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A paper based graphene-nanocauliflower hybrid composite for point of care biosensing

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ABSTRACT

Graphene paper has diverse applications in printed circuit board electronics, bioassays, 3D cell culture, and biosensing. Although development of nanometal-graphene hybrid composites is commonplace in the sensing literature, to date there are only a few examples of nanometal-decorated graphene paper for use in biosensing. In this manuscript, we demonstrate the synthesis and application of Pt nano cauliflower-functionalized graphene paper for use in electrochemical biosensing of small molecules (glucose, acetone, methanol) or detection of pathogenic bacteria (*Escherichia coli* O157:H7). Raman spectroscopy, scanning electron microscopy and energy dispersive spectroscopy were used to show that graphene oxide deposited on nanocellulose crystals was partially reduced by both thermal and chemical treatment. Fractal platinum nanostructures were formed on the reduced graphene oxide paper, producing a conductive paper with an extremely high electroactive surface area, confirmed by cyclic voltammetry and electrochemical impedance spectroscopy. To show the broad applicability of the material, the platinum surface was functionalized with three different biomaterials: 1) glucose oxidase (via chitosan encapsulation); 2) a DNA aptamer (via covalent linking), or 3) a chemosensory protein (via his linking). We demonstrate the application of this device for point of care biosensing. The detection limit for both glucose (0.08 ± 0.02 μM) and *E. coli* O157:H7 (1.3 ± 0.1 CFU mL^{-1}) were competitive with, or superior to, previously reported devices in the biosensing literature. The response time (6 sec for glucose and 10 min for *E. coli*) were also similar to silicon biochip and commercial electrode sensors. The results demonstrate that the nanocellulose-graphene-nanoplatinum material is an excellent paper-based platform for development of electrochemical biosensors targeting small molecules or whole cells for use in point of care biosensing.

Keywords: graphene, nanocellulose, point of care, biosensing

1. INTRODUCTION

A wide variety of paper-based biosensors such as colorimetric indicators (Suaifan et al. 2013), fluorescent/chemiluminescent immunoassays (Ge, Lei, and Zare 2012; Ellerbee et al. 2009), microfluidic arrays (Abe, Suzuki, and Citterio 2008; Chen et al. 2008; Liu and Crooks 2011), origami immunosensors (Li et al. 2014), and electrochemical biosensors (Dungchai, Chailapakul, and Henry 2009) have been developed. Development of conductive paper for electrochemical sensing based on conductive nanocarbon has been an area of extensive research for the last few decades. In electrochemical biosensing, the most common approach for fabricating sensors is to deposit a conducitve polymer or nanometal on the nanocarbon surface, facilitating adsorption of biorecognition agents and enhancing electron transport. Of the various metals used, nanoplatinum (nPt) has properties that make it particularly useful for biosensing (it is biocompatible, easily biofunctionalized, corrosion resistant, and stable).

Here, we demonstrate the synthesis and application of platinum nanocauliflower-graphene hybrids on nanocellulose paper for use in point of care (POC) biosensing. We demonstrate use of this device in amperometric biosensing with an oxidase and a RNA aptamer for detection of glucose of *Escherichia coli* O157:H7, respectively (**Fig 1**).

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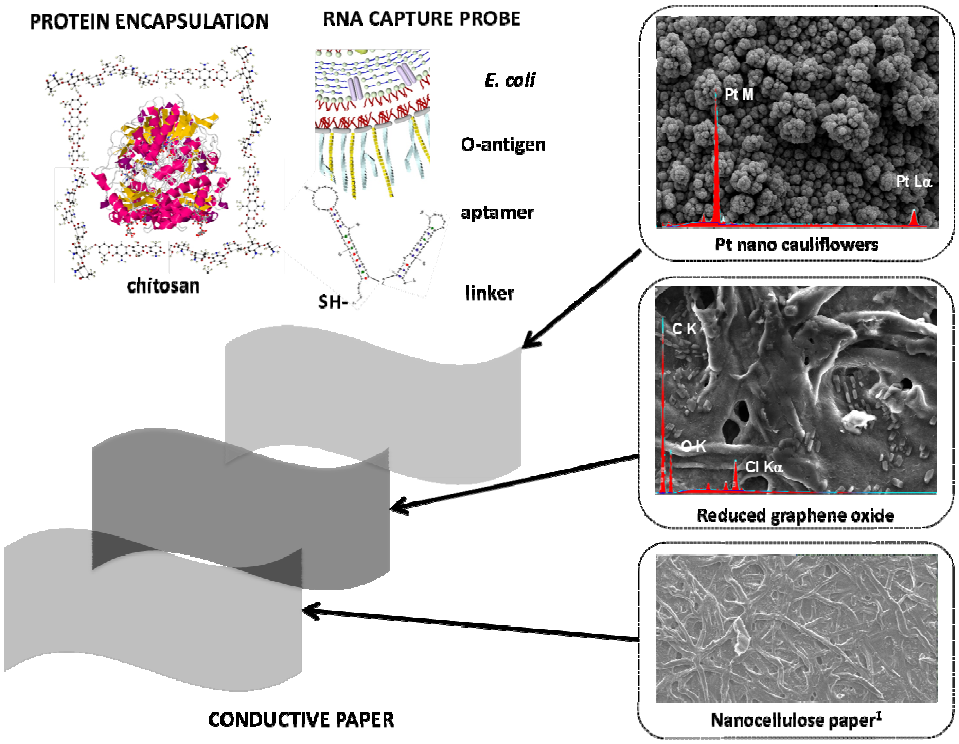

Figure 1. Conductive paper was prepared by decorating graphene-nanocellulose strips with platinum nano cauliflowers. The surface was functionalized with biomaterials (oxidase or RNA) for demonstration as a point of care biosensor.

2. METHODOLOGY

Preparation of conductive paper

Graphene-nanocellulose paper was prepared based on modification of the recipe developed by (Marr et al. 2015). A CNC layer was first formed by vacuum filtration of a 1:4 (slurry: water) mixture through a 7.0 cm cellulose acetate filter paper (50 mL total slurry volume). The filter with CNC retentate was removed and pressed between borosilicate glass slides, and then dried at 75°C for one hour. The CNC paper was then coated with 500 µL of conductive ink, and dried in a vacuum oven at 115°C for 2 hours. After this thermal reduction (referred to as thermally reduced graphene paper, or TRG), the material was immersed in 1mM ascorbic acid for one hour to chemically reduce the graphene oxide (referred to as ascorbic acid reduced graphene paper, or AARG). Finally, the samples were dried for 30 minutes at 75°C and cut into rectangular strips (1.2 cm by 0.5 cm). Platinum was electrodeposited on graphene-nanocellulose paper using pulsed sonoelectrodeposition at a frequency of 500 mHz for 180 sec at 10 V versus a bare platinum wire based on (Chaturvedi et al. 2014; Burrs et al. 2015). The conductive paper was fixed into a custom 3-D printed sample immobilization device with an open diameter of 2mm (see supplemental Figure S1).

Biofunctionalization of conductive paper

Glucose biosensors were prepared by functionalizing the nanoplatinum with glucose oxidase (GOx) entrapped in a chitosan hydrogel based on Burrs et al (Burrs et al. 2015). In summary, an aliquot (10 µL) of GOx (46mg/mL at a protein concentration of 138.4 kU/g) was mixed with 10 µL of chitosan (0.05% w/v), and vortex-agitated for 1 min. A 2 µL aliquot of enzyme-hydrogel suspension was drop-coated onto the surface of the device and dried at room temperature for 30 minutes. Aptasensors for detecting *E. coli* by O157:H7 by were prepared by dropcasting 2µL of thiolated 64-mer RNA aptamer that is known to bind the O-antigen (K_D = 110 nM) suspended in 10 mM TRIS EDTA buffer, pH 7.4). The aptamer was allowed to adsorb for 30 minutes at room temperature.

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Electrochemical Analysis

All electrochemical analysis was performed in a 3D printed sample immobilization device (see supplemental Figure S1). Cyclic voltammetry (CV) and DC potential amperometry, (DCPA) were carried out using a potentiostat (BASi, West Lafayette, USA). Electrochemical impedance spectroscopy (EIS) was conducted on a EA163 potentiostat and an eDAQ ERZ100 (eDAQ, Colorado Springs, USA).

3. RESULTS AND DISCUSSION

Conductive paper

Thermal reduction of the graphene-nanocellulose paper did not produce well-defined peak oxidative and reductive current for testing with ferrocyanide and also initial trials with the ferrocyanide/ferricyanide redox couple (**Fig 1A**). This indicates that the thermally reduced graphene oxide (TRG) paper had poor charge transfer. Chemical reduction of the graphene ink (AARG) increased the peak current response, but the change in redox peak was not significant ($p=0.31$; $\alpha=0.05$). When platinum nanocauliflowers were deposited on the AARG graphene, well-defined reversible redox peaks were apparanet, and the peak current increased by 160 ± 12 μA (**Fig 1A**). Representative Nyquist plots (**Fig 1B**) and Bode magnitude plots (**Fig 1C**) show that TRG and AARG nanocellulose paper displayed characteristic Nyquist plots, with a discernable charge transfer resistance (Rct) at low frequency impedance values (indicated by the semicircular region), and a diffusion limited region at high impedance values (indicative of Warburg impedance). However, EIS for the Pt nano cauliflower-decorated AARG paper did not have an obvious semicircular region, which indicates the charge transfer resistance was negligible. For all samples, the solution resistance did not change significantly as expected. The Bode magnitude plots in **Fig 2C** indicate that charge transfer in the TRG and AARG conductive paper were diffusion limited. Conversely, deposition of Pt nanocauliflowers caused a significant reduction in diffusion limited charge transport.

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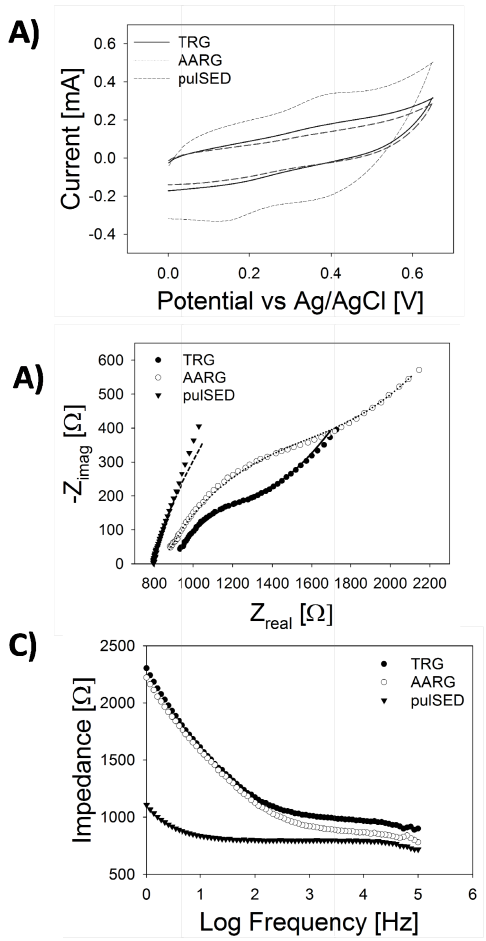

Figure 2. Electrochemical behavior of graphene-nanocellulose conductive paper for each treatment. Representative A) cyclic voltammograms at a scan rate of 100 mV/sec and switching potential of 650 mV, B) Bode magnitude plot, and C) Bode phase plot.

The average R_{ct} of Pt nano cauliflower (24 ± 6 Ω) material was significantly lower than any other material tested(ANOVA, p=0.010, α =0.05). In fact, the average R_{ct} was orders of magnitude lower than R_{ct} for TRG (980 ± 162 Ω) and AARG (1720 ± 206 Ω) paper. The average Warburg resistance for the TRG (1.7 ± 0.1 kΩ) and AARG (1.9 ± 0.2 kΩ) was significantly higher than the Pt nano cauliflower decorated graphene (1.1 ± 0.1 kΩ). This result, and the Bode phase plots indicate that diffusive transport limited the charge transfer for TRG and AARG conductive paper under the conditions tested. Although mixing and increase in operating temperature would likely reduce these effects for TRG and AARG nanocellulose paper, the Pt nano cauliflower device was highly efficient at room temperature and with no solution convection, which is a major advantage for application in POC biosensing.

Amperometric biosensing

Prior to testing for glucose, each treatment of the graphene-nanocellulose paper was tested for sensitivity, LOD and response time towards hydrogen peroxide using DC potential amperometry (DCPA) at + 500 mV (see supplemental Figure S9 for details). The average sensitivity (2.9 ± 0.4 mA mM^{-1}), LOD (0.2 ± 0.1 μM), and response time (6.5 ± 0.5 sec) are shown in **Fig 3A**; all values were within the range of laboratory electrodes and biochips functionalized with graphene-nanometal hybrid materials for detection of peroxide (Vanegas et al. 2014; Chaturvedi et al. 2014; Claussen et al. 2011). The average sensitivity, limit of detection and response time for enzymatic glucose biosensors prepared on the graphene nanocellulose paper was also tested using DCPA. A representative time-series plot of oxidative current for a Pt nanocauliflower decorated graphene paper device after stepwise addition of 50 μM glucose is sh3own in **Fig 3A** (arrows indicate addition of glucose). The average sensitivity for replicate biosensors (n=3) is

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shown in **Fig 3B;** average sensitivity was 3.59 ± 0.68 mA mM^{-1}, which is lower or within the range of laboratory electrochemical devices fabricated on glass, silicon, Katpon tape, and other materials. The average LOD (0.08 ± 0.02 μM), and response time (8.8 ± 1.8 sec) toward glucose are applicable for POC testing of glucose levels in saliva, tears, or diluted blood (approximately 10X dilution). The response time of the biosensor changed significantly within the testing range , but less than 10 sec for the range tested (and as low as 6 sec)

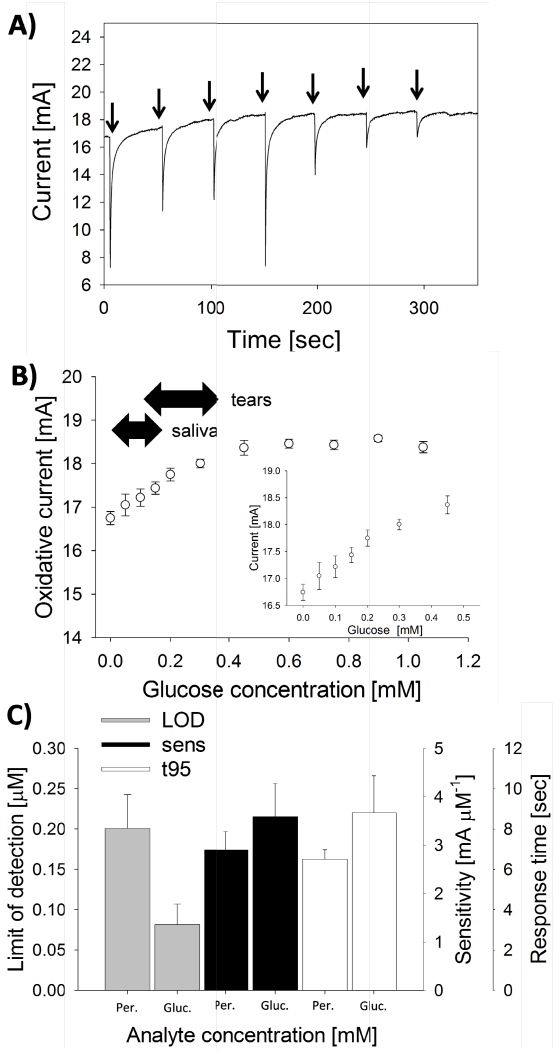

Figure 3. Glucose biosensor developed using the graphene-nanocellulose paper. DC potential amperometry was used to calculate the sensitivity, limit of detection (LOD) and response time (t95) A) Representative oxidative current response to injections of 50 μM glucose (indicated by vertical arrows). B) Average calibration curves for glucose derived from replicate DCPA tests (n=3). Inset shows linear range between 0 to 500 μM. C) Average LOD, sensitivity, and t95 for peroxide and glucose biosensors tested in buffer at 25 °C; Per.=hydrogen peroxide data, Gluc.= Glucose data. Error bars represent the standard error of the mean.

Bacteria detection

As a second demonstration of the versatile material, an aptasensor was developed for measuring *E. coli* O157:H7. **Fig. 4** shows a representative Nyquist plot (**Fig 4A**), Bode plot (**Fig 4B**) and the calibration curve obtained at 1 Hz using change in normalized impedance relative to baseline (**Fig 4C**). The average sensitivity value (n=3) was calculated to be 5.3 (log CFU mL^{-1}) from the linear regression curve. The detection limit was 1.3 ± 0.1 CFU mL^{-1} using the 3-sigma method, and linear range was from 1 to 10^5 CFU/mL with an average response time of 12 min (including 10 min for bacteria capture and 2 min for EIS). The performance values obtained in this study are

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comparable to, if not superior to, aptasensors published for the detection of *E. coli* O157:H7. Most recently, Li et al. (Ning et al. 2015) developed an impedance-based immunosensor for O157:H7 based on self assembled lectins with a LOD of 100 CFU mL^{-1}, a linear range from 10^2 to 10^7 CFU mL^{-1}, and a total detection time of 1 h. Fang et al. (Fang et al. 2014) developed a lateral flow biosensor based on aptamer-mediated strand displacement amplification with a detection limit of 10 CFU mL^{-1}; however, it required pre-enrichment steps and isothermal amplification prior to lateral flow test.

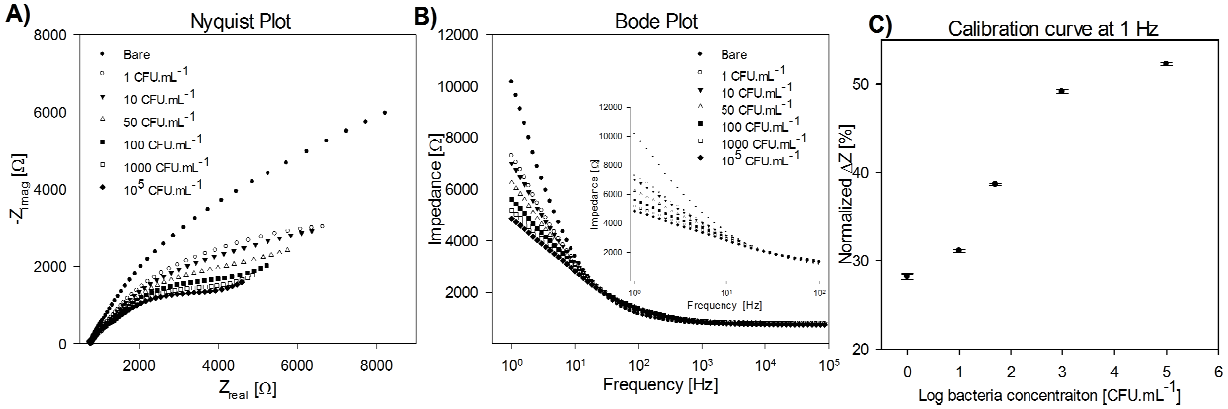

Figure 5. Representative A) Nyquist and B) Bode plots with an inset showing exploded view focusing on the lower frequencies for various E. coli O157:H7 concentrations (CFU mL-1). C) Calibration curve (normalized impedance change (%) vs. log bacteria concentration (CFU.mL-1) at 1 Hz for E. coli O157:H7 detection in PBS. All data represents the average of 3 repetitions.

5. CONCLUSIONS

For the first time, we develop fractal nanoplatinum (cauliflower-like) on graphene-nanocellulose paper, and the highly conductive structure is free of any discernable cracks or voids. Material analysis and electrochemical testing, together with the proof of concept testing show that the platinum nano cauliflower-functionalized graphene paper developed here is an extremely efficient material for use in electrochemical biosensing.

6. ACKNOWLEDGEMENTS

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