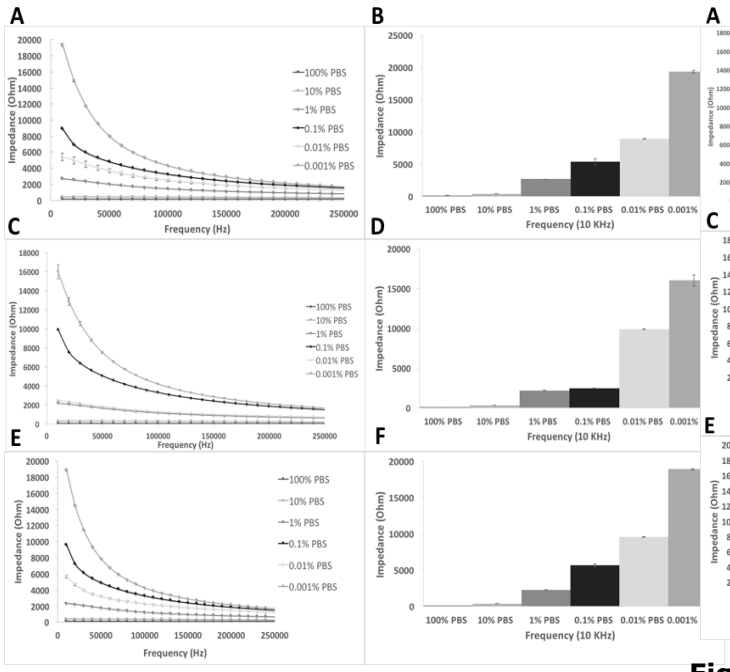


**Figure 3.** EIS characterization of varying concentrations of PEI on graphene electrode. (A) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 0.2% PEI deposited electrode. (B) Impedance offered by the 0.2% PEI modified electrode at 10 KHz. (C) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 0.5% PEI deposited electrode. (D) Impedance offered by the 0.5% PEI modified electrode at 10 KHz. (E) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 1% PEI deposited electrode. (F) Impedance offered by the 1% PEI modified electrode at 10 KHz.

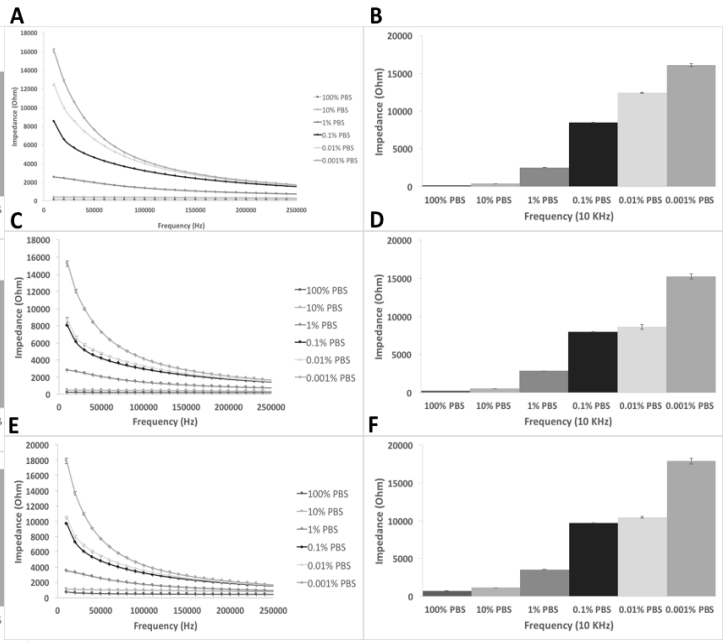


**Figure 4.** EIS characterization of graphene electrode functionalized with varying concentrations of PEI and 1% Glutaraldehyde (GA) with logarithmic concentrations of PBS. (A) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 0.2% PEI and GA deposited electrode. (B) Impedance offered by the 0.2% PEI and GA modified electrode at 10 KHz. (C) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 0.5% PEI and GA deposited electrode. (D) Impedance offered by the 0.5% PEI and GA modified electrode at 10 KHz. (E) Electrochemical impedance spectroscopy of logarithmic concentration of PBS diluted in DI on 1% PEI and GA deposited electrode. (F) Impedance offered by the 1% PEI and GA modified electrode at 10 KHz

## CONCLUSION

We have developed a novel graphene nanocomposite-based biosensor to capture and detect GFAP using an electrical technique. This could be used as an efficient, portable, and easy to use device to detect and monitor SCI.

## ACKNOWLEDGEMENTS

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