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# BIOCOMPATIBILITY AND ANTIBACTERIAL ACTIVITY OF CARBON DOTS IN VITRO

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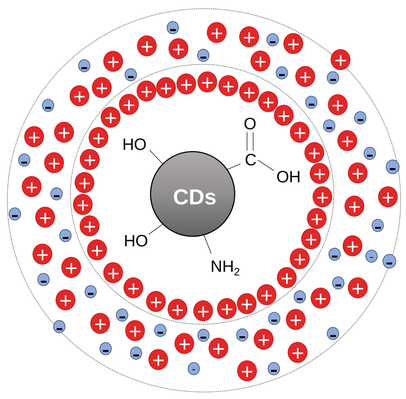
Carbon dots (CDs) are quasi-spherical carbonaceous nanoparticles less than 20 to 60 nm constituted by $sp^2$-$sp^3$ core and an irregular surface rich of polar functional groups that confers intriguing and tunable physico-chemical properties [1]. They display brilliant and excitation wavelength dependent photoluminescence, aqueous dispersibility, easy synthetic and functionalization conditions [2]. Recently great attention has been paid to the CDs, due their variegated physical-chemical properties that makes them appealing for multiple biomedical application. In this work we assessed the biocompatibility of sustainable CDs prepared, according to Sawalha et al method [3], recycling the discard of olive oil production. Firstly, the physico-chemicals properties of new synthetized CDs were evaluated. HRTEM analysis performed on the produced CDs confirm their presence with a range from 5 to 25 nm, UV-Vis spectroscopy showed broadband UV absorption and fluorescence spectroscopy an excitation wavelength dependent photoluminescence. Cell imaging displayed that CDs are mainly located in proximity of the cell membrane. Lastly, we assessed the biological properties of CDs. Biocompatibility studies were performed on two osteoblastic cell lines (U2-OS and hFOB) after 24 and 48 h of CDs incubation. Antibacterial activity was evaluated on a Gram+ bacterial strain *S. aureus*, after 24 h of culture in presence of CDs. Our results showed that Carbon dots exhibit a good biocompatibility up to 240 µg/mL, while at 360 µg/mL become cytotoxic. Differently, antibacterial assays showed that the bactericidal activity is slight at 240 µg/mL, but become more important at the concentration of 360 µg/mL. These results on CDs biosafety get the base for their promising application for biomaterial engineering. Further experiments may be performed for surface nano-functionalization obtaining new materials with CDs physico-chemical properties exploitable for many bio-applications.

## OPTICAL PROPERTIES

- Brilliant excitation wavelength dependent luminescence
- Photostability
- Tunable absorption

## BIOLOGICAL PROPERTIES

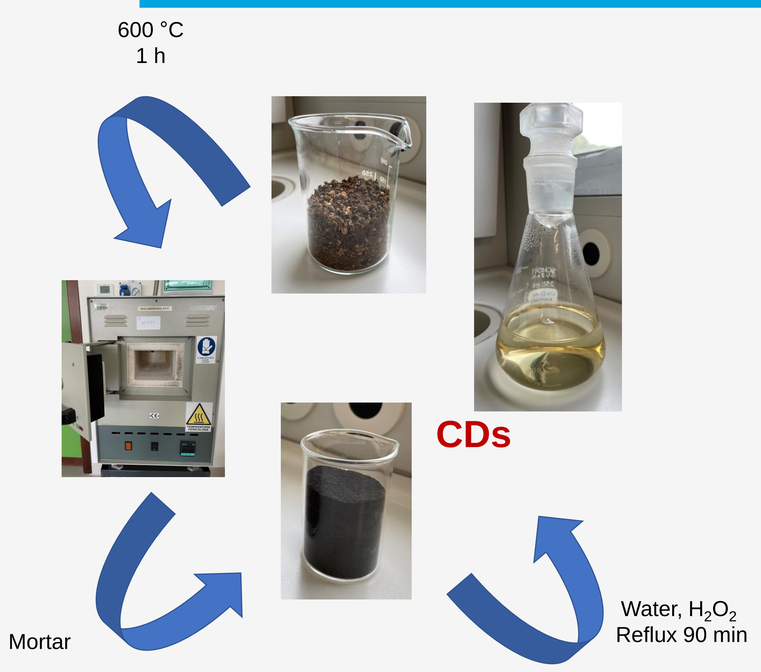
- Low toxicity
- Biocompatibility
- Useful tools for thenostics, drug delivery and bioimaging



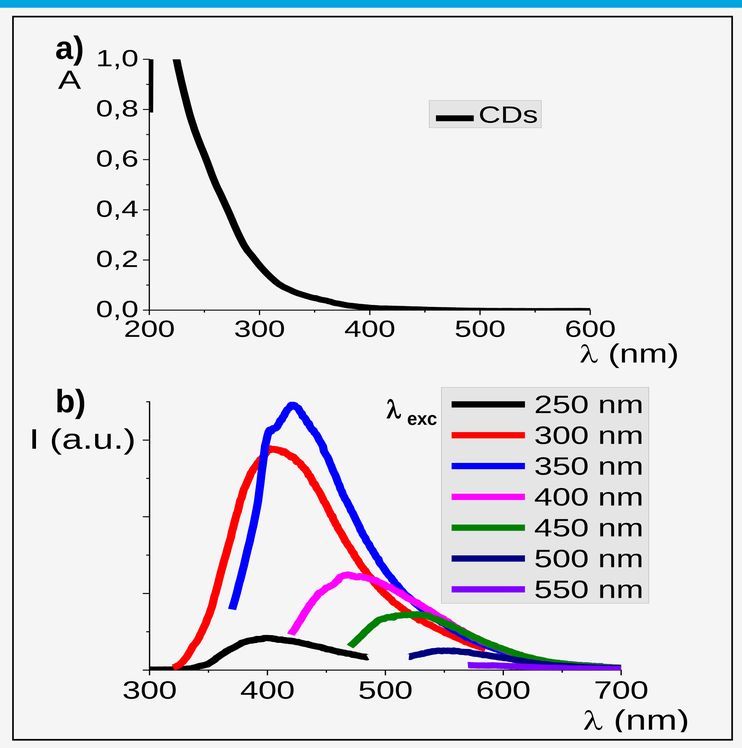

## CHEMICAL PROPERTIES

- Stability and inertness
- Water dispersibility
- Easy synthetic procedure
- Easy surface functionalization
- Tunable excited states: radiative decay (luminescence), ISC (photosensitizers), non-radiative decay (heat)
- Electrical conductivity

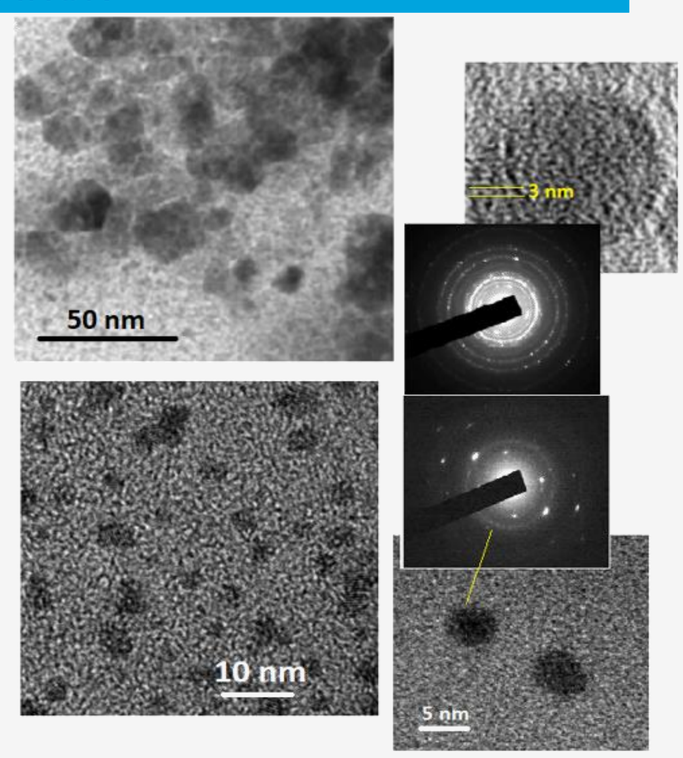
## SYNTHESIS AND PHYSICO-CHEMICAL CHARACTERIZATION




*Olive solid waste was washed with 80 °C water, dried, pyrolyzed for 1 h at 600 °C and fine powdered. The carbonized product was suspended in water and refluxed for 90 min with $H_2O_2$ (0,15 M). The supernatant was recovered and filtered (0,2 µm) obtaining Cdots water dispersion.*




*a) UV/Vis spectrum: broadband UV absorption. b) Excitation wavelength-dependent emission spectra recorded from 250 nm to 550 nm.*

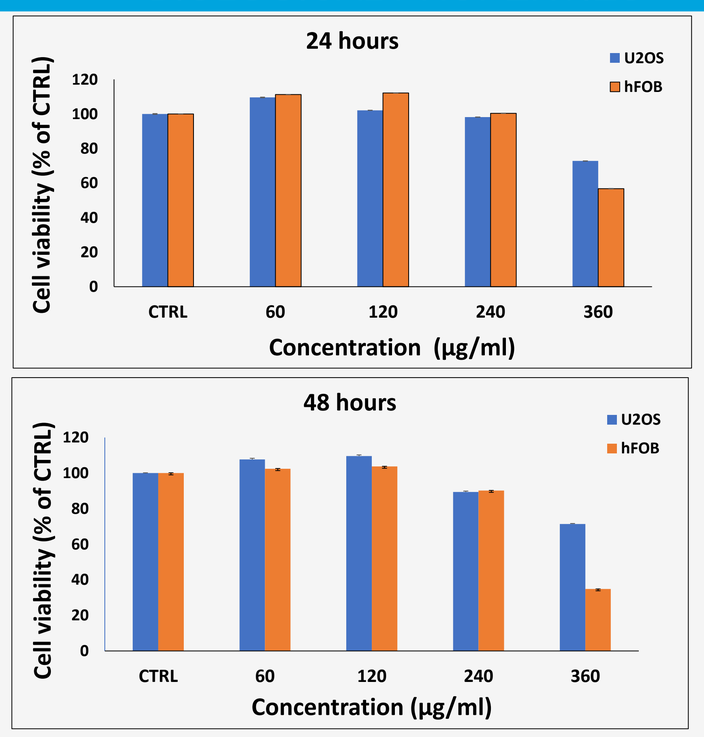



*TEM, HRTEM images and X-Ray diffraction pattern of CDs. NPs size distribution is from 5 to 25 nm. Interplanar spacing and X-Ray pattern confirms Carbon core.*

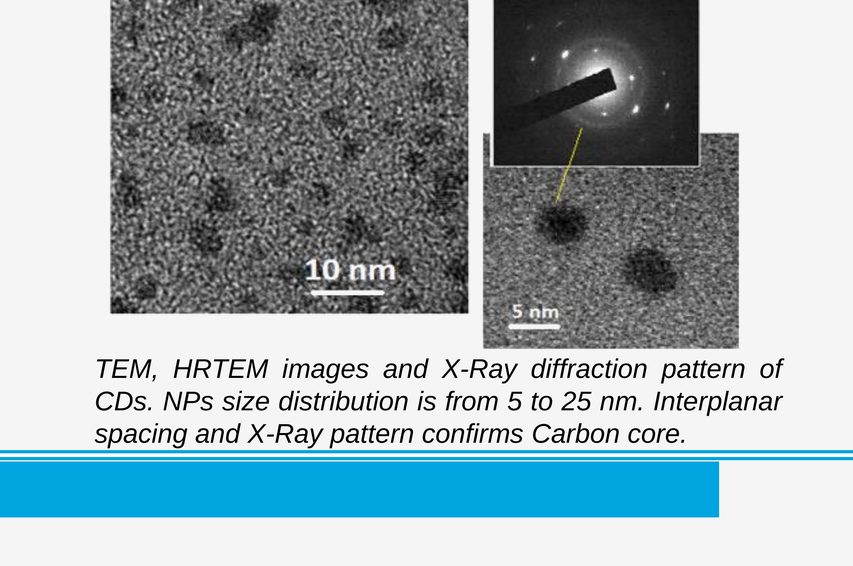
## BIOLOGICAL PROPERTIES




*Representative fluorescence images of U2-OS cells after 24 hours of incubation with CDs. a) Bright field; bar scale 100 µm. b) Blue fluorescence ($\lambda_{exc}$ = 365 nm, $\lambda_{em}$ = 430 nm) represent CDs emission mainly located in proximity of cell membrane; bar scale 100µm. c) Merge bright field + fluorescence; bare scale 50 µm.*




*Cell viability assay (XTT test) on U2-OS and hFOB cells after 24h and 48h of incubation with CDs.*




*Bacteria cells viability (XTT test) performed on S. aureus strain (Gram+) after 4, 8 and 24 hours post-incubation with CDs.*

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## REFERENCES

[1] Yuan, F. et al Nano Today, 2016, 11, 565-586
[2] Nocito, G. et al Cancers, 2021, 13, 1991
[3] Sawalha et al. Carbon, 2020, 167, 696-708

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