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Research Article

Vertically Aligned Graphene Prepared by Photonic Annealing for Ultrasensitive Biosensors

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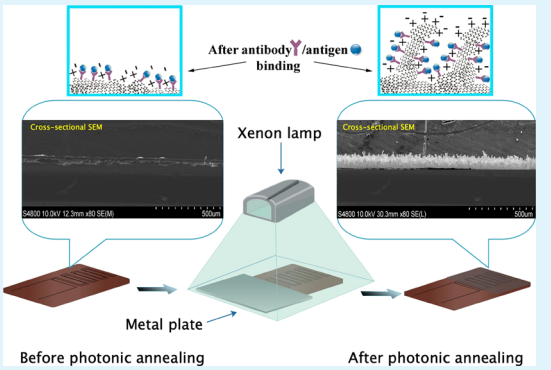
ABSTRACT: Graphene exhibits excellent physical, electronic, and chemical properties that are highly desirable for biosensing applications. However, most graphene biosensors are based on graphene lying flat on a substrate and therefore do not utilize its maximum specific surface area for ultrasensitive detection. Herein, we report the novel use of photonic annealing on a flexographically printed graphene−ethyl cellulose composite to produce vertically aligned graphene (VAG) biosensors for ultrasensitive detection of algal toxins in drinking water. These VAG structures, which maximized the specific surface area of graphene, were formed by partial removal of the polymeric binder upon applying intense pulsed light on the printed graphene. A label-free and low-cost VAG biosensor based on a non-faradaic electrochemical impedance spectroscopy technique was fabricated. The biosensor exhibited a limit of detection of 1.2 ng/L for microcystin-LR in local tap water. Such an ultrasensitive VAG biosensor is suitable for low-cost mass production using an integrated roll-to-roll flexographic printing with rapid photonic annealing technique.



KEYWORDS: *graphene, flexographic printing, photonic annealing, biosensor, algal toxins, non-faradaic EIS*

Recent advances in graphene-related nanomaterials have led to major technological advancement in solar energy harvesting and storage,[1] water desalination[2,3] electronics,[4] and so forth. The superiority in electrical conductivity, theoretical surface area,[5] biocompatibility, and multifunctionalities[6] of graphene are envisaged to provide extraordinary performance and affordable solutions for next-generation biosensing platforms.[7−11] Indeed, there has been significant research interest in developing graphene-based biosensors that can provide ultrasensitive and early alert detection. To date, most of these biosensors have been reported with graphene lying flat on an electrode substrate. Such configuration does not fully utilize the specific surface area of graphene for biosensing applications. To maximize its surface area, graphene has been shown to be grown vertically on a substrate via chemical vapor deposition.[12,13] However, the growth technique is costly, time-consuming, and requires complex processing steps. Therefore, it is of great interest to develop an ultrasensitive vertically aligned graphene (VAG) biosensor that is feasible for cost-effective mass production to bring the technology to mass market.

Roll-to-roll flexographic printing is a high throughput technique that allows high-speed selective patterning and direct deposition of materials (via ink formulations) without the use of expensive photolithography and high vacuum equipment.[14,15] Previously, the authors flexographically printed electrochemical enzymatic and impedimetric biosensors based on ZnO nanowires[16] and a nanotextured surface of ZnO thin films[17] on organic substrates, respectively. The use of organic substrates with the printing technique would significantly reduce the production cost. Flexographic printing of graphene nanoplatelets (GNPs) and carbon nanotubes has also been performed for application in dye-sensitized solar cells[18] and resistive heaters,[19] respectively.

In this work, flexographic printing was used to produce graphene-interdigitated electrodes using ink composed of ball-milled GNPs and ethyl cellulose (EC). The latter was used as a binder as well as a dispersion stabilizer in the graphene ink. The printed electrodes were then photonically annealed, which resulted in dense VAG at the electrodes after partial removal of EC. The annealing technique uses intense pulsed light to provide rapid (e.g., milliseconds) localized heating because of optical absorption at the deposited film, while limiting thermal damage to the substrate. It has gained much attention recently; for example, Paglia et al. employed photonic annealing to reduce inkjet-printed CuO nanocrystals on a polyethylene terephthalate (PET) substrate into a sintered copper film.[20] Arapov et al. observed that photonic annealing can enhance

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the conductivity of polymeric binder-containing graphene ink printed on a PET substrate using a stencil method.[21] The use of photonic annealing to produce a dense porous microstructure in printed graphene, consisting of nitrocellulose as a binder, for microsupercapacitor application was reported by Secor et al.[22] Das et al. reported rapid pulse laser annealing on inkjet-printed graphene for paper-based electronics and electrochemical devices.[23,24] To the best knowledge of the authors, photonic annealing has not been performed on flexographically printed graphene films, which produced VAG electrodes for biosensor. These graphene-based electrodes are potentially suitable for ultrasensitive biosensing applications because of their significantly increased surface area.

Herein, we report the novel use of photonic annealing on flexographically printed graphene films to produce ultrasensitive VAG biosensors, which are suitable for low-cost mass production by integrating roll-to-roll flexographic printing with a rapid photonic annealing technique. The VAG biosensors were based on a non-faradaic electrochemical impedance spectroscopy (EIS) technique, which offered label-free, rapid, and direct detection of biomarkers. Unlike faradaic EIS biosensors, they do not require redox species, and hence they are easy to operate and are well-suited for real-time monitoring. Any physical change at the biosensor because of biorecognition events, such as antigen−antibody binding, will result in the perturbation of double layer capacitance at the electrode surface leading to a phase shift between the input voltage and output current over a range of frequencies.[25,26] Functionalization of the biosensor was performed using the spray-coating technique to preserve the VAG structures. Key fabrication processes of the biosensor are illustrated in Figure S1. The VAG biosensors were functionalized to detect algal toxin microcystin-LR (MC-LR), a most widely occurring variant and well-known potent hepatotoxin, in drinking water because of increasing occurrence of harmful algal bloom events around the globe.[27] Algal toxins are posing enormous challenges to current water supply practice, in terms of both monitoring and treatment control.[28,29] Following a significant harmful algal bloom event, there is an urgent need to develop a cost-effective and quick turnaround monitoring technology.[30,31] In this context, the VAG biosensor in this work exhibited ultrasensitive detection of MC-LR and demonstrated excellent selectivity against other algal toxin variants and interfering compounds.

EXPERIMENTAL SECTION

Preparation of Graphene Ink for Flexographic Printing. GNPs with a diameter of 2−7 μm and a thickness of 2−10 nm were purchased from Advanced Chemicals Supplier (California, USA). GNPs (1 g) and 20 mL of dipropylene glycol methyl ether (DPGME) were mixed together and then ball-milled at 300 rpm using grinding balls with a diameter of 5 mm in a FRITSCH Ball Miller (FRITSCH, Idar-Oberstein, Germany) for 24 h to produce GNPs with fewer graphene layers. EC (2 g) was mixed with 20 mL of isopropanol (IPA) with 10 min ultrasonication to form EC solution. The 24 h ball-milled GNP mixture was mixed with the ready-made EC solution and then ball-milled for another 2 h to obtain a stable GNP−EC composite. HDPlas conductive carbon ink was purchased from HDPlas ink (Haydale Limited, Carmarthenshire, UK), and a solution was prepared by adding 41 g HDPlas powder into a 50/50 v/v IPA/DPGME solvent mixture.

A polyimide substrate was cleaned before printing by first dipping in IPA and then in acetone both with 10 min sonication and drying under nitrogen. Flexographic printing was carried out using a RK Flexiproof 100 printer (RK Print-Coat Instruments Ltd. Hertfordshire, UK). A flexible printing plate with the design of interdigitated electrodes was taped on a flexographic plate roller, while the polyimide substrate was fixed on an impression roller. The GNP ink was introduced between a doctor blade and an anilox roller (with a volume of 24 cm^3/m^2), which transferred the ink to the printing plate and was simultaneously printed onto the polyimide substrate. To ensure good conductivity, the substrate was first printed with a layer of HDPlas as a conductive base followed by the printing of the sensing layer of GNPs over the top. Between each printing cycle, the printed layer was blow-dried using a hair dryer. Interdigitated electrodes featuring an array of eight fingers (e.g., each finger has a length of 5.5 mm, a width of 1 mm, and a separation of 1 mm between each other) with a total working electrode area of 2.25 mm^2 were printed using the flexography technique.

Fabrication of VAG Biosensors. The flexographically printed graphene electrodes were photonically annealed using intense pulsed light from a PulseForge1300 photonic curing system (NovaCentrix, Texas, USA). The optimal photonic annealing condition was a single pulse with a radiant power of 3.3 kW/cm^2. Under this condition, EC was partially removed with maximum density of the VAG structure. The photonically annealed VAG surface was treated with oxygen plasma for 10 min (Henniker HPT-100, flow rate 20 s.c.c.m.), which was followed by incubation in water vapor for 1 h. Functionalization of the biosensor was performed using an IWATA Eclipse HP-CS Airbrush (IWATA Eclipse, Japan). The spray-coating conditions were optimized (e.g., spraying distance and compressed air pressure) to preserve the VAG structures of which the results are shown in Figure S4.

The following VAG biosensor preparation procedure is illustrated in Figure 1. After spray coating (3-aminopropyl)triethoxysilane

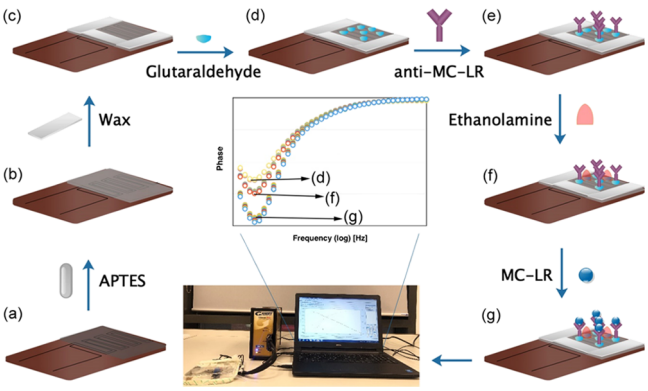

Figure 1. Surface functionalization procedure of the VAG biosensor from (a) to (g) and non-faradaic EIS measurement at the step (d), (f), and (g), respectively.

(APTES), the electrodes were annealed in nitrogen at 120 °C for 20 min. Previously, the annealing process has been shown to densify the APTES layer, which would provide the essential blocking properties.[32] A sensing area of 5 mm × 8 mm was marked by paraffin wax over the interdigitated area. A volume of 125 μL of 5% glutaraldehyde prepared in phosphate buffered saline (PBS) solution was sprayed onto the sensing area. PBS solution of 10 mM at pH 7.4 ± 0.1 was purchased from Fisher Scientific (Loughborough, UK). The same area was then spray coated with 100 μL MC-LR antibodies (20 μg/mL in PBS solution). As a result, two separate aldehyde groups of the glutaraldehyde on each end of carbon chains would form covalent bonds with the amine groups exposed on the APTES layer and those of MC-LR antibody (mouse IgG1, Alexi Biochemicals Inc), respectively. This was followed by spray coating with 125 μL ethanolamine (5 mM) in the same area to neutralize any aldehyde groups from the unreacted glutaraldehyde. Basic solution (pH 9.5) addition would also assist the removal of loosened antibodies on the VAG biosensor surface.[33]

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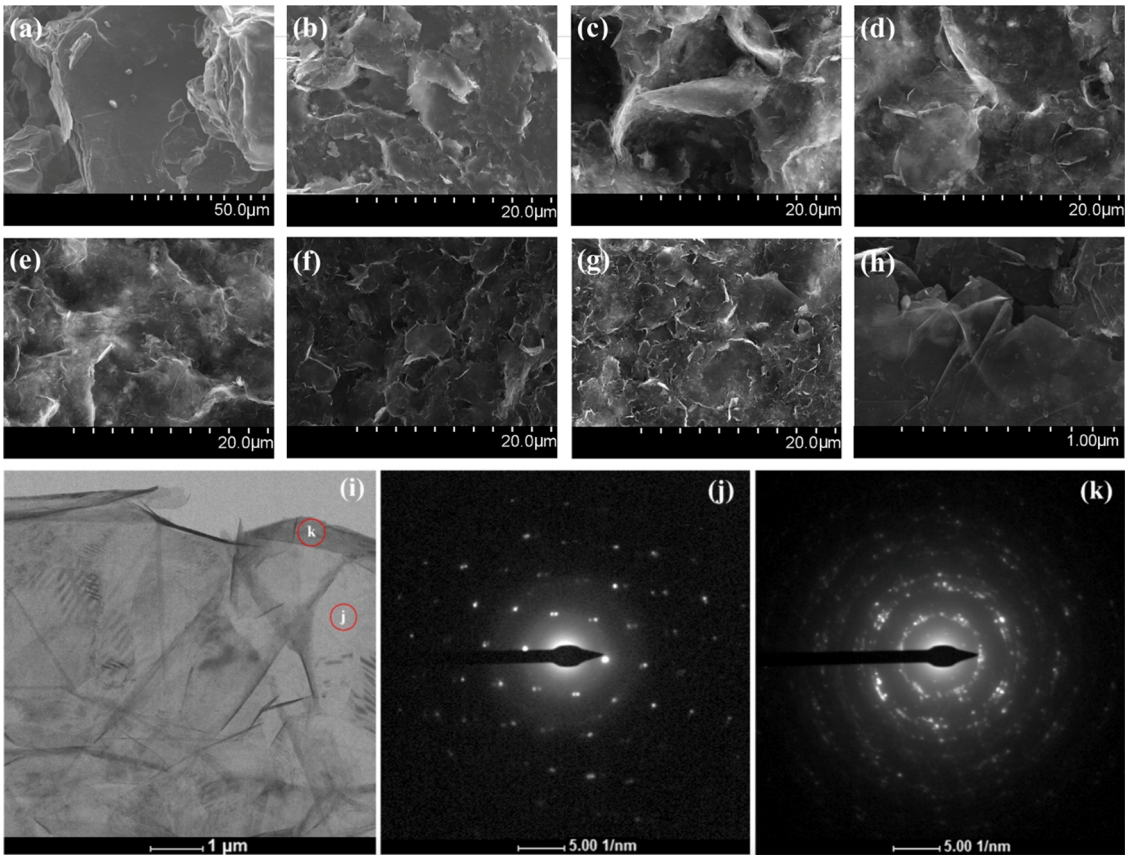

Figure 2. SEM images of GNPs after different durations of ball milling: (a) without ball milling, (b) after 4 h, (c) 8 h, (d) 12 h, (e) 16 h, (f) 20 h, and (g) 24 h ball milling (2.5 k magnification), and (h) higher magnification (i.e., 50 k) SEM image of 24 h ball milling. (i) TEM image of 24 h ball-milled GNPs for flexographic printing. (j) and (k) SAED patterns from regions marked accordingly in (i).

Characterization. Morphology of the printed graphene electrodes was investigated using a Hitachi S-4800 scanning electron microscope. An integrated energy dispersive X-ray (EDX) spectroscopy was applied to investigate the presence of APTES on the VAG surface via element mapping of silicon. An FEI TALOS F200X field-emission gun transmission electron microscope was employed to study the lattice structure of ball-milled GNPs. The structure and defects of VAG were studied using an inVia confocal Raman microscope (Renishaw). Surface functional groups at the VAG electrodes after plasma and water vapor treatments were investigated using Fourier transform infrared spectroscopy (FTIR, Perkin Elmer Spectrum 100) and X-ray photoelectron spectroscopy (XPS, Kratos Axis Supra) performed at room temperature. Surface roughness and topography of VAG biosensors were probed using white light interferometry (Veeco Wyko NT9300). Cyclic voltammetry (CV) and non-faradaic EIS were carried out on a Gamry Reference 600+ potentiostat.

Non-faradaic EIS Biosensing of MC-LR in Water. First, EIS measurement was carried out on a 100 μL of PBS, and the phase obtained was used as a baseline. The pure PBS was then replaced with 100 μL of PBS spiked with MC-LR (Enzo Life Sciences); after 15 min incubation, a further EIS measurement was carried out. The change in phase between the two EIS measurements was used as a non-faradaic biosensing response. The same procedure was repeated for five different concentrations ranging from 0.001 to 10 μg/L and a negative sample without any MC-LR.

To assess the specificity of detection, the VAG biosensors were incubated in a mixture containing 0.1 μg/L MC-RR, MC-LR, and MC-LW for 15 min. Non-faradaic EIS measurements using the same procedure were then performed on the mixture, and the results were compared with those of biosensors tested solely on MC-LR with the same concentration of 0.1 μg/L. Local tap water samples (Swansea, UK) were also spiked with MC-LR at the same concentration to validate the biosensor performance on real drinking water, which contains many different metal ions and chemicals (e.g., fluoride, chlorine, etc.).

RESULTS AND DISCUSSION

Morphology of Ball-Milled GNPs. GNPs were ball-milled to produce graphene sheets for the fabrication of graphene-interdigitated electrodes. Morphology of the GNPs after different durations of ball milling was studied using scanning electron microscopy (SEM). Figure 2a shows the SEM image of thick and multilayered GNPs prior to ball milling, indicating severe agglomeration. It can also be seen that the size of GNPs was reduced drastically as the ball milling duration increased from 4 to 24 h as shown in Figure 2b−g, respectively. A higher magnification image of GNPs after 24 h ball milling is shown in Figure 2h. The GNPs became much thinner with good transparency, which suggests that graphene sheets with fewer layers were produced. The transmission electron microscopy (TEM) image of GNPs after 24 h ball milling is shown in Figure 2i. It showed mono−/bi-layer thick graphene, which was almost transparent under the electron beam and consisted of wrinkles, creases, and overlapping sheets. Figure 2j,k show selected area electron diffraction (SAED) patterns acquired from locations 'j' and 'k' in Figure 2i, respectively. A distinctive hexagonal diffraction pattern shown in Figure 2j was associated with single or bi-layered graphene sheets. On the other hand, the multiple rotationally misaligned hexagonal patterns were observed in Figure 2k, indicating that some areas were still multilayer thick especially around the edges.[34] The 24 h ball-

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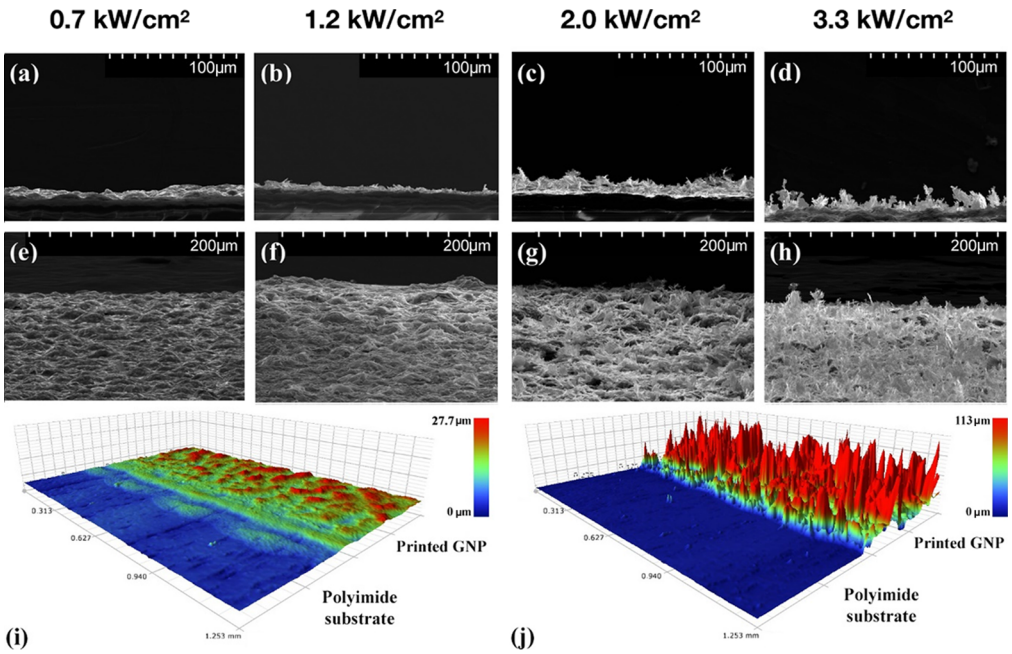

Figure 3. SEM images of VAG structures under different photonic annealing conditions: (a–d) cross-sectional and (e–h) tilted view of VAG after photonic annealing at an increased radiant power of 0.7, 1.2, 2, and 3.3 kW/cm^2, respectively; (i) and (j) are topographical mapping using the white light interferometry technique on the graphene electrode before and after photonic annealing, respectively.

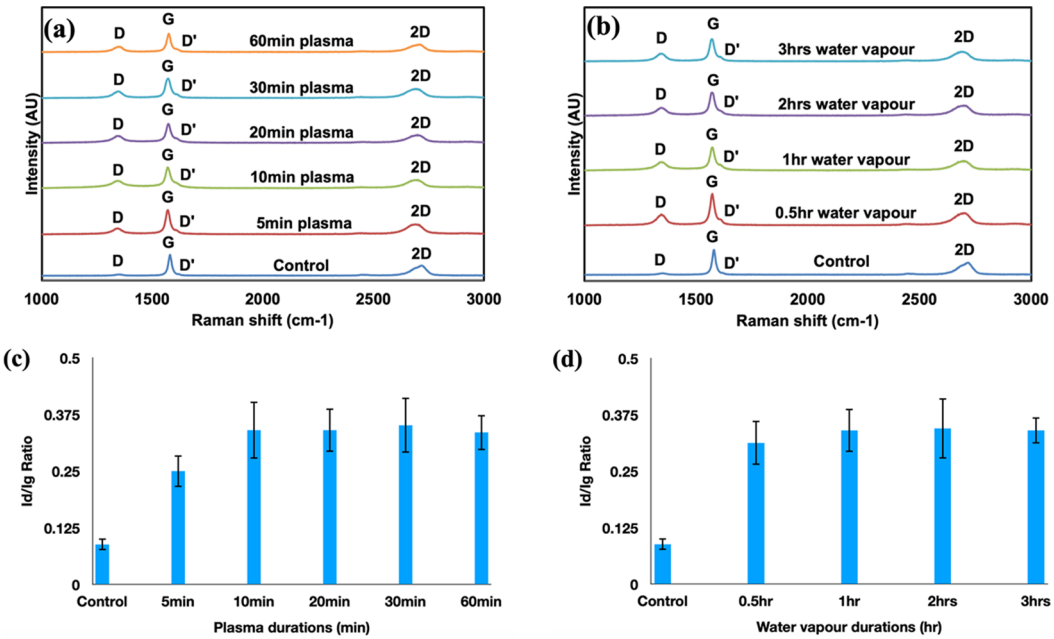

Figure 4. (a) Raman spectra of VAG after different durations of oxygen plasma treatment with 1 h water vapor incubation. (b) Raman spectra of VAG after 10 min oxygen plasma treatment with different durations of water vapor incubation. The ratios of I_D/I_G for each condition corresponding to (a) and (b) are shown in (c) and (d), respectively.

milled GNPs were used in the formulation of graphene ink suitable for flexographic printing of interdigitated electrodes.

2.2. VAG Characterization of Photonically Annealed Graphene Electrodes. Photonic annealing was performed on the flexographically printed graphene electrodes, and the morphology of the electrodes was studied at different radiant power densities. Cross-sectional and titled SEM images of the graphene electrodes after photonic annealing are shown in Figure 3a–h. When the power density was increased to 2.5 kW/cm^2, VAG began to appear at the electrode in Figure 3e,f. Density of the VAG structure reached maximum at 3.3 kW/cm^2 (shown in Figure 3g,h). Beyond 3.3 kW/cm^2, the graphene started to come off from the substrate. Total delamination of the graphene layer occurred at a power density greater than 5.4 kW/cm^2. The intense pulsed light resulted in localized heating at the printed graphene electrodes leading to rapid removal of polymeric binders (e.g., EC) used in the ink and subsequent formation of VAG. The optimal radiant power density was found to be 3.3 kW/cm^2, so denser VAG would provide a larger specific surface area. The effect of

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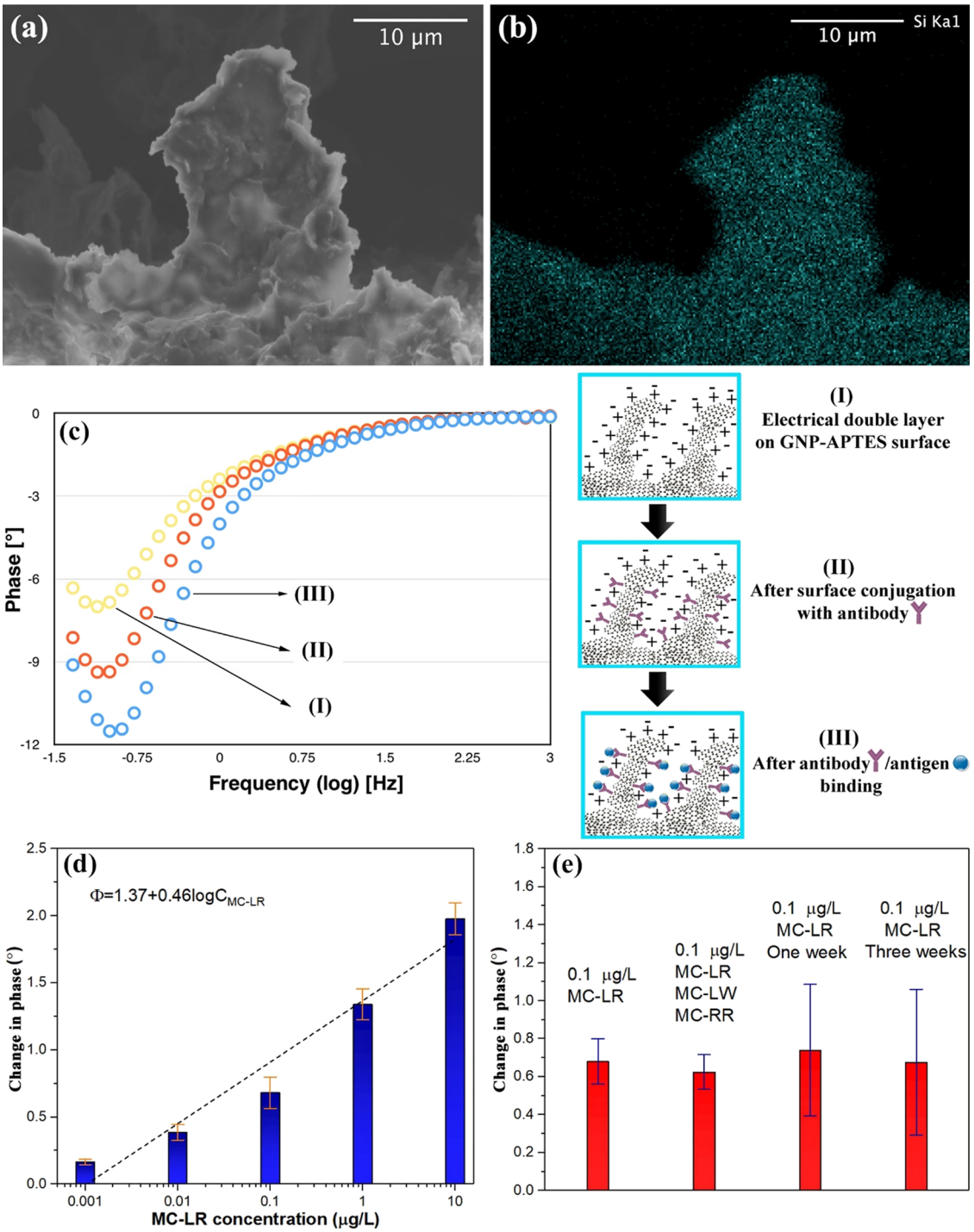

Figure 5. (a) High magnification cross-sectional SEM image and corresponding (b) EDX mapping of silicon on the VAG electrodes; (c) Bode plots from non-faradaic EIS measurements of the VAG biosensor after APTES coating, immobilization of antibody and MC-LR conjugation; (d) change in phase shift, Φ, at VAG biosensors with MC-LR concentrations (C_{MC-LR}) (error bars: SD, $n = 3$); (e) selectivity and storability tests of the VAG biosensors (error bars: SD, $n = 3$).

photonic annealing on surface topography of the printed graphene was characterized using white light interferometry as shown in Figure 3i,j. It further confirmed that photonic annealing has produced a dense forest-like structure of graphene at the surface of electrodes. An average surface roughness was measured to be 1.61 ± 0.14 μm before photonic annealing and significantly increased to 15.88 ± 2.12 μm after photonic annealing because of the formation of VAG.

Optimization of Hydroxylation on the Surface of VAG Biosensors. Prior to functionalization of the VAG for biosensing application, its surface was first treated with oxygen plasma coupled with water vapor incubation to generate hydroxyl groups, which were required to bond with APTES, and studied using Raman spectroscopy.[35] For 'control' or pristine graphene, there were two signature bands produced by the Stokes phonon energy shift in the Raman spectra: the G band (1580 cm^{-1}) represented the in-plane vibration of

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graphene lattice and the two-dimensional (2D) band (2690 cm^{-1}) represented a second-order overtone of a different in-plane vibration in Figure 4a,b.[36,37] The D (1350 cm^{-1}) and D' (1620 cm^{-1}) bands in the Raman spectra are generally related to intervalley and intravalley defect scattering, respectively. The intensity of these two bands increases when disorder or defects are introduced into the graphene structure.[38] Figure 4a shows Raman spectra of VAG treated with different durations of oxygen plasma but with the same duration of water vapor incubation, while Figure 4b shows VAG treated with the same plasma duration but with different durations of water vapor incubation. The I_D/I_G, D and G band intensity ratio, is useful to assess the extent of defect formation in graphene after plasma treatment.[39] The I_D/I_G ratio was found in Figure 4c,d to increase sharply and then began to plateau after 10 min of plasma treatment and 1 h of water vapor incubation, which was set as optimal surface treatment conditions for VAG biosensors. The increase of the I_D/I_G ratio was probably caused by hydroxyl groups generated at the surface of VAG. In typical FTIR analysis, hydroxyl groups are usually observed as a broad peak near 3500 cm^{-1} regions.[40] In this study (see Figure S2), a relatively sharp peak was apparent at ∼3676 cm^{-1}, and its intensity increased significantly after oxygen plasma and water vapor treatments. This further confirmed the generation of hydroxyl groups on the VAG surface, whereas slight shifting and sharpening of the peak could be due to the absence of hydrogen bonding.[41] Finally, the I_{2D}/I_G ratio of ∼1.42 and full width at half-maximum of the 2D peaks of ∼92 cm^{-1} indicated that optimized VAG at the electrodes consists of graphene mostly of more than five layers.[42,43] This observation was consistent with previous SAED analysis on the VAG.

Chemical bond analysis of the VAG before and after oxygen plasma/water vapor surface treatment was carried out using the XPS technique, which is shown in Figure S3a. There were two distinctive peaks around the binding energy of 284 and 532 eV related to C1s and O1s, respectively.[44] The C1s core level before and after surface treatment, shown in Figure S3b,c, respectively, was deconvoluted using two peaks. These peaks positioned at ∼284 and ∼ 285.6 eV generally belong to graphitic carbon (C−C) and carbonyl group (C═O), respectively.[45] The graphitic carbon (C−C) was attributed to the graphene, while the presence of carbonyl groups (C═O) was probably due to the graphene defects. Figure S3d,e show the deconvoluted O1s core level before and after surface treatment, respectively. The peak components consisted of C−OH bonds and carbonyl groups (C═O) at a binding energy of ∼532 and ∼ 530.9 eV, respectively.[26] Ratios of each oxygen-containing functional group to graphitic carbon (C−C) were calculated by integration of the corresponding peak areas. There was an increase in the hydroxyl group (C−OH) to C−C ratio of almost five times from 0.11 to 0.53 after surface treatments, hence indicating that hydroxyl groups were successfully generated on the VAG surface. On the other hand, the carbonyl group (C═O) to C−C ratio was found to increase barely from 0.1 to 0.14 after the same surface treatments. This suggests that the oxygen plasma-generated defects were hydroxyl group-dominated mostly at the edge of graphene sheets without breaking majority of σ- or π-bonds in the planar area,[46] which was also consistent with Raman and FTIR analysis of the same samples.

Surface Biofunctionalization and Non-faradaic EIS Biosensing of VAG Biosensors. For non-faradaic EIS measurements, a well-formed insulating self-assembly monolayer (SAM) is essential. A well-established silanization approach was employed for chemically functionalizing hydroxylated graphene surfaces with APTES as the SAM.[47] To preserve the structure of VAG, surface functionalization was performed using a spray-coating technique. Figure S5 shows EDX mapping of the silicon element, which can be found in APTES, and revealed uniform distribution of APTES on the surface of VAG after spray coating. Higher magnification cross-sectional SEM and the corresponding EDX mapping images of VAG after spray coating are shown in Figure 5a,b, respectively. It was evident that VAG structures remained intact after the spray-coating process, which demonstrated its suitability and reliability as an important surface functionalization technique for VAG biosensors. The effectiveness of APTES coating as an insulating SAM at the VAG was characterized electrochemically using the CV technique at ambient temperature. The SAM was found to block electron transfer effectively, as evidenced by CV scans shown in Figure S6, which did not exhibit any redox peak (i.e., Fe^{2+}/Fe^{3+}). This is indicative of a well-packed SAM formation (i.e., APTES) at the surface of VAG.[48]

In a typical non-faradaic EIS process, biomolecules are bound to the electrode and displace water molecules at its surface. This alters the double layer capacitance and dielectric constant at its surface.[49] Unlike capacitance measurement, the phase change during the EIS measurement is relatively independent of the biosensing area size.[50] Therefore, phase measurement is highly desirable as it provides better reproducibility on the biosensing results against manufacturing tolerances. In theory, the phase of impedance shifts toward −90° as a result of a more insulated interface between the electrode and solution, while it shifts toward zero for a non-insulated interface.[51] As shown in Figure 5c, the observed decrease in phase toward negative after antibody immobilization and then after binding with antigens (i.e., MC-LR) suggests that an additional layer was formed at each step that further hindered the access of currents or ions to the sensor surface.

The calibration curve of the VAG biosensor in Figure 5d was obtained by plotting the change in phase against MC-LR concentrations at a frequency of 167 mHz. The change in phase increased linearly (i.e., R^2 = 0.97) with the logarithm of MC-LR concentration over 5 decades between 0.001 and 10 μg/L. Saturation of response was not observed within the MC-LR concentration range. The biosensor exhibited a sensitivity (or the slope, m) of 0.46° ± 0.06° per decade for MC-LR in PBS performed in triplicate. It is worth noting that the biosensors showed no significant response (0.04 ± 0.006°) to just PBS samples (i.e., without MC-LR as negative sample). Hence, this confirms that the biosensing response was due to the binding of MC-LR at the electrodes. For comparison, biosensing results in Figure S7 obtained from printed graphene biosensors without photonic annealing revealed an inconclusive response. The limit of detection (LoD) for MC-LR detection was calculated to be 1.2 ng/L using the most common method,[55] which is well below 1 μg/L set by the World Health Organization[56] and also those obtained previously by others using mainly faradaic EIS-based biosensors as shown in Table 1. Two other most common freshwater algal toxin variants (i.e., MC-RR and MC-LW) were selected to assess the biosensor selectivity toward MC-LR detection. As shown in Figure 5e, the detection recovery rate

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Table 1. MC-LR Detection Results Using EIS Biosensors in Most Recent Studies

LoD (ng/L)	range (μg/L)	recovery in real water samples (%)	methods	references
	0.05–100		faradaic EIS	52
	0.05–100	92.7	faradaic EIS	53
4	0.01–100	94.1–98.1	faradaic EIS	54
2.3	0.005–100	93.5–98.2	faradaic EIS	41
1.2	0.001–10	98.4 ± 3.9	non-faradaic EIS	current study

for 0.1 μg/L MC-LR, MC-LW, and MC-RR mixtures in PBS was 91.8% of the same concentration of MC-LR on its own (i.e., without any interfering variants). These results demonstrated high selectivity of the VAG biosensor functionalized with monoclonal MC-LR antibody. Moreover, long-term stability of the biosensors was also examined as shown in Figure 5e. After one- and three-week storage in a freezer, 108.8 and 99.4% of initial responses in phase shift were obtained to detect 0.1 μg/L MC-LR in PBS, respectively. A large relative standard deviation could be caused by the gradual deterioration and loosening of immobilized antibodies after a long-term storage. The MC-LR detection performance was also validated using tap water samples in Swansea, UK (with total organic carbon of 0.91 mg/L, pH 7.8 and conductivity of 127.89 μs/cm), showing an excellent 98.4% recovery for the detection of 0.1 μg/L MC-LR.

CONCLUSIONS

An electrochemical impedimetric VAG biosensors have been reported for the ultrasensitive detection of algal toxins (i.e., MC-LR) in drinking water supply via the non-faradaic EIS technique. The biosensor, consisting of graphene-interdigitated electrodes, was fabricated using roll-to-roll flexographic printing of ball-milled GNP/EC ink and then followed by photonic annealing that partially removed the polymeric binder in the printed graphene. As a result, VAG structures were formed at the electrode surface, which provided a maximum specific surface area for ultrasensitive biosensing applications. A spray-coating technique was employed to functionalize the biosensor to preserve the VAG structures. Prior to immobilization of MC-LR antibodies on the biosensor, a uniform coverage of APTES, which acted as an insulating SAM for non-faradaic EIS biosensors, was spray-coated onto the VAG. The effectiveness of the spray-coated APTES was studied using EDX and CV techniques. The change in phase from the non-faradic EIS measurement was obtained at a frequency of 167 mHz. At this frequency, an optimal linear response from the antibody/antigen conjugation was observed at the VAG biosensors of which the correlation was subsequently established between the non-faradaic EIS phase change and logarithm of MC-LR concentration (i.e., 0.001–10 μg/L). An exceptional sensitivity of 0.46 degree per decade and a LoD of 1.2 ng/L were achieved with excellent reproducibility, selectivity, and stability. Finally, the VAG biosensor was validated using local tap water samples and experienced minimal matrix effects from other factors, such as metal ions, in the water. Such a low-cost, easy-to-use, and ultrasensitive VAG biosensor is ideal for large-scale early screening of contaminants in drinking water and detection of diseases in biomedical applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c08036.

Schematic illustration of the key fabrication process of the VAG biosensor, FT-IR and XPS results of GNP after oxygen plasma/water vapor treatments, high-magnification cross-sectional SEM images of APTES-coated graphene, top-down view SEM images and EDX mapping on GNPs, CV scan results, and biosensing results of non-photonic annealed electrodes (PDF)

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Author Contributions

†L.W. and W.Z. performed the experiments and analyzed the data, contributing equally to this work. K.S.T. and W.Z. designed and supervised the research. S.S. and D.D. assisted in flexographic printing and photonic annealing.

Notes

The authors declare no competing financial interest.

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