Nanoporous Carbon Immunosensor for Highly Accurate and Sensitive Clinical Detection of Glial Fibrillary Acidic Protein in Traumatic Brain Injury, Stroke, and Spinal Cord Injury

R. Salahandish[1,2], S. Khetani[2] & A. Sanati-Nezhad[2]

[1] York University, Toronto, Ontario, Canada; [2] University of Calgary, Calgary, Alberta, Canada

UHN
10th Annual Concussion Research Symposium
Update on Research and Care for the Concussion Spectrum of Disorders
Friday, April 21, 2023 | Hybrid: Zoom & BMO Education & Conference Centre

ABSTRACT

Elevated Glial fibrillary acidic protein (GFAP) in the blood serum is one of the promising bodily fluid markers for the diagnosis of central nervous system (CNS) injuries, including traumatic brain injury (TBI), stroke, and spinal cord injury (SCI). However, accurate and point-of-care (POC) quantification of GFAP in clinical blood samples has been challenging and yet to be clinically validated against gold-standard assays and outcome practices. This work engineered and characterized a novel Nanoporous Carbon screen-printed electrode with significantly increased surface area and conductivity as well as preserved stability and anti-fouling properties. This nano-decorated electrode was immobilized with the target GFAP antibody to create an ultrasensitive GFAP immunosensor and quantify GFAP levels in spiked samples and the serum of CNS injury patients. The immunosensor presented a dynamic detection range of 100 fg/mL – 10 ng/mL, limit of detection of 86.6 fg/mL, and sensitivity of 20.3 Ω mL/pg mm2 for detecting GFAP in the serum. Its clinical utility was demonstrated by consistent and selective quantification of GFAP comparable to the ultrasensitive single-molecule array (SIMOA) technology in 107 serum samples collected from TBI, stroke, and SCI patients. Comparing the diagnostic and prognostic performance of the immunosensor with the existing clinical paradigms confirms the immunosensor's accuracy as a potential complement to the existing imaging diagnostic modalities and presents a potential for rapid, accurate, cost-effective, and near real-time POC diagnosis and prognosis of CNS injuries.

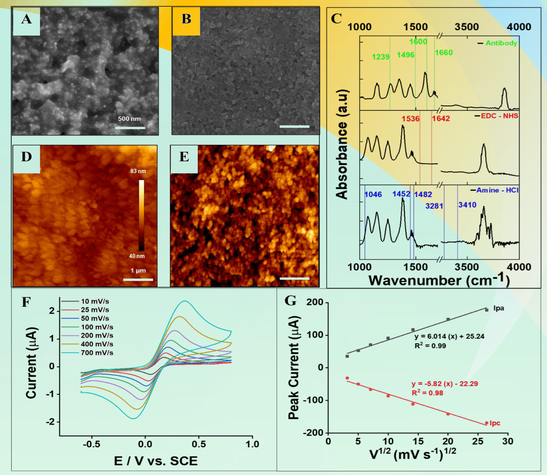
HIGHLIGHTS

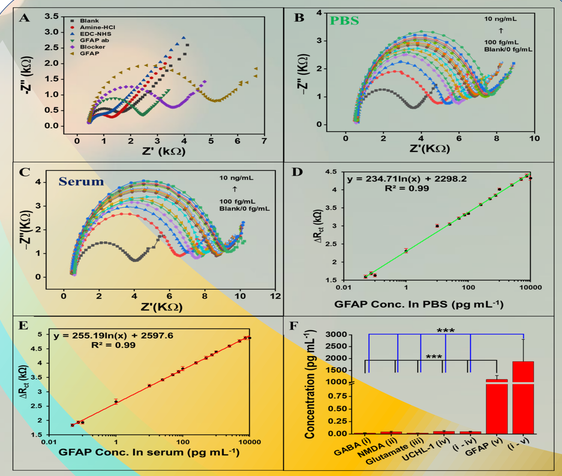
- An ultrasensitive Nanoporous Carbon Glial fibrillary acidic protein (GFAP) immunosensor with a wide detection range and very low limit of detection was engineered.
- The GFAP immunosensor quantified GFAP in the blood of patients with traumatic brain injury (TBI), stroke, and spinal cord injury (SCI).
- The clinical performance of the immunosensor was comparable to the most sensitive single-molecule array (SIMOA) assay and the existing clinical assessments practices.
- Measuring the change in GFAP concentration in the blood of patients during 6-month post-injury was predictive for monitoring the patient recovery.

RESULTS & DISCUSSION

Development and characterization of the GFAP immunosensor

Carbon screen-printed electrodes (C-SPEs) were treated with hydroxylamine (NH_2OH) and subject to CV undergoing electrochemical oxidation. These characterization tests confirmed that the flatted electrode structure is turned into a sponge-like porous structure. Furthermore, nano-porosities emerge after NH2OH treatment of the electrode resulting from the release of H2 in the lower reduction potentials on the surface of the working electrode in each cycle of the cyclic voltammetry. Therefore, NH2OH treatment adds Nitroso functional groups on the surface and increases the electrode's sensitivity by increasing its surface area. Additionally, interconnected porous channels enable electrolyte diffusion and promote faster electrochemical reaction kinetics.





Characterization of the GFAP immunosensing Assay

The sensitivity of the immunosensor was assessed by spiking GFAP into PBS and the human serum at GFAP concentrations ranging from 100 fg/mL to 10 ng/mL while measuring the EIS response in each medium. Specifically, the difference between the EIS signal recorded for the blocker and each GFAP concentration was measured, and the calibration equations were derived for each fluid. These linear responses showed that the GFAP immunosensor has more than sufficient sensitivity to measure GFAP at physiological levels of 1 pg/mL – 10 ng/mL found in individuals with and without CNS injuries. The selectivity of the immunosensor was examined to assess its suitability for detecting GFAP in clinical serum samples. Several interfering biomarker candidates known to increase alongside GFAP during CNS injury5 were spiked in the human serum and tested for their impact on the sensor's response. The concentration measured for all the spiked interfering markers was significantly lower compared to the concentration recorded for GFAP alone or together with the interfering molecules (P <0.001).

CONCLUSION

We developed a rapid, minimally-invasive, blood-based point-of-care immunosensor to ubiquitously detect GFAP in the serum of patients with CNS injuries (TBI, SCI, and stroke). This immunosensor performed superior to all previously reported GFAP immunosensors. The Nanoporous Carbon immunosensor enables ultrasensitive detection of GFAP in a wide operational range of 100 fg/mL - 10 ng/mL concentrations in PBS and the human serum. In addition, it selectively detects GFAP when tested with other biomarkers that are often released after the injury. The sensor allows for rapid (<30 min), portable, and streamlined detection of GFAP in the serum in three CNS injury conditions. The sensor quantified GFAP in 107 clinical samples and 44 healthy controls and the results were compared to the commercially available SIMOA assay to examine its clinical utility.