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

Cindy Dias [a,b*], Marisa P. Sária [a*], Nagamalai Vasimalai [a], Vânia Vilas-Boas [a], João Peixoto [b], Begoña Espiña [a]

[a] INL - International Iberian Nanotechnology Laboratory, Braga, 4715-330, Portugal
[b] CEB- Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal
The corresponding author, Email: begona.espina@inl.int
*Both authors contributed equally to the development of the work

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[2]. 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.

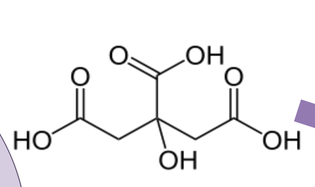
In vitro & *In vivo* testing

- *In vitro* toxicity was determinated 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.

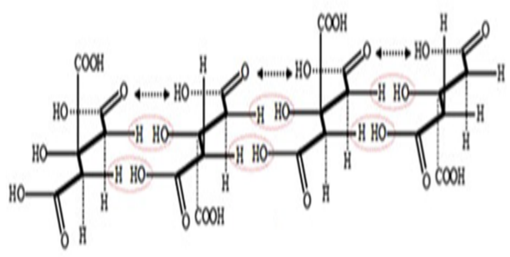
Confocal Imaging

Imaging of 4 and 80 hpf zebrafish embryos exposed to fruit-based c-dots for 2 h.

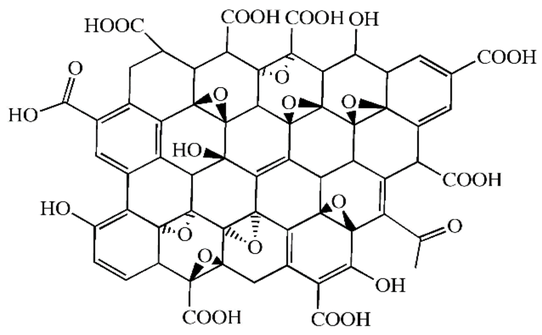
Results



Carbon source



Intermediate



c-dots

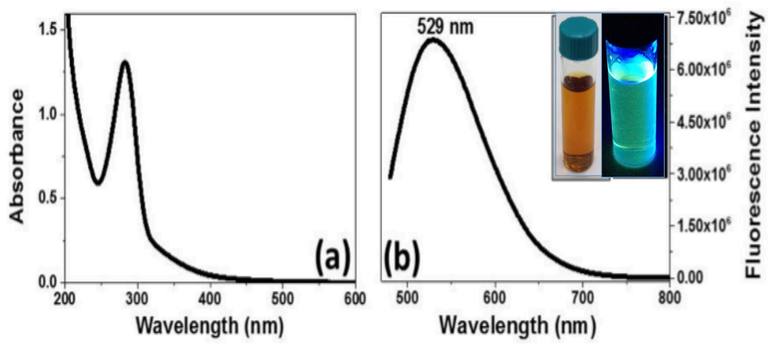


Figure 1. Carbon dots preparation (bottom up method) and a) absorption and (b) fluorescence spectra of avocado c-dots (λex**:** 470 nm and λem**:** 529 nm). *Inset*: Photographs of avocado c-dots under day light and UV light.

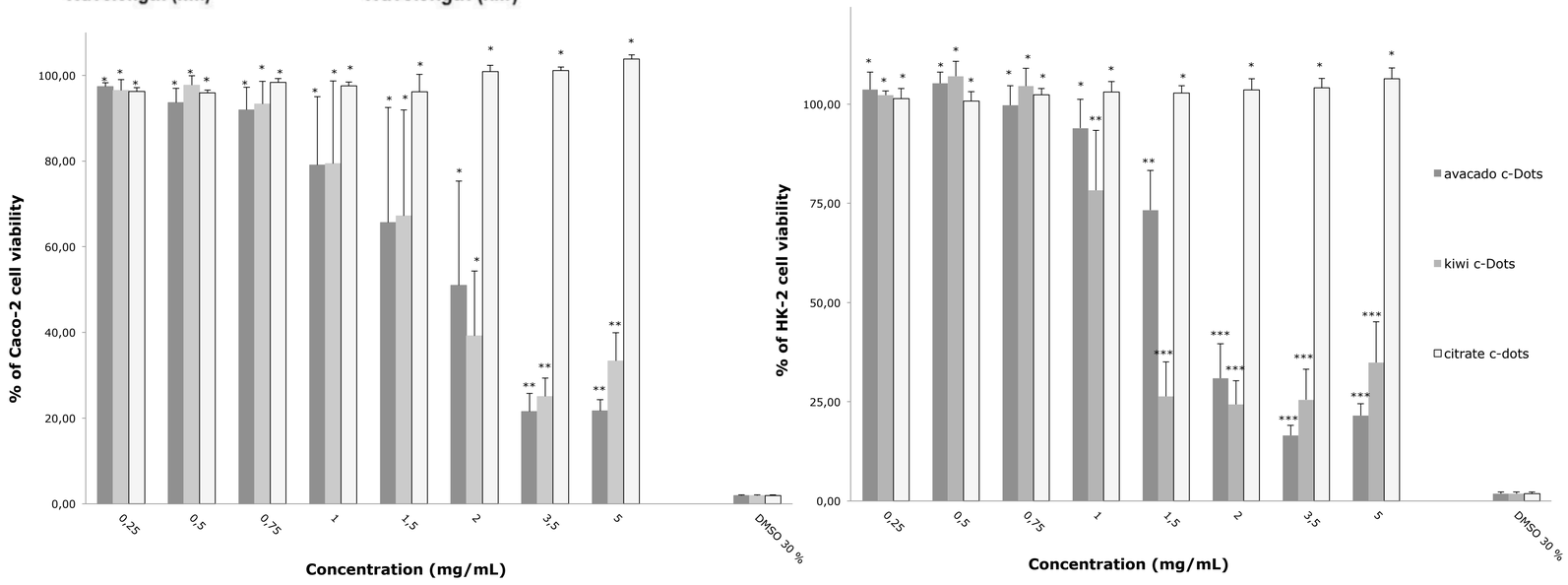


Figure 2. *In vitro* evaluation of fruit-based c-dots 48 h in and Caco-2 cells and HK-2 cell lines. Results are expressed as mean ± SEM of four and six independent experiments, respectively. Different letters indicate significant differences among treatments (P<0.05, one-way ANOVA).

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 LD_{50} 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.

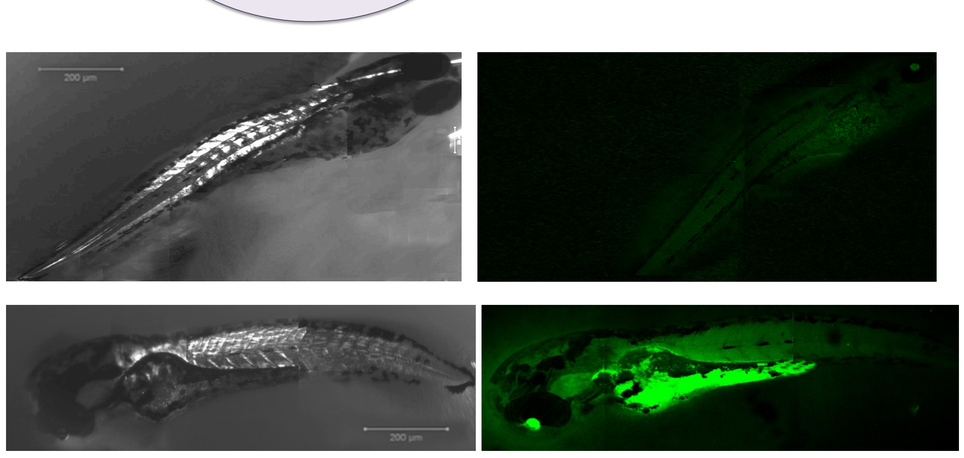


Figure 4. Transmission and fluorescence imaging of zebrafish embryos with 80 hpf with no c-dots (upper) and with 1 mg/mL of avocado c-dots (lower).

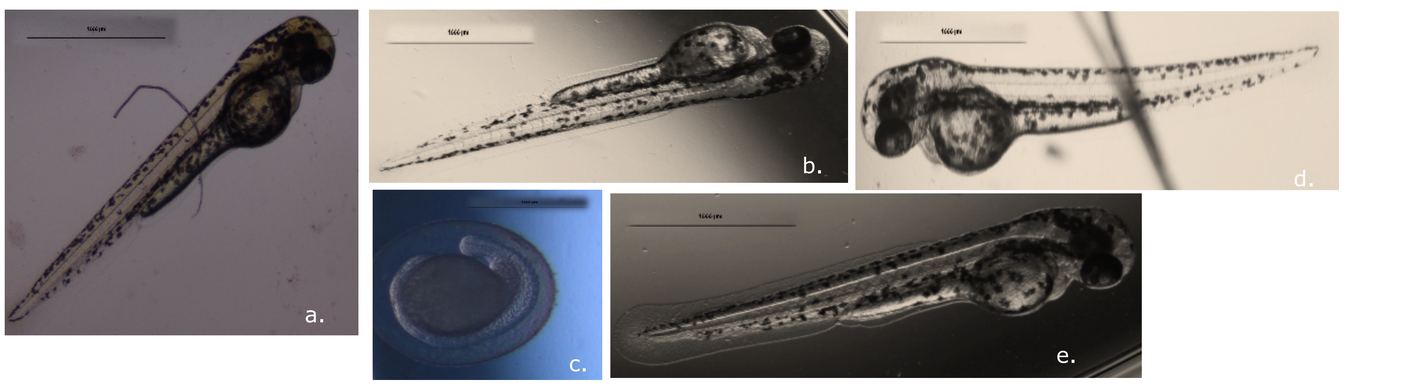


Figure 3. Zebrafish embryos at 56 hpf exposed to freshwater (i.e. experimental control) (a), kiwi c-dots 0.1 mg.mL-1 (b), kiwi c-dots 1.5 mg.mL-1 (c), avocado c-dots 0.1 mg.mL-1 (d) and avocado c-dots 1.5 mg.mL-1 (e).

In vivo data (morphological and physiological features analysis: mortality, developmental delays, phenotypical malformations, spontaneous movements, heart and hatching rates) demonstrated that 1.5 mg/mL of kiwi c-dots exerted more pronounced sublethal toxic effects, than avocado c-dots.

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 it’s higher quantum yield.

References

[1] Y. Wang, A. Hu, *J. Mater. Chem. C.* 2 (2010), 6921–6939.
[2] C. Li, et al, *J. Mater. Chem. B. 2* (2014) 4564–4571.

Acknowledgments

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