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Bifunctional Carbon Dots Derived From an Anaerobic Bacterium of *Porphyromonas gingivalis* for Selective Detection of Fe³⁺ and Bioimaging

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ABSTRACT

In this study, for the first time, *Porphyromonas gingivalis*, an anaerobic bacterium, was selected to synthesize carbon dots. The achieved *P.gingivalis*-carbon dots (Pg-CDs) exhibited strong fluorescence and high stability with capacity for dual function as Fe^{3+} sensor and intracellular imaging agent. The detection limit for Fe^{3+} was as low as $1.85\mu\text{M}$. On the other hand, the prepared Pg-CDs were an excellent candidate for biosensor with high biocompatibility.

INTRODUCTION

Fluorescent carbon-based dots (CDs), including carbon quantum dots and graphene quantum dots [1] have been an ongoing focal point of research for the past decade. As a new developed carbonaceous nanomaterials, CDs possess advantages of high chemical inertness, low toxicity and excellent biocompatibility over conventional quantum dots particularly semiconductor quantum dots which are heavy metal based with serious problem of toxicity [2-4]. CDs can be obtained from countless natural supplies as carbon source [5] by facile synthesis. Increasing scientific efforts have been made on the following: (A) continued attempts to find new carbon source suitable for synthesis of CDs; (B) designing effective approaches to improve their optical properties, the major and most essential properties of CDs which are determined by the starting materials[6], thus expanding their applications. A great expansion of carbon source has been seen in the last recent years. Carbon based or containing materials, such as plants, including fruit [7], leaves [8], vegetables, grass[9], and other natural biomass materials, such as hair fiber [10], goose feathers [11] and baked Lamb [12]were utilized to synthesize CDs. However, most of microorganisms, especially the bacteria, including aerobe and anaerobe, contributing significantly to carbon and nitrogen cycles in the ecosystems, have been paid less attention.

Iron (III) (Fe^{3+}) is considered as one of the most essential ions in biological metabolic systems, performing crucial functions in biochemical and biophysical processes, such as enzymatic catalysis, DNA and RNA synthesis, as well as cellular metabolism [13]. Various physiological disorders and disease are associated with iron deficiency or overload, such as anemia, cancer, diabetes, intelligence decline and so on [14]. Therefore, it is of great significance to develop effective probes for quantitative detection of Fe^{3+} .

Herein, in this study, we reported a preparation of a novel CDs from *P. gingivalis*, a gram-negative anaerobe, through one-step hydrothermal treatment. To the best of our knowledge, this is the first attempt so far anaerobe was selected as precursor for synthesis of CDs. As an individual and unique precursor, it is remarkable that *P. gingivalis* plays a crucial role not simply by serving as carbon source, but more importantly in providing heteroatoms of sulfur and nitrogen that

contributed to the improvement of optical properties of the obtained *P. gingivalis*-carbon dots (Pg-CDs).

P. gingivalis is recognized as the main cause of adult periodontitis [15] and also responsible for occurrence of various tumors[16]. Due to specific requirement for growth under anoxic conditions, *P. gingivalis* releases volatile and other sulfur compounds, such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide[17] through the metabolic pathway by degradation of sulfur-containing compounds including peptides and amino acids[18], explaining well why Pg-CDs synthesized by using *P. gingivalis* as an individual starting material can be carbon-rich and self-passivated with S and N co-dopants on the surface. The obtained Pg-CDs are composed of sufficient volume of nitrogen and adequate amount of sulfur. As a result, the synthesized Pg-CDs exhibited strong fluorescence and excellent stability. The absolute quantum yield is up to 4.23%. To our delight, the fluorescence can be quenched in the presence of Fe^{3+} . Thus, the obtained Pg-CDs can serve as sensing probe for selective detection of Fe^{3+} . The detection limit was as low as 1.85 μM . Furthermore, the Pg-CDs could internalize easily into the cells and have great potentials in biosensing and bioimaging. To the best of our knowledge, this is also the first report of bacteria-deprived CDs that possess bifunction for both metal ion detection and bioimaging [19-21].

It is known many previous co-doped CDs were fabricated from synthetic chemicals or organic compounds which were individually served as carbon or heteroatom donor[6]. Furthermore, most of man-made materials used for preparation of such CDs are highly toxic or corrosive, such as thiourea [22], guanidine thiocyanate[23] and H_2SO_4 [24] used as S source, guanidinium chloride [25] acted as N donor. Although these obtained dual doping CDs have high performance with great potentials toward wider applications, the involvement of toxic chemicals has given rise to main drawbacks of time consumption, toxicity and extra cost upon heteroatom doped CDs. There are a large number of bacteria, including anaerobic microbe on the earth, providing with abundant carbon source to synthesize CDs. Therefore, the production of carbon dots from bacteria is feasible, low-cost, non-hazard and environment friendly.

MATERIALS AND METHODS

Materials : *P. gingivalis* cells were purchased from Bena Culture collection (Beijing, China). Brain heart infusion medium were obtained from Solarbio Life Science (Beijing, China). 2.5 L Anaero Packs were acquired from Mitsubish Gas Chemical (Mitsubish, Janpan). Reagents of FeCl₃, PbCl₂, CoCl₂, BaCl₂, CrCl₂, ZnSO₄, CdCl₂, MnCl₂, LiCl, CaCl₂, MgCl₂ and CuCl₂ were obtained from Tianjin Fengchuan Chemical Reagent (Tianjin, China). AgNO₃ was purchased from Shanghai Xunda Fine Chemical (Shanghai, China). All reagents were of analytical grade and used as received. Double distilled water (ddH₂O) was used throughout all the duration of experiments.

Bacteria Preparation for synthesis of CDs : CDs were prepared via one-step hydrothermal carbonization of *P. gingivalis* cells. The bacterium was cultured on brain heart infusion agar which was supplemented with 5% goat blood and 10 mg/mL hemin, and incubated in cultivation pouch with Anaero Pack for 7-10 days at 37 °C. The cell culture was suspended with 8.5% saline solution prior to being scraped off from the blood agar plate. Subsequently, the bacterium cells, a total of 7×10^{15} cfu of *P. gingivalis* cells were collected from 8 blood agar plates, and centrifuged with 6000 rpm for 10 minutes to remove supernatant. After that, cells were washed with ddH₂O three times, then transferred into a Teflon-lined stainless-steel autoclave (50 mL), and heated at 180 °C for 24 h. After the reaction completed, the resultant light brown solution was filtered through a syringe filter membrane (0.22 μm) and then dried under vacuum.

Characterization: X-ray photoelectron spectroscopy (XPS) measurements were performed on a PHI5000 Versa probe-II (Shimazu Co., Japan). The morphologies of Pg-CDs were investigated by a Tecnai G2 TF30 S-Twin microscope transmission electron microscope (TEM, FEI Holland). The Ultraviolet-Visible absorption spectra were recorded by UV-5100H (METASH, Shanghai) spectrophotometer. The fluorescence spectrum was recorded by both F-7000 (Hitachi, Japan) fluorescence spectrophotometer and Flex Station 3 (Molecular Devices, USA) Multi-Mode Microplate Reader. Fourier transform infrared spectroscopy (FTIR) was acquired with Thermo Fisher NICOLLET iS10 FTIR spectrophotometer (USA).

Fluorescence detection of Fe³⁺: Total 13 kinds of metal ions solutions, including Cd²⁺, Zn²⁺, Mn²⁺, Ag²⁺, Mg²⁺, Cu²⁺, Ba²⁺, Li²⁺, Al³⁺, Ca²⁺, Pb²⁺, and Co²⁺, together with Fe³⁺ solutions with various concentrations were prepared. 2 mL (0.8 g/L) original solution of Pg-CDs was diluted in 8 mL ddH₂O for selectivity assays of Fe³⁺ detection. 50 µL Pg-CDs solution was placed into separate well, same amount of each metal ion solution (50µL) was dispersed into Pg-CDs solution with final concentration of 80 µg/mL. The effect of individual metal ions on the PL intensity of Pg-CDs was recorded by microplate reader at an excitation wavelength of 310 nm.

Sensitivity of Pg-Cds towards Fe³⁺ was evaluated at same experimental conditions. 50 µL of different concentration of Fe³⁺ was added into the aqueous solution of Pg-CDs with final concentration of 200 µg/mL. The changes of the fluorescence intensity upon the mixture were collected by microplate reader at the excitation wavelength of 310nm. All experiments were performed at room temperature.

Cellular toxicity test: Both cancer and normal cells were selected to investigate the effects of the prepared Pg-CDs on cell viability through MTS assay method. Hela cells ((cervical cancer cell line, adherent cells)) and BEAS-2B (Lung Normal Epithelial Cells) were cultivated in the Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum. 100µL of cells were seeded into 96-well plate at a density of 5000 cells per well. 100µL of Pg-CDs solution was added to each well with final concentrations reached to 12.5µg, 50µg, 100µg and 200µg, respectively. Taxol was used for positive control. Then the cells were cultivated at 37°C in a 5% CO₂ atmosphere for 48 hours. After removal of the medium, 20µL MTS solution and 100µL medium were added to each well. Three wells with the mixture of 20µL MTS and 100µL media without cells were used as control. The cells were incubated for another 4 hours. Finally, the absorbance was measured at 492 nm using MULTISKAN FC (Thermo Fisher, USA)

Cell Imaging: Hela cells were seeded in the Glass Bottom Dish with density of 5000 cells/cm² in 1 mL of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and then cultivated at 37°C in a 5% CO₂ atmosphere for 24 h. Then 31.25 uL of Pg-CDs solution were added into the cells with final concentration of 200µg/mL. After cultivation for another 24 hours, the culture medium was discarded and the cells were washed 3 times with phosphate buffer

saline (PBS). Finally, the cells in dish were soaked in PBS and observed on laser confocal scanning microscope. The cells without addition of Pg-CDs cultured under the same experimental conditions were used as the control.

RESULTS

Characterization of Pg-CDs

The High Resolution TEM (HRTEM) imaging was applied to show the microstructure of the prepared Pg-CDs. As shown in Fig. 1a, the individual dots showed uneven quasi-spherical shape with sizes from 3 to 7 nm, which were similar to previously reported S/N co-doped CDs [1, 22]. Furthermore, no obvious aggregation was observed, meaning high dispersity in water solution. The lattice fringes with a lattice space of 0.22 nm were clearly shown in the image (Fig. 1a inset), which was consistent with the lattice spacing of graphene[26]. The average diameter measured was 5.05nm (Fig. 1b). The X-Ray powder diffraction (XRD) pattern (Fig. 2a) exhibited a broad peak centered at about 28°, illustrating the turbostratic carbon structure of the synthesized Pg-CDs with graphitic and disordered carbons[27]. This was agreeable with the HRTEM result.

<Figure 1>

X-ray photoelectron spectroscopy (XPS) was employed to investigate the surface structure and elemental composition of the resultant Pg-CDs. The full range of XPS spectrum of Pg-CDs, as shown in Fig. 2b, revealed four typical peaks at 283.5, 398.1, 532.1 and 160.5 eV, which were associated with C1s, N1s, O1s and S2p, respectively. The elementary analysis of the Pg-CDs verified there were carbon, nitrogen and sulfur elements present on the surface, indicating the sulfur and nitrogen atoms were efficiently doped into carbon framework of the synthesized Pg-CDs. The Pg-CDs were mainly composed of carbon (71.3%), nitrogen (6.54%), oxygen (21.53%) and sulfur (0.63%).

<Figure 2>

The high-resolution of C1s shown in Fig. 3a demonstrated three main peaks at 283, 284.4 and

286.6 eV. The peaks centered at 283 and 286.6 eV were contributed to C=C, and C-O/C-S bonds, respectively. The binding energy peak at 284.4 eV was assigned to C-C bond, suggesting significant numbers of graphitic structures present on the surface of the prepared Pg-CDs [1]. Fig. 3b showed the deconvoluted peak of O1s and two kinds of oxygen species were displayed. The peak centered at 530 eV was ascribed to O-O groups, while the peak located at 531.2 was originated from C=O groups. The N1s spectrum (Fig. 3c) revealed two forms of N elements. The peak at 397.9 was assigned to the C-N binding while the small peak with the binding energy of 399.7 eV was contributed to the C-N-C binding group[28].

The S2p spectrum (Fig. 3d) exhibited two peaks at 160.8 and 162.2 eV, suggesting two sulfur species present on the surface of the prepared Pg-CDs. The contribution at 160.8 eV was ascribed to S2p_{3/2} site [23] while the main peak at 162.2 e V indicated the existence of the S-C-N groups[29].

<Figure 3>

The functional groups analysis of the resultant Pg-CDs was confirmed by FTIR spectra. As shown in Fig. 4a, the absorption peak at 3211 cm⁻¹ corresponded to the OH stretching vibration. The peaks at 1587 and at 1667 cm⁻¹ aroused from -NH groups and C=O stretching vibration, respectively [29]. The typical absorption peaks at 1071 and 704 cm⁻¹ were originated from -SO₃-symmetric vibration and C-S stretching vibration, respectively [30].The peak at 1405 cm⁻¹ originated from C-O-O groups and OH groups [29] while the absorption band at 1463 was due to the stretching vibration of C=C. The FTIR result spectra also exhibited C-H stretching vibrations at 2964 and the absorption peak at 1463 cm⁻¹ which suggested the presence of the amine III C-N groups [30].These results from FTIR analysis were in good accordance with XPS analysis data. From the above observation, it is clear that the surface of Pg-CDs contained abundant hydroxyl, carboxyl, amino and sulfonic groups, confirming nitrogen and sulfur atoms were successfully incorporated into the framework of Pg-CDs. The multitudinous functional groups formed on the surface of Pg-CDs maybe facilitate their distinct optical properties and offered selective probes for metal detection and bioimaging even without further surface passivation.

Optical properties of Pg-CDs

The UV-vis absorption and fluorescence emission spectra were used to evaluate the detailed optical properties of Pg-CDs. The Pg-CDs exhibited two obvious absorption bands at 209 nm and 267 nm as shown in Fig. 4b. The strong peak at 204 nm could be attributed to the π - π^* transitions of the C=O groups[31] while the shoulder peak at 267 nm is assigned to the n- π^* transitions of the C=O groups. The intensive blue fluorescence of the Pg-CDs dots under 365 nm UV light illustrated by the inset in Fig. 4b demonstrated their potential application in metal detection. The Pg-CDs displayed an excitation-dependent fluorescence behavior. As shown in Fig. 4c, when excited by different wavelength from 260 to 310 nm in 10 nm increment, the corresponding FL emission peaks decreased gradually and then increased till reaching to the maximum intensity at 310 nm of excitation wavelength. The FL intensity decreased once again and red shifted when the excitation wavelength increased from 320 to 350 nm, revealing the emission intensity and bandwidth were affected significantly by the excitation wavelength. The results demonstrated the optical performance of Pg-CDs relied heavily on the excitation wavelength owing to the various radiative sites as a result of abundant C, O, S and N elements-containing functional groups on their surface [1, 32]. The maximum FL emission intensity was achieved at excitation wavelength of 310 nm which was not consistent with that of absorption spectra, implying that the sizes of the dots were not uniform [36] which were agreeable with the result of HRTEM image.

Edinburgh fluorescence spectrometer (FLS920) with an integrating sphere was employed to calculate the quantum yield of the resultant Pg-CDs. The absolute quantum yield was measured as 4.83%. The decay curve of Pg-CDs in Fig. 4d was a bi-exponential function determined by lifetimes of 1.44 ns (16.35%) and 5.42 ns (83.65%) at 310 nm excitation, with an intensity weighted average time of 4.8 ns.

The fluorescence stability of Pg-CDs was studied by monitoring its changes upon exposure to wide range of temperature, pH values and ionic concentrations. Fig. 5a showed the FL intensity changed slightly when Pg-CDs were heated from 20 to 90 °C, indicating FL intensity was independent on temperature. As it is shown in Fig. 5b, the fluorescence intensity was not

significantly affected by the presence of 0 to 2000 μM NaCl, suggesting these CDs were highly resistant to ionic strength. As seen in Fig. 5c, the fluorescence remained almost constant with the pH value changing from 2 to 10 and dropped slightly when pH value ranged from 11 to 13. The above observation demonstrated the obtained Pg-CDs were highly stable, suggesting their unique fluorescence properties which can be exploited for various applications.

<Figure 5>

Detection of Fe^{3+}

As seen in Fig. 6a, PL intensity of the prepared Pg-CDs reduced tremendously upon interaction with Fe^{3+} . In contrast, there were no noticeable declines upon dispersion into the other common cations with the same concentration, indicating Pg-CDs responded remarkably to Fe^{3+} ions. Then we explored the sensitivity of Pg-CDs towards Fe^{3+} . The quenching effects of Fe^{3+} ions on fluorescence of the Pg-CDs were collected under various concentrations of Fe^{3+} . The Pg-CDs concentration was remained at 200 $\mu\text{g/ml}$. Fig. 6b showed the PL intensity decreased gradually with the increasing concentrations of Fe^{3+} from 0 to 500 μM , revealing that Fe^{3+} ions had significant influence on the fluorescence intensity of Pg-CDs. Fig. 6c demonstrated good linear relationships between fluorescence intensity and Fe^{3+} concentrations with a correlation coefficient R^2 of 0.96507. In which F_0 and F were the fluorescence emission peaks before and after interaction with Fe^{3+} , respectively. The following equation was found to express the relationship between quenching effect and the concentration of Fe^{3+} .

$$y=0.0026*x+0.82151$$

The detection limit for Fe^{3+} was estimated to be 1.85 μM based on $3\sigma/\text{slope}$ (where σ was the standard deviation, slope value was 0.0026 according to the above correlation equation), suggesting Pg-CDs was among the sensitive promising probes for detection of Fe^{3+} as shown in Table. 1

<Figure 6>

Mechanism for the fluorescence response of Pg-CDs to Fe^{3+}

To gain insight into the quenching mechanism, the FL response of Pg-CDs toward Fe^{3+} was

further investigated. Ion exchange or energy transfer between the sensor and ions were proposed as two main reasons for the fluorescence quenching [33]. As demonstrated in Fig. 5d, The red shift in the absorption spectrum of Pg-CDs was captured in the presence of Fe^{3+} , its peak overlaying with the emission peak of Pg-CD and Fe^{3+} , indicating the occurrence of energy transfer between Fe^{3+} and Pg-CDs, consequently leading to the FL quenching [34]. S and N two dopants endowed the prepared Pg-CDs with abundant functional groups including some chelating groups, such as hydroxyl and amine groups, which could interact easily with Fe^{3+} ions. [35, 36]. Although the amount of sulfur is less than nitrogen in the surface of Pg-CDs, compared with nitrogen, sulfur has smaller electro-negativity and larger atomic radius, the valence electrons of Sulfur are easier to lose, thus resulting in the alteration on the charge density of CDs and the release of the amine and/or hydroxyl groups, facilitating the coordination interaction between those groups and the Fe^{3+} ions. On the other hand, the surface co-doping may result in the suppression force on the surface of CDs which accompany and interfere with the fluorescence emission[37]. This can well explain why some reported S and N dual doped CDs displayed less quenching capability upon the addition of Fe^{3+} ions than the un-doped or sole doped CDs[38] as shown in Table1.

<Table 1>

Cell cytotoxicity

Compared with Taxol taken as the positive control in Fig.7 c and d, the cell viability treated with Pg-CDs remained over 80% even at relatively high dose of Pg-Cds ($200\mu\text{g}/\text{mL}$) (Fig.7 a and b), the prepared Pg-CDs exhibited low toxicity to both cancer cells and normal cells, indicating the Pg-CDs are an ideal material for bioimaging under physiological conditions with excellent biocompatibility.

<Figure 7>

Cell imaging

As expected, the obtained Pg-CDs were suitable for intracellular cell imaging. As shown in Fig.8 a1-a3 and b1-b3, under different excitation light of 408,488 and 555, the Hela cells in the presence of Pg-CDs exhibited bright blue, green, and red fluorescence, respectively. There was no

fluorescence observed in the control cells untreated with Pg-CDs. The Pg-CDs were internalized easily into the cells and distributed in the cytoplasm, mostly around the nucleus. These results revealed the prepared Pg-CDs still remain intense PL emission after being penetrated into the cytoplasm and have great application potential in bioimaging and biosensing due to their low cytotoxicity and high biocompatibility.

<Figure 8>

CONCLUSION

In this study, we provided directive evidence that the anaerobic bacterium of *P. gingivalis* was a good candidate for synthesis of CDs. XPS, FTIR, and TEM techniques were employed to study formation and characterization of Pg-CDs. Their fluorescence intensity was almost unchanged under various conditions of different temperature, pH value and extremely high ion concentrations, suggesting their attractive optical stability. To our delight, the prepared Pg-CDs held promising potential both in Fe^{3+} detection and bioimaging owing to their strong fluorescence and nontoxicity.

The total number of the bacteria and archaea existing in all environments on the Earth was presumed to be 1.2×10^{30} . With immense diversity, the microorganisms play vital roles in carbon and nutrient cycling, ecosystem health and sustainability[39]. Among the bacteria inhabited in animal (including human) microbiota, a remarkable proportion is anaerobic bacteria which can be classified to two types: strict and facultative, providing new opportunities of the continued growth in this exciting field of CDs.

Conflicts of interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Dong, Y., et al., *Carbon-Based Dots Co-doped with Nitrogen and Sulfur for High Quantum Yield and Excitation-Independent Emission*. Angewandte Chemie, 2013. **125**(30): p. 7954-7958.
2. Wang, H., et al., *Turn-on fluorescence probe for high sensitive and selective detection of Ag⁺ by L-glutathione capped CdTe quantum dots in aqueous medium*. Journal of Luminescence, 2016. **180**: p. 14-19.
3. Prathumsuwan, T., et al. *Preparation of carbon quantum dots from succinic acid and glycerol by hydrothermal technique for heavy metal detection and cell imaging*.
4. Shuai, et al., *In Situ Synthesis of Fluorescent Mesoporous Silica–Carbon Dot Nanohybrids Featuring Folate Receptor-Overexpressing Cancer Cell Targeting and Drug Delivery*. 2019. **v.11**(02): p. 193-205.
5. Ye, R., et al., *Coal as an abundant source of graphene quantum dots*. Nat Commun, 2013. **4**: p. 2943.
6. Lu, W., et al., *Comparative study for N and S doped carbon dots: Synthesis, characterization and applications for Fe(3+) probe and cellular imaging*. Anal Chim Acta, 2015. **898**: p. 116-27.
7. Chatzimitakos, T., et al., *Two of a kind but different: Luminescent Carbon Quantum Dots from Citrus Peels for Iron and Tartrazine Sensing and Cell Imaging*. Talanta, 2017. **175**.
8. Sachdev, A. and G. Packirisamy, *Green synthesis of multifunctional carbon dots from coriander leaves and their potential application as antioxidants, sensors and bioimaging agents*. Analyst, 2015. **140**.
9. Liu, S., et al., *Hydrothermal Treatment of Grass: A Low-Cost, Green Route to Nitrogen-Doped, Carbon-Rich, Photoluminescent Polymer Nanodots as an Effective Fluorescent Sensing Platform for Label-Free Detection of Cu(II) Ions*. Advanced Materials, 2012. **24**: p. 2037-41.
10. Sun, D., et al., *Hair fiber as a precursor for synthesizing of sulfur- and nitrogen-co-doped carbon dots with tunable luminescence properties*. Carbon, 2013. **64**: p. 424-434.
11. Liu, R., et al., *A Facile Microwave-hydrothermal Approach towards Highly Photoluminescent Carbon Dots from Goose Feather*. RSC Adv., 2014. **5**.
12. Wang, H., et al., *Presence of Fluorescent Carbon Nanoparticles in Baked Lamb: Their Properties and*

Potential Application for Sensors. Journal of Agricultural and Food Chemistry, 2017. **65**.

13. Guo, S., D.M. Frazer, and G.J. Anderson, *Iron homeostasis: transport, metabolism, and regulation*. Curr Opin Clin Nutr Metab Care, 2016. **19**(4): p. 276-81.
14. Torti, S.V. and F.M. Torti, *Iron and cancer: more ore to be mined*. Nat Rev Cancer, 2013. **13**(5): p. 342-55.
15. Socransky, S.S. and A.D. Haffajee, *The Bacterial Etiology of Destructive Periodontal Disease: Current Concepts*. J Periodontol, 1992. **63 Suppl 4S**: p. 322-331.
16. Liu, X.-b., et al., *The potential role of P.gingivalis in gastrointestinal cancer: a mini review*. Infectious Agents and Cancer, 2019. **14**(1): p. 23.
17. Van den Velde, S., et al., *Detection of odorous compounds in breath*. J Dent Res, 2009. **88**(3): p. 285-9.
18. Aydin, M. and C.N. Harvey-Woodworth, *Halitosis: a new definition and classification*. Br Dent J, 2014. **217**(1): p. E1.
19. Hua, X.W., et al., *Bacteria-derived fluorescent carbon dots for microbial live/dead differentiation*. Nanoscale, 2017. **9**(6): p. 2150-2161.
20. Lin, F., C. Li, and Z. Chen, *Bacteria-Derived Carbon Dots Inhibit Biofilm Formation of Escherichia coli without Affecting Cell Growth*. Front Microbiol, 2018. **9**: p. 259.
21. Hua, X.-W., et al., *Bacteria-derived fluorescent carbon dots for microbial live/dead differentiation*. Nanoscale, 2016. **9**.
22. Zhang, J., et al., *Rapid synthesis of N, S co-doped carbon dots and their application for Fe³⁺ ion detection*. Journal of Nanoparticle Research, 2018. **20**.
23. Qin, Z., et al., *One-pot synthesis of dual carbon dots using only an N and S co-existed dopant for fluorescence detection of Ag⁺*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2018. **208**.
24. Sun, D., et al., *Hair fiber as a precursor for synthesizing of sulfur- and nitrogen-co-doped carbon dots with tunable luminescence properties*. Carbon, 2013. **64**: p. 424-434.
25. Rong, M., et al., *One-pot solid phase pyrolysis synthesis of nitrogen-doped carbon dots for Fe³⁺ sensing and bioimaging*. Sensors and Actuators B: Chemical, 2017. **245**: p. 868-874.
26. Gao, S., et al., *Facile preparation of sulphur-doped graphene quantum dots for ultra-high performance ultraviolet photodetectors*. New J. Chem., 2017. **41**.
27. Shao, M., et al., *The synthesis of carbon nanotubes at low temperature via carbon suboxide*

disproportionation. Carbon, 2004. **42**(1): p. 183-185.

28. Yang, W., et al., *Carbon dots with red-shifted photoluminescence by fluorine doping for optical bio-imaging*. Carbon, 2017. **128**.
29. Xue, M., et al., *Sulfur and nitrogen binary doped carbon dots derived from ammonium thiocyanate for selective probing doxycycline in living cells and multicolor cell imaging*. Talanta, 2016. **150**: p. 324-30.
30. Yang, Z., et al., *Nitrogen-doped, carbon-rich, highly photoluminescent carbon dots from ammonium citrate*. Nanoscale, 2014. **6**(3): p. 1890-5.
31. Wang, B.-B., et al., *Single-step synthesis of highly photoluminescent carbon dots for rapid detection of Hg²⁺ with excellent sensitivity*. Journal of Colloid and Interface Science, 2019. **551**: p. 101-110.
32. Parambath, A., et al., *Toward quantitatively fluorescent carbon-based "quantum" dots*. Nanoscale, 2011. **3**: p. 2023-7.
33. Wu, M. and Y.-W. Yang, *A Fluorescent Pillarene Coordination Polymer*. Polymer Chemistry, 2019. **10**: p. 2980–2985.
34. Gao, J., et al., *N-doped carbon dots covalently functionalized with pillar[5]arenes for Fe³⁺ sensing*. Beilstein Journal of Organic Chemistry, 2019. **15**: p. 1262-1267.
35. Chan, K.K., S.H.K. Yap, and K.T.J.N.M.L. Yong, *Biogreen Synthesis of Carbon Dots for Biotechnology and Nanomedicine Applications*. 2018. **10**(4).
36. Praneerad, J., et al., *Multipurpose sensing applications of biocompatible radish-derived carbon dots as Cu²⁺ and acetic acid vapor sensors*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2019. **211**: p. 59-70.
37. Liang, J., et al., *Back Cover: Sulfur and Nitrogen Dual-Doped Mesoporous Graphene Electrocatalyst for Oxygen Reduction with Synergistically Enhanced Performance (Angew. Chem. Int. Ed. 46/2012)*. Angewandte Chemie International Edition, 2012. **51**(46): p. 11640-11640.
38. Hong, D., et al., *One-step hydrothermal synthesis of down/up-conversion luminescence F-doped carbon quantum dots for label-free detection of Fe³⁺*. Microchemical Journal, 2019. **151**: p. 104217.
39. Flemming, H.-C. and S. Wuerzt, *Bacteria and archaea on Earth and their abundance in biofilms*. Nature Reviews Microbiology, 2019: p. 1.

FIGURE CAPTIONS

Figure 1. Characterization of Pg-CDs. (a) TEM image, inset: lattice space; (b) Particle size distribution of Pg-CDs.

Figure 2. (a) XRD spectra of Pg-CDs. (b) XPS scanning spectrum of Pg-CDs: Four major peaks of C1s, O1s, N1s and S2p.

Figure 3. XPS corresponding high resolution scan of (a) C1s, (b) O1s, (c) N1s, and (d) S2p regions of Pg-CDs.

Figure 4. (a) FTIR spectrum of Pg-CDs; (b) UV-visible absorption spectra of Pg-CDs, inset: photograph of aqueous Pg-CDs solution under UV light and daylight; (c) Emission spectrum of Pg-CDs excited at progressively longer wavelength in 10 nm increment from 260 nm to 350 nm; (d) Fluorescence decay curve of Pg-CDs.

Figure 5. FL spectra of Pg-CDs under various conditions of different: (a) temperature; (b) NaCl concentration; (c) pH value; (d) UV-visible absorbance spectrum of Fe^{3+} and Pg-CDs+ Fe^{3+} , respectively.

Figure 6. (a) FL intensity peaks of Pg-CDs in the presence of 13 metal ions. Concentration: Fe^{3+} of 80 $\mu\text{g/mL}$ and metal ion of 900 μM ; (b) FL spectrum of Pg-CDs (Concentration of 200 $\mu\text{g/mL}$) with different concentration of Fe^{3+} : 0, 50, 100, 150, 200, 250, 300, 500 μM (From top to bottom); (c) The calibration curve of FL quenching effects and concentration of Fe^{3+}

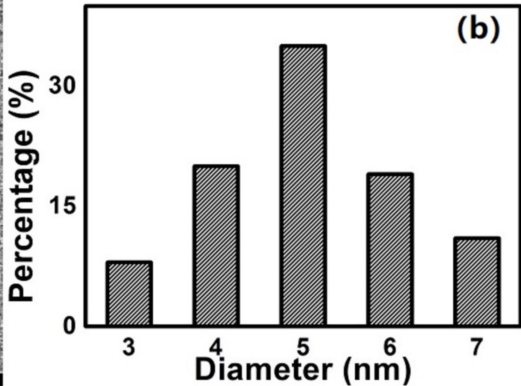
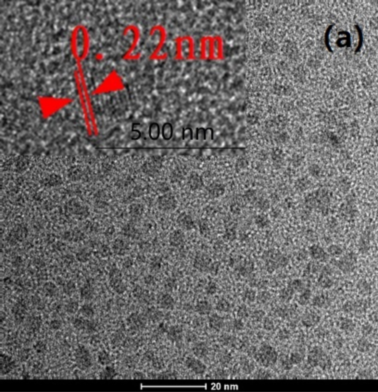
Figure 7. (a) The effects of Pg-CDs on cancer cell viability; (b) The effects of Pg-CDs on

normal cell viability ; The effects of Taxol on: (c) Cancer cell viability (d) Normal cells as positive control.

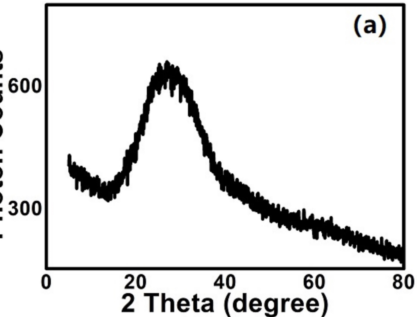
Figure 8. Confocal images of Hela cells incubated with Pg-CDs (a1-a3) and without Pg-CDs (b1-b3). Hela cells imaged by excitation wavelength at 408 (a1, b1), 488 (a2, b2) and 555 (a3, b3).

Table.1 Comparison of Non-doped, Sole-doped and bi-doped CDs for the determination of Fe^{3+}

CDs	Liner Range	Limit of detection	References
Non-doped CDs	0-0.18 mM	52nM	[28]
S-doped CDs	0-500 μM	0.1 μM	[39]
N-doped CDs	0-500 μM	0.2 μM	[40]
F-doped CDs	1-100 μM	0.01 μM	[27]
S/N co-doped	6.0–200 μM	0.80 μM	[6]
S/N co-doped CDs	μM	1.7 μM	[41]
S/N co-doped CDs	1.56-200 μM and 12.5-1000 nM	1.72 μM	[42]
S/N co-doped CDs	0-500 μM	1.85 μM	This work



Photon Counts



Photon Counts

