Facile synthesis of MoS_2 nanoflower-Ag NPs grown on lignin-derived graphene for Troponin I aptasensing

Mugashini Vasudevan[1,2], Sathaniswarman Remesh[1,2], Veeradasan Perumal[1,2,*], Pandian Bothi Raja[3], Mohammad Nasir Mohammad Ibrahim[3], Subash C.B. Gopinath[4,5], Saravanan Karuppanan[2] and Mark Ovinis[6]

[1]Centre of Innovative Nanostructures and Nanodevices (COINN), Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia

[2]Department of Mechanical Engineering, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia

[3]School of Chemical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia

[4]Institute of Nano Electronic Engineering, Kangar 01000 & Faculty of Chemical Engineering & Technology, Arau 02600, Universiti Malaysia Perlis (UniMAP), Perlis, Malaysia

[5]Micro System Technology, Centre of Excellence (CoE), Universiti Malaysia Perlis (UniMAP), 02600 Arau, Pauh Campus, Perlis, Malaysia.

[6]School of Engineering and the Built Environment, Faculty of Computing, Engineering and the Built Environment, Birmingham City University, B4 7XG, UK

*Corresponding email: veeradasan.perumal@utp.edu.my (Ph: +6010-3773747)

ABSTRACT

This article presents the development and application of a green lignin-derived graphene biosensor for Troponin I, a biomarker for Acute Myocardial Infarction (AMI). The graphene was synthesized from oil palm lignin through an optimized laser scribing process. While the three-dimensional nature of the laser-scribed lignin-derived graphene (3D LSG) is advantageous, it suffers from poor electrical conductivity due to the amorphous nature of lignin. Therefore, semi-conductive molybdenum disulphide (MoS_2) precursor with conductive green silver nanoparticles (Ag NPs) was added to 0.5, 1.0, 1.5, and 2.0 grams of 3D LSG to synthesize 3D LSG_MoS2_Ag NPs hybrids via an aqueous hydrothermal process. Morphological, physical, and structural analyses showed the presence of petal-like MoS_2 nanoflower with Ag NPs on the 3D LSG surface. The strong interrelation between 3D LSG, MoS_2, and Ag NPs was confirmed by X-ray spectroscopy, Raman spectroscopy and energy dispersive spectroscopy (EDS). Specifically, X-ray spectroscopy revealed the formation of O1s, Ag 3d, C1s, Mo 3d, and S2p in the 3D LSG_MoS2_Ag NPs-2.0 hybrid. Raman spectroscopy revealed an enhancement in the surface area of the 3D LSG_MoS_2_Ag NPs-2.0 hybrid, which enhances the detection sensitivity. The 3D LSG_MoS_2_Ag NPs hybrid was subsequently chemically modified and immobilised with an aptamer to interact with Troponin I on an impedimetric sensor. The 3D LSG_MoS_2_Ag NPs hybrid showed high analytical performance, high specificity, and a ~4-fold increment in selectivity, with a detection limit of 100 attomolar. This biosensor has a sensitivity of 31.45 µA mM^{-1} cm^{-2}, stability of 87%, with a relative standard deviation for reproducibility of 3.8%.

Keywords: *Laser scribed graphene; Biopolymer; Nanosensor; Electrochemistry; Biomarker; Acute Myocardial Infarction*

1.0 Introduction

According to the World Health Organisation (WHO), acute myocardial infarction (AMI) has one of the highest mortality rates, responsible for almost 17.9 million deaths every year [1]. Early treatment of AMI significantly improves prognosis, as it prevents additional damage to the heart. AMI is detected by evaluating the concentration of AMI biomarker (Troponin I) in the human blood system. As such, a quick, cost-effective, and precise AMI biosensor is needed to detect the AMI biomarker in patients experiencing AMI symptoms. The alternative is having to wait in line for the traditional clinical method such as electrocardiography (ECG), angiogram and blood test. In that regard, the development of an readily accesible biosensor to detect AMI is a promising approach to overcoming the high mortality rates [2]. Three main factors need to be considered to create a biosensor: the active material/substrate, transducer/bioelectrode,

and bioreceptor-target interactions [3]. Optimizing the active material/substrate is the most challenging factor and is anactive research area. The nanomaterials mainly used in AMI biosensors are conducting polymer [4], gold nanoparticles [5], boron nitride quantum dots [6] and zinc oxide [7]. Utilizing these nanomaterials improves the analytical performance of an AMI biosensor. However, these nanomaterials are chemical-based, expensive, difficult to work with, and not green.

The discovery of graphene by Geim and Novoselov has opened the doors of opportunities to create carbon-based biosensor nanomaterials [8–11]. Graphene is used for biological and chemical sensing due to its excellent electrical conductivity, physiochemical properties, and morphological structure [12]. The commonly used methods to synthesize graphene are chemical vapour deposition (CVD), and mechanical and chemical exfoliation. However, these methods need high temperatures, high energy, highly volatile precursors, increased usage of toxic chemicals, and are time-consuming [13,14]. Therefore, an alternative, simple, straightforward method, like laser scribing, was introduced to synthesize graphene. The laser scribing (LS) method is a direct one-step conversion method to produce graphene from polyamide films through pulsed ultraviolet laser scribing treatment [15]. The LS method rapidly produces graphene on a large scale in a short time with excellent productivity and reproducibility [16]. However, the fabrication of graphene from polyamide using the LS method has limitations in terms of harmfulness to the environment, requiring large resources, and forms large polyamide waste after the service lifetime.

Biomass is an ideal material to replace polyamide as an LS substrate, as lignin is an abundant renewable resource, cost-effective, biodegradable, and environmentally friendly [17]. In lignin-derived graphene through the LS method, biowaste lignin is converted into graphene under a visible CO_2 laser [18]. Lignin biomass has remarkable characteristics such as high thermal stability, high carbon content, biodegradability, excellent physical /chemical properties, and good stiffness. It is a natural reducing agent, making it an attractive green polymer material for biosensing applications [19]. Recent breakthroughs have enabled the development of microporous three-dimensional (3D) graphene from lignin biopolymer through the LS method. The unique interconnected sandwich network structure of 3D graphene from lignin possesses a large surface area, and high mechanical strength and is compatible with other materials. Even so, lignin-derived graphene does not have commercial-grade graphene electrical conductivity, due to the amorphous nature of lignin [20].

Silver nanoparticles (Ag NPs) are extensively used nanoparticles in biosensor applications due to their unique physical and chemical properties. Ag NPs have attracted significant attention as nanocomposites for surface modification of sensor electrodes. Ag NPs are

inexpensive, easy to obtain, have high conductivity, large surface area, high stability, and excellent biocompatibility [21]. In particular, the coupling of graphene with Ag NPs enhances the sensitivity of a biosensor. Furthermore, the adsorption of nanoparticles in graphene prevents the aggregation of graphene sheets [22]. The deposition of Ag NPs on graphene oxide will enhance the electrocatalytic performance and stability of biomolecules. It also overcomes the instability of Ag NPs and the electrical conductivity loss of graphene oxide [23,24]. In that regard, many biosensors were developed using graphene/silver nanoparticles as active material, such as glucose biosensors [25], sulfite biosensors [26], hydrogen peroxide biosensors [27], 4-nitrophenol biosensors [28], adenine and guanine biosensor [29]. However, the use of harmful chemicals and the high-energy consumption needed to synthesize Ag NPs limit their large-scale production.

Moreover, highly concentrated reductants and stabilizing agents are needed to reduce the Ag ions into Ag atoms, which then agglomerate into oligomeric clusters leading to the formation of colloidal Ag NPs [30]. The stabilizing agent play a crucial role in the formation of nanoparticles with controlled size and shape. Therefore, a green Ag NPs based biopolymer was introduced in biosensors due to the biocompatible, biodegradable, non-toxic, safe reagents, and decrease in the cost of energy. In a study, Prasad et al. synthesized Ag NPs from the fruits of *Phyllanthus acidus* decorated on reduced graphene oxide (Ag-rGO) to detect dopamine and uric acid [31]. Graphene oxide coupled with pear extract-based green-synthesised silver nanoparticles (Ag-NPs) have been used for the detection of tuberculosis (TB) treatment drugs, ethambutol (EMB) and pyrazinamide (PZA)[32].

In this work, transition metal and noble nanoparticles were added to oil palm biowaste lignin-derived graphene, creating a heterostructure hybrid with improved electrical conductivity, chemical reaction, mechanical stability, and electrocatalytic properties. A synergistic combination of molybdenum disulphide (MoS_2) and silver nanoparticles (Ag NPs) was fabricated on lignin-based 3D graphene creating a biosensor with a large surface area and enhanced electron transfer efficiency. / MoS_2 and 3D graphene formed a compact geometric structure owing to their similar sandwich structure with enhanced optical, electrical, and magnetic properties. The intrinsic, large surface area and conductive properties of Ag NPs and MoS2 improve the immobilisation of biomolecules. Furthermore, the 3D morphology of lignin-based graphene is an advantage for MoS2 NF 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 MoS2 NF with lignin-based 3D graphene in its precursor.

Ag NPs are a promising biocompatible metal for biomarker sensing as they generally conjugate with organic/inorganic materials that protect Ag NPs against agglomeration and improve their electrocatalytic activity and stability, leading to improved electrochemical activity and detection sensitivity [33]. Strong van der Waals force between Ag NPs causes severe aggregation and instability. To overcome the drawbacks of these materials, we developed a heterostructure hybrid of lignin-derived 3D LSG_MoS2_Ag NPs, via a hydrothermal method to grow MoS_2 nanoflower_Ag NPs on lignin-derived 3D graphene. The morphological, optical, physical, and analytical performance of lignin-derived 3D LSG_MoS2_Ag NPs for Troponin I biomarkers in human serum were investigated. This research provides new insights into understanding the electrical conductivity and probe-target interaction on advanced biopolymer graphene fabricated hybrid and advances the state of the art in Troponin I biosensing.

2.0 Materials & Methods

2.1 Materials

Lignin extracted from oil palm's empty fruit bunch was purified [34] for conversion to graphene. Chemicals such as 16-Mercaptohexadecanoic acid, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Silver Nitrate ($AgNO_3$), N-hydroxysuccinimide (NHS), Streptavidin, Phosphate Buffered Saline (PBS), and (3-Aminopropyl) triethoxysilane (APTES) were obtained from Sigma-Aldrich Co., Ltd. (USA). Ammonium Heptamolybdate Tetrahydrate, Thiourea, Potassium Hydroxide (KOH), Ethanolamine, and Ethanol Absolute were obtained from Merck & Co (USA). All these chemicals were used as received without additional purification. The Screen-Printed Carbon Electrode (SPCE) was purchased from Metrohm (Malaysia) Sdn. Bhd. The oligonucleotides were purchased from Avantis Laboratory (USA). A detailed description of the oligonucleotide sequence dilution and characterizations can be found in the supplementary information. The oligonucleotide sequences utilized in this current work are as follows:

Probe (Aptamer) – 5′ – CGT GCA GTA CGC CAA CCT TTC TCA TGC GCT GCC CCT CTT AAA AAA AAA AAA AAA AAA AAA A-3′; Biotin oligo linker – 5′ - / 5Biosg // iC6Sp / TTT TTT TTT TTT TTT TTT TT −3′; Target – Troponin I; Control – Troponin T; Human Serum of male AB plasma were purchased from Sigma-Aldrich Co., Ltd. (USA).

2.2 Synthesis of lignin-based laser-scribed graphene (3D LSG)

Lignin-derived graphene was produced via the laser scribing method [35]. Initially, 20% of the lignin solution was prepared by adding 10 grams of lignin powder in 50 millilitres of distilled water and stirred until a homogenous lignin solution formed. The homogenous lignin solution

was coated on a glass substrate and left to dry for 30 minutes in an oven at 50 °C. The dried lignin glass substrate was then inserted in a laser-scribed machine under optimized parameters of 70% laser power, 50% laser speed, and 500% pulse per inch. A CO_2 laser was used to penetrate a lignin-coated glass substrate forming graphene after 30 minutes and the graphene was placed in a desiccator to avoid contamination.

2.3 Synthesis of oil palm lignin-mediated silver nanoparticles (Ag NPs)

Biogenic Ag NPs were synthesised following the experimental procedure reported by M.J.Y. Tai et al.[34]. In brief, a 1000 ppm silver nitrate ($AgNO_3$) solution was added gradually to 1000 ppm of lignin solution under constant stirring conditions until the homogenous solution changed from dark brown to brownish black, which confirms the reduction of the silver ions to Ag NPs, aided by the lignin. The surface plasmon excitation of Ag NPs causes the colour changes of the solution. This show that the lignin extract could reduce aqueous silver ions to yield stable Ag NPs in water.

2.4 Synthesis of 3D LSG_MoS2_Ag NPs through a hydrothermal method

The 3D LSG_MoS2_Ag NPs were prepared accordingly to the previously reported method with a slight modification [36,37]. In brief, 0.6 grams of ammonium heptamolybdate tetrahydrate and 1.77 grams of thiourea were dissolved in 60 ml of distilled water in a beaker. Next, 30 ml of Ag NPs solution and 0.5 gram of 3D LSG were mixed in the same beaker. The resulting mixture solution was stirred for an hour until all the solutes were fully dissolved. Then, the mixture was transferred into a 250 ml Teflon-lined stainless-steel autoclave and heated at a temperature of 200 °C for 24 hours. After the hydrothermal process, the autoclave was left to cool for a few hours. The resultant product, black-coloured 3D LSG_MoS_2_Ag NPs hybrid, was collected thrice, in sequence, through centrifugation using distilled water and ethanol absolute. The 3D LSG_MoS_2_Ag NPs hybrid precipitate was then dried in an oven at 60 °C for 12 hours. A similar procedure was repeated by replacing 0.5 gram of graphene with 1.0 gram, 1.5 gram and 2.0 gram of 3D LSG separately. The 3D LSG_MoS_2_Ag NPs hybrid sample was classified as 3D LSG_MoS_2_Ag NPs-0.5, 3D LSG_MoS_2_Ag NPs-1.0, 3D LSG_MoS_2_Ag NPs-1.5 and 3D LSG_MoS_2_Ag NPs-2.0, according to the amount of 3D LSG added. Bare MoS_2 was fabricated using the same procedure without Ag NPs and 3D LSG.

2.5 Immobilisation of biotinylated aptamer on modified SPCE

Chemical modification on SPCE was conducted for aptasensor fabrication. Initially, 10 µL of potassium hydroxide (1 M, pH 9.2) was dropped on SPCE carbon surface and incubated for 5 minutes. Next, SPCE/KOH surface was incubated with 10 µL of 2% APTES for an hour to

activate the amine surface, followed by incubation with 10 µL of 3D LSG_MoS_2_Ag NPs hybrid for 30 minutes to form a strong covalent bond between amine and the hybrid. 1 mg in 1 ml proportion was then used to dilute functionalized 3D LSG_MoS_2_Ag NPs nanostructure. Next, 10 µL of a complex mixture (16-mercaptohexadecanoic acid, NHS, and EDC) was dropped and incubated for an hour to form a stable side reaction with a free functional group for detection of proteins, followed by 10 µL of streptavidin with 30 minutes incubation time. To cap the non-specific interaction sites, 10 µL of ethanolamine was dropped and incubated for 30 minutes. Finally, 10 µL of biotinylated aptamer was dropped and incubated for 15 minutes. Biotinylated aptamer was prepared by diluting 2.5 µL of aptamer and biotinylated oligo linker in separate 47.5 µL of pure water. After that, the mixture was combined to form 1 µM of biotinylated aptamer, which was annealed at 60 °C for 1 minute and cooled down at room temperature. The additional oligos in the linker act as a spacer. A buffer solution of 10 mM PBS at pH 7.4 was used to wash after each modification thoroughly. Impedance analysis was conducted to determine the chemical reaction of the assembled molecules on the surface-modified electrode [2,38]. The chemical modification is illustrated in Fig. 1.

2.6 Troponin I hybridization on biotinylated aptamer

The biotinylated aptamer-modified SPCE is now ready to interact with the Troponin I. Concentrations of Troponin I ranging from 100 pM to 100 aM were diluted through serial dilutions. 10 µL of all diluted Troponin I concentrations were dropped one at a time. Each concentration was incubated for 10 minutes and washed thoroughly with 10 mM PBS of pH 7.4 to remove the unbonded target. After washing, an impedance analysis was performed. The complete hybridized aptasensor was stored at 4 °C when not in use.

2.7 Determination of Troponin I in protein in Human Serum

A dilution factor of 1:10 000 was used to dilute the Troponin I solution by using the same serial dilution at concentrations ranging from 100 pM to 100 aM to form a Troponin I spiked sample, similar to section 2.5. The rest of the surface modifications and detection strategies employed were identical to the previous section.

2.8 Stability and Repeatability analyses

The stability of the hybridized electrode was determined every week by dropping 10 µL of buffer solution on the surface of the electrode. The repeatability of the electrode was examined by taking measurements using different electrodes from the same batch of preparation for results comparison of every electrode when the aptamer was immobilised on SPCE.

3.0 Results and discussion

Nanoscale 3D LSG was produced from oil palm lignin biopolymer through the laser scribing method with the CO_2 laser penetrating three layers of 20% oil palm lignin solution coated on a glass substrate at optimised parameters. To improve the properties in 3D LSG, MoS_2 and Ag NPs were combined through the hydrothermal method to form a hybrid. Multiple studies were conducted to investigate the growth of MoS_2 vertically on 3D LSG in the presence of Ag NPs. MoS_2 grew on 3D LSG due to its hydrophilic nature. The -OH on the surface of 3D LSG makes for a negative surface charge, which effectively binds with the positively charged Mo precursors by electrostatic interaction [39,40]. Furthermore, the 3D LSG produced through the laser scribing process exhibits a unique irregularly spaced structure with porous surfaces that enables MoS_2 to attach to the 3D LSG. A reducing agent was not needed because lignin has natural stabilizing and reducing properties [41]. Loading of Ag NPs on 3D LSG can effectively prevent agglomeration, and improved the dispersion/stability of the materials in physiochemical solutions. The formation of numerous soft Lewis base sites in the form of sulphur atoms in MoS_2 leads to a strong attraction to Ag+ ions, easily forming Ag-S bonds [42].

Lignin is composed of numerous functional groups, such as reductive aliphatic hydroxyls and phenolic hydroxyls. Each of these can potentially reduce or cap silver nanoparticle synthesis. Also, these functional groups serve as binding sites for Ag+ ions and act as a natural reducing agent. The phenolic hydroxyl group has high potential to reduce silver cations into silver nanospheres while the sulfonate groups of lignin induce the dispersion of the formed nanoparticles in an aqueous solution. Thus, the lignin stabilizes the nanoparticles and prevents agglomeration due to its three-dimensional network structure [43,44]. Furthermore, traditional methods used a reducing agent such as hydrogen peroxide and sodium borohydride to synthesize silver nanoparticles. These chemicals are toxic to the environment if not disposed of in a proper manner. We utilized a green silver nanoparticle by deriving from lignin biopolymer without using a reducing agent, as lignin has natural reducing properties. The lignin-derived silver nanoparticles have a uniformed spherical shape and no agglomeration, as shown in Fig. S2 (e-f). Also, it is biocompatible, biodegradable and friendly to the environment. The MoS_2_Ag NPs-decorated on 3D LSG offers unrivalled nanostructures that can heighten the separation efficiency of the electron-hole pairs having higher catalytic activity, sensitivity, and stability for Troponin I aptasensing.

3.1 Field-Emission Scanning Electron Microscopy (FESEM)

FESEM was used to study the general morphology of the as-synthesized MoS_2_Ag NPs-decorated on a 3D LSG hybrid. The 3D LSG_MoS_2_Ag NPs hybrid, synthesized by a

hydrothermal method at different doping amounts of 3D LSG, were classified as 3D LSG_MoS_2_Ag NPs-0.5, 3D LSG_MoS_2_Ag NPs-1.0, 3D LSG_MoS_2_Ag NPs-1.5, and 3D LSG_MoS_2_Ag NPs-2.0 respectively corresponding to the doping amounts of 3D LSG. The result in Fig. 2(a-b) shows the MoS_2 nanoflower is dominant with high density in 3D LSG_MoS_2_Ag NPs-0.5. The shape and size of MoS_2 nanoflower vary, with a diameter range of 0.5 to 2.5 µm. However, in Fig. 2c, clumps MoS_2 surrounding the 3D LSG surface were found. The growth of MoS_2 nanoflower vertically on the surface of 3D LSG is attributed to the high surface roughness shown in Fig. 2d [45]. More clumps with bigger diameters can be seen in Fig. 2(e & g), as the doping amount of 3D LSG increases. 3D LSG with mesopores has a large surface area which can boost the electrochemical performance and attracts MoS_2 to grow on its surface [46]. The close-up view in Fig 2 (f & h) reveals the incorporation of smaller MoS_2 nanoflower with a diameter range of 0.2 to 2.5 µm on the irregular shape of 3D LSG, forming an interconnected conductive network which ensures optimum convection during the heating process. A well-stacked MoS_2 nanoflower on 3D LSG was observed due to van der Waals interaction which overcomes the severe stacking issues of MoS_2 [47,48]. The TEM results are provided in the supplementary information (Fig. S1). The variation in the MoS2 size is due to the number of nanopetals curved around it. MoS2 is an interlayered nanomaterial that grew as a layer-by-layer pattern along the preferred curly growth direction during the hydrothermal process, forming several single MoS2 nanoflowers, with growth continuing until a critical thickness was reach, which then causes aggregation of MoS2 to occur. During the growth of smaller nanoflowers or petal shape grains, they absorb thermal energy and start coalescing or fusing to form bigger grains. The bigger and fluffy grains provide more void space and numerous active sites, which could buffer the volume changes during the intercalation-deintercalation process with added material. The smaller nanoflower provide short pathways and high kinetics for surface modification and aptamer immobilisation. The various size of nanoflower is also accompanied with the changes to their crystallinity, defects, thickness and surface area, which determines the specific capacity. Furthermore, sulfur deficiency could happen during hydrothermal treatment, which destabilize MoS2, altering the geometry from trigonal prismatic of 2H phase into octahedral of IT phase of MoS2 nanoflowers. The fluffiness of nanoflower was formed due to the lower stacking of nanosheets [49–51]. An observation of lignin nanoparticles on the surface of 3D LSG_MoS_2 with an enhanced magnification of FESEM (Fig.2 (f & h)) reveals that the lignin was not fully converted into graphene during the laser scribing process, leaving some lignin residues. However, these lignin residues are beneficial as a natural reducing and stabilizing agent, which improves the catalytic reaction. The 3D LSG_MoS_2_Ag NPs hybrid provides an efficient path for the chemisorption of biotinylated probe aptamer during immobilization and target (Troponin I)

interaction. The FESEM images of 3D LSG, MoS_2, Ag NPs and EDS spectrum of the 3D LSG_MoS_2_Ag NPs hybrids are shown in the supplementary information (Fig. S2 & S3).

3.2 X-ray diffraction (XRD)

X-ray diffraction (XRD) was conducted to examine the purity, crystallinity, and plane orientation of synthesized 3D LSG, Ag NPs, MoS_2, and 3D LSG_MoS2_Ag NPs hybrids at different 3D LSG doping amounts. As shown in Fig 3a, the 3D LSG diffraction peaks at broad 23.4° (0 0 2), 34.2° (1 0 0), and 45.6° (2 0 0) corresponding to the diffraction peaks of reduced graphene oxide. There is no obvious peak at (0 0 1), indicating a large amount of oxygen-content was removed during the laser scribing process [52,53]. In the XRD pattern of Ag NPs in Fig 3b, the lignin-based Ag NPs have high-intensity peaks at 32.3° (1 2 2) and 35.6° (1 1 1), indicating no defects and high crystallinity, compared to short peaks at 67.5° (2 2 0) and 75.7° (3 1 1). These peaks refer to crystal planes of Ag, which is in good agreement with face-centered cubic metallic Ag (JCPDS04-0783). Meanwhile, the peaks diffraction in Fig 3c indexed to MoS_2 reveal that MoS_2 is a well-defined hexagonal structure (JCPDS 37-1492), with diffraction peaks at 14.0° (0 0 2), 33.5° (1 0 0), 40.1° (1 0 3), 49.4° (1 0 5) and 58.9° (1 1 0). This observation show that MoS_2 are stacked, confirming the layered structure of MoS_2. The broadening of (1 0 3) and (1 0 5) is due to the formation of defects in the nanosized flower. In the pattern of 3D LSG_MoS2_Ag NPs hybrids, the diffraction peaks of MoS_2 were found in Fig. 3 (d & e) at 13.6° (0 0 2), 33.4° (1 0 0), 39.9° (1 0 3), 49.2° (1 0 5) and 58.5° (1 1 0), whereas in Fig. 3 (f & g) only diffraction planes of (1 0 0) and (1 1 0) with broader peaks were observed, which suggest that MoS_2 dominates the hybrids. However, the diffraction peaks of 3D LSG_MoS_2_Ag NPs hybrids were shifted slightly to the left compared to the peaks for MoS_2, indicating the successful blending of 3D LSG and MoS_2. The absence of 3D LSG and Ag NPs diffraction peaks in 3D LSG_MoS_2_Ag NPs hybrids suggests that the doped silver nanoparticles changed the original stacked structure of the 3D LSG and prevented stacking and re-stacking. The intercalations of MoS_2 and Ag NPs on 3D LSG were observed through TEM analysis [37,52,54–56].

3.3 Fourier-transform infrared spectroscopy (FT-IR)

FT-IR analysis was conducted to determine the functional groups of the prepared materials. Fig. 3 (h-n) depicts the FT-IR spectra of 3D LSG (Fig. 3h), Ag NPs (Fig. 3i), MoS_2 (Fig. 3j) and 3D LSG_MoS_2_Ag NPs hybrids at 3D LSG with 0.5 g (Fig. 3k), 1.0 g (Fig. 3l), 1.5 g (Fig. 3m) and 2.0 g (Fig. 3n) of doping. The absorption peaks of 3D LSG_MoS_2_Ag NPs hybrids at different doping amounts of 3D LSG are distinct at 3435.5, 1718.2, 1628.3, 1125.6, 652.5 cm^{-1}, which are assigned to O-H, C=O, C=C, C-O, and Mo-S groups, respectively. Peaks at 667.4,

and 758.2 cm^{-1} refer to asymmetric vibration of Mo-O, and the existence of stretching vibration of S-S bond suggests that MoS_2 was successfully loaded. A small peak between 500 – 600 cm^{-1} was observed and that attributed to the vibration of Ag-S peak [57]. The hidden short peak of Ag-S bond is due to very little Ag NP added in the MoS2 precursor. A peak at 1718.2 cm^{-1} (C=O bond) in 3D LSG_MoS_2_Ag NPs hybrids confirms the presence of Ag NPs in the hybrids. The broad O-H bond (hydroxyl) at 3435.5 cm^{-1} is similar for all fabricated materials and is caused by the adsorption of water molecules. However, the O-H bond that appeared in 3D LSG at 1434 cm^{-1} is absent in 3D LSG_MoS_2_Ag NPs hybrids, indicating a reduction process occurred during the hydrothermal process. The FT-IR analysis of 3D LSG_MoS_2_Ag NPs hybrids has validated the successful pairing of 3D LSG, MoS_2 and Ag NPs in the fabricated hybrids [58,59].

3.4 X-ray photoelectron spectroscopy (XPS)

XPS studies were performed to reveal the elemental composition and chemical present on the outmost layer of hydrothermally grown MoS_2_Ag NPs on 3D LSG surface. As expected, the wide XPS survey scan of the 3D LSG_MoS_2_Ag NPs-2.0 hybrid has photoelectron peaks of carbon (C), oxygen (O), molybdenum (Mo), sulphur (S) and silver (Ag) (Fig. 4a). The presents of Ag 3p peaks and the slight shift of S 2p peaks in XPS indicate the presence of an Ag-S bond [60,61]. The carbon, C1s spectrum (Fig.4b) of 3D LSG_MoS_2_Ag NPs have peaks at 284.8 (C=C and sp^2 carbon), 286.4 (C-O), 287.5 (C=O) and 289.2 eV (O-C=O). These peaks correspond to the presence of graphene and the interactions of carbon atoms with oxygen, forming carbonyl and epoxide functional groups found at the graphene edges. The epoxy functional groups determine the defects and disorder of graphene structure in the basal plane. The oxygen, O1s spectrum shows the phenolic functional groups found in the hybrid structure at a binding energy of 531.5 (C=O), 533.2 (C-O), and 535.4 (C-OH). The enlarged spectra of Mo 3d are in Fig. 4d and can be deconvoluted to binding energy peaks at 229.1, 232.5, and 236.1 eV, which are assigned to Mo^{4+} $3d_{5/2}$. Mo^{4+} $3d_{3/2}$ and Mo^{6+} $3d_{3/2}$, respectively. The formation of MoS_2 is identified from the presence of Mo^{4+}, however, the Mo^{6+} is from the surface oxidized and the unconverted molybdenum residues in the precursor forming Mo-O group at peaks 233.5, 234.2 and 236.1 eV. The Mo^{4+} peaks confirm the formation of 2H-MoS_2 (semiconductor), whereas a peak at 230.5 eV refers to 1T-MoS2 (metallic). S 2s orbital was also observed at 226.4 eV in Fig. 4d. The Ag peak found in the XPS spectrum of 3D LSG_MoS_2_Ag NPs-2.0 hybrid further confirms the successful attachment of Ag. The corresponding Ag 3d spectra observed from 3D LSG_MoS_2_Ag NPs-2.0 hybrid wide scan are enlarged in Fig. 4e. The Ag 3d XPS spectra consist of two peaks of Ag $3d_{5/2}$ and Ag $3d_{3/2}$, which correlate the binding energy peaks at 368.5 and 374.5 eV, attributed to zero valence state. The XPS spectra of three characteristics peaks of S 2p at 162 (S $2p_{3/2}$), 163.2 (S $2p_{1/2}$)

and 168.5 (S-O) eV in Fig. 4f reveal the presence of sulphur. The S-O bond appeared during the formation of MoS_2 nanoflower. The XPS analysis confirms the existence and interactions in the 3D LSG_MoS_2_Ag NPs-2.0 hybrid. In short, the conversion of lignin through laser scribing, suggests that graphene oxide is formed. To justify the findings, XPS was conducted to analyse the elemental composition, and chemical and electronic state of oxygen and carbon, with similar composition of commercial graphene oxide found in laser-scribed graphene. The XPS analysis of Mo revealed that the Mo present in the synthesized hybrid exhibits two kind of crystalline phases: hexagonal 2H phase and octahedral 1T phase. 1T-MoS2 displays much greater chemical/physical properties than natural semiconductor 2H-MoS2. The element composition of molybdenum (Mo), sulphur (S), and silver (Ag) showed that these elements are present in the synthesized hybrid as a supporting material in improving the physiochemistry of laser-scribed graphene. Additionally, Raman Spectroscopy was performed on the 3D LSG and 3D LSG_MoS_2_Ag NPs-2.0, as shown in supplementary Fig.S4. It shows that 3D LSG_MoS2_Ag NPs-2.0 hybrid has fewer defects, a large surface area, and greater crystallinity. The higher peaks intensity compared to 3D LSG of 3D LSG_MoS_2_Ag NPs-2.0 hybrid with the addition of MoS_2 and Ag NPs suggest an electron and hole recombination state on 3D LSG surface and the enhancement of graphitization degree attributed to the ablation of heat. The peaks for MoS_2 and broad 2D (presence of Ag NPs) were found in Fig. S4b, confirming the formation of 3D LSG_MoS_2_Ag NPs-2.0 hybrid [62–69]. This hybrid is the most appropriate candidate for further investigation of its analytical performances as a biosensor

3.5 Bio-sensing analyses on 3D LSG/MoS_2/Ag NPs/SPCE

Electrochemical Impedance Spectroscopy (EIS) has been employed to study the biosensing performance of surface-modified 3D LSG_MoS_2_Ag NPs on SPCE in terms of their electrical resistance and interfacial properties. Fig. 5a shows the Nyquist plot of the modified surface of 3D LSG_MoS_2_Ag NPs on SPCE. The Nyquist plots can be interpreted by using a fitting model from Randles equivalent circuit. The fitting model is composed of bulk electrolyte resistance (Ra) with a parallel combination of constant phase element (CPE), and charge transfer resistance (Rct) related to the diffusion of ions. The interaction between the interfacial layer of the electrode and electrolyte is represented by the diameter of the semicircle to the magnitude of charge transfer resistance [70,71]. The carbon-filled SPCE chip was initially treated with KOH at 1 M of pH 9.2 to activate the surface of the bare SPCE. In Fig. 5a, the lowest frequency region, the semicircle of APTES, in the impedance spectrum represents Rct =22K Ω. Proper functionalized surface modification over an electrode is needed to ensure a strong stacked bonding for aptamer-troponin I interactions.

APTES is a silane that supplies amino acids to functionalize oxide surface (-OH groups). The high surface energy of the oxide surface rapidly interacts and forms a covalent bond with silane molecules [72]. The APTES binds with the -OH group, leaving the amine group unreacted [73]. The amine group of APTES attached to the silver ions found in 3D LSG_MoS_2_Ag NPs hybrid, causing Rct to increase to 27K Ω. The fabricated 3D LSG_MoS2_Ag NPs hybrid attached to the silane layer through covalent interactions without disrupting the silane structure, as shown in Fig. 1. The Ag NPs linked on NH_2 groups of the APTES covalently [74]. The synthesized hybrid acts as a conductive diffusion pathway in accelerating the charge transfer and reveals the synergistic effect between 3D LSG, semi-conductive MoS_2 and noble metal Ag NPs. The intrinsic conductivity properties of 3D LSG_MoS_2_Ag NPs hybrid tend to have a high energy density, which boosts the electron transfer between the hybrid and electrode. However, the resistance of fabricated 3D LSG_MoS2_Ag NPs-2.0 hybrid was higher than the APTES, as shown in Fig. 5a.

The LSG and Ag NPs were synthesized from lignin biopolymer. Lignin is an amorphous nature biopolymer. The residues of lignin in LSG cause the resistance to be high. The high resistance was due to the unavoidable lignin residues present in LSG. The extra peaks found in Fig. S3 are due to the lignin impurities or residues found in the fabricated hybrid material. Several optimizations were conducted to produce high-quality graphene, such as optimizing the lignin concentrations, layers of lignin coated on the glass substrate, and the speed and power of the laser machine. Still, these were unable to remove the lignin residues. The lignin residues can be seen in FESEM in Fig 2. With the increased surface modification of the complex mixture, the Rct value increases. These modifications enable the thiol branch (-SH functional group) of 16-mercaptohexadecanoic acid to bind with Ag NPs of 3D LSG_MoS2_Ag NPs. In contrast, the carboxylic group binds with the amine group of streptavidin, causing the Rct value of complex mixture and streptavidin to be 38K Ω and 46K Ω respectively. NHS and EDC in the complex mixture acts as activators to activate and intensify the surface modified electrode [75]. Besides that, it is also used to stabilize the interaction between amine and carboxyl group (streptavidin). The large surface area of Ag NPs induces charge transfer in biochemical interactions.

Ethanolamine was added to the electrode, to block the unoccupied area of the complex mixture to prevent bio-fouling, causing an increase in Rct value to 54K Ω. Ethanolamine acts as a blocking agent to block the unreacted functional groups from the electrode surface that would otherwise compete with reacted functional groups [38]. Usage of blocking agent is necessary to stabilize the Aptamer during immobilisation process and to improve the biosensor's selectivity towards their specific targets. The unique four binding sites feature of streptavidin induces the biotinylated aptamer to bind extensively, forming a strong electrostatic

streptavidin-aptamer complex [76]. This complex formation was observed when the Rct value reached 67K Ω. The increment in Rct value of aptamer shows that its immobilisation on streptavidin is a success. Further, an increment in the Rct value is observed when the target (Troponin I) interacted electrode, as shown in Fig. 5c. These results suggest successful immobilisation and interaction of Troponin I on the aptamer-modified SPCE electrode. The increment in the EIS is induced by a relatively larger biomolecule complex formation on the electrode surface. Creating suitable surface chemistry to immobilise or capture biomolecules on the 3D LSG_MoS_2_Ag NPs will aid the detection and monitoring of various biomarkers.

The resistances and electrical conductivity of the 3D LSG_MoS_2_Ag NPs hybrids were determined through electrochemical impedance spectroscopy (EIS) measurements. Fig. 5b shows the Nyquist plot of four hybrid variations (3D LSG_MoS_2_Ag NPs-0.5, 3D LSG_MoS_2_Ag NPs-1.0, 3D LSG_MoS_2_Ag NPs-1.5 and 3D LSG_MoS_2_Ag NPs-2.0) that differ in 3D LSG doping amounts. The EIS of the surface-modified SPCE electrode was performed at room temperature. The diameter of the semicircle formed for each hybrid corresponds to the charge transfer resistances (Rct) caused by the redox reaction. The intersection of the semicircle at the X-axis indicates the equivalent series resistance (Rs) of the electrode materials. The Nyquist plot in Fig. 5b revealed that 3D LSG_MoS2_Ag NPs-2.0 has the smallest semicircle with the lowest Rct value of 28K Ω, due to ideal capacitive behaviour compared to other 3D LSG_MoS2_Ag NPs hybrids. The enrichment amount of 3D LSG directly affects the charge transfer and conductivity of 3D LSG_MoS2_Ag NPs-2.0 because the 3D LSG has electrical conductivity, which means electrons transfer happens when it is in use. Still, it is not up to standard commercial-grade graphene. Due to this, the addition of supporting materials like MoS2 and Ag NPs is required to improve the conductivity of the LSG.

Furthermore, the addition of 3D LSG aids in the aggregation-free growth of MoS2 on 3D LSG, which eventually increases the nucleation sites for MoS2 growth on 3D LSG and further accelerates the ionic conductivity, as more charge carriers are formed. In addition, 3D LSG has excellent enrichment ability for organic molecules and has an attractive chemical enhancement effect. The large number of MoS2 edges act as active catalytic sites, which leads to excellent electrical coupling between MoS2 and the underlying 3D LSG network. The synergistic effect of Ag NPs on MoS2 opens more interparticle gaps, which creates a conducive surface for the interaction of biomolecules. The addition of less than 2.0 gram of 3D LSG (3D LSG_MoS2_Ag NPs-0.5, 3D LSG_MoS2_Ag NPs-1.0 and 3D LSG_MoS2_Ag NPs-1.5), results in the repulsion of electron transfer, and the gradual loss of conductivity. The Nyquist plot highlights the improvement in ion diffusion and charge transfer characteristics within electrodes due to a higher 3D LSG loaded amount. The 3D LSG_MoS2_Ag NPs-2.0

hybrid is therefore the best hybrid material for immobilisation and interaction of probe and bio-target to detect Troponin I.

Fig. 5c shows the impedimetric semi-circle response of aptamer-modified 3D LSG_MoS_2_Ag NPs bio-electrode hybridized with concentrations of the complementary target (Troponin I) ranging from 100 attomolar (aM) to 100 picomolar (pM). The value of charge transfer resistance (Rct) increases significantly with increasing concentrations of Troponin I, due to a significant rise in bio-conjugation between aptamer and Troponin I. The resultant Rct confirms the successful detection of a linear range (100 aM to 100 pM) of Troponin I on aptamer-modified bio-electrode, demonstrating the superiority of the sensing elements. The excellent bio-sensing mechanism of 3D LSG_MoS_2_Ag NPs-2.0 is because 3D LSG has a large surface area with MoS_2 nanoflowers on its surface that provides a platform for high catalytic activity. Furthermore, the binding of Ag at the sulphur atom of MoS_2 improves the electrocatalytic capability of the hybrid. The addition of Ag NPs avoids the need for a stabilizer or reducing agent for biomolecular immobilisation on modified bio-electrode. Hence, the coupling of 3D LSG, MoS_2 nanoflower and Ag NPs accelerated the charge transfer properties, which resulted in unparalleled electrochemical performances.

Various concentrations of human serum were prepared to evaluate the impedimetric semicircle response of aptamer-modified bio-electrode that was able to hybridize with the specific Troponin I nucleic acid sequence under the same experimental conditions as the target in Fig. 5c. The Nyquist plot, as shown in Fig. 5d, shows a similar charge resistance increment with the increasing concentrations of the target in human serum, upon aptamer-target interaction. The similar semicircle trend with target concentrations revealed that the aptamer-modified 3D LSG_MoS_2_Ag NPs bio-electrode exhibits high specificity for Troponin I detection in human serum, with the presence of an abundance of other biomolecules in the human serum. The performance of the aptamer-modified 3D LSG_MoS_2_Ag NPs biosensor was differentiated and compared with previously reported biosensors for Troponin I detection. The values are given as supplementary information (Table S1) with the aptamer-modified 3D LSG_MoS_2_Ag NPs biosensor more selective compared to other studied biosensors.

3.6 Analytical performances of 3D LSG_MoS_2_Ag NPs_SPCE biosensor

The sensitivity of the 3D LSG_MoS_2_Ag NPs_SPCE biosensor and linear correlation of differences in the charge transfer resistance, g with the logarithm of Troponin I concentrations (Fig. 6a) was investigated. It was observed that Rct increases linearly with increasing Troponin I concentrations from 100 aM to 100 pM. The Rct at aptamer for immobilized bio-electrode and hybridized bio-electrode were found to be directly proportional to the logarithm of target concentrations, with a linear equation of y = 3.95286E4 + 3.62927E4x, (R^2 = 0.9876). The

linear regression, R^2 shows that the sensor has good linearity with target concentration increment. The sensitivity was calculated using the following equation [77]:

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

with respect to the logarithm of Troponin I concentration, as shown in Fig. 6a. It was observed that the Rct linearly increases as the Troponin I concentrations increase from 100 pM-100 aM and thereafter saturated further. The sensitivity of 3D LSG_MoS_2_Ag NPs_SPCE biosensor is 31.45 µA mM^{-1} cm^{-2} for a SPCE active surface area of 0.1257cm^2 [2]. The limit of detection (LOD) of the 3D LSG_MoS_2_Ag NPs biosensor was 100 aM, estimated using a signal-to-noise ratio of more than 3σ (where σ is the standard deviation of the blank solution, n=5). The selectivity of 3D LSG_MoS_2_Ag NPs biosensor was evaluated by dropping human serum on aptamer-modified 3D LSG_MoS_2_Ag NPs bio-electrode in the presence of various proteins. Fig. 6b shows that the aptamer-modified 3D LSG_MoS_2_Ag NPs bio-electrode hybridized with Troponin I only, with a rise in Rct values compared to immobilisation of Troponin T and other proteins. The charge transfer resistance displayed a ~4-fold increase in selectivity, as the aptamer only captures Troponin I, although other biomolecules were present.

Troponin I level in normal healthy human blood is <0.04 ng/ml and >0.40 ng/ml after an AMI, well within the limit of detection for the current biosensor. The aptamer is designed to capture or interact with the specific Troponin I target. Further, the blocking step prevents the non-specific attachment of biomolecules on the sensing surface. Every material added to the SPCE chip has its own incubation time. After the incubation time, the extra and unbounded material will be washed before taking the impedance reading. Through washing, the interference of the electrode surface will be avoided and could prevent the resistance increment on the electrode surface. Furthermore, selectivity test on the developed biosensor was conducted using human serum, as shown Fig. 6b. The selectivity test demonstrated that the designed aptamer captures only Troponin I in human serum, even when other biomolecules are present. As a result, the developed biosensor has a high selectivity. The 3D LSG_MoS_2_Ag NPs biosensor showed good repeatability with a relative standard deviation (RSD) of 3.8% for five parallel devices prepared under a similar procession procedure, as shown in Fig. 6c. The shelf-life of the electrode was investigated by checking the stability of a hybridized electrode for six weeks stored at a temperature 4 °C. The stability analysis shows that the sensor used in the current work is stable and exhibits stability of 87% after six weeks (Fig. 6d).

4.0 Conclusion

A biopolymer-based hybrid for biosensing has been reported in this study, with large active surface area, high sensitivity, and excellent electrical characteristics. The combination of 3D LSG with MoS_2 and Ag NPs resulted in an exceptional active material/substrate. MoS_2 and Ag NPs were introduced to enhance the poor conductivity of 3D LSG. Morphological observation and structural analyses revealed petal-like MoS_2 with embedded Ag NPs on the 3D LSG surface. Impedimetric analysis revealed that the aptasensor attained an attomolar level of detection with excellent selectivity detection of Troponin I. In addition, 3D LSG_MoS_2_Ag NPs hybrid has remarkable features, which can enhance the immobilisation of aptamer due to its large surface area enabling the capture of more Troponin I biomolecules. Furthermore, biopolymer-based 3D LSG forms a high-affinity alternative material to non-renewable graphene, with the addition of MoS_2 and Ag NPs improving the interaction between the aptamer and Troponin I. This biopolymer-based biosensor will open new pathways in developing green-based biosensors for non-invasive diagnostic systems. Laser scribing is a promising one-step method for producing lignin-derived graphene. However, due to lignin residues in the final product, lignin-derived graphene has slightly higher resistance than high pristine graphene. In our work, we have optimised the process to produce high-quality graphene by optimising lignin concentrations, layers of lignin coated on glass substrate, laser machine speed and power. However, there are still minor lignin residues present when we fabricate at larger scale. As a result, we concluded that the laser scribing technique is still in its early stages of development and that many parameters need to be optimised before high purity graphene can be produced with no lignin residues. A proper method to convert lignin into 100% graphene through laser scribing will be explored in future to obtain residues-free laser scribed graphene.

Acknowledgements

The authors acknowledge the Ministry of Higher Education Malaysia for supporting the research with Fundamental Research Grant Scheme (FRGS) (FRGS/1/2020/TKO/UTP/03/7) and Universiti Teknologi PETRONAS (UTP) for the opportunity to carry out the research in Nanotechnology Research Laboratory and Dye Solar Cell Laboratory.

Author Contributions

Fabrication and development of hybrid microstructures were conducted by M.V. Experiments and drafted manuscript were carried out by M.V with the help of S.R, P.B.R, M.N.M.I, S.C.B.G,

M.O, and S.K proof-read the manuscript. V.P supervised the work. All authors analyzed the results and contributed to the discussion presented in the manuscript.

Additional Information

The authors declare no competing financial interests

References

[1] K. Reddy, Recent advances in the diagnosis and treatment of acute myocardial infarction, World J. Cardiol. 7 (2015) 243. https://doi.org/10.4330/wjc.v7.i5.243.

[2] M. Vasudevan, M.J.Y. Tai, V. Perumal, S.C.B. Gopinath, S.S. Murthe, M. Ovinis, N.M. Mohamed, N. Joshi, Highly sensitive and selective acute myocardial infarction detection using aptamer-tethered MoS₂ nanoflower and screen-printed electrodes, Biotechnol. Appl. Biochem. (2020). https://doi.org/10.1002/bab.2060.

[3] V. Perumal, U. Hashim, Advances in biosensors: Principle, architecture and applications, J. Appl. Biomed. 12 (2014) 1–15. https://doi.org/10.1016/j.jab.2013.02.001.

[4] I. Lee, X. Luo, J. Huang, X.T. Cui, M. Yun, Detection of cardiac biomarkers using single polyaniline nanowire-based conductometric biosensors, Biosensors. 2 (2012) 205–220. https://doi.org/10.3390/bios2020205.

[5] Y. Tan, Y. Wang, M. Li, X. Ye, T. Wu, C. Li, Enhanced photoelectrochemical immunosensing of cardiac Troponin I based on energy transfer between N-acetyl-L-cysteine capped CdAgTe quantum dots and dodecahedral Au nanoparticles, Biosens. Bioelectron. 91 (2017) 741–746. https://doi.org/10.1016/j.bios.2017.01.040.

[6] M. Lüt, N. Atar, Development of cardiac troponin-I biosensor based on boron nitride quantum dots including molecularly imprinted polymer, Biosens. Bioelectron. 126 (2019) 418–424. https://doi.org/10.1016/j.bios.2018.11.016.

[7] N.R. Shanmugam, S. Muthukumar, S. Prasad, Ultrasensitive and low-volume point-of-care diagnostics on flexible strips – a study with cardiac troponin biomarkers, Nat. Publ. Gr. (2016) 1–10. https://doi.org/10.1038/srep33423.

[8] J. Peña-Bahamonde, H.N. Nguyen, S.K. Fanourakis, D.F. Rodrigues, Recent advances in graphene-based biosensor technology with applications in life sciences, J. Nanobiotechnology. 16 (2018) 1–17. https://doi.org/10.1186/s12951-018-0400-z.

[9] M. Pumera, Graphene in biosensing, Mater. Today. 14 (2011) 308–315. https://doi.org/10.1016/S1369-7021(11)70160-2.

[10] S. Luo, X. Chen, Y. He, Y. Gu, C. Zhu, G.H. Yang, L.L. Qu, Recent advances in graphene nanoribbons for biosensing and biomedicine, J. Mater. Chem. B. 9 (2021) 6129–6143. https://doi.org/10.1039/d1tb00871d.

[11] P. Joshi, R. Mishra, R.J. Narayan, Biosensing applications of carbon-based materials, Curr. Opin. Biomed. Eng. 18 (2021) 100274. https://doi.org/10.1016/j.cobme.2021.100274.

[12] W.Q. Wang, H.Y. Yue, Z.M. Yu, S. Huang, S.S. Song, X. Gao, E.H. Guan, H.J. Zhang, Z. Wang, Synthesis of graphene/carbon nanofiber for electrochemical determination of levodopa in the presence of uric acid, Ionics (Kiel). 25 (2019) 2835–2843. https://doi.org/10.1007/s11581-018-2801-2.

[13] S.S. Shams, R. Zhang, J. Zhu, Graphene synthesis: A Review, Mater. Sci. Pol. 33 (2015) 566–578. https://doi.org/10.1515/msp-2015-0079.

[14] K.A. Madurani, S. Suprapto, N.I. Machrita, S.L. Bahar, W. Illiya, F. Kurniawan, Progress in Graphene Synthesis and its Application: History, Challenge and the Future Outlook for Research and Industry, ECS J. Solid State Sci. Technol. 9 (2020) 093013. https://doi.org/10.1149/2162-8777/abbb6f.

[15] M.R. Bobinger, F.J. Romero, A. Salinas-Castillo, M. Becherer, P. Lugli, D.P. Morales, N. Rodríguez, A. Rivadeneyra, Flexible and robust laser-induced graphene heaters photothermally scribed on bare polyimide substrates, Carbon N. Y. 144 (2019) 116–126. https://doi.org/10.1016/j.carbon.2018.12.010.

[16] A.A. Lahcen, S. Rauf, T. Beduk, C. Durmus, A. Aljedaibi, S. Timur, H.N. Alshareef, A. Amine, O.S. Wolfbeis, K.N. Salama, Electrochemical sensors and biosensors using laser-derived graphene: A comprehensive review, Biosens. Bioelectron. 168 (2020). https://doi.org/10.1016/j.bios.2020.112565.

[17] D.A. Baker, T.G. Rials, Recent advances in low-cost carbon fiber manufacture from lignin, J. Appl. Polym. Sci. 130 (2013) 713–728. https://doi.org/10.1002/app.39273.

[18] Y. Lei, A.H. Alshareef, W. Zhao, S. Inal, Laser-Scribed Graphene Electrodes Derived from Lignin for Biochemical Sensing, ACS Appl. Nano Mater. 3 (2020) 1166–1174. https://doi.org/10.1021/acsanm.9b01795.

[19] S.L. Burrs, S. Jairam, D.C. Vanegas, Z. Tong, E.S. McLamore, Lignin and silicate based hydrogels for biosensor applications, Smart Biomed. Physiol. Sens. Technol. X. 8719 (2013) 87190H. https://doi.org/10.1117/12.2016285.

[20] M. Stanisz, Ł. Klapiszewski, T. Jesionowski, Recent advances in the fabrication and application of biopolymer-based micro- and nanostructures: A comprehensive review, Chem. Eng. J. 397 (2020) 125409. https://doi.org/10.1016/j.cej.2020.125409.

[21] A.E. Kartika, H. Setiyanto, R.V. Manurung, S. Nurul, A. Jenie, Silver Nanoparticles Coupled with Graphene Nanoplatelets Modi fi ed Screen-Printed Carbon Electrodes for Rhodamine B Detection in Food Products, ACS Omega. 6 (2021) 31477–31484. https://doi.org/10.1021/acsomega.1c03414.

[22] R. Ghanbari, R. Safaiee, M.H. Sheikhi, M.M. Golshan, Graphene Decorated with Silver Nanoparticles as a Low- Temperature Methane Gas Sensor, ACS Appl. Mater. Interfaces. 11 (2019) 21795−21806. https://doi.org/10.1021/acsami.9b00625.

[23] J. Shin, K. Kim, J. Yoon, J. Jo, W.A. El-said, J. Choi, Silver Nanoparticle Modified Electrode Covered by Graphene Oxide for the Enhanced Electrochemical Detection of Dopamine, Sensors. 17 (2017) 2771. https://doi.org/10.3390/s17122771.

[24] M. Cobos, I. De-La-pinta, G. Quindós, M.D. Fernández, M.J. Fernández, Graphene oxide–silver nanoparticle nanohybrids: Synthesis, characterization, and antimicrobial properties, Nanomaterials. 10 (2020) 376. https://doi.org/10.3390/nano10020376.

[25] H. V Tran, N.D. Nguyen, C.T.Q. Tran, L.T. Tran, T.D. Le, H.T.T. Tran, B. Piro, C.D. Huynh, T.N. Nguyen, N.T.T. Nguyen, H.T.M. Dang, H.L. Nguyen, L.D. Tran, N.T. Phan, silver nanoparticles-decorated reduced graphene oxide : A novel peroxidase-like activity nanomaterial for development of a colorimetric glucose biosensor, Arab. J. Chem. 13 (2020) 6084–6091. https://doi.org/10.1016/j.arabjc.2020.05.008.

[26] W. Sroysee, K. Kongsawatvoragul, P. Phattharaphuti, Enzyme-immobilized 3D silver nanoparticle / graphene aerogel composites towards biosensors, Mater. Chem. Phys. 277 (2022) 125572. https://doi.org/10.1016/j.matchemphys.2021.125572.

[27] H. Wu, Hai; Huang, Deqian; Jin, Xiaoyan; Luo, Chunhua; Dong, Qiujing; Sun, Bangjin; Zong, Ruimei; Li, Jingcao; Zhang, Lei; Zhang, Silver nanoparticles/polyethyleneimine/graphene oxide composite combining with surfactant film for the construction of electrochemical biosensor., R. Soc. Chem. (2016) 1–7. https://doi.org/10.1039/C6AY00377J.

[28] N. Ahmad, A.S. Al, F. Rizwan, W. Manawwer, A. Anis, Synthesis of silver nanoparticles decorated on reduced graphene oxide nanosheets and their electrochemical sensing towards hazardous 4 - nitrophenol, J. Mater. Sci. Mater. Electron. 31 (2020) 11927–11937. https://doi.org/10.1007/s10854-020-03747-3.

[29] K. Huang, L. Wang, H. Wang, T. Gan, Y. Wu, J. Li, Y. Liu, Talanta Electrochemical biosensor based on silver nanoparticles – polydopamine – graphene nanocomposite for sensitive determination of adenine and guanine, Talanta. 114 (2013) 43–48.

https://doi.org/10.1016/j.talanta.2013.04.017.

[30] Y. Khan, M. Qasimnasar, M. Numan, Z.K. Shinwari, Biomimetic Synthesis of Silver Nanoparticles for Breast Cancer Therapeutics Biomimetic Synthesis of Silver Nanoparticles for Breast Cancer Therapeutics and Its Mechanism, Int. J. Nanotechnol. Nanomedicine. 3 (2018) 1–9.

[31] S. Prasad, S. Sathish, J.K.K. Kumar, Green synthesis of silver nanoparticles decorated reduced graphene oxide nanocomposite as an electrocatalytic platform for the simultaneous detection of dopamine and uric acid, Mater. Chem. Phys. 252 (2020) 123302. https://doi.org/10.1016/j.matchemphys.2020.123302.

[32] T. Mulaudzi, N. Mayedwa, E. Iwuoha, Green Method Synthesised Graphene-Silver Electrochemical Nanobiosensors for Ethambutol and Pyrazinamide, Processes. 8 (2020) 879.

[33] F. Gao, T. Fan, S. Ou, J. Wu, X. Zhang, J. Luo, N. Li, Y. Yao, Y. Mou, X. Liao, D. Geng, Highly efficient electrochemical sensing platform for sensitive detection DNA methylation, and methyltransferase activity based on Ag NPs decorated carbon nanocubes, Biosens. Bioelectron. 99 (2018) 201–208. https://doi.org/10.1016/j.bios.2017.07.063.

[34] M.J.Y. Tai, V. Perumal, S.C.B. Gopinath, P.B. Raja, M.N.M. Ibrahim, I.N. Jantan, N.S.H. Suhaimi, W.W. Liu, Laser-scribed graphene nanofiber decorated with oil palm lignin capped silver nanoparticles: a green biosensor, Sci. Rep. 11 (2021) 1–9. https://doi.org/10.1038/s41598-021-85039-2.

[35] N.N. Loganathan, K.R. Munusamy, V. Perumal, B.R. Pandian, Laser scribed graphene from oil palm lignin for supercapacitor applications, J. Water Environ. Nanotechnol. 6 (2021) 356–366. https://doi.org/10.22090/jwent.2021.04.006.

[36] G. Ma, H. Xu, M. Wu, L. Wang, J. Wu, F. Xu, A hybrid composed of MoS2, reduced graphene oxide and gold nanoparticles for voltammetric determination of hydroquinone, catechol, and resorcinol, Microchim. Acta. 186 (2019). https://doi.org/10.1007/s00604-019-3771-4.

[37] M. Vasudevan, M.J.Y. Tai, V. Perumal, S.C.B. Gopinath, S.S. Murthe, M. Ovinis, N.M. Mohamed, N. Joshi, Cellulose acetate-MoS2 nanopetal hybrid: A highly sensitive and selective electrochemical aptasensor of Troponin I for the early diagnosis of Acute Myocardial Infarction, J. Taiwan Inst. Chem. Eng. 118 (2021) 245–253. https://doi.org/10.1016/j.jtice.2021.01.016.

[38] K. Gobalu, M. Vasudevan, S.C.B. Gopinath, V. Perumal, M. Ovinis, Molybdenum disulphide/cellulose acetate nanofiber composite on screen printed electrodes for detecting cardiac Troponin by electrical impedance spectroscopy, Cellulose. 28 (2021). https://doi.org/10.1007/s10570-021-03911-w.

[39] D. Gupta, V. Chauhan, R. Kumar, A comprehensive review on synthesis and applications of molybdenum disulfide (MoS2) material: Past and recent developments, Inorg. Chem. Commun. 121 (2020). https://doi.org/10.1016/j.inoche.2020.108200.

[40] D. Chen, W. Chen, L. Ma, G. Ji, K. Chang, J.Y. Lee, Graphene-like layered metal dichalcogenide/graphene composites: Synthesis and applications in energy storage and conversion, Mater. Today. 17 (2014) 184–193. https://doi.org/10.1016/j.mattod.2014.04.001.

[41] C. Thulluri, S.R. Pinnamaneni, P.R. Shetty, U. Addepally, Synthesis of Lignin-Based Nanomaterials/Nanocomposites: Recent Trends and Future Perspectives, Ind. Biotechnol. 12 (2016) 153–160. https://doi.org/10.1089/ind.2015.0022.

[42] X. Li, Jingchen; Zheng,Jie; Yu, Yadong; Su, Zhen; Zhang, Lihui; Chen, Facile synthesis of rGO-MoS2-Ag nanocomposites with long-term antimicrobial activities, Nanotechnology. (2019) 0–27. https://iopscience.iop.org/article/10.1088/2053-1583/abe778.

[43] Y. Li, D. Yang, P. Li, Z. Li, Lignin as a multi-functional agent for the synthesis of Ag nanoparticles and its application in antibacterial coatings, J. Mater. Res. Technol. 17 (2022) 3211–3220. https://doi.org/10.1016/j.jmrt.2022.02.049.

[44] S. Hu, Y. Lo Hsieh, Silver nanoparticle synthesis using lignin as reducing and capping agents: A kinetic and mechanistic study, Int. J. Biol. Macromol. 82 (2016) 856–862. https://doi.org/10.1016/j.ijbiomac.2015.09.066.

[45] T.T.T. Phan, T.T.H. Nguyen, H.T. Huu, T.T. Truong, L.T. Nguyen, V.T. Nguyen, V.A. Tran, T.L. Nguyen, H.L. Nguyen, V. Vo, Hydrothermal Synthesis of MoS2/rGO Heterostructures for Photocatalytic Degradation of Rhodamine B under Visible Light, J. Nanomater. 2021 (2021). https://doi.org/10.1155/2021/9941202.

[46] W. Zhang, Y. Lei, Q. Jiang, F. Ming, P.M.F.J. Costa, H.N. Alshareef, 3D Laser Scribed Graphene Derived from Carbon Nanospheres: An Ultrahigh-Power Electrode for Supercapacitors, Small Methods. 3 (2019). https://doi.org/10.1002/smtd.201900005.

[47] M. Govindasamy, S.M. Chen, V. Mani, M. Akilarasan, S. Kogularasu, B. Subramani, Nanocomposites composed of layered molybdenum disulfide and graphene for highly

754 sensitive amperometric determination of methyl parathion, Microchim. Acta. 184 (2017)
755 725–733. https://doi.org/10.1007/s00604-016-2062-6.

756 [48] F. Pan, J. Wang, Z. Yang, L. Gu, Y. Yu, MoS2-graphene nanosheet-CNT hybrids with
757 excellent electrochemical performances for lithium-ion batteries, RSC Adv. 5 (2015)
758 77518–77526. https://doi.org/10.1039/c5ra13262b.

759 [49] Y.B. Li, Y. Bando, D. Golberg, MoS2 nanoflowers and their field-emission properties,
760 Appl. Phys. Lett. 82 (2003) 1962–1964. https://doi.org/10.1063/1.1563307.

761 [50] Y. Lu, X. Yao, J. Yin, G. Peng, P. Cui, X. Xu, MoS2 nanoflowers consisting of
762 nanosheets with a controllable interlayer distance as high-performance lithium ion
763 battery anodes, RSC Adv. 5 (2015) 7938–7943. https://doi.org/10.1039/c4ra14026e.

764 [51] A.R. Fareza, F.A.A. Nugroho, V. Fauzia, Facile synthesis of 1T-MOS2 nanoflowers
765 using hydrothermal method, Mater. Sci. Forum. 1028 MSF (2021) 173–178.
766 https://doi.org/10.4028/www.scientific.net/MSF.1028.173.

767 [52] N. Sharma, V. Sharma, Y. Jain, M. Kumari, R. Gupta, S.K. Sharma, K. Sachdev,
768 Synthesis and Characterization of Graphene Oxide (GO) and Reduced Graphene
769 Oxide (rGO) for Gas Sensing Application, Macromol. Symp. 1700006 (2017) 1–5.
770 https://doi.org/10.1002/masy.201700006.

771 [53] J.R. Anasdass, P. Kannaiyan, Palladium nanoparticle-decorated reduced graphene
772 oxide sheets synthesized using Ficus carica fruit extract : A catalyst for Suzuki cross-
773 coupling reactions, PLOS ONE 13(2) E0193281. (2018) 1–10.

774 [54] S.K. Abdel-aal, A.I. Beskrovnyi, A.M. Ionov, R.N. Mozhchil, A.S. Abdel-rahman,
775 Structure Investigation by Neutron Diffraction and X-Ray Diffraction of Graphene
776 Nanocomposite CuO – rGO Prepared by Low-Cost Method, Phys. Status Solidi A.
777 2100138 (2021) 1–9. https://doi.org/10.1002/pssa.202100138.

778 [55] A. Kumar, M.S. Aathira, S. Jain, Silver doped reduced graphene oxide as promising
779 plasmonic photocatalyst for oxidative coupling of benzylamines under visible light
780 irradiation., New J. Chem. (2019) 1–8. https://doi.org/10.1039/C9NJ00852G.Volume.

781 [56] J. Chen, Y. Xia, J. Yang, B. Chen, Fabrication of monolayer - MoS 2 / rGO hybrids with
782 excellent tribological performances through a surfactant-assisted hydrothermal route,
783 Appl. Phys. A. 430 (2018) 1–8. https://doi.org/10.1007/s00339-018-1843-7.

784 [57] R. Zamiri, H.A. Ahangar, A. Zakaria, G. Zamiri, M. Shabani, B. Singh, J.M.F. Ferreira,
785 The structural and optical constants of Ag2S semiconductor nanostructure in the Far-

Infrared, Chem. Cent. J. 9 (2015) 4–9. https://doi.org/10.1186/s13065-015-0099-y.

[58] C.R.- Graphene, M.A. Aldosari, K. Bin, B. Alsaud, A. Othman, M. Al-hindawi, N.H. Faisal, R. Ahmed, F.M. Michael, M.R. Krishnan, Microwave Irradiation Synthesis and Nanoparticle Nanocomposites and Their Anti-Microbial Activity, Polymers (Basel). 12, 1155 (2020) 1–16.

[59] R. Vinoth, I.M. Patil, A. Pandikumar, B.A. Kakade, N.M. Huang, D.D. Dionysios, B. Neppolian, Synergistically Enhanced Electrocatalytic Performance of an N - Doped Graphene Quantum Dot-Decorated 3D MoS 2 − Graphene Nanohybrid for Oxygen Reduction Reaction, ACS Omega. 1 (2016) 971–980. https://doi.org/10.1021/acsomega.6b00275.

[60] F. Bensebaa, Y. Zhou, Y. Deslandes, E. Kruus, T.H. Ellis, XPS study of metal-sulfur bonds in metal-alkanethiolate materials, Surf. Sci. 405 (1998).

[61] T. Laiho, J.A. Leiro, J. Lukkari, XPS study of irradiation damage and different metal-sulfur bonds in dodecanethiol monolayers on gold and platinum surfaces, Appl. Surf. Sci. 212–213 (2003) 525–529. https://doi.org/10.1016/S0169-4332(03)00462-8.

[62] M. Ren, J. Bao, P. Wang, C. Wang, Y. Ao, Titanium Phosphate Nanoplates Modified With AgBr @ Ag Nanoparticles : A Novel Heterostructured Photocatalyst With Significantly Enhanced Visible Light Responsive Activity, Front. Chem. 6 (2018) 1–9. https://doi.org/10.3389/fchem.2018.00489.

[63] F.T. Johra, J. Lee, W. Jung, Facile and safe graphene preparation on solution based platform Journal of Industrial and Engineering Chemistry Facile and safe graphene preparation on solution based platform, J. Ind. Eng. Chem. 20 (2014) 2883–2887. https://doi.org/10.1016/j.jiec.2013.11.022.

[64] A. Ganguly, S. Sharma, P. Papakonstantinou, J. Hamilton, Probing the Thermal Deoxygenation of Graphene Oxide Using High-Resolution In Situ X-ray-Based Spectroscopies, J. Phys. Chem. 115 (2011) 17009–17019.

[65] F. Ghasemi, S. Mohajerzadeh, A Sequential Solvent Exchange Method for Controlled Exfoliation of MoS 2 suitable for Phototransistor Fabrication, ACS Appl. Mater. Interfaces. (2016). https://doi.org/10.1021/acsami.6b07211.

[66] Y. Lei, P. Guo, M. Jia, W. Wang, J. Liu, One-step photodeposition synthesis of TiO 2 nanobelts / MoS 2 quantum dots / rGO ternary composite with remarkably enhanced photocatalytic activity, J. Mater. Sci. (2020). https://doi.org/10.1007/s10853-020-05067-7.

[67] L. Zou, R. Qu, H. Gao, X. Guan, X. Qi, C. Liu, Z. Zhang, X. Lei, Results in Physics MoS 2 / RGO hybrids prepared by a hydrothermal route as a highly e ffi cient catalytic for sonocatalytic degradation of methylene blue, Results Phys. 14 (2019) 102458. https://doi.org/10.1016/j.rinp.2019.102458.

[68] Y. Wang, M. Zhen, H. Liu, Interlayer-expanded MoS 2 / graphene composites as anode materials for high-performance lithium-ion batteries, J. Solid State Electrochem. (2018).

[69] U. Hydrothermalmethod, Synthesis Of Mos 2 / Graphene Nanocomposite By Facile Synthesis Of Mos 2 / Graphene Nanocomposite By Facile Ultrasonic-Assisted Hydrothermalmethod, Vietnam J. Sci. Technol. 6 (2019) 703–713. https://doi.org/10.15625/2525-2518/57/6/13955.

[70] H.S. Magar, R.Y.A. Hassan, A. Mulchandani, Electrochemical Impedance Spectroscopy (EIS): Principles, Construction, and Biosensing Applications, Sensors. 21, 6578 (2021) 1–21. https://doi.org/10.3390/s21196578.

[71] N.O. Laschuk, E.B. Easton, O. V Zenkina, Reducing the resistance for the use of electrochemical impedance spectroscopy analysis in materials chemistry, RCS Adv. 11 (2021) 27925–27936. https://doi.org/10.1039/d1ra03785d.

[72] M. Sypabekova, A. Hagemann, D. Rho, S. Kim, Review : 3-Aminopropyltriethoxysilane (APTES) Deposition Methods on Oxide Surfaces in Solution and Vapor Phases for Biosensing Applications, (2023).

[73] S.F. Chin, L.S. Lim, S.C. Pang, M.S.H. Sum, D. Perera, Carbon nanoparticle modified screen printed carbon electrode as a disposable electrochemical immunosensor strip for the detection of Japanese encephalitis virus, Microchim. Acta. 184 (2017) 491–497. https://doi.org/10.1007/s00604-016-2029-7.

[74] M. Roushani, K. Ghanbari, An electrochemical aptasensor for streptomycin based on covalent attachment of the aptamer onto a mesoporous silica thin film-coated gold electrode, Microchim. Acta. 186 (2019) 0–8. https://doi.org/10.1007/s00604-018-3191-x.

[75] M. Vasudevan, M.J.Y. Tai, V. Perumal, S.C.B. Gopinath, S.S. Murthe, M. Ovinis, N.M. Mohamed, N. Joshi, Cellulose acetate-MoS<inf>2</inf> nanopetal hybrid: A highly sensitive and selective electrochemical aptasensor of Troponin I for the early diagnosis of Acute Myocardial Infarction, J. Taiwan Inst. Chem. Eng. 118 (2021). https://doi.org/10.1016/j.jtice.2021.01.016.

[76] S.C.B. Gopinath, V. Perumal, S.R. Balakrishnan, M.K. Md Arshad, T. Lakshmipriya, R.

Haarindraprasad, U. Hashim, Aptamer-based determination of ATP by using a functionalized impedimetric nanosensor and mediation by a triangular junction transducer, Microchim. Acta. 184 (2017) 4425–4431. https://doi.org/10.1007/s00604-017-2485-8.

[77] S.R. Balakrishnan, U. Hashim, G.R. Letchumanan, M. Kashif, A.R. Ruslinda, W.W. Liu, P. Veeradasan, R. Haarindra Prasad, K.L. Foo, P. Poopalan, Development of highly sensitive polysilicon nanogap with APTES/GOx based lab-on-chip biosensor to determine low levels of salivary glucose, Sensors Actuators, A Phys. 220 (2014) 101–111. https://doi.org/10.1016/j.sna.2014.09.027.

Figure legends

Figure 1: Schematic illustration of the steps involved in the synthesis of aptamer-based 3D LSG_MoS2_Ag NPs hybrid structure on screen printed carbon electrode (SPCE).

Figure 2: FESEM images of 3D LSG_MoS2_Ag NPs hybrid structure. (a) Low and (b) high magnifications images of 3D LSG_MoS2_Ag NPs-0.5; (c) Low and (d) high magnifications of 3D LSG_MoS2_Ag NPs-1.0; (e) Low and (f) high magnifications of 3D LSG_MoS2_Ag NPs-1.5 and (g) Low and (h) high magnifications of 3D LSG_MoS2_Ag NPs-2.0. The scanning electron microscope uses a focused beam of high-energy electrons up to 30 kV.

Figure 3: XRD image of (a) 3D LSG, (b) Ag NPs, (c) MoS2 nanoflower, (d) 3D LSG_MoS2_Ag NPs-0.5; (e) 3D LSG_MoS2_Ag NPs-1.0; (f) 3D LSG_MoS2_Ag NPs-1.5 and (g) 3D LSG_MoS2_Ag NPs-2.0. Showing the semi-crystalline peaks of fabricated nanostructures. FT-IR spectra image of fabricated (h) 3D LSG, (i) Ag NPs, (j) MoS2 nanoflower, (k) 3D LSG_MoS2_Ag NPs-0.5; (l) 3D LSG_MoS2_Ag NPs-1.0; (m) 3D LSG_MoS2_Ag NPs-1.5 and (n) 3D LSG_MoS2_Ag NPs-2.0. Absorption regions are shown from 400 to 3700 cm−1.

Figure 4: (a) Survey scan of XPS core level spectra taken on 3D LSG_MoS2_Ag NPs. The binding energy of (b) carbon, C1s; (c) oxygen, O1s; (d) molybdenum, Mo3d; (e) silver, Ag3d and (d) sulphur, S2p electrons.

Figure 5: (a) Nyquist plot on surface modifications on SPCE electrode. (b) Nyquist plot of 3D LSG_MoS2_Ag NPs hybrid nanofibers at 0.5, 1.0, 1.5 and 2.0 g on SPCE electrodes. c) Analysis of the limit of detection. Different concentrations of the target (100 aM to 100 pM) through impedance spectroscopy analysis were shown. (d) Impedance spectra measurement. It is to identify the selectivity of the biosensor. Human serum in the presence of other biomolecules was tested.

Figure 6: (a) Linear regression curve. Different concentrations of the target with a linear equation of ΔRct; (b) A bar diagram on the selectivity of the biosensor and its Rct value; (c) Reproducibility of the biosensor using 5 different SPE electrodes (d) Stability of the biosensor. Tested for 6 weeks against Rct is shown.