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Organic carbon nano-particle fluorescent probe from plant sources for cell bio-imaging: Its application in medical and biomedical industries

ABM Sharif Hossain
Biology Department, Faculty of Science, Hail University, Hail, KSA, Saudi Arabia
Biotechnology Program, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

Abstract
Bio-imaging components are employed in a variety of studies of inflammatory diseases, plant and cancer cell biology, diabetes, neuroscience and developmental biology. Different types of bio imaging methods like microscopy detection method, organic carbon nanoparticle fluorescent probe, digital image analysis and flow cytometry (cell bio imaging) as analytical and diagnostic tools in research and health care are an effective and innovative technology. Organic carbon nanoparticle fluorescent probe keeps a role in live cell imaging and medical imaging methods such as whole body analysis. The review study was carried out employing different innovative research data to attain the latest modern technology in the organic carbon nanoparticle fluorescent probe from plant sources for cell bio imaging and its application in medical and biomedical industries. However, research data on natural dye from plant sources like colorful leaf, flower, excellent chlorophyll based algae and cyan bacteria etc. based pigment instead of synthetic dye have been noted which are more effective for cell bio imaging in human (cancer cell), animal and plant cell. Moreover, fluorescent nanoparticle-based bio imaging probes data have been highlighted from different innovative research data in cell bio imaging applied in cell biology which have advanced labeling technology and expected to generate new medical diagnostic tools based on their superior brightness and photo-stability compared with conventional molecular probes. Finally it can be summarized that organic carbon nanoparticle fluorescent probe from plant sources for cell bio imaging is more effective for cell bio imaging and can be applied in medical and biomedical industries.

Keywords: carbon nanoparticle, fluorescent probe, cell bio imaging, medical, biomedical

1. Introduction

Fluorescent probe keeps a significant role due to their extraordinary application in cell bio-imaging. Nowadays, researchers are using natural dye from plant sources (like colorful flower, excellent chlorophyll based algae and cyan bacteria etc.) based pigment instead of synthetic dye due to the more effective for cell bio imaging for human (cancer cell), animal and plant cell. Das *et al.* (2017) [14] stated that utilization of the fluorescent molecules for bio imaging and biomedical applications has attracted considerable attention over the past few decades. It is being used different types of fluorescent molecules such as fluorescent proteins (Betzig *et al.* 2006) [2], highly conjugated polymers, fluorescent inorganic materials, dye etc. However, they have many disadvantages which many times limit their practical applications in imaging purposes and they are not at all cost effective. Most of the organic dyes are hydrophobic and unstable in biological media. Some molecules show photo-bleaching property during prolonged experimental period. Quantum dots with high yield fluorescence intensity are toxic to living organisms due to their heavy metal toxicity and inorganic fluorescent materials are non-biodegradable. Some new organic (plant sources) fluorescent probes (OFP), which should be nature friendly, Scientists are giving much effort to discover new dyes for fluorescence imaging to investigate and understand the phenomena more precisely.

Das *et al.* (2017) [14] reported that organic beet root extracted fluorescent dye as an efficient pigment for effective cell imaging. They also reported that different types of human cells showed very good bio-image in fluorescence cell imaging in case of all types of cells and also less time consuming to detect cell and remains stable for sufficiently long period. Haydar *et al.* (2016) [10] evaluated fluorescent carbon nanoparticle-based probes and reported that visible emission are biocompatible, environment friendly and most suitable for various biomedical applications. They also reported that red fluorescent carbon nanoparticle-based nano-bio conjugates (<25 nm hydrodynamic size) and their application as fluorescent cell labels are applicable. Hydrophobic carbon nanoparticles are synthesized via high temperature colloid-chemical approach and transformed into water-soluble functional nanoparticles via coating with amphiphilic polymer followed by covalent linking with desired biomolecules (Li *et al*. 2009) [11].

Susanta *et al.* (2013) [16] evaluated the fluorescent nanoparticle-based bio imaging probes and reported that they have advanced labeling technology and were expected to generate new medical diagnostic tools based on their superior brightness and photo stability compared with conventional molecular probes. They also mentioned that it had been made in fluorescent semiconductor nano crystal-based biological labeling and imaging, the presence of heavy metals and the toxicity issues associated with heavy metals have severely limited the application potential of these nanocrystals fluorescent carbon nanoparticle-based, alternative, nontoxic imaging probe that is suitable for biological staining and diagnostics. They recommended a chemical method to synthesise highly fluorescent carbon nanoparticles 1-10 nm in size; these particles exhibit size-dependent, tunable visible emission. These carbon nanoparticles have been transformed into various functionalized nano probes with hydrodynamic diameters of 5-15 nm and have been used as cell imaging probes.

Das *et al.* (2017) [14] studied the fluorescent carbon dots, zero-dimensional nanomaterials with surface ligand and reported that extensively studied done for past few years in bio-labeling or fluorescence-based live cell assays. They also reported that synthetic organic dyes have been used as cell tracking materials and found severe limitations; fluorescent carbon dots may pave the way to bio-labeling and cell imaging. They reported that green fluorescent carbon dots have been synthesized from a green source like bacteria *Escherichia coli* without any sort of covalent or ionic modifications. They mentioned that these gram-derived carbon dots are unique with respect to synthetic commercial cell-tracking dyes as they are non-toxic, cell internalization occurs quickly, and they have excellent bio conjugation with bacterial cells. The objective of this review study was to put recent research dada on carbon red fluorescent nanoparticle probe for cell bio imaging.

2. Materials and Methods

Application methods of the fluorescence dye for cell imaging

1. Breast cancer cell imaging (Das *et al.* 2017)) [14] Accorting to Das *et al.* (2017)) [14] breast cancer cell imaging has been taken MDAMB-231 (breast cancer) cell line. Cell imaging was done after incubation of cells with beet root extracted fluorescent (BREF) dye functionalized particles and with only BREF dye. First 1 mg of cobalt ferrite particles functionalized with BREF dye was dissolved in 1 ml of de-ionized water and sonicated to disperse the particles for further use (Dey *et al*. 2017) [3]. The cancer cells were seeded in 12 wells plate and incubated overnight. After that the cells were further incubated for 24 hrs to check the interaction of cells with dye functionalized particles in first 6 wells and with dye except particles in 2nd 6 wells. The experimental protocol was fixed by addition of dye fuctionalized particles in different doses (10-50 µl/well) in first 6 wells where one well of first row of the first 6 wells was considered as control. In the next 2nd set of 6 wells the order was maintained same as in 1st 6 wells but the differences was, here the direct beet root extracted dye solution (10 µl of BREF dye was dissolved in 1 ml of deionized water without attaching with the NPs) was added taking first one well as control and next 5 wells treating with different doses of dye (10-50 µl/well).
   To dissolve the formazan crystals, the cells were incubated for 30 min with 50 ml of DMSO after removing the medium. Then these colored solutions were allowed for absorbance measurement by UV-VIS spectrometer at 575nm wavelength and the results were compared with the control. The absorption and emission spectra were taken with the help of the UV-Vis and fluorescent spectrophotometer for different concentrations of BREF dye with varying the amount from 10 - 70 µl each in 3ml of water and taking the solution in a quartz cuvette.
2. Green component to photo luminescent carbon dots for imaging of bacteria (Escherichia coli) (Das *et al.* 2017) [1] According to Arpita *et al.* (2017) [1], bio labeling or fluorescence-based live cell assays has been used. In this work, green fluorescent carbon dots have been synthesized from a green source, gram negative bacteria, without any sort of covalent or ionic modifications. These gram-derived carbon dots are unique with respect to synthetic commercial cell-tracking dyes. Physicochemical features like the tunable luminescence property, high degree of water solubility and low toxicity, towards various environments (wide range of pH, high ionic strength) were studied.
3. According to Haydar *et al.* (2016) [10] red fluorescent carbon nanoparticle-based nano bio conjugates (<25 nm hydrodynamic size) and their application as fluorescent cell labels were used. Hydrophobic carbon nanoparticles were synthesized using high temperature colloid-chemical approach and transformed into water-soluble functional nanoparticles by coating with amphiphilic polymer followed by covalent linking with desired biomolecules. Carbon nanoparticles were functionalized with polyethylene glycol, primary amine, glucose, arginine, histidine, biotin and folic acid. These functional nanoparticles were excited with blue/green light (400–550 nm) to capture their emission spanning from 550 to 750 nm. Arginine and folic acid functionalized nanoparticles have been demonstrated as fluorescent cell labels where blue and green excitation has been used for imaging of labeled cells. The presented method could be extended for the development of carbon nanoparticle-based other bio imaging probes.
4. According to Susanta *et al.* (2013) [15], fluorescent nanoparticle-based imaging probes have been made using fluorescent semiconductor nano crystal-based biological labeling and imaging, the presence of heavy metals and the toxicity issues associated with heavy metals have severely limited the application potential of these nanocrystals. They have developed a chemical method to synthesise highly fluorescent carbon nanoparticles 1-10 nm in size; these particles exhibit size-dependent, tunable visible emission. These carbon nanoparticles have been transformed into various functionalised nanoprobes with hydrodynamic diameters of 5-15 nm and have been used as cell imaging probes.

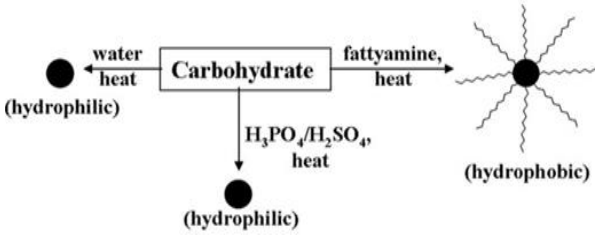
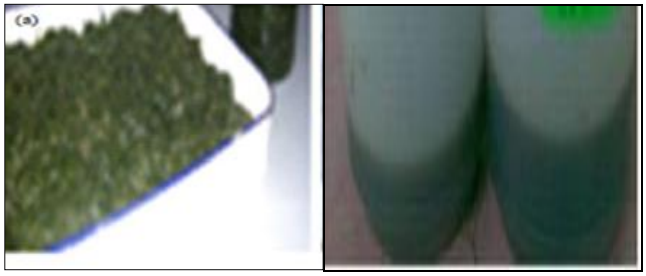


Fig 1: Synthesis of fluorescent carbon nanoparticle.

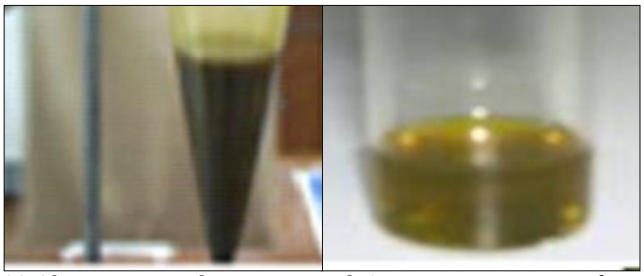
5. Different controlled carbonization approach of carbohydrate in producing fluorescent carbon nanoparticle (Susanta *et al.* 2013) [15]. Xian *et al.* (2017) [18] studied that Washington State University has created an injectable dye that illuminates molecules with near infrared light through deep inside the body. They used cancer cell. Xian *et al.* (2017) [18] investigated the dye's unique properties like Near infrared light passes right through the human body, made it almost invisible and made dyes that respond to this color of light perfect for bio-imaging (Samanta *et al*, 2012, Niu *et al.* 2015) [16,13]. Fluorescent bio-imaging was the visualization of cells and other biological tissues marked with a dye that glows when activated by light. Available dyes for bio-

imaging fluoresce in the visible spectrum with wavelengths used between 400-600 nanometers. There were several practical issues with these dyes. Like molecules in amino acids, hemoglobin and other natural tissues and fluids also fluoresce when illuminated with colors of light in this region of the spectrum, such as green and blue.

6. According to Hossain *et al.* (2008) [8] fresh water algae (*Spirogyra* sp.) were collected from the surrounding area of University of Malaya, Malaysia and Hail University, KSA (Fig. 2a). Carbon nanoparticles were prepared followed by the protocol (Hossain 2015a) [6]. Then Carbon nanoparticles pigments mixture from the algae was extracted following the method established by Santhanam (2004) [18, 7] and modified by Hossain *et al.* (2008) [8]. The carbon nanoparticles pigments from algae were extracted by hexane and diether (b). The mixture was then kept aside for 48 h to settle down (c). The carbon nanoparticles pigments were separated after filtration the residues.



(a) Fresh algae, (b) Carbon nanoparticle after 12 hours



(c) Algae nanoparticle pigments with (as pigments extraction from algae) (oil extraction after 48 h)

Fig 2: Photograph shows nanoparticle pigment (green) extraction from algae

(a) Bougainvillea flower (b) Carbon nanoparticle as pigments (red) extraction

Fig 2: Photograph shows nanoparticle pigment (red) extraction from bougainvillea flower

According to Hossain (2013) [9], carbon nanoparticles pigment (red) extraction from bougainvillea flower was done at Hail University, KSA (Fig 2b). Pigments was extracted following the method established by Hossain (2013) [9]. The pigments were extracted. Carbon nanoparticles were prepared followed by the protocol (Hossain, 2015b, Hossain and Uddin. 2018) [7, 4]. The nanoparticle mixture was then kept aside for 24 h to settle down. The carbon nanoparticles pigments were collected after filtration residues. These red pigments nanoparticle can be used for natural carbon nanoparticle fluorescent probe for cell bio-imaging of plant, animal and human (cancer cell).

3. Result and Discussion

1. Breast cancer cell imaging has been taken MDAMB-231 (breast cancer) cell line by Das *et al.* (2017) [14]. Imaging of various type of cells were investigated. The BREF dye has been applied to different types of cells, like breast cancer cell line (MDAMB-231), WBC, leukemia and Squamous epithelium. It was observed that BREF dye fictionalized NPs give very good fluorescent image. They stated that different types of cell imaging after tagging them with BREF dye. GFP range excited images for WBC type cells after staining them with BREF. RFP range excited for MDAMB-231 breast cancer cell stained with BREF dye. RFP range excited three different images of leukemia cell after treating them with BREF dye.
2. Arpita *et al.* (2017) [1] established carbon dots in a biolabelling assay with its other physicochemical features like the tunable luminescence property, high degree of water solubility and low toxicity, towards various environments. They introduced a new perspective on the commercialization of carbon dots as a potential alternative to synthetic organic dyes for fluorescence-based cell-labeling assays.
3. Haydar *et al.* (2016) [10] reported that blue, green, purple and red nanoparticles have been demonstrated as fluorescent cell labels where blue and green excitation has been used for imaging of labeled cells and got brightness more than others color for bio imaging probes.

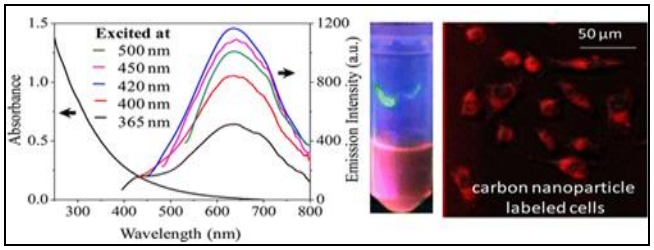


Fig 3: Absorbance of nanoparticles at different wavelength and carbon nanoparticles cell labeled (Haydar *et al.* 2016) [10].

4. According to Susanta *et al.* (2013) [15] fluorescent nanoparticle-based imaging probes has been described. FCN blue, green, yellow and red showed more brightness than uncolored one.

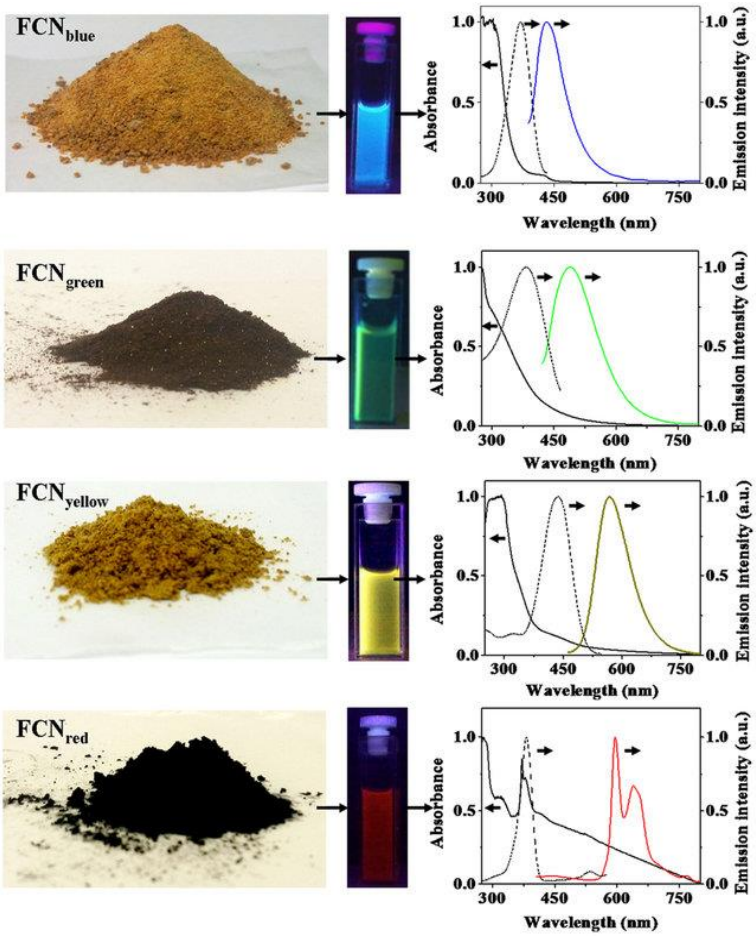


Fig 4: Fluorescent carbon nanoparticle optical's property.

Digital image of gram scale solid FCN samples, digital images of their solutions under appropriate excitations and their absorption, excitation, and emission spectra [Susanta *et al.* (2013)] [15].

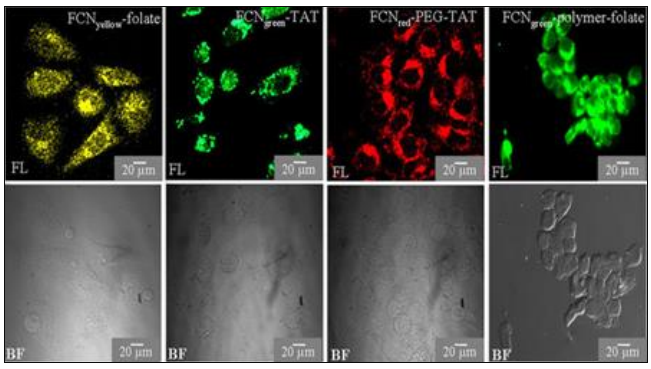


Fig 5: Carbon nanoparticle as fluorescent cell label. Cells are imaged under bright field (BF) and fluorescence (FL) mode with confocal or Apotome microscope [Susanta *et al.* (2013)] [15].

5. Xian *et al.* (2017) [18] studied in Washington State University and have created an injectable dye that illuminates molecules with near infrared light through deep inside the body (Yuan *et al.* 2012) [19]. They reported that the new dye would help medical researchers track the progression of a wide array of diseases, such as cancer (Xian and Ralph, 2017) [11]. Xian *et al.* (2017) [18] published a new study detailing the dye's unique properties near infrared light passes right through the human body, making it almost invisible and making dyes that respond to this color of light perfect for bio-imaging. They reported that Washington Red is a unique and inexpensive dye that I see being put into use in many areas." It is used to observe disease biomarkers and other biological compounds in real time and helps doctors identify and treat blood clots, heart disease, tumors and other dangerous abnormalities.

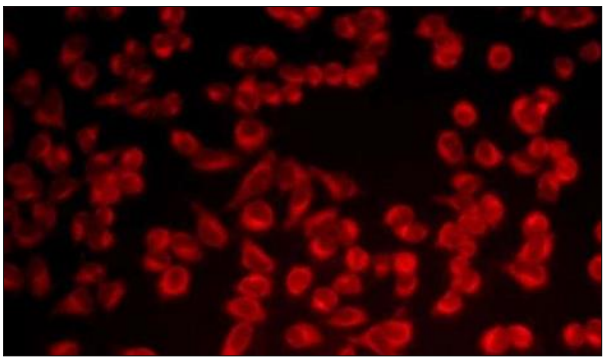


Fig 6: Chemists develop novel Washington Red dye for bio-imaging, WSU (Washington State University, 2017. Xian *et al.* (2017) [18].

6. According to Hossain *et al. (*2008) [8] and Hossain (2013) [9] [Table 1].

Table 1: Observation of green and red pigment from algae and bougainvillea flower.

	Green pigment (Chlorophyll) content	Red pigment (Carotinoid) content	pH content	Color observation
Green nanoparticle (algae)	Chl a 8.6 µg Chl. b 4.5 µg	0	4.0	Deep green (high color)
Red nanoparticle (bougainvillea)	0	3.4 µg	3.6	Deep red (high color)

Hossain (2013) & 2015a) [9, 6] reported that green pigments of algae contained 8.6 and 4.5µg chlorophyll a and b content and pH was 4.0. He also recommended that carbon nanoparticle fluorescent probe for cell bio imaging can be used from the green algae (Hossain, 2015b) [7] followed by the protocol of Hossain and Uddin (2016) [5]. Red pigment (carotine) was 3.4 and pH was 3.5 of bougainvillea flower (Hossain, 2013) [9]. He also recommended that carbon nanoparticle fluorescent probe for cell bio imaging can be used from the bougainvillea flowers followed by the protocol of Hossain and Uddin (2016) [5]. These carbon nano-particles can be used as fluorescent probe for cell bio imaging in the medical and biomedical application.

4. Conclusion

It can be concluded that natural carbon nanoparticle fluorescent probe from plant sources (like colorful flower, excellent chlorophyll based algae and cyanobacteria etc) was better than synthetic dye for cell bio imaging in human (cancer cell), animal and plant cell. It would be an attracted considerable attention in medical and biomedical applications.

5. Acknowledgement

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