A Quadruplet 3-D Laser Scribed Graphene/MoS_2, Functionalised N_2-doped Graphene Quantum Dots and Lignin-based Ag-nanoparticles for Biosensing

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ABSTRACT

Troponin I is a protein released into the human blood circulation and a commonly used biomarker due to its sensitivity and specificity in diagnosing myocardial injury. When heart injury occurs, elevated troponin Troponin I levels are released into the bloodstream. The biomarker is a strong and reliable indicator of myocardial injury in a person, with immediate treatment required. For electrochemical sensing of Troponin I, a quadruplet 3D laser-scribed graphene/molybdenum disulphide functionalised N_2-doped graphene quantum dots hybrid with lignin-based Ag-nanoparticles (3D LSG/MoS_2/N-GQDs/L-Ag NPs) was fabricated using a hydrothermal process as an enhanced quadruplet substrate. Hybrid MoS_2 nanoflower (H3 NF) and nanosphere (H3 NS) were formed independently by varying MoS_2 precursors and were grown on 3D LSG uniformly without severe stacking and restacking issues, and characterized by morphological, physical, and structural analyses with the N-GQDs and Ag NPs evenly distributed on 3D LSG/MoS_2 surface by covalent bonding. The selective capture of and specific interaction with Troponin I by the biotinylated aptamer probe on the bio-electrode, resulted in an increment in the charge transfer resistance. The limit of detection, based on impedance spectroscopy, is 100 aM for both H3 NF and H3 NS hybrids, with the H3 NF hybrid biosensor having better analytical performance in terms of linearity, selectivity, repeatability, and stability.

Keywords: Biopolymer; molybdenum; nitrogen-graphene; nanoparticles; nanosensor

1.0 Introduction

According to the American Heart Association, around 19.1 million loss of life globally were attributed to cardiovascular disease (CVD) in 2020 [1]. Timely diagnosis is essential to reduce the mortality rate of CVD patients. CVD is closely related to the presence of Troponin I biomarkers in human body fluids [2,3]. It is a protein found in human blood that is generally released in the blood when the heart muscle is injured or damaged following a heart attack with concentrations in the blood stream depending entirely on the injury level in the heart muscles [4]. It is a biomarker for detecting heart injury in patients because it is specific for myocardial injury. Quantitative detection of this biomarker is vital in early treatment and clinical testing.

Biosensors combine a biological component (antibodies, enzymes, proteins, and nucleic acid) with a detector, which detects various specific targets according to the signal change when biological components interact with the target [5], and are quick, accurate, low cost, and meet the clinical test requirement.

In particular, graphene-based sensors have an excellent specific surface area, electronic properties, electron efficiency, strong mechanical strength, and flexibility. As such, carbon-based material like graphene has been actively researched due to these physical and chemical properties [6]. It is a one-atom-thick material consisting of sp^2- bonded carbon with a honeycomb structure. Traditional methods of producing graphene include chemical vapor deposition (CVD) growth, mechanical exfoliation of graphite, or exfoliation of graphite oxide, with varying levels of defects and functionalities [7,8]. These methods use excessive temperature, energy, volatile precursors, toxic chemicals and is time-consuming [9]. Furthermore, conventional graphene is made from non-renewable resources. The depletion of coal reduces the future availability of graphene. The defects in graphene are not a drawback because heterogenous electron shifting in sp^2 carbons structure does not happen at the graphene's basal plane, but at the edges and defect areas.

Computer-controlled laser scribing with optimized laser parameters, which converts a biopolymer such as lignin into graphene[10–12], is a viable alternative for producing graphene. Lignin is an abundant natural resource which is biodegradable, biocompatible, sustainable, and low-cost [13,14]. The lignin content in biowaste materials varies significantly, with empty oil palm fruit bunches among those with the highest lignin content (Table S1). Lignin, such as kraft lignin and organosolv lignin, have been used in flexible biosensor devices by depositing lignin on a polyamide sheet [15]. Laser scribing is inexpensive method for transforming biopolymer lignin into porous graphene using CO_2 laser scribing. Biopolymer-derived graphene synthesized through the CO_2 laser scribing technique is known as laser-scribed graphene (LSG). LSG is an ideal substrate/platform for interface biological components as it can be synthesized quickly in large amounts, has a carbon structure with a large and permeable morphology and excellent mechanical toughness. However, it has poor electrical properties due to the amorphous nature of lignin.

To enhance the eletrical properties of biopolymer-derived graphene, transition metal dichalcogenide (TMD) nanomaterials, specifically molybdenum disulfide (MoS_2) are considered as it has excellent biocompatibility and simple fabrication methods [16] . MoS_2 has a layered structure with sulfur (S) atoms forming the top and bottom layers, while the molybdenum (Mo) atoms are arranged in a hexagonal pattern. It is stable in aqueous solutions and has no dangling bonds on its surface [17]. Changes in the size and thickness of MoS_2 affects the reactive edge sites. The main parameters that influence the morphology and properties of MoS_2, which can vary from nanosheets or nanospheres to flower-like hierarchical structures, are the hydrothermal reaction time and temperature [18], and the precursor used. The precursor determine the crystallinity and morphology of the LSG and MoS_2 composite. This combination of LSG and MoS_2 has a synergistic effect with properties surpassing those of each nanomaterial [19]. By encapsulating MoS_2 in LSG, MoS_2 has improved biocompatibility properties, while LSG has better electron efficiency and higher electrochemical signal, making it well-suited for enhancing redox signals in the biosensing application. These synergistic characteristics enhance the detection capabilities in sensing, resulting in higher sensitivity and selectivity [20]. T unique sensing platform, by combining the specific structure of graphene and the surface alteration of MoS_2, is investigated.

In the present study, we have synthesized a quadruplet hybrid that consists of 3D LSG, MoS_2, N_2-doped graphene quantum dots (N-GQDs) and lignin-derived Ag-nanoparticles (L-Ag NPs) via a simple hydrothermal method by varying MoS_2 precursors. Graphene quantum dots (GQDs) are nanometer-sized graphene with unique properties which improve biosensing in terms of sensitivity (high surface area), biocompatibility, stability (under a wide range of environmental conditions), and ease in functionalization. This is the first reported work using N-GQDs, L-Ag NPs, and 3D LSG/MoS_2 to detect Troponin I biomarkers. N-GQDs have exceptional optical and electronic properties, and many reactive sites, making N-GQDs a powerful tool in analytical sensing [21]. Doping the low toxicity, biocompatible and hydrophilic N-GQDs on 3D LSG/MoS_2 structure covalently improves the electrochemically active surface area interaction with electroactive analytes. Green-based L-Ag NPs were incorporated in 3D LSG/MoS_2/N-GQDs for an efficient bonding process during the surface modification of aptamer [22,23]. The L-Ag NPs were infused as their features are similar to gold nanoparticles.Furthermore, L-Ag NPs, which improve the firmness of biological components during biosensing applications, are renewable, environmentally friendly, and inexpensive . The surface, structural, element composition, and sensing interactions of the fabricated three-dimensional LSG/MoS_2/N-GQDs/L-Ag NPs hybrids for troponin I detection were investigated. The findings offer an insight into the electroconductivity and aptamer-Troponin I detection of this green lignin-derived graphene-based hybrid biosensor. Thus, the current work contributes to advancing Troponin I biosensing by expanding the boundaries of current knowledge and technology.

2.0 Experimental Procedures

2.1 Materials

Lignin was extracted from palm oil biowaste and subsequently purified to obtain lignosulfonate [24]. The lignosulfonate was further utilized for the synthesis of graphene [10]. Chemical reagents including silver nitrate ($AgNO_3$), N-hydroxysuccinimide (NHS), (3-Aminopropyl) triethoxysilane (APTES), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Phosphate Buffered Saline (PBS), Streptavidin, and 16-Mercaptohexadecanoic acid were procured from Sigma-Aldrich (USA). Potassium Hydroxide (KOH), Molybdenum (VI) Oxide powder, Ethanol absolute, L- cysteine, Thiourea, Ethanolamine, and Ammonium Heptamolybdate Tetrahydrate were obtained from Merck & Co (USA). Citric acid-1-hydrate was purchased from Bendosen, Laboratory Chemicals, while ethylenediamine was obtained from QREC, Grade AR, (Asia) Sdn. Bhd, Malaysia. These purchased reagents were used in their original form without further purification,. Metrohm (Malaysia) Sdn. Bhd. supplied the Screen-Printed Carbon Electrode (SPCE). Oligonucleotides used in the study were bought from Avantis Laboratory (USA). additional details on oligonucleotide sequences dilution are available in the supplementary information and the sequence are provided below:

Probe (Aptamer) – 5′–CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTAAAAAA

AAAAAAAAAAAAAAAAAA-3′;

Biotin oligo linker – 5′-/5Biosg//iC6Sp/TTTTTTTTTTTTTTTTTTTT −3′;

Target – Troponin I, Human Serum of male AB plasma and Control – Troponin T were purchased from Sigma-Aldrich (USA).

2.2 Lignin extraction process

Lignin was extracted using a soda pulping process with aqueous sodium hydroxide (NaOH) [24]. The process begins with 500 g of oil palm waste mixed with 30% (w/v) NaOH and 4 L of water in a 10 L stainless steel rotary digester unit. The ratio of biomass to water is 1:8 (w/v). The mixture is heated to 170 °C under a 10-15 bar pressure for 3 hours. After cooking, a filter is used to separate the resulting mixture into pulp and black liquor. The black liquor undergoes additional vacuum filtration to remove excess pulp residues. The black liquor, which has high alkalinity with a pH of approximately 12.80, is then treated with 20% v/v H_2SO_4 to reduce its pH to 2. This process precipitates lignin and is further processed by centrifugation at 3500 rpm for 10 minutes to remove excess water. It is then dried in an oven at 50 °C for 4 days to eliminate moisture and ground into a powder. Next, the lignin powder was purified by refluxing it with n-pentane using a Soxhlet apparatus for 6 hours at 37°C. This step helps remove lipophilic non-lignin materials such as wax and lipids. After refluxing, the lignin was filtered and washed with acidified water to remove excess n-pentane. Finally, the purified lignin was dried in an oven for 24 hours. The process involves digestion, filtration, precipitation, purification and drying to obtain lignin in powder form.

2.3 Fabrication of three-dimensional lignin scribed graphene (3D LSG)

To synthesize 3D LSG, laser scribing [25] was used. Initially, a 20% concentration lignin solution was prepared by dissolving a few grams of lignin powder in a beaker filled with distilled water and mixed well until a uniform solution was obtained. The well-mixed lignin solution was carefully applied onto a glass platform . The glass platform was then dried for half an hour at 50 °C. The dried lignin-coated on a glass platform was subsequently subjected to laser scribing using 75 % laser power, 50% laser speed, and 500 pulse per inch (PPI), resulting in the formation of graphene after 30-minutes. The graphene was stored in an air-tight container to prevent contamination..

2.4 Preparation of N_2-doped graphene quantum dots (N-GQDs)

N-GQDs were prepared by a method reported elsewhere [26], with minor modifications. Briefly, the hydrothermal method was used by diluting citric acid (CA) and ethylenediamine (EDA) as carbon and nitrogen precursors. A detailed description of the preparatiom is given in supplementary information. The by-product of N-GQDs was further diluted into 1000 ppm.

2.5 Preparation of lignin-derived Ag-nanoparticles (L-Ag NPs)

L-Ag NPs derived from lignin were synthesized using the experimental procedure described in [10]. In short, a solution of silver nitrate ($AgNO_3$) with a concentration of 1000 ppm was incrementally introduced into a solution of lignin with a concentration of 1000 ppm while continuously stirred. The initially dark brown homogeneous solution transitioned to a brownish-black color, indicating the conversion of Ag ions into Ag NPs, facilitated by lignin. L-Ag NPs induce changes in the solution's color due to surface plasmon excitation. The lignin extract effectively reduced aqueous Ag ions, producing persistent Ag NPs dispersed in water.

2.6 Preparation of 3D LSG/MoS_2 NF/N-GQDs/L-Ag NPs hybrid through hydrothermal process

The preparation of the 3D LSG/MoS2 NF/N-GQDs/L-Ag NPs hybrid followed a slightly modified procedure reported in [27,28]. 60 ml of distilled water, 0.6 grams of ammonium heptamolybdate tetrahydrate, and 1.77 grams of thiourea were dissolved in a beaker. Subsequently, 2.0 grams of 3D LSG, along with 30 ml of N-GQDs and 30 ml of L-Ag NPs solution, were added in the same beaker with ultrasonication for 30 minutes. Next, the mixture was transferred into a Teflon-lined stainless-steel autoclave and heated at 200 °C for 24 hours. At the end of the process, the black-coloured 3D LSG/MoS2 NF/N-GQDs/L-Ag NPs hybrid was collected and washed thrice using distilled water and absolute ethanol through centrifugation. The residue of the 3D LSG/MoS2 NF/N-GQDs/L-Ag NPs hybrid was subsequently dryed in an oven set at 60 °C for 12 hours. The 3D LSG/MoS2 NF/N-GQDs/L-Ag NPs hybrid was named H3 NF. A similar procedure was repeated without 3D LSG, N-GQDs, and L-Ag NPs solution to produce bare molybdenum disulfide nanoflower (MoS_2 NF).

2.7 Preparation of 3D LSG/MoS_2 NS/N-GQDs/L-Ag NPs hybrid through hydrothermal process

The following steps were performed to synthesize the 3D LSG/MoS2 NS/N-GQDs/L-Ag NPs hybrid: Firstly, 0.25 g of molybdenum (VI) oxide powder was mixed in 20 ml of distilled water and subjected to 20 minutes of sonication. In parallel, 0.3 g of L-cysteine was dissolved in 50 ml of distilled water and agitated for 30 minutes on a hot plate. The molybdenum (VI) oxide and L-cysteine solutions were combined in a 100 ml beaker. Subsequently, 2.0 grams of 3D LSG, along with 30 ml of N-GQDs and 30 ml of L-Ag NPs solution, were added to the same beaker. The mixture underwent an additional 30 minutes of ultrasonication. The resulting solution was transferred to a Teflon-lined stainless-steel autoclave and subjected to 200°C for 16 hours. The mixture was subsequently left to cool naturally before removied from the oven. The resulting black product washed thrice using absolute ethanol and distilled water. The residue of the 3D LSG/MoS2 NS/N-GQDs/L-Ag NPs hybrid was dried at 45°C overnight. The 3D LSG/MoS_2 NS/N-GQDs/L-Ag NPs hybrid was named H3 NS. A similar procedure was repeated without 3D LSG, N-GQDs, and L-Ag NPs solution to produce bare molybdenum disulfide nanosphere (MoS_2 NS).

2.8 Aptamer immobilisation and Troponin I interaction

The aptamer immobilisation and Troponin I interaction were done separately for both hybrids. For the fabrication of the aptasensor, the SPCE was chemically modified. Firstly, 10 µL of 1M KOH (pH 9.2) was added onto the working area surface of the SPCE and allowed to incubate for several minutes [29]. Subsequently, the SPCE/KOH surface was treated with 10 µL of 2% APTES and allowed to rest for 1 hour to activate the amine surface, followed by30-minute conditioning with 10 µL of either H3 NF hybrid or H3 NS hybrid on the amine surface. The functionalized hybrid was diluted in a 1 mg in 1 ml proportion. Next, 10 µL of a complex mixture containing 16-mercaptohexadecanoic acid, NHS, and EDC was added onto the surface and incubated for 1 hour to facilitate a secondary reaction involving an unoccupied functional group suitable for protein detection. Subsequently, 10 µL of streptavidin was added and incubated for 30 minutes.10 µL of ethanolamine was introduced and allowed to undergo incubation for a duration of half an hour to hinder unspecific binding sites. Lastly, 10 µL of biotinylated aptamer was added and conditioned for 15 minutes. Thorough washing with buffer solution was performed for every functionalization step.

Electrochemical impedance spectroscopy (EIS) was used to study the chemical reaction of the constructed elements on the modified surface electrode [30,31]. Fig. 1 depicts the step-by-step surface functionalisation on SPCE surface area. The target (Troponin I) concentrations between 100 pM and 100 aM were primed via systematic dilutions.

Subsequently, 10 µL of each prepared Troponin I concentration was sequentially added to the electrode surface. Each concentration was conditioned for 10 minutes and rinsed gently with buffer solution to remove the unreacted target. Following the rinsing step, the impedance was analysed.

A similar procedure was followed for the interactive analysis using human serum, with a 1:10,000 dilution factor to assess the biosensor's capability to interact with actual samples. The stability of the interacted electrode was evaluated weekly by applying 10 µL of buffer solution onto the electrode working area. The repeatability of the biosensor was assessed by comparing the results obtained from several SPCEs within the same batch, where the aptamer was immobilised on the electrode working area.

2.9 Characterisation methods

The H3 NF and H3 NS hybrids' surface morphology was characterized using a variable pressure Field Emission Scanning Electron Microscopy (VP-FESEM) (Carl Zeiss SUPRA55 VP, Gemini). The nanoscale imaging was obtained using TEM. High resolution TEM (HRTEM) images of H3 NF and H3 NS hybrids was carried out using a HITACHI HT 7830 series up to 120 kV. Sample preparation was performed by dispersing H3 NF and H3 NS hybrids in double distilled water and sonicated for 10 minutes before dropping the sample on the TEM grid. Powder X–ray diffraction (XRD) was conducted using X'Pert3 Powder & Empyrean, PANalytical with a Cu Kα radiation (λ=1.54 Ǻ) was used to study the crystallization and structural properties of H3 NF and H3 NS hybrids. The XRD pattern was recorded in the range of 10° to 80° operating at a voltage of 40 kV and a current of 30 mA. The fabricated H3 NF and H3 NS hybrids were also analyzed using XPS (Thermo Scientific K-Alpha) to determine the elemental composition and the chemical and electronic state of the atoms within H3 NF and H3 NS hybrids. Fourier Transform Infrared Spectroscopy (FT-IR) (Brand: Pelkin Elmer Inc, Spectrum One/ BX) was used to identify the functional groups in the prepared materials. The wave range is from 7800 – 350 cm^{-1} at resolution from 0.5 cm cm^{-1} to 64 cm^{-1}. Electrochemical Impedance spectroscopy (EIS) measurements were made with a Metrohm Multi Autolab M204 Potentiostat/Galvanostat. All measurements were done at room temperature.

3.0 Results and discussion

The synergistic quadruplet hybrid involving 3D LSG, N-GQDs, MoS$_2$, and L-Ag NPs was successfully tested for biosensing. The properties of 3D LSG have always gained the attention of researchers, and in this paper, a conjugation of three supporting materials has been used. The synthesis of H3 NF and H3 NS hybrids by varying the surface morphology of MoS$_2$ was used to study the physiochemical, electrical conductivity, and analytical performance in Troponin I biosensing application. The bonding process between 3D LSG, N-GQDs, MoS_2, and L-Ag NPs involves complex chemistry. The laser scribing process used to fabricate the 3D LSG generates a distinctively irregular, porous, and large surface area, facilitating the attachment of N-GQDs and MoS_2 on the 3D LSG surface. The inherent stabilising and reducing properties of lignin avoids the need for a reducing agent. The surface of 3D LSG is enriched with diverse oxygen-containing functional groups. These functional groups ease the attachment of the supporting nanomaterials or nanoparticles on the 3D LSG surface. The 3D LSG and MoS_2 are attached through a covalent bond forming a synergistic nanocomposite [32,33]. MoS_2 and 3D LSG formed a compact geometric structure owing to their similar sandwich structure, with enhanced optical, electrical, and magnetic properties. Additionally, the negative surface charge resulting from the -OH on the 3D LSG surface allow an effective electrostatic interaction with the positively charged Mo precursors, leading to their binding [34,35]. In contrast, the MoS_2 surface has good adsorption capacity for N-GQDs, primarily through van der Waals interactions and the Mo atom binds with the N atom of N-GQDs [36,37]. Ag NPs were added to the hybrids to enhance material dispersion and stability and facilitate surface modification for probe-target interactions [38].

Lignin residues were still present after the laser scribing in converting lignin into graphene, which causes the 3D LSG to have high resistance. The high resistance was due to the presence of sodium and sulphur in the residues during the pulping process. The presence of the lignin residues in 3D LSG can be observed in EDS in Figure 2 (d & h). As a result, we concluded that the laser scribing technique needs to be fine-tuned and its parameters need to be optimized before high-purity graphene can be produced without lignin residues. We could convert lignin into 100% graphene through a laser scribing process by utilizing only a thin layer of 20% lignin coated on a glass substrate. Secondly, the developed biosensor can detect the Troponin I biomarker at higher specificity and has the potential for multiplex detection. In addition, our study focused on H3 NF and H3 NS hybrids and their analytical performance in Troponin I biosensing and compared these findings with prior work, as shown in Table S2.

Each nanomaterial added in forming quadruplets plays a role in biosensing. The size, shape, chemical composition, surface morphology, aggregation, agglomeration, charge-discharge and solubility of a material can greatly influence its relationship with biomolecules. The intrinsic, large surface area and conductive properties of Ag NPs and MoS_2 improve the immobilisation of biomolecules. Furthermore, the 3D morphology of lignin-based graphene is an advantage for MoS_2 to grow vertically, providing more space for Ag NPs bonding and simultaneously improving the graphene's physiochemical properties, which improves the analytical performance of synergistic hybrid in detecting Troponin I. The fabricated hybrid overcomes the severe stacking issues in MoS_2 NF with lignin-based 3D graphene. Ag NPs are a promising biocompatible metal for biomarker sensing. They generally conjugate with organic/inorganic materials that protect Ag NPs against agglomeration and improve their electrocatalytic activity and stability, improving electrochemical activity and detection sensitivity [39]. N-GQDs were chosen to augment the electroconductivity of LSG due to the amorphous nature of LSG, which allows it to maintain its inherent electrical properties. N-GQDs can improve the kinetics of electron transfer, as nitrogen modifies the electronic configuration of GQDs [40]. N-GQDs possess a quantum-sized structure with a large surface area, which enables a large density of functional groups to be deposited on its surface, especially during layer-by-layer surface modification through the chemical process [41], allowing for effective interactions between aptamer and Troponin I. Furthermore, N-GQDs improve chemical binding to anchor nanoparticles, enhancing electrocatalytic activity and stability.

3.1 Surface Morphology

Fig. 2 shows the morphological structure of the fabricated H3 NF and H3 NS hybrids examined through field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The 3D LSG, MoS_2 NF, MoS_2 NS, N-GQDs, and L-Ag NPs, which were prepared separately, exhibited a well-defined structure, as shown in Fig. S1. The FESEM image in Fig. 2a demonstrates the formation of MoS2 NF on a 3D LSG porous surface. The porous and large outer area of 3D LSG provides ideal conditions for the loading of MoS_2 NF. As for the magnified image of H3 NF in Fig. 2b, a well-structured flake-like MoS_2 grown on 3D LSG were observed. A few spherical structures are also observed in Fig. 2b, confirming the adhesion of Ag NPs on the active site of flake-like MoS_2. The porous structure of 3D LSG with various pore diameters with active edges induces the growth of MoS_2. To further confirm the formation of MoS_2 NF on 3D LSG incorporated with N-GQDs and L-Ag NPs, TEM analysis was conducted, as shown in Fig. 2c. The numerous delayered MoS_2 NF and 3D LSG are intercalated with each other, which results in the formation of a strong network hybrid. Meanwhile, the energy-dispersive X-ray (EDX) profile in Fig. 2d and elemental mapping in Fig. S2 prove that elements such as carbon (C), oxygen (O), nitrogen (N), molybdenum (Mo), sulphur (S), and silver (Ag) are uniformly distributed on the surface of H3 NF hybrid. However, the surface morphology of H3 NS appears dense, thicker, rougher, with irregular shape and size, as shown in Fig. 2e, compared to the bare structure of 3D LSG, MoS_2 NS, N-GQDs, and L-Ag NPs as can be seen in Fig. S1. The structures found in Fig. 2e were highly porous, consisting of wrinkled sheets, clumps, and flakes, forming a strong intercalated bonded microstructure due to the hydrothermal treatment conducted to form H3 NS. Furthermore, small spherical shape morphology N-GQDs with various diameters and L-Ag NPs affixed on

3D LSG/MoS_2 NS surface can be seen in Fig. 2f. The finding of H3 NS was further validated through TEM analysis (Fig. 2g), EDX profile (Fig. 2h) and elemental mapping (Fig. S3).

3.2 X-ray diffraction (XRD)

Fig. 3a shows the XRD patterns of 3D LSG, MoS_2 NF, MoS_2 NS, N-GQDs, L-Ag NPs, H3 NF, and H3 NS. The diffraction peaks observed for 3D LSG corresponds to the diffraction peaks of graphene oxide, specifically peaks of 23.4° (002), 32.19° (002), 34.2° (100), 45.5° (200), 47.25° (102). Well-resolved diffraction peaks appeared at 23.4°, corresponding to the (0 0 2) plane and indicating the good arrangement of the interlayer distance of graphene and oxygen reduction [42,43]. A minor diffraction peak at an angle of 47.69° corresponds to the (102) plane of the rGO structure [44]. There is no (001) peak in 3D LSG, indicating that the laser scribing process has effectively removed much oxygen from the material [45,46]. Additional peaks in 3D LSG are present due to unconverted lignin impurities. This conclusion is supported by the EDX profile shown in Figure 2, which provides evidence of the presence of lignin residues in the sample. As for MoS_2 NF, the diffraction peaks denote that it has a well-defined hexagonal structure (JCPDS 37-1492), with peaks at 14.0° (002), 34.0° (100), 40.1° (103), 48.2° (105), and 59.0° (110), suggesting that MoS_2 is stacked and confirming its layered structure. The broadening of the (103) and (105) peaks can be attributed to the occurrence of imperfection within the nanoscale flower structure [47,48]. The void of additional peaks in the MoS_2 NF diffraction pattern suggests that the MoS_2 synthesized are pure, with no detectable impurities. Furthermore, the absence of the (002) plane in the MoS_2 NS indiates that the stacking of the (002) plane in MoS_2 NS is greatly hindered, resulting in an extremely thin and spherical-shaped MoS_2. The diffraction peaks of the MoS_2 NS were observed at angles of 26.2°, 32.7°, 36.8°, 41.7°, 53.6°, 57.5°, 60.7°, 66.8°, 72.8°, and 79.4°, corresponding to (11-1), (10-2), (200), (103), (31-1), (100), (130), (021), (311), and (040) [30] suggesting that the MoS_2 NS possesses a unique crystalline structure with inhibited stacking of certain planes. The MoS_2 NS has three primitive crystal systems, which are tetragonal, rhombohedral, and monoclinic. After Rietveld refinement, the molybdenite and rosickyite percentage in MoS_2 NF is 96.3 and 3.7 wt%, respectively. As for MoS_2 NS, molybdenite (43.6 wt%), molybdenum dioxide (30.1%), and sulfur (26.3 wt%). X'pert Highscore software was used to quantify the phase percentages of molybdenite, molybdenum dioxide, rosickyite, and sulfur in MoS_2 NF and MoS_2 NS. The choice of precursor can significantly impact the resulting structural behaviour of the MoS_2 material. For instance, using MoO_3 (MoS_2 NS) as the precursor powder leads to the formation of MoS_2 through a chemical reaction with sulfur during thermal treatment [49]. Reducing MoO_3 to MoO_2 is an important step in this process, as it prepares the precursor material for the subsequent reaction with sulfur [50]. Once the MoO_2 is formed, it reacts with sulfur to form MoS_2. By carefully controlling the reaction conditions, it is possible to tune the properties of the resulting MoS2 material, such as its size, shape, and crystallinity. The broad peak at around 23° is characteristic of the (0 0 2) diffraction of N-GQDs [51]. The XRD pattern of Ag NPs reveals prominent peaks at 32.3° (1 2 2) and 35.6° (1 1 1), indicating the absence of defects and demonstrating the strong crystal lattice of lignin-based Ag NPs. In comparison, the shorter peaks at 67.5° (2 2 0) and 75.7° (3 1 1) correspond to specific crystal planes of Ag. These peaks confirm that the Ag NPs are face-centered cubic metallic Ag (JCPDS04-0783). The structural pattern of H3 NF hybrid has peaked at 9.0° (0 0 2), 18.2°, 28.3°, 33.5° (1 0 0), 34.2° (1 0 0), 40.1° (1 0 3), 47.0° (1 0 2), 48.2° (1 0 5), 58.0° (1 1 0) and 78.0° (3 1 1). Five peaks cantering at 9.0°, 33.5°, 40.1°, 48.2°, and 58.0° correspond to a crystal plane of MoS_2 NF, revealing that MoS_2 is dominant in the H3 NF hybrid. The (0 0 2) plane peak in H3 NF was slightly shifted to the left compared to the (0 0 2) plane peak in MoS_2 NF, suggesting that a new layered structure and a wider interlayer spacing have been formed. The alteration of (002) plane revealed that the MoS_2 are extremely stacked onto each other [52]. Furthermore, the peaks that appeared at 34.2° and 47.0° correspond to the presence of 3D LSG, while the peak at 78.0° confirms Ag NPs are attached to the H3 NF hybrid. Conversely, a broad (0 0 2) plane that corresponds to the existence of N-GQDs, 3D LSG and MoS_2 NS (1 1 -1) was observed in the H3 NS hybrid. An obvious peak at 36.8° (2 0 0) and 41.7° (1 0 3) confirms the presence of MoS_2 NS in the H3 NS hybrid. Furthermore, a dual peak at 32.5° and 34.1° confirms that MoS_2 NS is well conjugated on 3D LSG that functionalized with N-GQDs.

3.3 Fourier Transform-Infrared (FT-IR) spectroscopy

FT-IR spectra of 3D LSG, MoS_2 NF, MoS_2 NS, N-GQDs, L-Ag NPs, H3 NF, and H3 NS are displayed in Fig 3b. The absorption peak of H3 NS and H3 NS hybrids have almost similar peaks. The wide O-H bond (hydroxyl) observed in the 3500 – 3300 cm^{-1} range is consistent across all synthesized materials and originated from the uptake of aqua molecules. The MoS_2 NF peak around 3100 cm^{-1} in FT-IR indicates the existence of hydroxyl groups on the surface or within the MoS_2 NF [53]. The major absorption peaks of H3 NS and H3 NF hybrids are distinct at 1630.3,1360.8,1125.4, and 751 cm^{-1}, which are the C=C, C-N, C-O, and Mo-S groups. In contrast, the absence of the C=O bond observed at 1724 cm^{-1} in the hybrids, which is present in 3D LSG, suggests a reduction process occurred during the heating technique. The oxygen-rich functional groups were highly removed from lignin during the laser scribing process, forming a reduced LSG. However, the short peak observed at 1724 cm^{-1} shows that there is still a C=O bond in LSG due to the presence of lignin residues [54,55]. The weak peak of C=O bond in LSG indicates the insignificant amount of oxidation of LSG [56]. During the hydrothermal process, the functional group was further reduced, reducing C=O bonds and producing shorter intensity C-O bond in H3 NF and H3 NS. The C=C group in hybrids confirm the presence of 3D LSG. Furthermore, the short peak at 1360.8 cm^{-1} implies that the C-N bond is present in the hybrid structure and nitrogen atoms are successfully attached to the hybrid structure. The C-O bond (1125.4 cm^{-1}) peak indicates the presence of an oxygen functional group in H3 NF and H3 NS hybrids that corresponds to the presence of an alkoxy group [54]. However, it is important to consider that the same peak could also be attributed to the epoxy group (C-O-C) in the Graphene/MoS_2/Ag or Graphene/MoS_2/N-GQDs nanocomposite [38,57]. Furthermore, there is a possibility that the peak at 1125.4 cm^{-1} could be related to the syringyl ring of L-Ag NPs during the synthesis of silver nanoparticles (Ag NPs) from lignin. Lignin is a complex organic compound found in plant cell walls. During the synthesis of Ag NPs from lignin, the hydroxyl groups and -OCH_3 in lignin might undergo oxidation to form chromophores, such as quinone and α-carbonyl groups, which could contribute to the observed peak [10,58]. In summary, the peaks at 1125.4 cm^{-1} in the spectra might indicate the presence of different functional groups, such as oxygen-containing groups like alkoxy and epoxy groups or the presence of chromophores formed during the redox reaction involved in the synthesis of Ag NPs from lignin. The FT-IR analysis of H3 NF and H3 NS hybrids has verified the successful conjugation of 3D LSG, MoS_2, N-GQDs, and Ag NPs in the synthesized conjugated material [59,60].

3.4 Raman Spectroscopy

As shown in Fig. 3c, Raman spectroscopy was used to detect the nonpolar vibrations of fabricated hybrids. The Raman spectra of 3D LSG, H3 NF, and H3 NS show three characteristic peaks corresponding to the D, G, and 2D graphene bands. The 3D LSG revealed its peaks at 1349, 1575, and 2670 cm-1, which refers to the vibration mode of graphene [61]. The D band can be attributed to graphene's disorder or edge folding degree. In contrast, the G band is due to the first-order scattering of the stretching vibrations E_{2g} mode observed for the sp^2 carbon domains. Hence, the 2D band is called the stacking order of graphene layers and the boundary phonon at the Brillouin zone [62]. The H3 NF hybrid shows the characteristics bands at 1362 cm-1 (D band), 1577 cm-1 (G band), and 2500 – 3200 cm-1 (broad 2D band), whereas the H3 NS reveals its peaks at 1364 cm-1 (D band), 1585 cm-1 (G band) and 2500 – 3200 cm-1 (broad 2D band). Compared with 3D LSG, the peaks of hybrids showed some peak shifts. The shift of the G band was due to the structural distortion of the hybrids caused by the variation in bond distance of C=C. Interestingly, an additional peak at 882 cm-1 (H3 NF) and 871 cm-1 (H3 NS) is due to the terminal stretching vibration of hexagonal Mo=O of MoO_3 [63–65]. The presence of MoO_3 in hybrids may be due to the unreacted and partially oxidised MoS_2 formed after exposure to air or during a hydrothermal process. The intensity peak of MoO_3 of H3 NS is higher than H3 NF. I_D/I_G was calculated to estimate the disorder of carbon-based components. The 3D LSG, H3 NF, and H3 NS have

I_D/I_G values at 0.79, 0.83, and 0.76, which suggest that H3 NS is more highly ordered and has fewer defects than the 3D LSG and H3 NF. The I_D/I_G value for the 3D LSG is smaller than the H3 NF hybrid, indicating the delocalised π conjugation is partially destroyed and combined with supporting materials during the hydrothermal process [66]. Furthermore, the synthesized materials' crystalline size (La) can be estimated using the Tuinstra-Koeing relation [67], where the λ is equivalent to 514 nm.

$$L_a\ (\text{nm}) = (2.4 \times 10^{-10})\ \lambda^4\ (I_D/I_G)$$

The L_a of 3D LSG, H3 NF, and H3 NS was assumed to be 13.23 nm, 13.90 nm, and 12.73 nm, respectively. The H3 NF hybrid shows greater crystallinity owing to the surface-enhanced Raman scattering activity of Ag NPs. Furthermore, the dominancy of MoS_2 NF reflects higher crystallinity than H3 NS, as proven using XRD [68–72].

3.5 X-ray photoelectron spectroscopy (XPS)

Elemental arrangement and oxidation states of H3 NF and H3 NS hybrids were studied through XPS analysis. The XPS survey scan (Fig. 4a) of the resulting H3 NF and H3 NS hybrids exhibits C, N, Mo, S, O, and Ag elements. The result is aligned with the EDX mapping in Fig. S2 & Fig. S3. Figs. 4(b-k) displayed the result of C 1s, Mo 3d, S 2p, N 1s, and Ag 3d spectra of H3 NS and H3 NF hybrids, respectively. The four binding energies assigned by C 1s spectrum can be observed in Fig 4 (b-c). In general, the peaks centered at 291.1 eV (O-C=O), 288.7 eV (C=O), 286.5 eV (sp^3 / C-N), and 284.9 eV (sp^2, C=C / C-C) are found in H3 NS hybrid whereas the slightly similar peaks are displayed in H3 NF hybrid at 290.6 eV (O-C=O), 288.2 eV (C=O), 286.3 eV (sp^3 / C-N) and 284.6 eV (sp^2, C=C / C-C) [37,73,74]. The presence of graphene and its interaction with oxygen, resulting in carbonyl and epoxide functional groups at the edges of the graphene, correspond to the observed peaks. The epoxy functional groups determine the defects and disorders in the graphene structure on the basal plane. The Mo 3d XPS range of hybrids is characterized by several contributions associated with different oxidization states and structural phases of the Mo species in the hybrids. Fig. 4 (d-e) revealed the presence of Mo^{4+} in both hybrids, representing a semiconducting 2H phase of MoS2, where the $3d_{5/2}$ and $3d_{3/2}$ binding energies were 229.0 eV and 233.2 eV for H3 NS and 228.8 eV and 233.0 eV for H3 NF [75,76]. The deconvolution of the Mo 3d spectrum resulted in additional peaks at 230.4 eV (H3 NS) and 230.1 (H3 NF) appearing below the corresponding peaks of the 2H phase attributed to the 1T-MoS_2 phase and MoO_2 phonon modes. The peaks at 234.8 eV (H3 NS) and 235.0 eV (H3 NF) were attributed to the Mo $3d_{3/2}$ due to the presence of Mo^{5+}. It is worth noting that some amount of Mo^{6+} was also found, for which the H3 NS hybrid has a binding energy of 232.0 eV and 234.8 eV. In contrast, the H3 NF hybrid has a binding energy of 232.0 eV and 235.0 eV for Mo $3d_{5/2}$ and Mo $3d_{3/2}$ [66,77].

The Mo^{6+} attributed to a small amount of MoO_3 [78,79]. Additional Mo-O orbital at 228.2 eV was observed in the H3 NS hybrid due to the use of molybdenum (VI) oxide as the precursor. The peak at 226.5 eV (H3 NS) and 226.1 eV (H3 NF) in the low energy region of the Mo 3d spectrum corresponds to S 2s [79]. Moreover, the XPS spectrum of S 2p in Fig. 4 (f-g) can be deconvoluted into several peaks. The 168.6 eV (H3 NS) and 169.1 eV (H3 NF) may be attributed to S^{4+}, generated by incomplete oxidation of S^{2-} and might also be due to the formation of $C\text{-}SO_2$ [80]. The strong peaks of S $2p_{1/2}$ and S $2p_{3/2}$ for MoS2 can be observed at 164.2 eV and 162.0 eV for H3 NS, while 162.9 eV and 161.8 eV for H3 NF [81]. The S species observed in the S 2p region of the H3 NS hybrid were referred to as bridging C-S-C structures (Binding energies = 163.1 eV and 165.5 eV to the S $2p_{3/2}$ and $2p_{1/2}$, respectively). The N 1s spectrum in Fig 4 (h-i) is deconvoluted into four components. The peak centered at 395.5 eV (H3 NS) and 394.8 eV (H3 NF) is related to Mo $3p_{3/2}$, whereas the rest of the peaks noted at 398.7 eV, 400.6 eV, and 403.7 eV of H3 NS hybrid and the peaks assigned at 398.8 eV, 400.6 eV, and 402.6 eV correspond to pyridinic-N, pyrrolic-N, and graphitic-N, respectively [82,83]. Pyrrolic-N refers to the nitrogen atom (part of a pyrrole ring), a five-membered aromatic ring containing four carbon atoms and one nitrogen atom. On the other hand, Pyridinic-N refers to the nitrogen atom that is part of a pyridine ring, with a six-membered aromatic ring containing five carbon atoms and one nitrogen atom. The potential advantage of higher pyrrolic-N content in a hybrid includes enhancement in surface area, electron transfer efficiency, and reactivity. Similarly, having higher pyridinic-N content in a hybrid improves thermal stability and mechanical properties and increases electron mobility. The peak at 396.42 eV in the H3 NF hybrid can be assigned to the Mo-N bonds of molybdenum nitrides [84]. The presence of Mo-N bonds suggests that the hydrothermal synthesis of MoS2 and N-GQDs/L-Ag NPs/3D LSG involves a strong adhesive interaction, attributed to the interaction between the p-orbitals of the N-dopants and the transition metal Mo, resulting in the formation of robust Mo-N bonds through a strong coordination bond [54,85]. The nanoflower morphology in H3 NF hybrid has a large surface area with a three-dimensional structure that induces the attachment of N-GQDs easily compared to H3 NS hybrid. Furthermore, after Rietveld refinement through X'pert Highscore software, the weight percentage of molybdenum in H3 NS is significantly lesser than the H3 NF, as confirmed by the EDX result in Figure 2. This may due to no obvious attachment of Mo-N bonds, as shown in XPS for H3 NS hybrid. The successful integration of N-GQDs onto the 3D LSG/MoS2/L-Ag NPs framework is verified by the various types of C−N bond formation detected in the N 1s spectra. It is well-known that the N_2-doped graphene material can boost the electrical conductivity and the reaction efficiency through the conjugation of N-GQDs onto the 3D LSG/MoS2/L-Ag NPs structure [86]. Thus, successful incorporation of Ag into the H3 NS and H3 NF hybrids is further supported by the presence of an Ag peak in the XPS spectrum, as shown in Fig. 4(j-k). The corresponding Ag 3d spectra were detected from the wide scan of the H3 NS and H3 NF hybrids. The Ag 3d XPS spectra have Ag $3d_{5/2}$ and Ag $3d_{3/2}$, with binding energy peaks at 368.3 and 374.2 eV, indicating the presence of Ag in its zero-valence state [87].

3.6 Bio-sensing analyses on H3 NF and H3 NS hybrids

To prove the feasibility of this biosensing strategy, electrochemical impedance spectroscopy (EIS) was conducted to study the interface properties of H3 NF and H3 NS hybrids on the surface-modified SPCE electrode separately. Fig. 5 depicts the Nyquist plots of H3 NF (a-c) and H3 NS (d-f) hybrids aptamer-troponin I modified electrodes. Nyquist plots generally exhibit a semi-circular pattern at higher frequencies representing the resistance load transfer (Rct). This resistance is associated with the transfer of carriers from the electrode to the PBS solution. At lower frequencies, the plots show a short linear component of the diffusion-limited phase. The Nyquist plots can be analyzed by applying a model based on Randles' equivalent circuit, which incorporates the bulk electrolyte resistance (Ra) in combination with a parallel constant phase element (CPE) and charge transfer resistance (Rct). The charge transfer resistance (Rct) is associated with the diffusion of ions [88,89]. The magnitude of the charge transfer and the diameter of the semicircle on the plot provide insights into the interaction between the interfacial layer of the electrode and the electrolyte. The Nyquist plot of H3 NF (Fig. 5a) and H3 NS (Fig. 5d) hybrids shows layer-by-layer surface modifications with similar semicircle trends.

The SPCE electrodes were initially treated and modified with KOH / APTES to activate and functionalised the electrode surface to ensure a strong conjugation of the fabricated hybrids . The APTES forms a binding interaction with the -OH group during the treatment, leaving the amine group unaffected. The L-Ag NPs present in H3 NF and H3 NS hybrids bind with the unreacted amine group of APTES, forming a covalent interaction that showed an Rct value of 30K Ω (H3 NF) and 28K Ω (H3 NS), respectively [90–92]. The hybrids enhance the charge transfer process by serving as a conducive diffusion pathway due to the combined effect of the conductive 3D LSG, N-GQDs, MoS_2, and noble metal L-Ag NPs. With the addition of a complex mixture in surface modification, the Rct values increase as the thiol branch (-SH functional group) of 16-mercaptohexadecanoic acid binds with unoccupied L-Ag NPs of H3 NF and H3 NS hybrids.

On the other hand, the carboxylic group forms a bond with the amine group of streptavidin, resulting in Rct values of the complex mixture and streptavidin of H3 NF hybrid at 42K Ω and 64K Ω, and 36K Ω and 45K Ω for H3 NS hybrid at. The NHS and EDC in the complex mixture form two additional branches of the carboxylic group for more streptavidin attachment. Furthermore, streptavidin has four unique active sites, an advantage for biotin aptamer affixation. To prevent biofouling, ethanolamine was introduced during the surface modification process to hinder the presence of unoccupied functional groups on the electrode surface, which could otherwise compete with the occupied functional groups. The blocking agents, with Rct values of 66K Ω (H3 NF) and 55K Ω (H3 NS), were utilised to stabilize the aptamer during immobilization and enhance the biosensor's selectivity towards specific targets. The unique properties of streptavidin, with its four binding sites, promote extensive binding of the biotinylated aptamer, forming a strong electrostatic streptavidin-aptamer complex [93]. This complex formation was observed when the Rct value reached 63K Ω (H3 NF) and 50K Ω (H3 NS). The increment of the Rct value of biotinylated aptamer compared to streptavidin is attributed to the successful immobilisation of biotinylated aptamer with streptavidin. The aptamer-modified electrode is now prepared to capture the specified target for the interaction study.

The aptamer-troponin I hybridisation impedimetric semicircle response was investigated by varying the Troponin I concentration between 100 attomolar (aM) and 100 picomolar (pM), as shown in Fig. 5b (H3 NF) and Fig. 5e (H3 NS). The Rct values increase significantly with an increase in Troponin I concentrations, attributed to a substantial increase in bio-conjugation between the aptamer and Troponin I. The Rct values confirm the successful detection of Troponin I within a linear range of (100 aM to 100 pM) on the aptamer-modified bio-electrode. This demonstrates the exceptional performance and effectiveness of the sensing elements employed in the detection process. The remarkable biosensing mechanism of H3 NF and H3 NS hybrids is due to the large surface area of the 3D LSG, which features MoS_2 NF and NS that offers a platform for high electrical and catalytic activity. The N-GQDs improve the intrinsic features, and electrocatalytic activity provides more active sites and stability. Additionally, incorporating of L-Ag NPs eliminates the requirement for a chemical stabilizer or reducing agent in immobilising of biomolecules on the modified bio-electrode. As a result, the synthesized H3 NF and H3 NS hybrids enhance the charge transfer mechanism, leading to exceptional electrochemical performance.

A similar serial dilution under the same experimental condition was performed for human serum. Human serum dilution was prepared and analysed through impedance spectroscopy to investigate the ability of aptamer-modified H3 NF and H3 NS bio-electrode to hybridise with specific Troponin I nucleic acid with the presence of numerous biomolecules in human serum, as shown in Fig. 5c (H3 NF) and Fig. 5f (H3 NS). The Nyquist plot shows a similar Rct increment trend for Troponin I concentrations. As the concentration of human serum increases, the Rct value also increases, indicating an interaction between the aptamer and Troponin I in human serum and highlighting the high specificity of the developed biosensor. Compared to previously reported literature, the obtained analytical performance of the aptamer-modified H3 NF and H3 NS biosensors with graphene-based biosensors, shown in Supplementary Table S2, have the lowest LOD. The developed H3 NF hybrid biosensor has better analytical performance in terms of linearity, selectivity, repeatability, and stability compared to H3 NS.

3.7 Analytical performance of developed biosensors

The sensitivity of the H3 NF and H3 NS biosensors enables the detection of Troponin I at concentrations as low as 100 aM. Fig. 6 (a&e) demonstrates the linear regression analysis, with R2 values of 0.9870 (H3 NF) and 0.9766 (H3 NS), indicating a strong linear relationship between the sensitivity of the biosensors and the incremental concentration of Troponin I. The following equation was used to determine the sensitivity of the developed biosensor [94]:

$$\text{Sensitivity} = \frac{\text{Slope of calibration plot, m } (\mu\text{A mM}^{-1})}{\text{Working Area, A } (\text{cm}^{2})}$$

The SPCE has a working area (A) of 0.1257 cm^2, and the sensitivity of the biosensor was measured as 25.0 μA $mM^{-1}cm^{-2}$ for H3 NF and 16.8 μA mM^{-1} cm^{-2} for H3 NS. Human serum was introduced on the aptamer-modified bio-electrodes, along with various proteins to evaluate the selectivity of the H3 NF and H3 NS biosensors. Fig. 6 (b&f) demonstrates that the aptamer-modified H3 NF and H3 NS bio-electrodes specifically hybridized with Troponin I, leading to an increase in Rct values compared to immobilisation of troponin T (control) and other proteins. The Rct values showed an approximately four-fold enhancement in selectivity, as the aptamer selectively captured only Troponin I, even in the presence of numerous biomolecules. The H3 NF and H3 NS biosensors exhibited good repeatability, with a relative standard deviation (RSD) of 2.58% and 4.23%, respectively, for five parallel bio-electrodes prepared using the same procedure (Fig. 6 (c&g)). The shelf-life of the bio-electrode was assessed by studying the stability of the hybridized bio-electrode over six weeks at 4 °C. The stability analysis revealed that the biosensor remained stable, exhibiting stability of 95% (H3 NF) and 93% (H3 NS) after six weeks (Fig. 6 (d&h)).

4.0 Conclusion

The authors synthesized a quadruplet 3D LSG/MoS_2/N-GQDs/L-Ag NPs hybrid by varying the MoS_2 precursor followed by a simple hydrothermal process. The variation in MoS_2 precursor and the doping of N-GQDs and Ag NPs on 3D LSG improved the understanding of the fundamental relationship between nanostructures in terms of physiochemical, electrical, and analytical Troponin I detection. There is a slight difference in the analytical performance of the

Troponin I biosensor based on these two widely used MoS_2 precursors. The synergistic effect of the synthesized quadruplets enhances the electron transfer, stabilises the sensing systems, and overcomes the drawbacks faced as individual nanomaterials. L-Ag NPs provide overall stability to the system and form a bonding platform during the immobilisation of the aptamer. No significant cross-reactivity was observed in this study when the biosensor was tested with human serum. The H3 NF and H3 NS hybrid biosensors are excellent potential point-of-care devices for early identification of Troponin I biomarkers.

Figure legends

Figure 1: Schematic illustration of synthesised 3D LSG/MoS_2/N-GQDs/L-Ag NPs hybrid on the modified surface for Aptamer – Troponin I interaction.

Figure 2: FESEM, TEM, and EDX images of fabricated hybrids. (a) Less magnifications, (b) Enhanced magnifications, (c) TEM and (d) EDX results of H3 NF; (e) Less magnifications, (f) Enhanced magnifications, (g) TEM and (h) EDX of H3 NS hybrid.

Figure 3: XRD result (a), FT-IR image (b), and Raman spectroscopy image (c). It comprises synthesized 3D LSG, MoS_2 NF, MoS_2 NS, N-GQDs, L-Ag NPs, H3 NF, and H3 NS for XRD and FT-IR. For Raman Spectroscopy, H3 NF and H3 NS hybrids were compared with 3D LSG.

Figure 4: (a) XPS survey scan of H3 NS and H3 NF hybrids compared with 3D LSG. The H3 NS hybrid binding energy of (b) carbon, C1s; (d) molybdenum, Mo3d, (f) sulphur, S2p, (h) nitrogen, N1s; and (j) silver, Ag 3d, while the H3 NF hybrid binding energy of (c) carbon, C1s; (e) molybdenum, Mo3d, (g) sulphur, S2p, (i) nitrogen, N1s; and (k) silver, Ag 3d

Figure 5: Nyquist plots of H3 NF and H3 NS hybrids. (a&d) Layer by layer modification on SPCE; (b&e) Limit of detection (LOD) determination. Impedance analysis on various target concentrations between 100 aM and 100 pM were revealed; (c&f) Impedance study to investigate the selectivity and human serum analysis on a developed biosensor.

Figure 6: Analytical performance of H3 NF and H3 NS biosensor. (a&e) Linearity result; (b&f) Selectivity test via impedance Rct value; (c&g) Reproducibility of 5 individually developed biosensor and Rct bar chart diagram; (d&h) 6 weeks biosensor's stability check are displayed.

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Author Contributions

Fabrication and development of nanocomposites were conducted by M.V. Experiments and drafted manuscript were carried out by M.V with the help of P.B.R, M.N.M.I, S.C.B.G, H.L.L, M.O, S.K, N.A, P.C.A and R.S.K proof-read the manuscript. V.P supervised the work. All authors analysed the results and contributed to the discussion presented in the manuscript.

Additional Information

The authors declare no competing financial interes

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