Fruit-based carbon dots as fluorescent probes: in vitro and in vivo toxicity evaluation

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The recent discovery of carbon dots has opened a new family of exciting nanoscale materials for diagnostic approaches and drug delivery. Carbon dots (c-dots) emerge as a suitable replacement to metal-based quantum dots due to their higher biocompatibility, aqueous solubility, small size and high photoluminescence [1,2]. In addition, the possibility of using fabrication methods based on natural sources, such as fruits, turns this nanodots much more attractive, since they can accommodate the fruits therapeutic benefits[3]. In order to ensure safety in their application and in the environment, information on their toxicological profile both in vitro and in vivo is critical. We used in vitro cell viability tests as proficient tools to evaluate toxicity and to assess optimal concentrations to be used in bioimaging, and zebrafish Danio rerio (Hamilton, 1822) as in vivo model for toxicological investigation given its swift and peculiar development with transparent embryos developing ex-utero, allowing for a real-time analysis of the induced effects.

Objectives

• Gain insight into the toxicological profile of novel fruit-based carbon dots using both in vitro and in vivo models;
• Investigation of the bioimaging potential of novel fruit-based carbon-dots as diagnostic tools.

Methods

Fruit-based c-dots: synthesis and characterization

C-dots have been synthesized from kiwi and avocado fruits, by one-pot green hydrothermal method, and characterized for their photoluminescence properties.

In vitro & In vivo testing

• In vitro toxicity was determined by measuring the metabolic rate of HK-2 (normal human cell line) and Caco-2 cells (cancer human cell culture line) via PrestoBlue® assay upon 48 h exposure to fruit-based c-dots.
• In vivo tests were performed using ZET (zebrafish embryo toxicity) protocol following animal experimentation ethical concerns according to the Council of Europe, Directive 86/609/EEC.

Results

In vivo evaluation of fruit-based c-dots 48 h in and Caco-2 and HK-2 cell lines. Results are expressed as mean ± SEM of four and six independent experiments, respectively. Different letters indicate significant differences among treatments (P<0.05, one-way ANOVA).

In general, it was noted that fruit-based c-dots induced more cytotoxicity to normal epithelia HK-2 cells than to Caco-2 as proved by the higher LD50 values obtained for these adenocarcinoma cell line. Cytotoxicity was more evident for concentrations above 1.5 mg/mL for both human cell lines. Citrate c-dots were used as a commercial source control group.

Conclusions

• Citrate c-dots did not induce any significant effect on cellular viability suggesting that the inhibition effect on cellular growth can be attributed to the different source employed for the c-dots synthesis.
• In vivo toxicity analyses using zebrafish embryos rendered agreeable correlation with in vitro results. In both tests, fruit-based c-dots were more toxic for concentrations above 1.5 mg/mL with kiwi c-dots revealing a more toxic profile than avocado c-dots.
• A low retention of both c-dots in zebrafish embryos with 4 hpf could indicate that the chorion acts as a physical obstacle. Avocado c-dots were more retained and present a higher luminescence intensity, in agreement with its higher quantum yield.

References


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