明志科技大學材料工程系108學年四技專題製作競賽

An electrochemical RNA-biosensor based on a graphene oxide/graphene electrode for miRNA-21 detection

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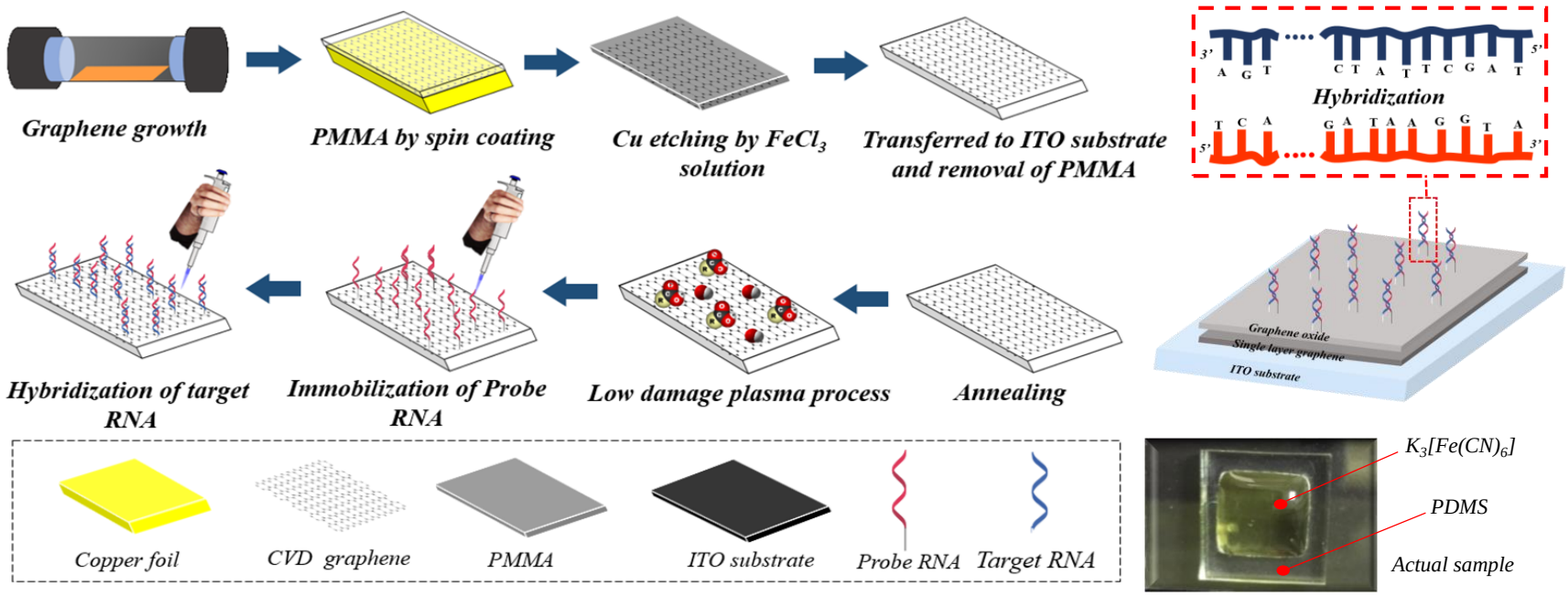
Introduction

In this research, a graphene oxide-graphene layered electrode was developed for the detection of the carcinogenic sequence microRNA-21(miRNA-21). The electrode is convenient, highly sensitive, and allows fast detection. The preparation of graphene oxide was achieved by subjecting the double-layer graphene to a low-damage plasma treatment (LDPT) .LDPT can shield the high energy particles ,i.e. electrons, ions, vacuum ultraviolet(VUV) and photons by a complementary filter to prevent structural damage and only allow low energy radicals to diffuse through the filter to reach onto graphene surface and then react with graphene. The high electron transfer activity was attributed to the high conductivity nature of graphene. A probe (ssRNA) was further immobilized onto the surface of the graphene oxide-graphene double-layer electrode via a hydrogen bond and then hybridized with its target RNA (cRNA). The change of peak current due to the hybridized dsRNA could be used for the quantitative sensing of miRNA-21 concentration.

Experimental procedure



Result & Discussion

Raman Spectra

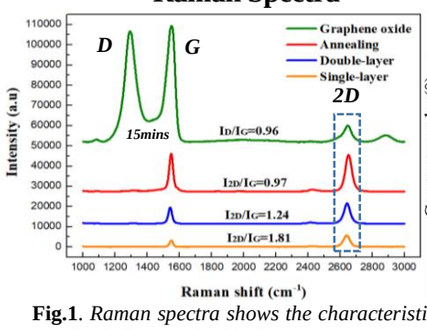


Fig.1. *Raman spectra shows the characteristics for single layer、double layer、annealing and plasma treatment graphene.*

Contact angle

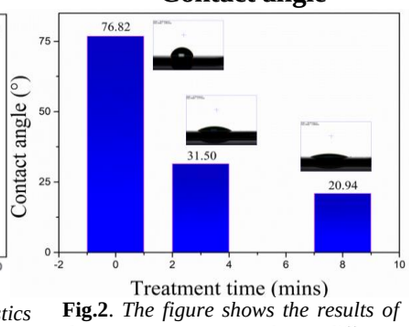


Fig.2. *The figure shows the results of the water contact angle at different treatment.*

Cyclic voltammetry

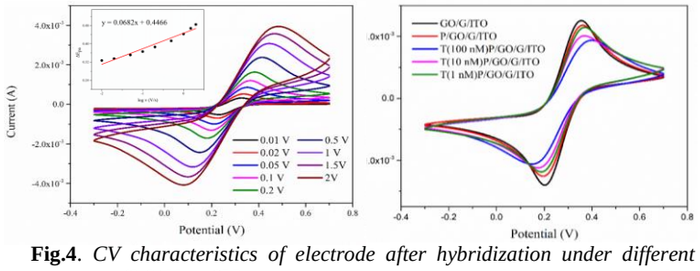


Fig.4. *CV characteristics of electrode after hybridization under different scan rates(0.01V to 2V) to observe the change in reduction potential, then calculated the electron transfer constant rate is about 0.13, and under different miRNA-21 concentration(1、10、100nM), as the concentration increases, it shows a lower current response.*

Chronocoulometry

	ΔQ	Γ_0	Γ_{DNA}
O/H-LDPT (15mins)	2.36×10^{-6}	2.45×10^{-11}	2.01×10^{12}

Table.1 *electrode surface probe densities.*

$$\Gamma_0 = \Delta Q / F$$
$$\Gamma_{DNA} = \Gamma_0 (z/m) N_A$$

N_A: Avogadro number($6.023\times10^{23} mol^{-1}$)
F:Farady constant(96485C/eqiuv)
Γ_{DNA}: the Probe density in molecules cm^{-2}
z:the charge of the redox molecule,3 for $Ru(NH_3)_6^{3+}$
Γ_0:The amount of surface-confined $Ru(NH_3)_6^{3+}$ near the electrode.
m:The number of bases in the MB-hairpin or DNA inducer I, 30 bases in this case.

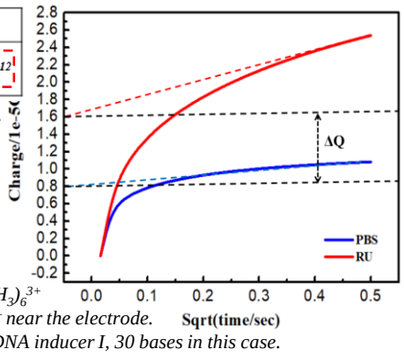
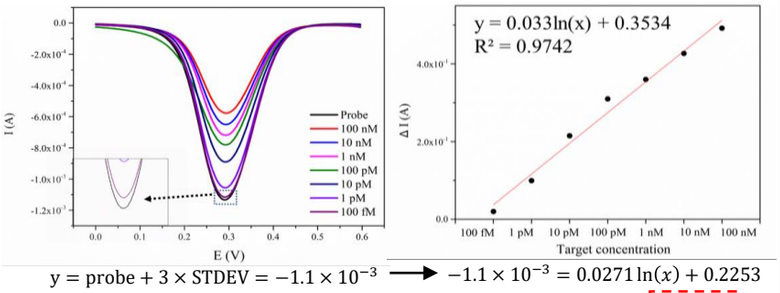


Fig.3. *Chronocoulometric response curves for the probe-mounted electrode in the absence (blue curve) and presence (red curve) of 50 μM $Ru(NH_3)_6^{3+}$.*

Differential pulse voltammetry



$$y = probe + 3 \times STDEV = -1.1 \times 10^{-3} \longrightarrow -1.1 \times 10^{-3} = 0.0271 \ln(x) + 0.2253$$

So the ln(x)=-10.67 $\quad LOD = e^{-10.67} = 2.46 \times 10^{-5}$ n(M)=24.6p(M)

Fig.5. *DPV recorded after hybridization with different concentrations of target cRNA varying from 10^{-7} to 10^{-13} M and detection limit of 24.6 fM as determined by three times standard deviation of zero DNA concentration.*

Conclusion

1.A graphene oxide/graphene double-layer electrode has been developed and its application in electrochemical biosensor detection of miRNA-21 has been demonstrated.
2.Through Chronocoulometry, we can find the probe density as high as 2.1×10^{12} for 15 mins LDPT, it is advantageous to hybridize more target on electrode surface.
3.Through CV in different concentration, we discovered the higher concentration, the lower current response, which proves that the target hybridization was successful.
4.Through DPV, LOD is calculated as low as 24.6fM by three times standard deviation, it is expected that can be actually used for cancer sequence detection in the future.