Nano-fibre optic probe for label-free bioimaging detection

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ABSTRACT

We present 2D nanomaterial-functionalised fibre optic probes for the bioimaging detection of antibodies. Graphene oxide (GO) provides an ideal platform for biophilic binding interface due to its high surface-to-volume ratio and abundant oxygen functional groups. After functionalisation and bio-immobilisation, the fluorescence images reveal that GO-fibre optic probe exhibits significantly stronger fluorescence intensity compared to bare fibre probes. This GO-fibre optic probe can be further developed as a point-of-care medical device for biomedical imaging and portable bio-detection.

Keywords: Graphene oxide, optical fibre, bioimaging, fluorescence

1. INTRODUCTION

Graphene oxide (GO) possesses the excellent optoelectronic and chemical properties, making it as ideal material for various biosensing and biomedical applications [1]. The hydrophilicity nature and abundant oxygen-functional groups facilitate the GO to be easily deposited on sensor surface and bio-functionalised with various molecules, such as proteins, DNA, RNA, and cells, demonstrating high binding capacity and sensing efficiency of biosensors. Recently, the functionalised GO has been developed to fabricate biosensors for cancer diagnostics, label-free immunosensing, drug delivery, and bioimaging in living cells [2-5].

In this work, we report a highly effective platform based on GO-functionalised fibre optic probes for the enhanced biomedical imaging. The activated GO overlay significantly improved the ability of miniaturised fibre probes to facilitate biomolecule interactions, enabling biomolecule immobilisation for biomedical imaging applications [6]. The surface morphology and fluorescence effects were analysed using Scanning Electron Microscopy (SEM) and confocal optical microscopy. The proposed GO-fibre optic probe demonstrated the great potential for biosensing and bioimaging applications.

2. METHODS

2.1 Deposition of GO nanosheets

As shown in Figure 1, the cylindrical silica surface of fibre optic probe was firstly cleaned with acetone for 30 minutes to remove any organic contaminant, rinsed thoroughly with deionized (DI) water and dried. It was then immersed in a 1.0 M NaOH solution at room temperature for 1 hour to enrich the number of silanol (Si–OH) groups on fibre surface, followed by washing three times with ethanol and DI water and drying.

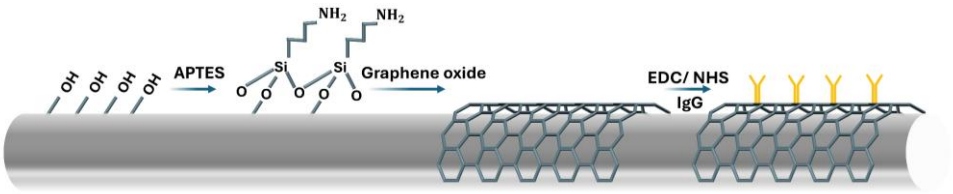

Figure 1. Schematic of GO nanosheets deposition and bio-functionalisation on cylindrical fibre probe.

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After the alkaline treatment, a silanisation process was applied. The alkaline-treated fibre probe was incubated in a freshly prepared 5% (v/v) (3-Aminopropyl)triethoxysilane (APTES) ethanol solution at room temperature for one hour, where the Si–O–Si bonds were formed via the reaction between APTES and surface hydroxyl groups [7]. The fibre probe was then rinsed thoroughly with ethanol to remove unreacted residuals and put into an oven baking at 70°C for 30 minutes to stabilise the APTES monolayer.

After APTES silanisation, the fibre probe was immersed in a 1.0 mg/mL GO aqueous solution contained in a custom-made mini-bath which was heated at 42°C for one hour, allowing GO nanosheets were deposited on fibre surface when the aqueous solution was gradually evaporated. The deposition process was repeated by adding fresh GO solution. The GO-coated fibre probe was baked at 70 °C for one hour to consolidate the GO coating.

2.2 Immobilisation of antibodies on GO-fibre surface

The GO-coated fibre probe was immersed into a mixture of 20 mM N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 40 mM N-Hydroxysuccinimide (NHS) in 1×phosphate buffered saline (PBS, pH 7.4) for one hour, followed by incubating into an antibody solution of Goat anti-rabbit IgG (Alexa Fluor® 488) with a concentration of 0.002 mg/mL for one hour to enable the covalent binding. The 2D aromatic structure of GO provided an excellent platform for the immobilisation of biomolecules. EDC was mixed with NHS to generate reactive esters before being applied to the GO to activate its carboxyl groups. These esters readily reacted with the primary amine groups of antibodies to form a covalent immobilisation. This approach ensured the efficient covalent binding of anti-IgG, preserving its structural integrity and functional binding sites while preventing desorption. The process ensured a uniform distribution of biomolecules on GO surface, emphasising the effectiveness of pre-mixed EDC/NHS chemistry for bio-functionalisation of sensor surfaces.

Similarly, following above-described processes, an optical fibre probe (without GO-coating) was used to immerse into the EDC/NHS mixture for one hour then incubated into anti-IgG solution for one hour.

3. RESULTS AND DISCUSSION

3.1 Surface morphological characterisation

The surface morphologies of probes were characterised by SEM at 500× magnification. The ridges and laminae characteristics of GO-coating were evident in Figure 2a, spreading uniformly over the optical fibre and forming a homogeneous layer over the entire fibre cylindrical surface. In contrast, the bare fibre exhibited a negligible change in surface roughness, maintaining a smooth surface with only minimal imperfections (Figure 2b). Due to the presence of GO overlay, the surface irregularities and features were more pronounced.

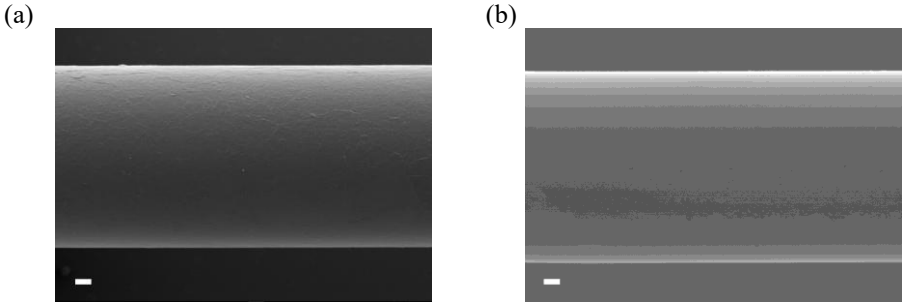

Figure 2. SEM images of (a) GO-coated fibre probe and (b) bare fibre probe (500× magnification, scale bar: 10 μm).

3.2 Confocal imaging of different probes

The confocal microscopy was employed to exam three probes: (a) GO-fibre optic probe bound with anti-IgG, (b) optical fibre probe (without GO coating) bound with anti-IgG, and (c) bare fibre probe.

As the images shown in Figure 3, the GO-fibre optic probe exhibited the strong and uniform green fluorescence, indicating the successful immobilisation of dense and consistent anti-IgG on probe surface due to the enriched oxygen-functional

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groups and hydrophilicity nature of GO nanomaterials. The optical fibre probe (without GO coating) under the confocal microscopy exhibited very limited green fluorescence, showing the poor attachment of antibodies due to the lack of functional groups on probe surface for efficient biomolecular binding [8]. As a contrast, an untreated bare fibre probe (without GO-coating, without binding of antibody) showed negligible fluorescence, demonstrating their inherent inability to support biomolecular attachment.

It has been confirmed that the GO nanocoating and the surface chemistry development (i.e. EDC/NHS) facilitated the fibre optic probes for effective biomolecular binding, hence the enhancement of fluorescence detection.

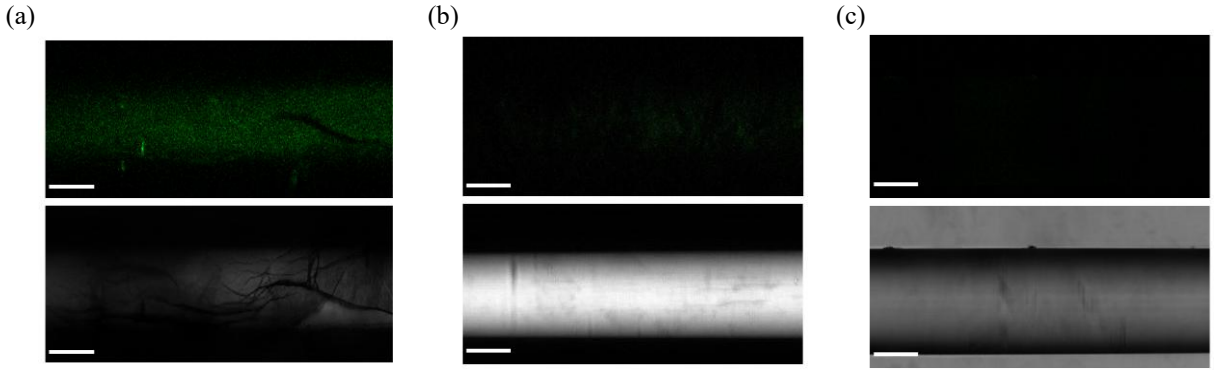

Figure 3. Confocal microscopy observations of fluorescent images (top) and differential interference contrast images (bottom): (a) GO-fibre optic probe bound with anti-IgG, (b) optical fibre probe (without GO-coating) bound with anti-IgG, and (c) bare fibre probe (Scale bar: 50 μm).

4. CONCLUSION

In this work, we proposed a GO nanosheets coated fibre optic probe with enhanced fluorescence performance for the detection of antibodies. The results indicated that the GO nanocoating with enriched functional groups and the EDC/NHS played the critical role for immobilisation of biomolecules by improving biomolecular binding efficiency and enhancing the reliable fluorescence. The proposed GO-fibre optic probe can be further adapted as bioimaging platform opening the potential for label-free detection, medical imaging, and early diagnostics.

ACKNOWLEDGEMENTS

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