In Vivo Fluorescence Imaging Of Fruit Fly With Soluble Quantum Dots

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Abstract

The ability of multi colour fluorescence imaging with water soluble carbon quantum dots (WSCQDs) in organisms and biological tissues has been explored using Drosophila melanogaster (fruit flies). Here we present strategies to visualize different developmental stages and their various internal organs in vivo and in vitro condition with multiple, distinct colors. Their viability and growth were not reduced by oral quantum dots ingestion. We demonstrate a new methodology in the field of bioimaging by using synthesized water soluble carbon quantum dots (WSCQDs) that will bring a revolution in the history of biomedical science.

Keywords: Noninvasive, bioimaging, carbon, quantum dots, water soluble, fruit fly

Introduction:

Exploration of quantum dots in biological systems got attention ever since its discovery. The role of WSCQDs, in biological systems and its implications are currently under evaluation primarily on the work interfacing chemistry, physics and biology. The emergence of fluorescence carbon nanoparticles/ dots shows high potential in biological labeling, bioimaging, and other different optoelectronic device applications (Batalov et al., 2009; Selvi et al., 2008; Mochalin and Gogotsi 2009). These carbon nanoparticles are biocompatible and chemically inert, (Lim et al., 2009 ; Kong et al., 2005) which has advantages over conventional cadmium-based quantum dots (Medintz, et al., 2005). However, these application of fluorescent carbon nanoparticles are poorly studied compared with other carbon or cadmium based materials. In addition, the understanding of the uses of fluorescence character in carbon nanoparticle is far from sufficient. (Zhou et al., 2007; Zhao et al., 2008). For example, the understand dynamic processes in live cells, such as intercellular and intracellular trafficking, microstructure remains unclear. To prove these difficult imaging tasks, a robust water soluble QDs are needed. Several synthesis strategies have been used, such as surface functionalization with water-soluble ligands (Sun et al., 2006),
silanization (Cao et al., 2007) and encapsulation within block-copolymer micelles (Liu et al., 2007). Here, we investigated the photo physical properties of water-soluble CQDs prepared by a synthesis method based on Common routes in making fluorescent water soluble carbon quantum dots followed by oxidation of carbon soot (collected from waste plant materials burning) with nitric acid (Ray et al., 2009).

We observed that these small carbon particles enter into cells without any further functionalization and the fluorescence property of the particles can be used for fluorescence-based cell-imaging applications. The ability of multi color fluorescence imaging with WSCQDs in organisms and biological tissues has been explored by using Drosophila melanogaster (fruit flies). In vivo emission imaging has made detailed study of a biological species by fluorescence microscopy.

Therefore we show all the in vivo images of various internal organs through out the developmental phases of Drosophila melanogaster using a new fluorescent material like WSCQDs and tally with a control experiment. We successfully acquire in vivo images of the developing three larval stages till the adult hood by using the minimally invasive imaging modality of ordinary fluorescence microscopy. The whole-body imaging of a probe in real time means that the efficacy of therapeutic treatments can be seen directly without the need for any invasive procedure.

**Experimental Procedures / Materials and Methods:**

**Synthesis of water soluble Carbon Particles:**
Carbon soot 50 mg (collected from burning waste plant materials) was mixed with 30 ml of 5 M nitric acid in a 50ml three-necked flask. It was then refluxed at 140 °C for 10 h with magnetic stirring. After that, the black solution was cooled and centrifuged at 8000 rpm for 7 min to separate out unreacted carbon soot. The light brownish-yellow supernatant was collected, which shows green fluorescence under UV exposure. The aqueous supernatant was mixed with acetone (water/acetone volume ratio was 1:3) and centrifuged at 16000 rpm for 10 min. The black precipitate was collected and dissolved in 30 ml of water. The colorless and nonfluorescent supernatant was discarded. This step of purification separates excess nitric acid from the carbon nanoparticles. This concentrated aqueous solution, having almost neutral pH, was taken for further use. The same synthesis technique was also performed for 18 h of reflux. The supernatant obtained from the 18 h reflux, was dark yellow. We weighed the unreacted carbon soot, which was removed as precipitate, in order to find out the yield of soluble carbon nanoparticles.

The weight was ∼50 mg for the 18 h reflux times (yield ∼22%). This solution has particles having sizes ranging from 20 to 220nm and is called as-synthesized carbon quantum dots (CQDs) (see Scheme 1). Figure-2 shows AFM and TEM images of WSCQDs.

**Fly cultured with water soluble CQDs:**
Flies were of the Canton S strain (obtained from the laboratory of Dr Pradip Singha, Department of biological science, IIT Kanpur) that had been reared in the laboratory for many generations. Stocks were maintained in an room temperature at 20- 25°C under a L14:D10 photoperiod in 250-ml bottles on WSCQDs mixed 60g of a cornmeal–agar medium seeded with yeast. Cornmeal agar medium was made according to a recipe modified from (Lewis, 1960).
Agar (8.00g) and 0.5mg WSCQDs mixed water (1000 ml) were added to a saucepan and heated until boiling. 50 grams of organic cornmeal, 40g dextrose, 25g dried yeast were mixed together and added when the agar was boiling. The mixture was simmered for 5 min and then removed from the heat and allowed to cool to at room temperature. 2.5 gms of Nipagin and 9 ml propionic acids in 15 ml of 95% ethanol were then added and stirred into the food. Flies that were to be used in experiments were reared as follows. Twenty virgins adult were assigned randomly to media containing vial. Vials were plugged with cotton wool bungs and placed in a room temperature / incubator at 20-25°C under a L14:D10 photoperiod (the lights came on at 08:00 GMT).

Drosophila were treated for two- three days before to lay eggs. These eggs were allowed to grow under WSCQDs treated food to complete their life cycle.

Another set of experiment (control) has done without any WSCQDs, others conditions were same like treated and their life cycle was monitored. The organism and their all life cycle stages were washed thrice with the sterilized PBS (pH 7.4) for fluorescence microscopy (LEICA DC200).

**Fluorescence microscopy:**
Images of life cycle stages of drosophila were captured by using a Leica inverted microscope (Leica DC200, Leica microscopy system ltd, CH-9435, Heerbrugg) with an attached RS Photometrics Sensys camera, KAF1401E G1. The intensity of fluorescence was quantified by using the 488, 561 and 633nm band pass (BP) emission filter functions of the Leica microsystem imaging solution software (Leica Q fluoro version V1.0a, Leica microsystem imaging solution ltd, Germany).

**Result and Discussion:**

The fruit fly Drosophila melanogaster is one of the most valuable organisms in genetic and developmental biology studies. Transgenic methods are in use to image with bleachable organic fluorophore or fluorescent protein, full image of all the stages of the life cycle of the living wild organism is lacking. WSCQDs have a high emission range fluorescence property. The fluorescence property of the particles were used to track their position in cells using a conventional fluorescence microscope. We acquire in vivo images of the eggs through all the larval stages till adult hood under oral ingestion (figure-3). Figure-4. showed multicolored fluorescence images providing clearer internal structure of Drosophila. In contrast, the fluorescence signals of the cells without addition of the CQDs were invisible in control experiments. Furthermore, these images reveal WSCQDs bind to the cells, but it is nonspecific. The possible mechanisms are that the WSCQDs with surface carboxylic acids bind on the surface of cells (Jessica et al., 2007; Liu and Vu, 2007). It indicated that the water-soluble CQDs bind on the surface of cells. Since the surface of WSCQDs were the functional carboxylic Group was free, which can be easily coupled with amine groups the surface cell of an organism, such as proteins, peptides and amino acids. In fact, one cell membrane carries numerous proteins and one protein typically bind on numerous water soluble CQDs.
We observed the viability rate of the both control and treated organisms were same. While both of them completed their life cycle within 12-14 days. Their behavioral pattern is same with the normal fly. So WSCQDs does not show any toxic effect during the life cycles of Drosophila).

**Conclusion:**

Before our these experiments a non-invasive method to create an image of a body structure from a laboratory animal using relatively simple equipment is not known. Bio–imaging began since the discovery of X-rays by Roentgen in 1895. The magnetic resonance imaging (MRI) technique has been introduced to overcome the relatively high permeability of X-rays and its deleterious effects on biological tissue. The imaging can noninvasively monitor cellular or genetic activity and subsequently use the results to track gene expression, the spread of disease, or the effect of a new drug in vivo. Our imaging process could give in vivo multicolor fluorescence images. Water soluble carbon quantum dots will become key probes for multicolor fluorescence microscopy. It is suitable for long term imaging because it is not photo bleaching. Also it has not cytotoxic effect. The whole-body imaging of a probe in real time means that the efficacy of therapeutic treatments can be seen directly without the need for any invasive procedure. Our approach can be used for milligram-scale to bio imaging. These fluorescence imaging process can useful for medical applications. These process can obtain in vivo images of cells without any invasive surgery. Also these process have the potential in biomedical applications where cadmium-based quantum dots show toxic effects. However, synthetic methods of these particles need to be much more advanced so that large quantities of these particles with different emission colors were easily prepared.

**Acknowledgements:**

T. K. M., N.P. and M.S. are grateful to Prof. R. Gurunath, Prof. S. Sarkar and Prof. B. Prakash, IIT Kanpur for providing necessary laboratory facilities. N.P. T.K.M thanks NIT Agartala for providing a fellowship. Thanks to Prasenjit Samanta and Santanu Mondal of D.A.V college, Kanpur, for helping us.

**References:**


Figure Legends

Figure 1. Schematic diagram of WSCQDs treated drosophila (fluorescing) and untreated drosophila (not fluorescing).

Figure 2. AFM topography images of water soluble C-Dots(left) and HRTEM image C.Dots (right).

Figure 3. Fluorescence images of various developmental stages of drosophila treated with WSCQDs. From left to right egg, larva, pupa, female imago and male imago respectively.

Figure 4. Various internal organs of D. melanogaster larva treated with water soluble quantum dots. In vivo image, merge of three lights (488, 561 and 633nm). Where at-atrium, bn-brain, as-anterior spiracle, tc-trachea, pxpharynx, sd-salivary duct, sg-salivary gland, Ep-esophagus, pvv-proventiculus, gc-gastric ceaca, mg-midgut, mi-mid intestine, gd-gonad, utr-ureter, mt-malpighian tubule, hg-hind gut, as-anus. Scale bar 0.5mm.