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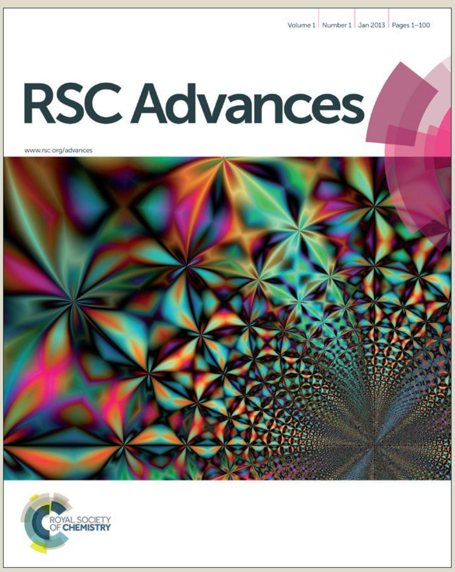

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COMMUNICATION

Theranostic carbon dots derived from garlic with efficient anti-oxidative effect towards macrophages

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Luminescent garlic carbon dots with superior photostability are synthesized via microwave assisted heating. The garlic dots are biocompatible, low toxicity and can be used as a benign theranostic nanoparticle for bioimaging with efficient anti-oxidative effect towards macrophages.

Two of the keystones in modern food technology are nutrition value and safety of the ingredients. While fundamental research into nanoscience has demonstrated the potential of nanotechnology in food safety and improved nutrition composition, nanofood is still a hotly debated topic due to potential risky effects on human health and environment.[1] Considerable development in the engineering of consumable nanoparticles either for curing diseases or for delivery of flavours, probiotics or nutrition has taken place and *in vitro* and *in vivo* testing of these nanoparticles have been taken place[2, 3]. Several biodegradable nanoparticles have entered clinical trials, however, the number of marketed nanoparticles is still small. To alleviate the misunderstanding of the public, that nanoparticles generally are toxic, it is an attractable strategy to focus on nanoparticles present in naturally prepared food.

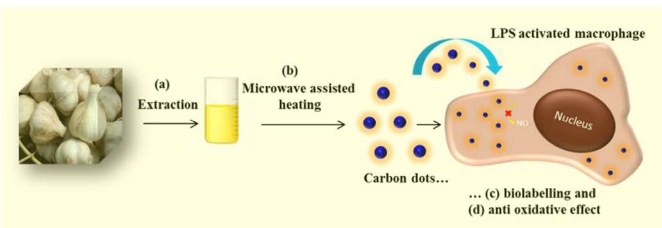
Fluorescent carbon nanodots (CNDs) have recently attracted growing interest as novel photoluminescent nanomaterials in a broad range of bio- related applications such as in bioimaging and sensors due to their highly attractive properties such as high photochemical stability, relatively high quantum yield, low toxicity, good biocompatibility ease of preparation and low cost.[4-7] In comparison with the traditional semiconductor quantum dots comprising of toxic heavy metals such as Cd^{2+} or Pb^{2+}, or fluorescent noble metallic nanoclusters, CNDs are less toxic and highly biocompatible and are therefore considered to be much safer for biological uses.

CNDs have been synthesized by various methods such as chemical carbonisation,[8-12] thermal carbonisation,[13-15] or microwave pyrolysis of hydrocarbons.[16, 17] Several studies have reported on the use of biocompatible and inexpensive starting materials such as glucosamine,[18] ascorbic acid,[19] orange juice,[20] mangoes,[21] ginger[22] and green tea,[23] for the synthesis of fluorescent CNDs. The discovery of CNDs in food products heated at high temperatures implies that these nanoparticles are safe and more likely to be used for biological applications. Further, the surface functional groups of these CNDs, containing carboxylic and alcoholic groups allow for easy conjugation with several therapeutics, making them the preferred candidate for a variety of biological applications. Since the ancient times, people from all over the world have considered garlic (Allium sativum L.) as a valuable condiment with strong healing properties.[24] Garlic has been used to cure a variety of ailments such as heart disease, infections and preventive measure for cancers.[25, 26] It is well known that garlic compounds can have an anti-inflammatory effect by inhibiting the oxidative stress-induced activation of nuclear factor-kappa B (NF-κB),[27] which is associated in the expression of pro inflammatory enzymes such as nitric oxide synthase (NOS) and cyclooxygenase-II (COX-II). Several studies have shown that garlic extracts and its associated organosulfur compounds can significantly suppressed nitric oxide production by lipopolysaccharide (LPS) stimulation, accompanied by suppression in inducible nitric oxide (iNOS) expression and iNOS activity.[28-30]

Herein we prepared multi-coloured luminescent CNDs from garlic (Scheme 1a, b) and demonstrate that our garlic CNDs are readily taken up by macrophages enabling fluorescence bioimaging of their cell bodies and with negligible toxicity (c). Notably, we demonstrated that macrophages treated with our garlic CNDs significantly reduced LPS induced NO production compared with LPS treated cells (d).

The garlic CNDs were prepared via microwave heating and the CNDs purified by dialysis were analysed for elemental atoms and chemical bonds using X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectrometry (FTIR). A XPS survey scan of the garlic CNDs clearly shows the presence of carbon (58.1 ± 0.3 %), nitrogen (6.9 ± 0.2 %), oxygen (34.1 ± 0.3 %) and potassium

(0.9 ± 0.3 %) (Figure 1a). The presence of potassium found in the analysis is because garlic is highly rich in minerals such as potassium.[31] The high resolution C1s XPS spectra (Figure 1b) were fitted and assigned into four peaks at the binding energies (BE) of 285 eV (C–C/C–H), 286.4 eV (C–N), 286.8 eV (C–O), 288 eV (C=O/ N–C=O) and 289.5 eV (C(=O)–O) while the N1s XPS spectra (Figure 1c) were assigned to the corresponding peaks at binding energies (BE) of 402 eV (NH_3^+) and 400 eV (C–N/N–C(=O).[32] FTIR analysis shown in Figure 1d confirmed the peaks at around 3400, 3000, 1700, and 1100 cm^{-1} are assigned for the vibrations of O–H/N–H, C–H, C=O and C–O bonds, and the peak at around 1400 cm^{-1} is assigned to sp2–CH in olefinic configuration. Due to the presence of hydroxyl and carboxylate groups on the CNDs the garlic dots have good water solubility. These data revealed that the functionality groups of garlic were maintained from raw garlic into the final composition of garlic CNDs under microwave heating.



Scheme 1. Schematic diagram of (a) extraction of garlic, (b) synthesis of fluorescent garlic carbon dots, (c) cell imaging using garlic carbon dots and (d) anti-oxidative effect of garlic carbon dots on LPS induced macrophages.

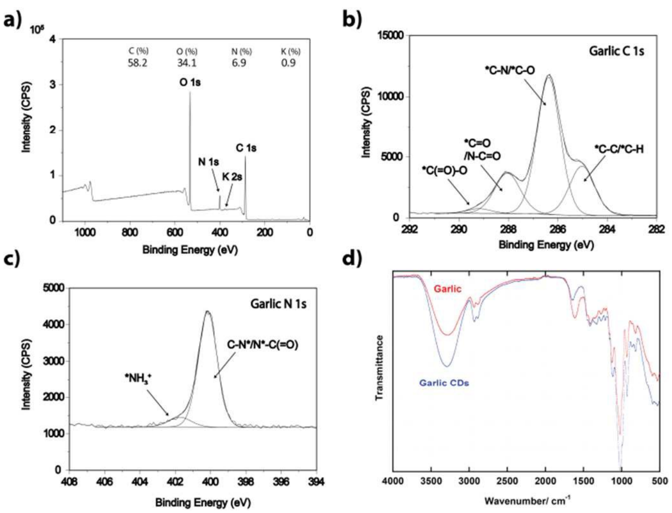


Figure 1. a) XPS survey scan of garlic CNDs, b) XPS high resolution survey scan 1s C1s, c) XPS high resolution N1s survey scan and d) FTIR of pure garlic and garlic CNDs.

The size and morphology of the garlic CNDs were observed by transmission electron microscopy (TEM) and atomic force microscopy (AFM). Dynamic light scattering (DLS) data shows that the garlic CNDs are approximately 3 nm (PDI=0.314) and the TEM image clearly indicates that the formed CNDs were spherical in shape with average diameters approximately 5 nm, with no sign of aggregation (Figure 2a,b). The AFM data in Figure 2c and d illustrate the topographic morphology and the height distribution of the GQDs. The height profile shows that the thickness of the CNDs is approximately 3 nm.

The UV-Vis absorbance spectrum of our garlic CNDs shown in Figure 3a, has a typical absorption of an aromatic π system (π→π* transition) at approximately 230 nm in the UV region.[33, 34] The broad absorption spectrum with a gradual change up to approximately 600 nm indicates the existence of band tail by defect states.[34, 35] To further explore the optical properties of our garlic CNDs, fluorescence emission spectra of the garlic CNDs were recorded at various excitation wavelengths from 340 to 460 nm with 20 nm increments (Figure 3b). With increasing excitation wavelength, the maximum peak of fluorescence gradually shifts towards longer wavelength accompanied by a decrease in the fluorescence intensity. The fluorescence properties of the CNDs arise due to the recombination of electron–hole pairs in localized sp2-hybridized carbon clusters with crystalline and amorphous structures,[36, 37] while the excitation wavelength dependent fluorescence properties are attributed to different hybridized compositions, structures and emissive trap sizes as well as dots of different sizes and shapes.[4] Upon excitation with a 488 nm laser, our garlic CNDs showed emissions at 520 nm (blue), 555 nm (green) and 580 nm (red) (Figure 3b, inset) and using quinine sulphate as a reference the quantum yield of our garlic CNDs was calculated to be approximately 5% (Figure S1).

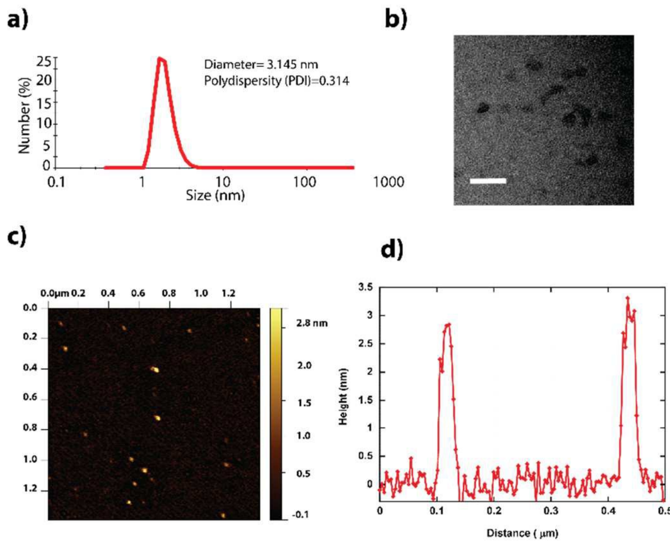


Figure 2. a) DLS data, b) TEM image, scale bar = 20 nm , c) AFM image and d) height profile of garlic CNDs.

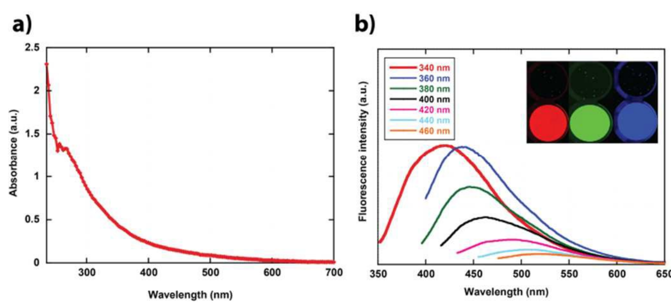


Figure 3. a) UV-vis absorption spectrum of garlic CNDs and b) fluorescence spectra of the CNDs with excitation wavelengths from 340 to 460 nm in 20 nm increments (inset: photographs of garlic CNDs (bottom row) and garlic extract (top row) scanning

the C-dots solution using Typhoon Trio+ with excitation wavelength set at 488 nm.

The stability of fluorescence nanomaterials is an important feature to consider for several bio-related applications. To investigate the photostability of our garlic derived CNDs, we compared their photoluminescence with a commercially available fluorescence dye, fluorescein, under continuous excitation with a laser operating at 488 nm. Our garlic CNDs appeared to have an excellent photostability, with fluorescence intensity remaining stable for at least 14 minutes, while the fluorescence intensity of fluorescein only preserved only 60% of the initial intensity in the same period (Figure 4). Moreover, our garlic derived CNDs also exhibited excellent stability over a period of two months with no sign of degradation (data not shown).

Considering the ease in the preparation of garlic CNDs, its high photoluminescence, excellent stability and biocompatibility owing to its origin, we investigated its potential as a biological label for cell imaging. First the inherent cytotoxicity of the garlic CNDs was evaluated with macrophages using AlamarBlue assay. The viabilities of macrophages were examined upon incubation of garlic CNDs for 24 h with different concentrations as shown in Figure 5. There is no significant decrease in the cell viabilities even at the highest concentration of 2 mg/mL, indicating good biocompatibility and low toxicity of the garlic derived CNDs. The images of macrophages treated with and without garlic CNDs for 4 h are shown in Figure 6. Bright field images of the cells revealed that after treatment with the garlic CNDs, the cells retained their morphology, further indicating that the CNDs were not toxic to the cells (Figure 6 a, column 1). In addition to the bright field images, blue, green and red fluorescent images of the cells clearly revealed vivid cell uptake/interaction of the CNDs (Figure 6 a, columns 2-4). The fluorescent images revealed that the CNDs were located in the cytoplasmic regions of the cells, similar to what has been reported in the literature.[38, 39] The control images did not show any auto fluorescent with excitation(Figure 6 b, columns 2-4). These results confirmed that our garlic CNDs can be used as fluorescent probe in cell labelling and tracking.

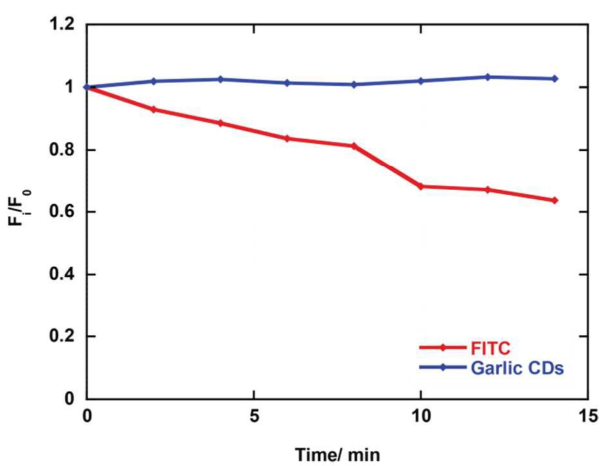


Figure 4. Photostability FITC and garlic CNDs exposed to a laser operating at 488 nm at different time intervals.

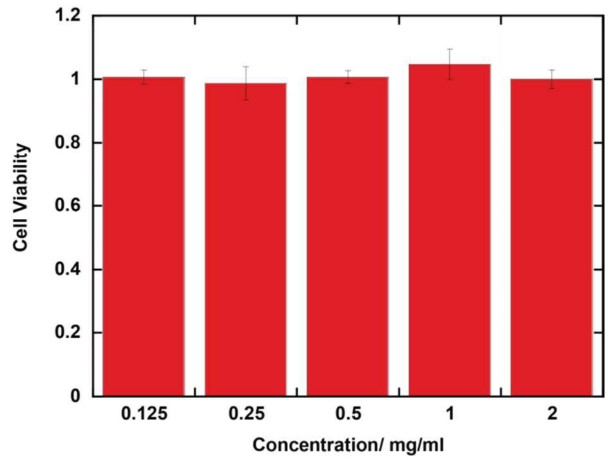


Figure 5. Viability of macrophages as a function of garlic CNDs concentration.

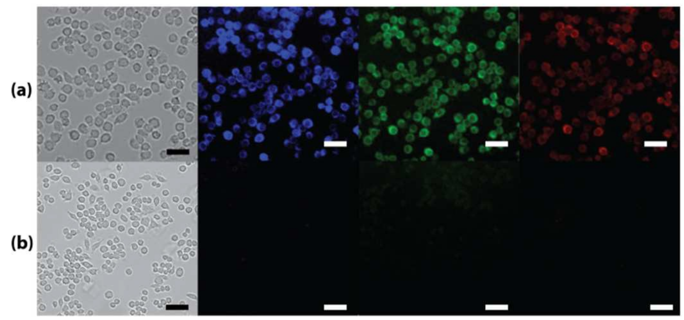


Figure 6. Bright field and fluorescence imaging of macrophages incubated with a) garlic dots, and b) garlic extract, scalebar = 20 μm.

Macrophages play a central role in the initiation and development of various inflammatory diseases by releasing proinflammatory cytokines and mediators. LPS is the major cell wall membrane of the Gram-negative bacteria and is a potent activator of monocytes and macrophages. In general, LPS activates macrophages through different pathways including the nuclear factor kappa B (NF-κB),[40, 41] which triggered the expression of nitric oxide synthase (iNOS) [28] , leading to the production of high level of radicals such as NO and causing oxidative stress and tissue damage. We next investigated the inhibitory effect of our garlic CNDs on lipopolysaccharide (LPS) induced nitric oxide production in macrophages. Treatment of macrophages with LPS strongly increased the NO production, whereas, pre-incubation of garlic and garlic CNDs with LPS activated macrophages significantly reduce LPS-induced NO production, indicating the potential therapeutic effect of garlic Cdots towards antioxidative stress (Figure 7). Although, it has been shown by Kang and coworkers that heat treatment of garlic will significantly reduce the potent anti inflammatory effect,[30] the present study demonstrate that extracting the garlic at 37 °C, followed by short-term microwaving to form garlic CNDs maintains the biological active substances responsible for the anti inflammatory effect of garlic.

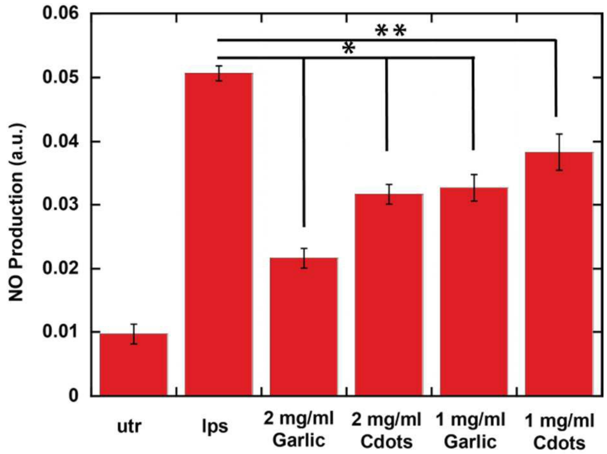


Figure 7. Effect of garlic and garlic CNDs on lipopolysaccharide (LPS) induced NO production in macrophages. Results are mean ± SD, *p < 0.0001 and ** p < 0.001.

Conclusions

In summary, we have successfully synthesized highly fluorescent and bioactive garlic carbon dots via microwave assisted heating. No surface modification was required in the preparation of the fluorescent dots. The garlic dots are highly water soluble, with excellent photo stability and biocompatibility, making them extremely suitable for applications in bioimaging. The garlic nanodots are effectively taken up by macrophages can also effectively reduced their NO production upon LPS activation suggesting that our garlic nanodots may be able to monitor and reduce inflammation at the same time.

Notes and references

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† Footnotes 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.

Electronic Supplementary Information (ESI) available: [experimental section, Figure S1 **Figure S1**. Integrated fluorescence intensity vs. absorbance of the quinine sulphate and garlic Cdots,]. See DOI: 10.1039/c000000x/

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