In this study, a novel signal-increase electrochemiluminescence (ECL) biosensor has been developed for the detection of glucose based on graphene quantum dot/glucose oxidase (GQD/GOx) on Ti foil. The proposed GQD with excellent ECL ability is synthesized through a green one-step strategy by the electrochemical reduction of graphene oxide quantum dot. Upon the addition of glucose, GOx can catalytically oxidize glucose and the direct electron transfer between the redox centre of GOx and the modified electrode also has been realized, which results in the bio-generated H$_2$O$_2$ for ECL signal increase in GQD and realizes the direct ECL detection of glucose. The signal-increase ECL biosensor enables glucose detection with high sensitivity reaching 5×10$^{-6}$ mol l$^{-1}$ in a wide linear range from 5 × 10$^{-6}$ to 1.5 × 10$^{-3}$ mol l$^{-1}$. Additionally, the fabrication process of such GQD-based ECL biosensor is also suitable to other biologically produced H$_2$O$_2$ system, suggesting the possible applications in the sensitive detection of other biologically important targets (e.g. small molecules, protein, DNA and so on).

1. Introduction

Graphene quantum dot (GQD), a newly promising zero-dimensional (0D) graphene material, not only shows the similar ability to graphene (e.g. high electron mobility, good chemical inerntness and eco-friendly nature) but also possesses many unique merits such as excellent biocompatibility, tuneable bandgap and outstanding photoluminescence/chemiluminescence, owing to its
strong quantum confinement effect and pronounced edge effect [1–4]. Recently, particular interest has been developed in electrochemiluminescence (ECL) ability of GQD due to its promising use in biosensing and bioimaging [5–11].

However, to date, the synthesis of GQD with the ECL property is still at an inchoate stage, not to mention its application in ECL biosensor. Meanwhile, current methods for the ECL GQD production are primarily via scissoring differently huge carbon materials, such as graphene oxide [12,13], XC-72 carbon black [14,15] or coal [16], into small graphene pieces through chemical means [17,18]; nevertheless, these methods often require complex and harsh synthetic procedures, involve the use of toxic organic reagents and, most importantly, generate GQD in large size. Thus, a facile and green approach to synthesize small-sized GQD with the ECL property is still an exigent demand.

Here, we first present a facile one-step strategy for the green synthesis of small-sized ECL GQD based on the electrochemical reduction of graphene oxide quantum dot (GOQD). Moreover, as reported, the ECL intensity of other traditional quantum dots can be linearly enhanced with assistance from H₂O₂ [19–22]; moreover, H₂O₂ can be biologically produced by various oxidases and their corresponding substrates [23,24]. In this work, glucose oxidase (GOx) has been chosen as a model oxidase to catalyse glucose for the generation of H₂O₂ and the direct electron transfer between the redox centre of GOx and the modified electrode also can be realized. More interestingly, the increasing concentration of the bio-generated H₂O₂ is well linear with the successive ECL enhancement of GQD, indicating the possible fabrication of an ECL biosensor. To the best of our knowledge, nearly no related work has been reported, and we would like to point out that this is the first report on using the bio-generated H₂O₂ for ECL increase in GQD and realizing the direct ECL detection of glucose. In addition, the fabrication process of such GQD-based ECL biosensor is also suitable to other biologically produced H₂O₂ system, suggesting the possible applications in the sensitive detection of other biologically important targets (e.g. small molecules, protein, DNA and so on).

2. Experimental procedure

2.1. Reagents
Titanium (Ti) foil (99.8%, 0.127 mm thickness) and GOx (Aspergillus niger, 100 U mg⁻¹) were purchased from Aldrich. GOQD was prepared according to the previous work of Zhu et al. [25]. D-Glucose was purchased from Shanghai Sangon and dissolved in 0.067 mol l⁻¹ pH ~7 phosphate buffer solution (PBS) to form a 1 mol l⁻¹ glucose stock solution. All other reagents were of analytical grade and used without further purification. Ultrapure water was used throughout the experiments.

2.2. Instruments
Fourier transform infrared spectroscopy (FTIR, FD-5DX), Raman spectroscopy (Labram-010 with a 632.8 nm laser), photoluminescence spectroscopy (PL, Thermo Fisher Scientific Lumina system) and X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific K-Alpha 1063 system). Electrochemical measurements were carried out on the three-electrode CHI 660D electrochemistry workstation (Chenhua Instrument Inc., China) using modified Ti foil (0.5 × 1.0 cm) as a working electrode, Pt foil as a counter electrode and saturated calomel electrode (SCE) as a reference electrode. ECL measurements were performed on MPI-E multifunctional chemiluminescent analyser (Xi’an Rimax Electronics Co. Ltd, China).

2.3. Preparation of GQD/GOx hybrid
Prior to modification, Ti foil (0.5 × 0.5 cm) was ultrasonically cleaned in acetone and ethanol solution for 15 min, respectively. The cleaned Ti foil was then immersed into the prepared 1 mg ml⁻¹ GOQD solution and subjected to cyclic voltammetric scanning from −1.4 to +1.0 V at 50 mV s⁻¹ for 10 cycles under stirring. Then, 5 µl of 10 mg ml⁻¹ GOx solution was dip-coated onto the modified Ti foil surface using a syringe. After drying at room temperature, the GQD/GOx was obtained. To obtain excellent electrochemical properties, the above experimental conditions were optimized.
3. Results and discussion

3.1. Characterization and evaluation of GQD and GQD/GO

As reported, GOQD with plenty of oxygenous functional groups on the surface shows excellent water-solubility, while the solubility of GQD was just the opposite [25,26]. Inspired by this, it is logical that the insoluble GQD is likely to be directly prepared on the electrode as long as the soluble GOQD can receive the electron and be electroreduced via the direct contact with the electrode surface. In this work, we have successfully prepared GQD through electroreduction of GOQD, and relatively evincive studies (e.g. cyclic voltammetry, FTIR, Raman, XPS and PL) have been discussed in detail in the electronic supplementary material, figures S1–S5. To the best of our knowledge, this is the first report on using the solubility difference of GOQD and reduced GOQD for the direct preparation of a GQD film.

Electronic supplementary material, figure S6(A) shows the cyclic voltammograms (CVs) of GQD on Ti foil and GQD/GOx on Ti foil in N2-saturated 0.067 mol l$^{-1}$ pH ~7 PBS, respectively. No obvious peak was detected for GQD, while the GQD/GOx showed a pair of stable and quasi-reversible redox peaks, which was due to the direct electron transfer between the redox centre of GOx and the modified electrode; moreover, the cyclic voltammetric current of GQD/GOx was much larger than that of GQD, indicating the higher conductivity and the larger surface-to-volume of GQD/GOx. Based on Faraday’s Law $\Gamma = \frac{Q}{(nFA)}$ [27], where the surface coverage is $\Gamma$, the charge amount is $Q$, the transferred electron number is $n$, Faraday’s constant is $F$ and the effective electrode area is $A$. The $F$ of electroactive GOx was estimated to be $3.4 \times 10^{-9}$ mol cm$^{-2}$ at GQD/GOx-modified Ti foil, which was about 1200-fold larger than the value obtained on the bare electrode surface [28], meaning the excellent biocompatibility and good adsorbability of GQD for GOx. Electronic supplementary material, figure S6(B) presents the CVs of GQD/GOx on Ti foil at different scan rates. The redox peak potentials of GOx, respectively, shifted in both negative and positive directions; meanwhile, the pair of reversible redox peak currents enhanced successively with the increasing scan rates from 0.05 to 0.5 V s$^{-1}$, suggesting a well reversible and surface-controlled electron transfer process between GOx and the electrode.

3.2. ECL behaviours and mechanism

Figure 1 displays the ECL–potential curves of bare Ti foil (black line), GQD on Ti foil (blue line) and GQD/GOx on Ti foil (red line) in 0.067 mol l$^{-1}$ pH ~7 PBS with 0.1 mmol l$^{-1}$ H$_2$O$_2$.

![ECL–potential curves](image)

Figure 1. ECL–potential curves of bare Ti foil (black line), GQD on Ti foil (blue line) and GQD/GOx on Ti foil (red line) in 0.067 mol l$^{-1}$ pH ~7 PBS with 0.1 mmol l$^{-1}$ H$_2$O$_2$.
To learn more about the ECL emission mechanism of GQD, the CVs and ECL of GQD on Ti foil have been examined in 0.067 mol l\(^{-1}\) pH \(\sim 7\) PBS with N\(_2\)-saturated and 0.1 mmol l\(^{-1}\) H\(_2\)O\(_2\), respectively. As shown in figure 2a, an irreversible reduction process at around \(-1.05\) V could be observed in N\(_2\)-saturated solution, which was ascribed to the injection of an electron into GQD to generate reduced state GQD. In 0.1 mmol l\(^{-1}\) H\(_2\)O\(_2\) solution, another reduction peak at around \(-0.55\) V also could be detected except for the original reduction peak of GQD (approx. \(-1.05\) V), owing to the reduction of H\(_2\)O\(_2\). Meanwhile, in figure 2b, due to the non-existence of any coreactant in N\(_2\)-saturated solution, there was no evident ECL emission during the cathodic ECL process; however, in 0.1 mmol l\(^{-1}\) H\(_2\)O\(_2\) solution, the ECL signal appeared beyond \(-0.55\) V, increased significantly after \(-1.05\) V and achieved the maximum value at around \(-1.53\) V, which agreed well with the respective electron injection voltages of H\(_2\)O\(_2\) and GQD in previous CVs. Accordingly, the possible ECL emission mechanisms were shown as below

\[
\text{GQD} + e^- \rightarrow \text{GQD}^{**},
\]

(3.1)

\[
\text{H}_2\text{O}_2 + 2\text{GQD}^{**} \rightarrow \text{GQD}^+ + 2\text{OH}^- \]

(3.2)

and

GQD\(^+\) \(\rightarrow\) GQD + \(h\nu\).

3.3. Detection of glucose

H\(_2\)O\(_2\) can be biologically produced by various oxidases and their corresponding substrates [23,25]. In this work, GO\(_x\) and glucose have been chosen as a pair of model oxidase and substrate to generate H\(_2\)O\(_2\). Meanwhile, the bio-generated H\(_2\)O\(_2\) concentration increases with the augmentation of glucose concentration, leading to the ECL enhancement of GQD. Thus, a novel ECL biosensor can be fabricated by monitoring the ECL increase in GQD.

Figure 3a shows the ECL–potential curve of GQD/GO\(_x\) for different concentrations of glucose \((\times10^{-6}\) mol l\(^{-1}\)): (A) 5, (B) 10, (C) 15, (D) 50, (E) 50, (F) 100, (G) 150, (H) 500, (I) 1000 and (J) 1500 and (b) the calibration curve for glucose determination.

\[
y = 124.47 + 10.51x \quad R^2 = 0.997
\]
increasing glucose concentration. As shown in figure 3b, in the range of $5 \times 10^{-6}$–$1.5 \times 10^{-3}$ mol l$^{-1}$, the ECL intensity revealed a linear relationship with the logarithm of glucose concentration with a correlation coefficient of 0.997, and the detection limit (LOD) was $5 \times 10^{-6}$ mol l$^{-1}$ ($S/N = 3$). Reproducibility and stability of this ECL biosensor were also tested. The ECL response of five identical QGD/GOx-modified electrodes to $5 \times 10^{-5}$ mol l$^{-1}$ glucose exhibited a relative standard deviation (r.s.d.) of 5.1%, suggesting the acceptable reproducibility of this ECL biosensor. Moreover, to validate the stability of the biosensor under the storage condition (0.067 mol l$^{-1}$ pH~7 PBS, 4°C), the ECL responses to $5 \times 10^{-5}$ mol l$^{-1}$ glucose were recorded during one month at 2-day intervals. The proposed biosensor could retain about 89% of its original ECL response, resulting from the excellent chemical stability of the QGD/GOx hybrid and the good bioactivity of GOx immobilized on GQD for a long time.

4. Conclusion

In this work, a facile one-step strategy for the green synthesis of small-sized ECL GQD is first proposed. The obtained GQD shows good bioactivity to GOx, and the direct electron transfer between GOx and the modified electrode surface has been realized. Interestingly, the ECL intensity of GQD is linearly enhanced with assistance from biologically produced H$_2$O$_2$ via a GOx bio-catalysing glucose system; moreover, the bio-generated H$_2$O$_2$ concentration increases with the augmentation of glucose concentration. Thus, a novel ECL biosensor for glucose detection has been fabricated by monitoring the ECL increase in GQD. Additionally, the fabrication of this proposed biosensor also breaks a new path to the sensitive detection of other biologically important targets (e.g. small molecules, protein, DNA and so on) based on such bio-enhanced ECL systems.

Data accessibility. All data are included in the article and the electronic supplementary material. Authors’ contributions. S.Y. and S.W. designed the study and performed the experiments. Y.L. collected and analysed the data. B.X. wrote the paper. M.C. reviewed and edited the manuscript. All authors read and approved the manuscript.

Competing interests. There are no conflicts to declare.

Funding. This work was supported by the National Natural Science Foundation of China (grant nos. 21504085, 11605163, 21604075), Foundation for Special Talents in the China Academy of Engineering Physics (grant nos. TP02201503, TP02201704), the Sichuan Science and Technology Development Foundation for Young Scientists (grant no. 2017JQ0050) and the Development Foundation of Radiochemistry (grant no. XK909) from the China Academy of Engineering Physics.

References

1. Ponomarenko LA, Schedin F, Katsnelson MI, Yang R, Hill EW, Novoselov KS, Geim AK. 2008 Chaotic Dirac billiard in graphene quantum dots. Science 320, 356–358. (doi:10.1126/science.1154663)
2. Gupta S, Smith T, Banaszak A, Boeckl J. 2017 Computer-aided design of graphene quantum dot electroluminescent and sensitive electrocatalytic glucose sensor development. Nanomaterials 7, 301–321. (doi:10.3390/nano7100301)
3. Liu F, Jang MH, Ha HD, Kim JH, Cho YH, Seo TS. 2013 Facile synthetic method for pristine graphene quantum dots and graphene oxide quantum dots: origin of blue and green luminescence. Adv. Mater. 25, 3657–3662. (doi:10.1002/adma.201300233)
4. Yang HM, Liu WY, Ma C, Zhang Y, Wang X, Yu JH, Song XR. 2014 Gold–silver nanocomposite-functionalized graphene based electrochemiluminescence immunosensor using graphene quantum dots coated porous PPy nanochains as labels. Electrochim. Acta 123, 470–476. (doi:10.1016/j.electacta.2014.01.014)
5. Sun JH, Wu L, Wei WY, Xu XG. 2013 Recent advances in graphene quantum dots for sensing. Mater. Today 16, 433–442. (doi:10.1016/j.mattod.2013.10.020)
6. Li LL, Wu GH, Yang GH, Peng J, Zhao JW, Zhu JJ. 2013 Focusing on luminescent graphene quantum dots: current status and future perspectives. Nanoscale 5, 4015–4039. (doi:10.1039/c3nr3849e)
7. Xu YH, Liu JQ, Gao CL, Wang EK. 2014 Applications of carbon quantum dots in electrochemiluminescence: a mini review. Electrochim. Commun. 44, 151–154. (doi:10.1016/j.elecom.2014.08.032)
8. Lei J, Hu H. 2011 Fundamentals and bioanalytical applications of functional quantum dots as electrogenerated emitters of chemiluminescence. Trend. Anal. Chem. 30, 1351–1359. (doi:10.1016/j.trac.2011.04.010)
9. Tian KL, Nie F, Luo K, Zheng XH, Zhang JH. 2017 A sensitive electrochemiluminescence glucose biosensor based on graphene quantum dot prepared from graphene oxide sheets and hydrogen peroxide. J. Electroanal. Chem. 801, 162–170. (doi:10.1016/j.jelechem.2017.07.019)
10. Salahiha F, Hosseini M, Ganjali MR. 2018 Enhanced electrochemiluminescence of luminol by an in situ silver nanoparticle-decorated graphene dot for glucose analysis. Anal. Methods 10, 508–514. (doi:10.1039/C7AY02375H)
11. Zhou C, Chen YM, You X, Dong YQ, Chi YW. 2017 An electrochemiluminescent biosensor based on interactions between a graphene quantum dot–sulfite co-reactant system and hydrogen peroxide. ChemElectroChem 4, 1783–1789. (doi:10.1002/celc.201600921)
12. Li LL, Ji J, Fei R, Wang C, Lu Q, Zhang JH, Jiang L-P, Zhu J-J. 2012 A facile microwave avenue to electrochemiluminescent two-color graphene quantum dots. Adv. Funct. Mater. 22, 2971–2979. (doi:10.1002/adfm.201200166)
13. Lu JJ, Yan M, Ge L, Ge SG, Wang SW, Yan JX, Yu JH. 2013 Electrochemiluminescence of blue–luminescent graphene quantum dots and its application in ultrasensitive aptasensor for adenosine triphosphate detection. Biosens. Bioelectron. 47, 271–277. (doi:10.1016/j.bios.2013.03.039)
14. Dong YQ, Chen CO, Zeng XT, Gao LL, Cui ZM, Yang HB, Guo CX, Chi YW, Li CM. 2012 One-step and high yield simultaneous preparation of...
single- and multi-layer graphene quantum dots from CX-72 carbon black. J. Mater. Chem. 22, 8764–8766. (doi:10.1039/c2jm03650a)

15. Dong YQ, Tian WR, Ren SY, Dai RP, Chi YW, Chen GN. 2014 Graphene quantum dots/l-cysteine coreactant electrochemiluminescence system and its application in sensing lead(II) ions. ACS Appl. Mater. Inter. 6, 1646–1651. (doi:10.1021/am404552s)

16. Dong YQ, Lin JP, Chen YM, Fu FF, Chi YW, Chen GN. 2014 Graphene quantum dots, graphene oxide, carbon quantum dots and graphite nanocrystals in coals. Nanoscale 6, 7410–7415. (doi:10.1039/C4NR01482K)

17. Liu Q, Wang K, Huan J, Zhu GB, Qian J, Mao HP, Cai JR. 2014 Graphene quantum dots enhanced electrochemiluminescence of cadmium sulfide nanocrystals for ultrasensitive determination of pentachlorophenol. Analyst 139, 2912–2918. (doi:10.1039/c4an00307a)

18. Lu Q, Wei W, Zhou ZX, Zhou ZX, Zhang YJ, Liu SQ. 2014 Electrochemiluminescence resonance energy transfer between graphene quantum dots and gold nanoparticles for DNA damage detection. Analyst 139, 2404–2410. (doi:10.1039/c4an00307a)

19. Wang CZ, Yifeng EF, Fan LZ, Wang ZH, Liu HB, Li YL, Yang SH, Li YL. 2007 Directed assembly of hierarchical CdS nanotube arrays from CdS nanoparticles: enhanced solid state electrochemiluminescence in H2O2 solution. Adv. Mater. 19, 3677–3681. (doi:10.1002/adma.200701386)

20. Zhou IG, Booker C, Li RV, Sun XJ, Sham TK, Ding ZF. 2010 Electrochemistry and electrochemiluminescence study of blue luminescent carbon nanocrystals. Chem. Phys. Lett. 493, 296–298. (doi:10.1016/j.cplett.2010.05.030)

21. Jiang H, Ju HK. 2007 Electrochemiluminescence sensors for scavengers of hydroxyl radical based on its annihilation in CdSe quantum dots film/ peroxide system. Anal. Chem. 79, 6690–6696. (doi:10.1021/ac0707601)

22. Zou GZ, Ju HK. 2004 Electrogenerated chemiluminescence from a CdSe nanocrystal film and its sensing application in aqueous solution. Anal. Chem. 76, 6871–6876. (doi:10.1021/ac0490012)

23. Ding SH, Gao BH, Shan D, Sun YM, Gronier S. 2013 TiO2 nanocrystals electrochemiluminescence quenching by biological enlarged nanoparticle and its application for biosensing. Biosens. Bioelectron. 39, 342–345. (doi:10.1016/j.bios.2012.07.065)

24. Razmi H, Mohammad-Rezaei R. 2013 Graphene quantum dots as a new substrate for immobilization and direct electrochemistry of glucose oxidase: application to sensitive glucose determination. Biosens. Bioelectron. 41, 498–504. (doi:10.1016/j.bios.2012.09.009)

25. Zhu SJ et al. 2012 Surface chemistry routes to modulate the photoluminescence of graphene quantum dots: from fluorescence mechanism to up-conversion bioimaging applications. Adv. Funct. Mater. 22, 4732–4740. (doi:10.1002/adfm.201201499)

26. Volarevic V et al. 2014 Large graphene quantum dots alleviate immune-mediated liver damage. ACS Nano 8, 12 098–12 109. (doi:10.1021/nn502466z)

27. Murray RW. 1986 Electroanalytical chemistry. New York, NY: Marcel Dekker.

28. Zhang J, Feng M, Tachikawa H. 2007 Layer-by-layer fabrication and direct electrochemistry of glucose oxidase on single wall carbon nanotubes. Biosens. Bioelectron. 22, 3036–3041. (doi:10.1016/j.bios.2007.01.009)

29. Zhu SJ et al. 2012 Surface chemistry routes to modulate the photoluminescence of graphene quantum dots: from fluorescence mechanism to up-conversion bioimaging applications. Adv. Funct. Mater. 22, 4732–4740. (doi:10.1002/adfm.201201499)