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# Synthesis of Carbon Quantum Dot from Cabbage with Down- and Up-Conversion Photoluminescence Properties: Excellent Imaging Agent for Biomedical Application

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Al-Mahmnur Alam[a], Byung-Yong Park[b,**], Zafar Khan Ghouri[a], Mira Park[c], Hak-Yong Kim[a,c,*]

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) during cell viability experiment against HaCaT cell, an immortalized non-tumerogenic human keratinocyte cell. Subsequently, CQD treated cells displayed three distinguished blue, green and red colour under a confocal microscope during in-vitro imaging technique. 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 application.

## 1. Introduction

Carbon quantum dots (CQD) have been emerged as attractive material for numerous applications because of their favourable tunable multi-colour photoluminescence (PL) properties, high chemical stability, low toxicity, biocompatibility, and easy fictionalization [1]. The capability of displaying excitation dependent PL emission has made the material excellent candidate for cell imaging agent in biomedical application, analytical probe for selective detection of inorganic molecules and in optoelectronic devices [2-4]. Therefore, synthesis of high quality CQD with environmentally benign technique from low cost source is extremely necessary. There are many organic compounds have already been reported for the synthesis of CQD for instance, polybasic acid, glucose, sucrose, glycol, glycerol, chitosan using various method such as hydrothermal, laser ablation, electrochemical oxidation, microwave irradiation, hot injection, and pyrolysis [5]. Hydrothermal method is considered to be a simple, direct, and efficient among the reported techniques. The above mentioned procedures, except hydrothermal noticeably suffer from some degree of drawbacks because most of those involved in 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 precursor and low toxicity of the product for biological application. There are already 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 the cases to synthesis CQD. The downsides of the reported results were consumption of expensive bio-precursor and usage of organic solvents, especially ethanol and dichloro-methane along with water during synthesis. These drawbacks limit their applications in environmental, biological and life sciences. It is, therefore,

highly anticipated to explore an easily available and cheap carbon source for the synthesis of high quality CQD with excellent PL behaviour through enviro-friendly green technique. In the search of exploring 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 the reliable biocompatibility test in a living cell environment.

It is worth to mention 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 size, charge, coating ligands, oxidative, photolytic and mechanical stability of II-IV QD can contribute to the 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 nanomaterial such as CQD, nanodiamonds, carbon nanotubes and graphene QD, are regarded as appropriate alternative candidates of aforementioned semiconducting II-VI QD because of their chemical stability, biocompatibility, high water-solubility, sufficient fluorescence quantum yield (QY) and low toxicity against living cell [19-23]. There are already few reports available in literature regarding application of CQD as cell imaging agent in biomedical research [24-28].

In this context, we have opted cabbage as new and cheaply abundant biomaterials 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 new biomaterials and 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 as derived CQD are highly functionalized with oxygen rich hydroxyl group 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. Environment friendly synthesis of CQD

A cabbage was collected from local market and washed several times with ultrapure water. It was then cut into pieces and placed into domestic fruit-juicer for further processing to conveniently use in hydrothermal reactor. The measured amount of as obtained smashed-cabbage was treated at 140˚C for 5 hours in hydrothermal reactor. The reactor was allowed to cool naturally. The dark brown solution was collected through vacuum filtration and then centrifuged at 12000 rpm for 15 min to separate the less-fluorescent larger CQD. Next, the obtained CQD containing solution was dialyzed against ultrapure water using tubular dialysis membrane (MWCO~1kDa) for 48 hours and finally the purified CQD solution was concentrated to approximately 5mg/mL using a vacuum oven operating at 60°C. The procedure was illustrated schematically in Fig.1.

### 2.3. 1. 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) and streptomycin (100 µg/ml medium). The cell suspension was kept in the incubator (Thermo Electron Corporation, USA) at 37°C, 5% $CO_2$; and 95% humidity.

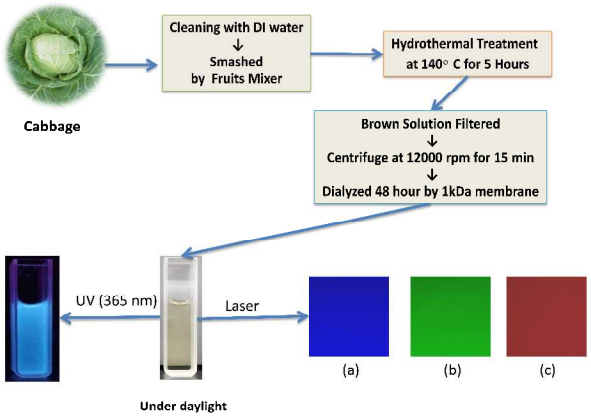


Fig.1. Schematic presentation of the synthesis procedure of CQD from cabbage with the hydrothermal treatment and visible blue emission under UV; and (a) blue, (b) green and (c) red emission under a confocal microscope when excited by 405, 480 and 543 nm laser respectively.

The cytotoxicity of CQD was evaluated by the 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assays. Briefly, HaCaT cells were treated with different doses of 100µl (1000, 700, 500, 300, 100 and 20 µg/ml) of the CQD solutions in wells and were incubated for 24 hours. The cells attached to each plate were treated with freshly prepared 20 µl of 2.5 mg/ml 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. A detail of cytotoxicity experiment was presented in ESI.

### 2.4. Sample preparation for bioimaging

HaCaT cells were dispersed in 12 replicate wells to a total volume of 200 µL/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 selected different concentrations for 24 h and washed with the PBS buffer prior to capture confocal fluorescence microscopic images.

### 2.5. Instrument for characterization

Transmission electron microscopy (TEM) and high resolution TEM (HRTEM) images were captured by using Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. Fourier transform infrared spectroscopy (FTIR) of samples was recorded on 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 (hυ=1486.6 eV). PL emission measurements were performed using LS50B Luminescence Spectrometer (Edinburgh Instruments, UK). UV-Vis absorption was measured on a TU-1810 UV–Vis Spectrophotometer (Pgeneral, China). PL life time decay profile was measured using 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 discussions

### 3.1. Yield of CQD

The yield of CQD was calculated to be 7.076% as 353.8 mg product was obtained from 5 g of cabbage leaves. The percentage of yield was compared with other reported results based on natural sources-derived CQD and listed in Table S1 (ESI). It was observed that the yield from orange peel, soybean ground, food waste and tomato was 12.3, 1, 0.12 and 12.5% respectively. The highest percentage of yield was 12.5% and lowest one 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 can be feasible.

### 3.2. Physiochemical characterization

The morphology of the CQD was assessed by TEM and HRTEM image. TEM image (Fig.2a) clearly revealed that CQD derived from cabbage was found to be 2−6 nm in diameter with narrow size distribution, mono-dispersed and well separated from each other with a spherical morphology in aqueous media. HRTEM (Fig.2a-inset) image showed the partially crystalline structure of 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 were presented in Figure 2b and demonstrated a wider peak centred at around 22.0 degree having an interlayer spacing of

0.395 nm, which is broader than that of graphite (0.34 nm). The oxygen containing groups existed on the surface of CQD might have played role to enhance the interlayer distance [30].

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.

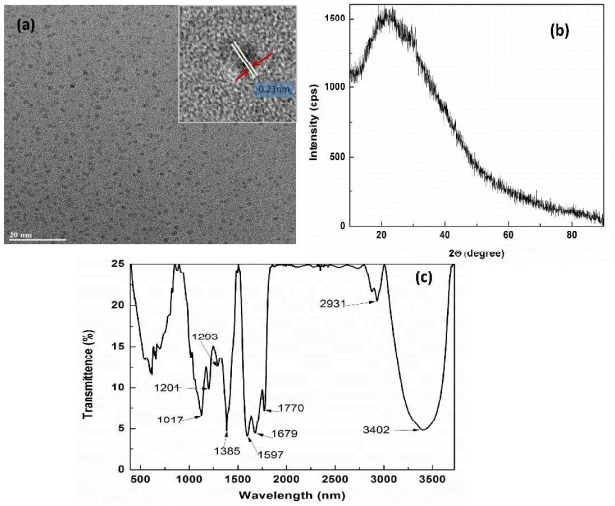


Fig.2. Physiochemical properties of CQD: (a) TEM image and HRTEM image (inset); (b) XRD spectrum and (c) FTIR spectrum of the resulting CQD.

The characteristic bands peaks 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) group which makes 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 C1s, N1s and O1s respectively. The atomic ratio of C1s, N1s and O1s was 66.5%, 4.61% and 28.73% respectively. The high-resolution C1s spectra (Fig.3b) demonstrated the presence of 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 N1s 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 atom. On the other hand, the high resolution spectra of O1s (Fig.3d) showed the major peaks at 531.49 and 532.97 eV, corresponding to C=O, and C–OH/C–O–C groups, respectively.

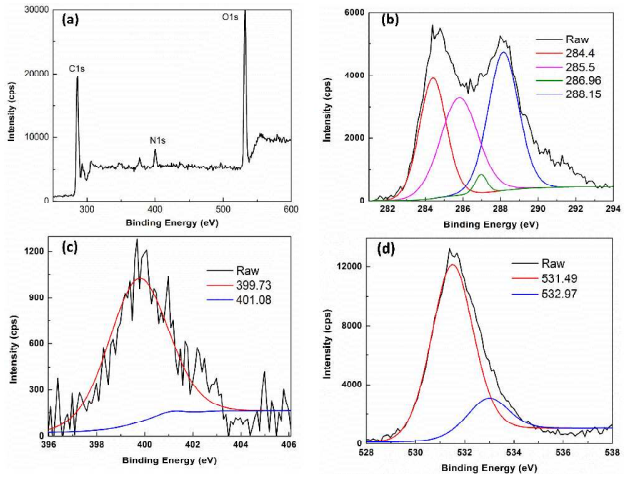


Fig.3. XPS spectra of CQD: (a) survey spectrum; (b) C1s spectra; (c) N1s spectra and O1s spectra.

These results supported the existence of plenty of oxygen-containing groups and consistent with other reported spectroscopic results of CQD [24-27]. The results also indicated the presence of nitrogen on the surface of CQD in the form of

amine molecule as 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 with oxygen containing group, but also nitrogen. The self-passivated oxygen and nitrogen containing CQD might be resulted from sequential dehydration, polymerization, carbonization and surface-passivation in presence of carbonaceous organic materials such as carbohydrates, sugars, fatty acids, protein and amino acid 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 CQD contributed to enhance the physiochemical and optical properties of 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 colour during excitation at 405, 488 and 543 nm laser 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 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* transition respectively with a tail extending to the visible range and the results are well aligned with the other reported work [25-27]. It was observed that 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 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 were 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 wavelength of 405, 488 and 543 nm respectively under 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 about 16.5%. Quinine sulphate was used as standard reference material for the determination of QY of CQD. The emission and absorption spectra of quinine sulfate and CQD were provided respectively in Fig.S1 (ESI) along with details of the parameter involved for the calculation of QY in Table S2.

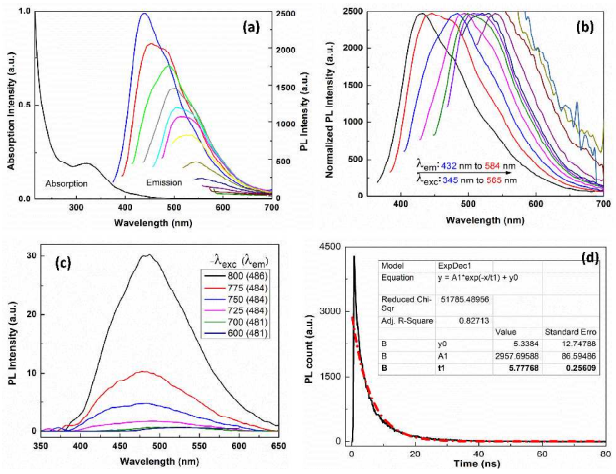


Fig.4. Optical properties of CQD: (a) UV-visible absorption and emission spectra of as prepared CQD dispersed in water at excitation wavelengths progressively increasing from 345 to 565 nm; (b) Normalized PL emission spectra where emission wavelength shifting from 432 to 584 nm; (c) Up-conversion PL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

The interesting up-conversion PL emission resulted from conversion of near infrared (NIR) light into shorter wavelength emissions was observed with strong signal at around 485 nm when the sample was excited at the wavelengths of 600 nm to 800 nm (Figure. 4c). The fixed emission intensity 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 at 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 the 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 wavelength, 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 at NIR range. Up-conversion PL is potentially safe and advantageous technique over conventional PL for cell imaging because biological tissues possess optical transparency window in the NIR range of 700-1100 nm. [36]. Therefore up-conversion PL property of as-derived CQD can be a promising candidate of alternative of lanthanide based up-conversion material utilized for cell imaging and tracking in biomedical application as well as for optoelectronic devices.

The PL decay profile of the CQD was obtained by TCSPC technique through recording the transitions at 450 nm emission while 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 indicating 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 for over a period of four weeks. Effect of pH on PL emission intensity at 450 nm was observed by adding acidic or basic solution 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 5days, indicating that the resulting CQD have excellent resistance to photo bleaching property. 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 agent in the cell environment is one of the most essential requirements for biomedical application. 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 for 24h as shown in Fig.5.

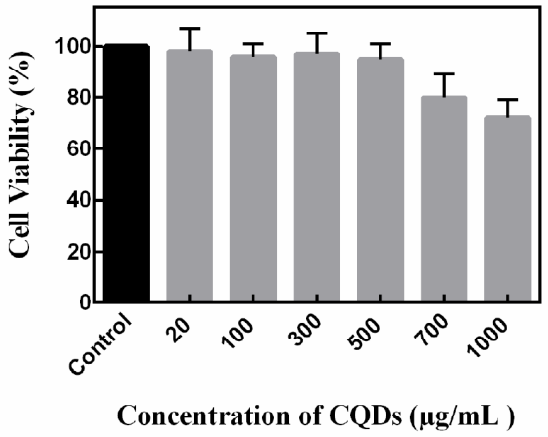


Fig.5. Cytotoxicity of CQD against human HaCaT cells at increasing concentrations from 0 to 1000 µg/ml.

The results indicated that the relative cell viability was still more than 90% after 24 h exposure in CQDs at concentration

of 700 µg/ml. The cell viability decreased by 22% when the administered dose of CQD was 1000 µg/ml. These observations clearly revealed that CQDs did not exert potential toxicity at a concentration below 700 µg/ml 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 treated by cabbage-derived CQD in the concentration range of 20-500 µg/ml and administered dose for cell imaging was 500 µg/ml in this study. The both of concentration range for more than 90% cell viability and administered dose were higher than some other works and competitive with others among the surveyed data. Therefore, the CQDs derived from 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. The target cells were introduced with CQD of a safe dose at a concentration of 500 µg/ml and imaging result was recorded by confocal fluorescence microscope.

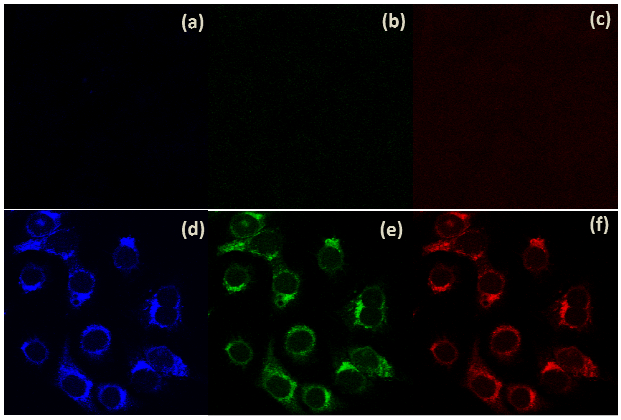


Fig.6. Confocal Fluorescence Microscopy images of HaCaT cells excited by 405 nm, 488 nm and 543 nm laser: Untreated cells (a) to (c) and treated cells (d) to (f) with CQD at concentration of 500 µg/ml.

The fluorescence images of CQD treated cells clearly showed that multicolour CQD was capable to label 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 confocal microscope. On the other hand, there was no emission of colour detected from the untreated cell during same laser excitation as shown in Fig.6(a-c). The result revealed the capability of cabbage derived CQD to penetrate into the cell membrane easily but did not enter into 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, drug delivery, biosensor, and 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 the cabbage as new, cheap and readily available natural source of carbon. The partially crystalline CQD showed strong and stable PL at 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 an immense potential of the material for biomedical application. 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 both in vitro and in vivo studies for early disease detection and rapid screening.

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## Notes and references

[a]Department of BIN fusion Technology, Chonbuk National University, Jeonju 561-756, South Korea. Corresponding Author: Tel: +82-63-270-2351. E-mail Address: khy@jbnu.ac.kr

[b]Department of Veterinary Anatomy, College of Veterinary Medicine and Bio-safety Research Institute, Chonbuk National University, Jeonju, 561-756, South Korea. **Co-corresponding Author: parkb@jbnu.ac.kr

[c]Department of Organic Materials and Fiber Enginering, Chonbuk National University, Jeonju 561-756, South Korea.

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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, T. Imae, *Ind. Eng. Chem. Res.,* 2013, 52, 15673.

8 J. Wang, C-F. Wang and S. Chen, *Angew. Chem. In. Ed. Engl.,* 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, 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, 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, Article number: 1473.