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Synthesis of carbon quantum dots from cabbage with down- and up-conversion photoluminescence properties: excellent imaging agent for biomedical applications†

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Carbon quantum dots (CQD) with down and up-conversion photoluminescence (PL) properties have been synthesized through low-temperature carbonization in a facile one step green method from cabbage as the natural source of carbon. The physiochemical and optical properties of the resultant CQD were performed using transmission electron microscopy, confocal laser scanning microscopy and various spectroscopic methods. The CQD with a quantum yield of 16.5% demonstrated excellent solubility and stability in aqueous media, superior resistance to photo bleaching, consistent PL within a biological pH range, excitation-dependent down conversion and excitation-independent up-conversion PL along with large stock shift behaviour. The purified CQD exhibited low cytotoxicity at higher concentration (500 μg ml^{-1}) during the cell viability experiment against HaCaT cell, an immortalized non-tumerogenic human keratinocyte cell. Subsequently, CQD treated cells displayed three distinguished blue, green and red colours under a confocal microscope during *in vitro* imaging. Due to the advantages of green synthesis, high biocompatibility, excellent optical properties, low cytotoxicity and good cellular imaging outcome, the cabbage derived CQD showed considerable promise in biomedical applications.

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1. Introduction

Carbon quantum dots (CQD) have emerged as attractive materials for numerous applications because of their favourable tunable multi-colour photoluminescence (PL) properties, high chemical stability, low toxicity, biocompatibility, and easy functionalization.[1] Their capability of displaying excitation-dependent PL emission has made the material an excellent candidate for a cell imaging agent in biomedical applications, an analytical probe for selective detection of inorganic molecules and for use in optoelectronic devices.[2–4] Therefore, synthesis of high quality CQD with environmentally benign techniques from a low cost source is essential. There are many organic compounds that have already been used for the synthesis of CQD, for instance, polybasic acid, glucose, sucrose, glycol, glycerol, and chitosan, using various methods such as hydrothermal, laser ablation, electrochemical oxidation, microwave irradiation, hot injection, and pyrolysis.[5] The hydrothermal method is considered to be a simple, direct, and efficient one among the reported techniques. The above mentioned procedures, except for hydrothermal, noticeably suffer from some drawbacks because most involve several steps, harsh chemical reaction, and post-treatment for surface passivation, which limit their wide applications.

Alternatively, the synthesis of CQD from natural sources has earned popularity because of the abundance of carbon precursors and the low toxicity of the product for biological application. There are already a few reports available for synthesis of CQD from natural sources, such as grape juice, orange juice, orange peel, eggs, strawberry juice, soy milk, soy ground, and cocoon silk.[6–13] The convenient hydrothermal method has been used in most of these cases to synthesise CQD. The down sides of the reported methods were consumption of expensive bio-precursors and usage of organic solvents, especially ethanol and dichloro-methane, along with water during the synthesis. These drawbacks limit their applications in environmental, biological and life sciences. It is, therefore, highly desired to explore an easily available and cheap carbon source for the synthesis of high quality CQD with excellent PL

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behaviour through environmentally friendly green techniques. In search of easily available natural precursors coupled with green synthesis of CQD, herein, we report a very simple and low cost synthesis of highly luminescent bio-friendly CQD coated with natural linkers from cabbage by one step hydrothermal treatment. Ultrapure water was the only solvent from 'before starting synthesis' to 'dispersing and storing' of CQD. The resulting high quality CQD showed excellent physiochemical and optical properties. Finally the product was applied for bio imaging after performing a reliable biocompatibility test in a living cell environment.

It is worth mentioning that quantum dots (QD), specially semiconducting II–VI QD, are already considered to be potential candidates as luminescent imaging probes and labels in biomedical application ranging from drug delivery to cell imaging. There are many reports of using semiconductor QD for imaging purposes in lymph nodes, tumour-specific receptors, and malignant tumour detectors.[14–16] Numerous studies have reported that the size, charge, coating ligands, oxidative, photolytic and mechanical stability of II–IV QD can contribute to cytotoxicity in the long run because of the leakage of heavy metal ions from the core caused by photolysis and oxidation.[17,18] On the other hand, carbon-based nanomaterials, such as CQD, nanodiamonds, carbon nanotubes and graphene QD, are regarded as appropriate alternative candidates to the aforementioned semiconducting II–VI QD because of their chemical stability, biocompatibility, high water-solubility, sufficient fluorescence quantum yield (QY) and low toxicity against living cells.[19–23] There are already a few reports available in the literature regarding the application of CQD as cell imaging agents in biomedical research.[24–29]

In this context, we have selected cabbage as a new and cheaply abundant biomaterial to synthesis high quality CQD for the alternative of heavy metal based QDs for biomedical application. Certainly cabbage is cheaper than other reported sources, such as orange, grapes, strawberry, eggs, and soya milk. Therefore, the choice of a new biomaterial and a one-step low temperature synthesis technique has paved the way to large scale synthesis of high quality CQD without hazardous chemicals for biomedical application. The surfaces of the as derived CQD are highly functionalized with oxygen rich hydroxyl groups as well as nitrogen and do not necessitate further modification. The resultant CQD was applied successfully for the imaging of HaCaT cells, an immortalized non-tumerogenic human keratinocyte cell.

2. Materials and methods

2.1. Chemicals

All required chemicals were purchased from Sigma-Aldrich, Korea and were used as received. Ultrapure water (18.2 MΩ cm^{-1}) from a Milli-Q ultrapure system was used in this study.

2.2. Environmentally friendly synthesis of CQD

A cabbage was collected from a local market and washed several times with ultrapure water. It was then cut into pieces and placed into a domestic fruit-juicer for further processing to conveniently use in a hydrothermal reactor. The measured amount of as obtained smashed-cabbage was treated at 140 °C for 5 hours in a hydrothermal reactor. The reactor was allowed to cool naturally. The dark brown solution was collected through vacuum filtration and then centrifuged at 12 000 rpm for 15 min to separate the less-fluorescent larger CQD. Next, the obtained CQD containing solution was dialyzed against ultrapure water using a tubular dialysis membrane (MWCO ~ 1 kDa) for 48 hours and finally the purified CQD solution was concentrated to approximately 5 mg mL^{-1} using a vacuum oven operating at 60 °C. The procedure was illustrated schematically in Fig. 1.

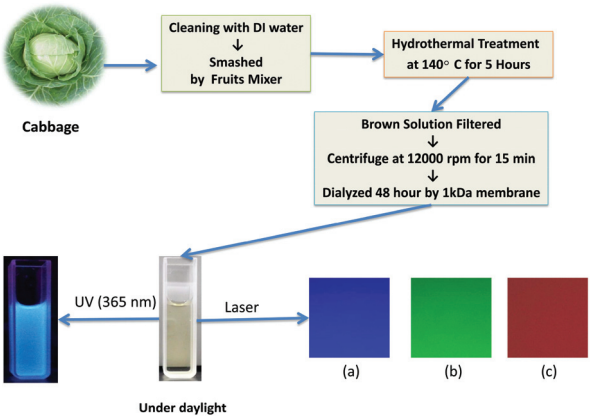

Fig. 1 Schematic presentation of the synthesis of CQD from cabbage with the hydrothermal treatment and visible blue emission under UV; and (a) blue, (b) green and (c) red emissions under a confocal microscope when excited by 405, 480 and 543 nm lasers, respectively.

2.3. Cell culture and cytotoxicity experiment

HaCaT cells were cultured in Defined K-SFM serum free medium containing 10% fetal bovine serum (FBS), penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1}). The cell suspension was kept in an incubator (Thermo Electron Corporation, USA) at 37 °C, 5% CO_2; and 95% humidity.

The cytotoxicity of CQD was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, HaCaT cells were treated with different concentrations of 100 μl (1000, 700, 500, 300, 100 and 20 μg ml^{-1}) of the CQD solutions in wells and were incubated for 24 hours. The cells attached to each plate were treated with 20 μl of freshly prepared 2.5 mg ml^{-1} MTT after incubation and were incubated further to allow formation of violet-coloured formazan. The supernatant was then carefully removed and the formazan was dissolved with the help of DMSO before the absorbance was measured at 570 nm with a microplate reader. Details of the cytotoxicity experiment are presented in ESI.†

2.4. Sample preparation for bioimaging

HaCaT cells were dispersed in 12 replicate wells to a total volume of 200 μL per well and maintained at 37 °C under a 5% CO_2 and 95% air atmosphere in incubator for 24 h. The

culture medium was removed, and the cells were incubated in culture medium containing the CQD at the selected concentrations for 24 h and washed with the PBS buffer prior to capturing the confocal fluorescence microscopic images.

2.5. Instrument for characterization

Transmission electron microscopy (TEM) and high resolution TEM (HRTEM) images were captured by using a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. Fourier transform infrared (FTIR) spectra of samples were recorded using a BRUKER VECTOR-22 spectrometer. The X-ray photoelectron spectroscopy (XPS) measurements were performed on a Thermo Scientific K-Alpha KA1066 spectrometer using a monochromatic Al-Kα X-ray source ($h\nu$ = 1486.6 eV). PL emission measurements were performed using an LS50B Luminescence Spectrometer (Edinburgh Instruments, UK). UV-Vis absorption was measured using a TU-1810 UV–Vis Spectrophotometer (PGeneral, China). The PL life time decay profile was measured using a Time Correlated Single Photon Counting (TCSPC) Spectrometer (FLS920, Edinburgh Instruments, UK). The confocal microscopic images were obtained using a confocal laser scanning microscope (LSM 510META; Carl Zeiss).

3. Results and discussion

3.1. Yield of CQD

The yield of CQD was calculated to be 7.076% as 353.8 mg of product was obtained from 5 g of cabbage leaves. The percentage yield was compared with other reported results based on natural sources-derived CQD and the comparison is listed in Table S1 (ESI†). It was observed that the yields from orange peel, soybean ground, food waste and tomato were 12.3, 1, 0.12 and 12.5%, respectively. The highest percentage yield was 12.5% and the lowest was 0.12% among the surveyed reports. Therefore, the yield of CQD using the method reported in this study is competitive and large-scale synthesis of CQD is feasible.

3.2. Physiochemical characterization

The morphology of the CQD was assessed by TEM and HRTEM imaging. The TEM image (Fig. 2a) clearly revealed that the CQD derived from cabbage were found to be 2–6 nm in diameter with a narrow size distribution, mono-dispersed and well separated from each other, with a spherical morphology in aqueous media. The HRTEM (Fig. 2a-inset) image showed the partially crystalline structure of the CQD with a lattice parameter of 0.21 nm, which may be attributed to the sp^2 (1120) graphitic crystal phase of graphene. The typical XRD data of CQD are presented in Fig. 2b and demonstrated a wider peak centred at around 22.0 degree with an interlayer spacing of 0.395 nm, which is broader than that of graphite (0.34 nm). The oxygen containing groups on the surface of the CQD might have played a role in enhancing the interlayer distance.[30]

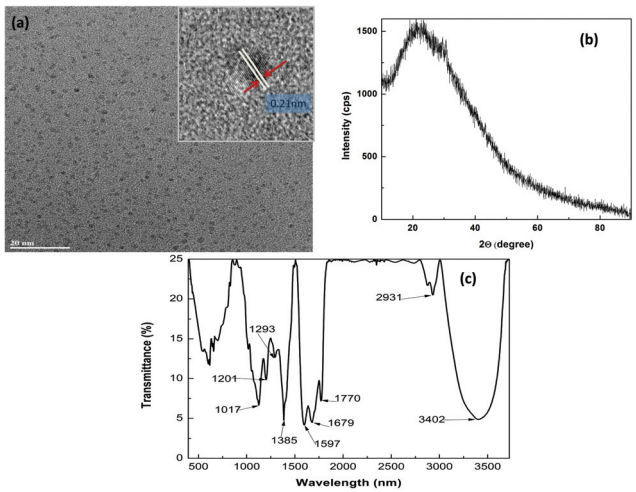

Fig. 2 Physiochemical properties of CQD: (a) TEM image and HRTEM image (inset); (b) XRD spectrum and (c) FTIR spectrum of the resulting CQD.

An FT-IR spectrum was recorded to study the functional groups on the surface of CQD as shown in Fig. 2c. The broad absorption band located at 3418 cm^{-1} was assigned to compounds with hydroxyl group (–OH), which implies the existence of a large number of residual hydroxyl groups on the surface. The absorption bands at 2930 and 2880 cm^{-1} were assigned to C–H stretching, which may arise due to methyl or methylene groups associated with the aliphatic hydrocarbons present in the cabbage.

The characteristic bands at 1715 and 1664 cm^{-1} were ascribed to C=O stretching vibration in carboxyl group and C=C stretching, respectively.[31] The absorption band at 1590 cm^{-1} indicated the amide (CONH) bending. Moreover, an absorption band at 1209 cm^{-1} can be assigned to symmetric stretching modes of C–O–C from either ether or epoxy. Therefore, the surface functional groups on the CQD were predicted to be hydroxyl (–OH), carboxyl (C=O), amide ($CONH_2$) and epoxide/either (C–O–C) groups, which makes the CQD highly dispersible in water. The available hydrophilic groups in the CQD may provide an insight into the luminescence mechanisms along with promoting the environmental friendliness of the products to be applied in biological environments.[32]

The XPS results were demonstrated for further confirmation of the functional groups and content of atoms in CQD. The XPS survey spectrum exhibited three peaks at 285, 399 and 532 eV (Fig. 3a), which are attributed to C 1s, N 1s and O 1s, respectively. The atomic ratio of C 1s, N 1s and O 1s was 66.5%, 4.61% and 28.73%, respectively. The high-resolution C 1s spectra (Fig. 3b) demonstrated the presence of a C=C/C–C bond with a binding energy at 284.4 eV; C–OH/C–O–C at 285.85 eV; C–O/C–N at 286.96 eV; and O–C=O at 288.18 eV. The high resolution N 1s spectra (Fig. 3c) indicated the major peak at 399.73 eV and 401.08 eV, corresponding to C–N and N–H or C=N pyridine-like and pyrrolic/amide nitrogen atoms.

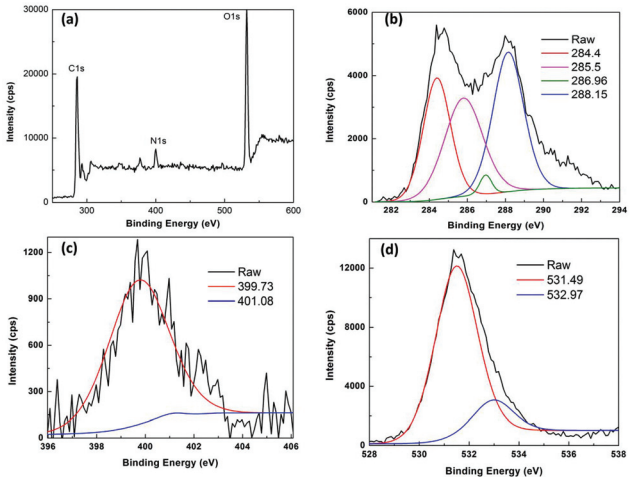

Fig. 3 XPS spectra of CQD: (a) survey spectrum; (b) C 1s spectra; (c) N 1s spectra and (d) O 1s spectra.

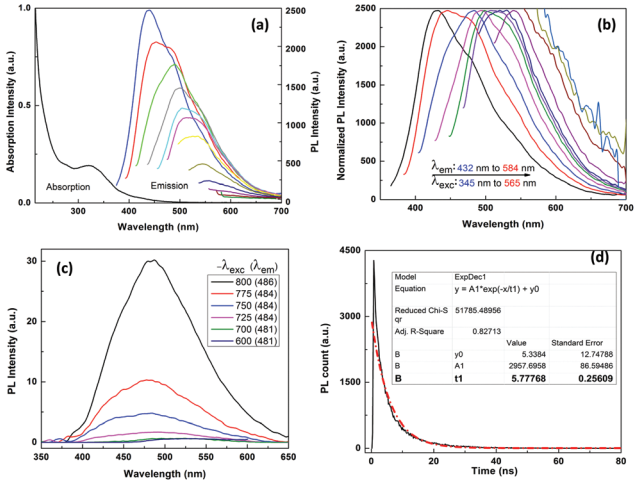

Fig. 4 Optical properties of the CQD: (a) UV-visible absorption and emission spectra of as prepared CQD dispersed in water at excitation wavelengths progressively increasing from 345 to 565 nm. (b) Normalized PL emission spectra where emission wavelength shifts from 432 to 584 nm. (c) Up-conversion PL emission spectra at excitation wavelengths from 600 to 800 nm. (d) PL life time data of the CQD by TCSP method.

On the other hand, the high resolution spectra of O 1s (Fig. 3d) showed major peaks at 531.49 and 532.97 eV, corresponding to C═O, and C–OH/C–O–C groups, respectively.

These results support the existence of plenty of oxygen-containing groups and are consistent with other reported spectroscopic results for CQD.[24–27] The results also indicate the presence of nitrogen on the surface of CQD in the form of an amine molecule as a passivating agent along with oxygen. Therefore, one step synthesis of CQD from cabbage by hydrothermal treatment was a substantial finding because the surface of CQD was passivated by not only an oxygen containing group, but also nitrogen. The self-passivated oxygen and nitrogen containing CQD might result from sequential dehydration, polymerization, carbonization and surface-passivation in the presence of carbonaceous organic materials, such as carbohydrates, sugars, fatty acids, proteins and amino acids in the cabbage leaves, during the hydrothermal treatment.[30] The presence of nitrogen as a doping element in the sphere might play an important role behind the high luminescence of CQD by inducing an upward shift in the Fermi level and electrons in the conduction band.[33] It is already reported that both nitrogen doping and surface functionalization with the amine group enhance the QY of CQD significantly.[34] Therefore, the combined chemistry of oxygen and nitrogen containing groups in the CQD contributed to enhancing the physiochemical and optical properties of the as derived CQD.

3.3. Optical characterization

The purified CQD exhibited strong blue emission under UV light (365 nm), as well as blue, green and red colours during excitation under 405, 488 and 543 nm lasers, respectively, under a confocal laser microscope (Fig. 1). The CQD also performed interesting up-conversion PL along with down-conversion PL properties simultaneously in the experiments. The related optical properties, such as UV-Vis absorption, emission spectra, QY, fluorescence confocal microscopic images and the PL life time profile, were recorded to illustrate the details of the optical features.

As shown in Fig. 4a, CQD showed absorption peaks in the UV region at around 276 and 320 nm, which are attributed to π–π* and n–π* transitions, respectively, with a tail extending to the visible range, and the results are well aligned with other reported work.[25–27] It was observed that the CQD demonstrated excitation dependent down-conversion PL emission. The emission spectra were shifted to longer wavelength with gradual decrease of intensity as a result of gradual shifting of excitation wavelength. The strongest emission peak was shifted from 432 to 584 nm with a gradual decrease of intensity as the excitation wavelength moves from 345 to 565 nm, as shown in Fig. 4a. The normalized PL emission spectra of shifting are also presented in Fig. 4b. Therefore, the CQD was observed to display different colours, predominantly blue, green and red when the sample was laser-excited at the wavelengths of 405, 488 and 543 nm, respectively, under a confocal microscope, as shown in Fig. 1. The self-passivated oxygen and nitrogen containing functional groups on the surface of CQD might be responsible for the efficient PL by trapping excitons under excitation and the radiative recombination of those surface-trapped excitons.[35] The QY of the down-conversion PL was estimated to be about 16.5%. Quinine sulphate was used as the standard reference material for the determination of the QY of the CQD. The emission and absorption spectra of quinine sulfate and CQD are provided in Fig. S1 (ESI†), along with details of the parameters involved for the calculation of QY in Table S2.†

The interesting up-conversion PL emission resulted from conversion of near infrared (NIR) light into shorter wavelength

emissions was observed with a strong signal at around 485 nm when the sample was excited at the wavelengths of 600 nm to 800 nm (Fig. 4c). The fixed emission intensity was found to be enhanced at around 485 nm wavelength with the gradual shifting of excitation wavelengths from 600 nm to 800 nm. The results indicated the excitation independent up-conversion characteristic during excitation in the NIR region. The conventional PL imaging technique involves Stokes-shifted emission using excitation in the short wavelengths, such as ultraviolet (UV) or blue-green visible spectral ranges, and it has some limitations, such as: (i) low signal-to-background ratio caused by auto-fluorescence and strong light scattering from biological tissues; (ii) low penetration depth of UV and visible excitation emission light in biological tissues; and (iii) possible DNA damage and cell demise because of long-term exposure to short wavelengths, particularly UV excitation.[36] On the contrary, up-conversion PL not only allows for deeper light penetration and reduced photo-bleaching, but also offers lower auto-fluorescence, reduced light scattering, and phototoxicity because of anti-stokes emission during excitation in the NIR range. Up-conversion PL is potentially safe and advantageous technique over conventional PL for cell imaging because biological tissues possess an optical transparency window in the NIR range of 700–1100 nm.[36] Therefore the up-conversion PL property of the as-derived CQD can be a promising candidate as an alternative to lanthanide based up-conversion materials utilized for cell imaging and tracking in biomedical applications, as well as for optoelectronic devices.

The PL decay profile of the CQD was obtained by using the TCSPC technique through recording the transitions at 450 nm emission while the sample was excited at 375 nm by a laser diode. The PL lifetime data was found to be well-fitted to an exponential function, as described in Fig. 4d, and indicates that the observed lifetime (t) is 5.78 ns for CQD. The result showed consistency with other reported results.[25,37,38]

The PL stability of the obtained CQD was also investigated. The CQD-containing aqueous solution was seen without any floating or precipitation of the particles in a glass vial at room temperature over a period of four weeks. The effect of pH on PL emission intensity at 450 nm was observed by adding acidic or basic solutions and the strongest emission intensity was detected in the pH range from 5 to 8 with a slight blue shift, as shown in Fig. S2a (ESI†). The PL emission intensity decreased slowly (~6%) at 450 nm (Fig. S2b, ESI†) under continuous UV irradiation at 365 nm for 5 days, indicating that the resulting CQD have excellent resistance to photo bleaching. Therefore, the remarkable feature can be attributed to their small particle size, a large number of hydroxyl groups on the surface and the electrostatic repulsions between the particles. These results revealed that the CQD derived from cabbage is a potential candidate for cell labelling and drug delivery in biomedical applications.

3.4. Cytotoxicity and cell imaging

Biocompatibility and low cytotoxicity of bio-imaging agents in the cell environment is one of the most essential requirements for biomedical applications. Therefore, the potential cytotoxicity and biocompatibility of the CQD were evaluated against HaCaT cells by MTT assay. The cell viability test result was obtained after incubation of the cells in CQD solution in the concentration range of 0–1000 μg ml^{-1} for 24 h, as shown in Fig. 5.

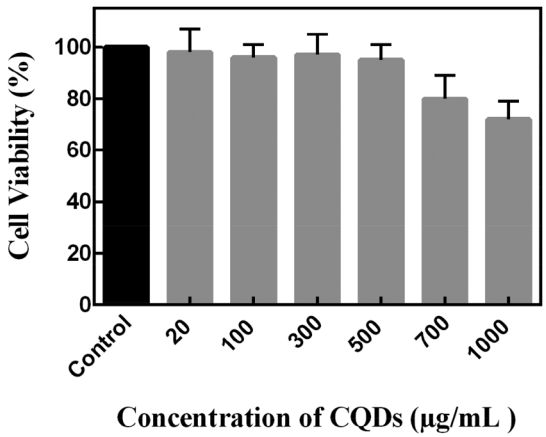

Fig. 5 Cytotoxicity of CQD against human HaCaT cells at increasing concentrations from 0 to 1000 μg ml^{-1}.

The results indicated that the relative cell viability was still more than 90% after 24 h exposure in CQD at a concentration below 700 μg ml^{-1}. The cell viability decreased by 22% when the administered dose of CQD was 1000 μg ml^{-1}. These observations clearly revealed that CQDs did not exert potential toxicity at a concentration below 700 μg ml^{-1} over a long incubation period of 24 h. The result in terms of cell viability against toxicity exerted by CQD at high concentration was remarkable. This result was compared with other reported works, as illustrated in Table S3 (ESI†). It was found that cell viability was more than 90% after treatment by cabbage-derived CQD in the concentration range of 20–500 μg ml^{-1} and the administered dose for cell imaging was 500 μg ml^{-1} in this study. Both the concentration range for more than 90% cell viability and the administered dose were higher than some other works and competitive with others among the surveyed data. Therefore, the CQD derived from a natural source can be potentially safe for *in vitro* and *in vivo* imaging applications.

In this context, *in vitro* cellular uptake of CQD by human HaCaT cells was performed to image the individual cells. CQD was introduced to the target cells at a safe dose at a concentration of 500 μg ml^{-1} and the imaging results were recorded by confocal fluorescence microscope.

The fluorescence images of CQD treated cells clearly show that the multicolour CQD was capable of labelling the cells. As shown in Fig. 6, the blue (Fig. 6d), green (Fig. 6e) and red (Fig. 6f) colour was pleasantly visible because of the excitation dependent PL feature of the multicolour CQD in the areas of the cell membrane and cytoplasm of the cells when the sample was excited with 405, 488 and 543 nm laser simultaneously under a confocal microscope. On the other hand,

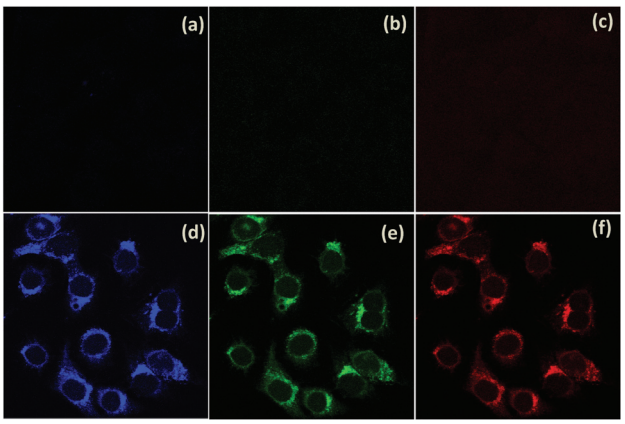

Fig. 6 Confocal fluorescence microscopy images of HaCaT cells excited by 405 nm, 488 nm and 543 nm laser: untreated cells (a) to (c) and treated cells (d) to (f) with CQD at a concentration of 500 μg ml^{-1}.

there was no emission of colour detected from the untreated cells with the same laser excitation, as shown in Fig. 6(a–c). The result revealed the capability of the cabbage derived CQD to penetrate into the cell membrane easily but not to enter the nuclei. The low cytotoxicity and biocompatibility of the CQD was further confirmed as no significant morphological damage of the cells was observed. These results suggested that the obtained CQD have promising applications in cell labelling, for drug delivery, as biosensors, and in other potential biomedical fields.

4. Conclusions

This study demonstrated a facile and green approach for the synthesis of high quality luminescent CQD using low temperature hydrothermal treatment from cabbage as a new, cheap and readily available natural source of carbon. The partially crystalline CQD showed strong and stable PL in the biological pH range with excitation dependent down-conversion and excitation independent up-conversion properties. The CQD exhibited excellent photostability and low cytotoxicity against living cells. The *in vitro* bioimaging results displayed the immense potential of the material for biomedical applications. Therefore, bio-friendly CQD derived from edible vegetables are well suited for cellular imaging and would be a replacement for fluorescent dyes and heavy metal based QDs in both *in vitro* and *in vivo* studies for early disease detection and rapid screening.

Acknowledgements

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Notes and references

1 A. M. Alam, Y. Liu, M. Park, S. J. Park and H. Y. Kim, *Polymer*, 2015, **59**, 35.
2 J. Li and J. J. Zhu, *Analyst*, 2013, **138**, 2506.
3 H. Choi, S. J. Ko, Y. Choi, P. Joo, T. Kim, B. R. Lee, J. W. Jung, H. J. Choi, M. Cha, J. R. l. Jeong, I. W. Hwang, M. H. Song, B. S. Kim and J. Y. Kim, *Nat. Photonics*, 2013, **7**, 732.
4 Y. L. Zhang, L. Wang, H. C. Zhang, Y. Liu, H. Y. Wang, Z. H. Kang and S. T. Lee, *RSC Adv.*, 2013, **3**, 3733.
5 H. Huang, Y. Xu, C. J. Tang, J. R. Chen, A. J. Wang and J. J. Feng, *New J. Chem.*, 2011, **38**, 784.
6 S. Sahu, B. Behera, T. K. Maiti and S. Mohapatra, *Chem. Commun.*, 2012, **48**, 8835.
7 A. Prasannan and T. Imae, *Ind. Eng. Chem. Res.*, 2013, **52**, 15673.
8 J. Wang, C.-F. Wang and S. Chen, *Angew. Chem., Int. Ed.*, 2012, **51**, 9267.
9 H. Huang, J. J. Lv, D. L. Zhou, N. Bao, Y. Xu, A. J. Wang and J. J. Feng, *RSC Adv.*, 2013, **3**, 21691.
10 S. Liu, J. Tian, L. Wang, Y. Zhang, X. Qin, Y. Luo, A. M. Asiri, A. O. Al-Youbi and X. Sun, *Adv. Mater.*, 2012, **24**, 2037.
11 C. Zhu, J. Zhai and S. Dong, *Chem. Commun.*, 2012, **48**, 9367.
12 W. Li, Z. Yue, C. Wang, W. Zhang and G. Liu, *RSC Adv.*, 2013, **3**, 20662.
13 W. Li, Z. Zhang, B. Kong, S. Feng, J. Wang, L. Wang, J. Yang, F. Zhang, P. Wu and D. Zhao, *Angew. Chem., Int. Ed.*, 2013, **52**, 1.
14 B. Ballou, L.-A. Ernst, S. Andreko, T. Harper, J.-A. Fitzpatrick, A.-S. Waggoner and M.-P. Bruchez, *Bioconjugate Chem.*, 2007, **18**, 389.
15 P. Diagaradjane, J. M. Orenstein-Cardona, N. E. Colón-Casasnovas, A. Deorukhkar, S. Shentu, N. Kuno, D. L. Schwartz, J. G. Gelovani and S. Krishnan, *Clin. Cancer Res.*, 2008, **14**, 731.
16 L. F. Qi and X. H. Gao, *ACS Nano*, 2008, **2**, 1403.
17 M. V. Yezhelyev, L. Qi, R.-M. O'Regan, S. Nie and X. Gao, *J. Am. Chem. Soc.*, 2008, **130**, 9006.
18 R. Hardman, *Environ. Health Perspect.*, 2005, **114**, 165.
19 H. Li, J. Zhai, J. Tian, Y. Luo and X. Sun, *Biosens. Bioelectron.*, 2011, **26**, 4656.
20 F. Liu, M. Jang, H. D. Ha, J. Kim, Y. Cho and T. S. Seo, *Adv. Mater.*, 2013, **25**, 3657.
21 W. Shi, Q. Wang, Y. Long, Z. Cheng, S. Chen, H. Zheng and Y. Huang, *Chem. Commun.*, 2011, **47**, 6695.
22 X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker, L. Gearheart, R. Ray and W. A. Scrivens, *J. Am. Chem. Soc.*, 2004, **126**, 12736.

23 Y. Lin, L. Zhang, W. Yao, H. Qian, D. Ding, W. Wu and X. Jiang, *ACS Appl. Mater. Interfaces*, 2011, **3**, 995.
24 H. Yan, M. Tan, D. Zhang, F. Cheng, H. Wub, M. Fan, X. Mab and J. Wang, *Talanta*, 2013, **108**, 59.
25 X. Zhang, S. Wang, C. Zhu, M. Liu, Y. Ji, L. Feng, L. Tao and Y. Wei, *J. Colloid Interface Sci.*, 2013, **397**, 39.
26 S. Y. Park, H.-U. Lee, E. S. Park, S. C. Lee, J. W. Lee, S. W. Jeong, C. H. Kim, Y.-C. Lee, Y.-S. Huh and J. Lee, *ACS Appl. Mater. Interfaces*, 2014, **6**, 3365.
27 Y. C. Lu, J. Chen, A. J. Wang, N. Bao, J.-J. Feng, W. Wanga and L. Shao, *J. Mater. Chem. C*, 2015, **3**, 73.
28 H. Li, X. He, Y. Liu, H. Huang, S. Lian, S. Lee and Z. Kang, *Carbon*, 2011, **49**, 605.
29 L. Cao, X. Wang, M. J. Meziani, F. S. Lu, H. F. Wang, P. J. Luo, Y. Lin, B. A. Harruff, M. Veca, D. Murray, S. Y. Xie and Y. P. Sun, *J. Am. Chem. Soc.*, 2007, **129**, 11318.
30 B. Yin, J. Deng, X. Peng, Q. Long, J. Zhao, Q. Lu, Q. Chen, H. Li, H. Tang, Y. Zhang and S. Yao, *Analyst*, 2013, **138**, 6551.
31 S. Mitra, S. Chandra, T. Kundu, R. Banerjee, P. Pramanik and A. Goswami, *RSC Adv.*, 2012, **2**, 12129.
32 Y. Liu, N. Xiao, N. Gong, H. Wang, X. Shi, W. Gu and L. Ye, *Carbon*, 2014, **68**, 258.
33 Y. Wang and A. Hu, *J. Mater. Chem. C*, 2014, **2**, 6921.
34 M. K. Barman, B. Jana, S. Bhattacharyya and A. Patra, *J. Phys. Chem. C*, 2014, **118**, 20034.
35 Y. P. Sun, B. Zhou, Y. Lin, W. Wang, K. A. S. Fernando and P. Pathak, *J. Am. Chem. Soc.*, 2006, **128**, 7756.
36 G. Chen, H. Qiu, P. N. Prasad and X. Chen, *Chem. Rev.*, 2014, **114**, 5161.
37 S. Chandra, S. H. Pathan, S. Mitra, B. H. Modha, A. Goswamic and P. Pramanik, *RSC Adv.*, 2012, **2**, 3602.
38 S. K. Bhunia, A. Saha, A. R. Maity, S. C. Ray and N. R. Jana, *Sci. Rep.*, 2013, **3**, 1473.