Carbon nanodots as delivery system for Squaraines: an in vitro study to investigate their Photodynamic activity

Carbon nanodots as delivery system for Squaraines: an *in vitro* study to investigate their Photodynamic activity

Cosmin Butnarasu,[a] Edoardo Di Gioia,[a] Giorgia Delsoglio,[a] Giorgia Chinigò,[b] Alessandra Fiorio Pla,[b] Nadia Barbero,[c] and Sonja Visentin [a]

[a] Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università degli Studi di Torino, 10125, Italia
[b] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, 10123, Torino
[c] Dipartimento di Chimica, Centre for Nanostructured Interfaces and Surfaces, Università degli Studi di Torino, 10125 Torino

Introduction

Photodynamic therapy (PDT) is a well-studied therapy for cancer and various non-malignant diseases. PDT employs the combination of a photosensitizer, light, and molecular oxygen, to selectively target cells *via* cytotoxic activity [1]. Porphyrin are the most well-known and used photosensitizers in the history of PDT however, their application highlighted some limitations, such as their complex composition and their low rate of light absorption.

For these reasons, extensive efforts have been devoted to the development of different near-infrared (NIR) photosensitizers. Polymethine dyes (i.e. squaraines and cyanines) deserve to be counted among innovative potential photosensitizers (PSs) because they offer numerous advantages such as providing NIR compounds with absorption that perfectly match the phototherapeutic window (650-850 nm). Moreover, they possess high absorption coefficients, bright fluorescence and photostability in organic media [2]. However, in physiological conditions, their chemical instability and self-aggregation properties limit their widely applications. In this context, the incorporation of these dyes in nanoparticles (NPs) is extremely important.

Carbon dots are an emerging family of nano-systems displaying a range of fascinating properties. They are characterized by high aqueous solubility, robust chemical inertness, easy functionalization, high resistance to photobleaching, low toxicity and good biocompatibility [3]. For these reasons they have been studied and applied in numerous fields such as biological labelling, bioimaging and drug delivery.

Aims

Squaraines have shown good characteristics suitable for PDT, however their instability in physiological conditions limit their application. In this study, we aimed at improving the aqueous solubility of a photodynamic active squaraine, Br-Sq-C4, through complexation with carbon nanodots without altering its properties. The photodynamic activity of the obtained complexes has been assessed through *in-vitro* PDT tests (Figure 1).

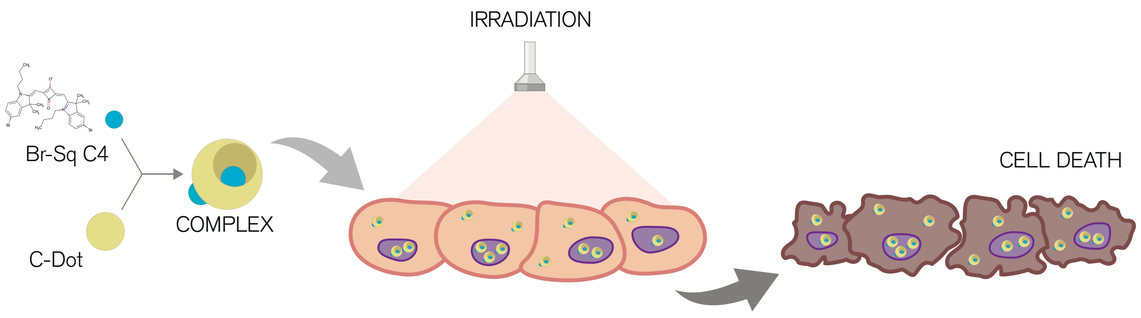

Figure 1: Scheme of in vitro PDT test.

Synthesis and characterization of a C-dots library

In this study a bottom-up microwave-based synthesis approach was used, and all the reactions were performed in a single-mode Biotage Initiator 2.5 (Uppsala, Sweden) (Figure 2c), using 2 - 5 mL vials. In this way, by just changing the reagents, different types of C-dots with different properties were synthetized (Table 1). The synthesis results were purified using Sephadex G12 columns and solids were obtained through rotavapor evaporation.
Successively, characterization of the so-obtained C-dots was assessed with TEM (Figure 2 a, b) and UV-vis spectroscopy. At this point in time, only the CD1 sample has been used for the next steps of the study.

Table 1	C precursor	N precursor	Solvent	MW settings	UV-Vis peak (nm)
CD1	Citric Acid	Urea	Water	140°C; 5min	331
CD2	Sucrose	Urea	Water	160°C; 30min	276
CD3	Citric Acid	Alanin	Water	140°C; 1h	340
CD4	Citric Acid	Ethylendiammine	Water	140°C; 10min	350
CD5	Citric Acid	DMF	DMF	160°C; 30min	242

Table 1: Library of C-dots

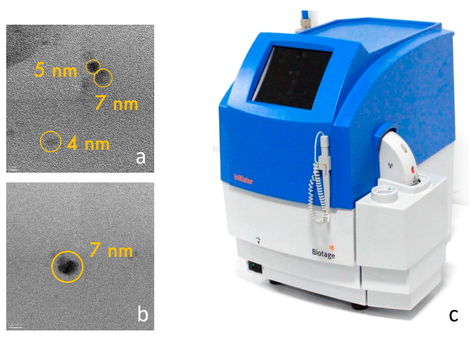

Figure 2: (a) and (b) TEM images of CD1 sample; (c) Biotage Initiator 2.5 (Uppsala, Sweden); (d) Results of complexes purification.

Complexation of C-dots and squaraines

C-dots and squaraines were incubated under stirring dark conditions for 24h. After that time, a large number of aggregates could be seen, and therefore aliquots of 96% ethanol were added in order to obtain a homogenous solution ready to be purified with a Sephadex column.
After the separation we could observe 3 phases with different colors (Figure 2d): uncomplexed squaraines (blue), uncomplexed C-dots (brown), C-dots squaraines complexes (green) (Figure Sephadex).

In vitro PDT test

We assessed the photodynamic activity of C-dot - squaraines complexes by evaluating the cellular survivability of MCF-7 cell lines with and without a 15 min irradiation. This type of irradiation is possible thanks to the use of a compact LED array-based illumination system produced by Cicci Research s.r.l. (Figure 3), specifically for in vitro PDT on cells grown in the standard multiwell plates (96-wells). The proposed illumination system includes a RED-LED array (light source with excitation wavelength: 640 nm, and voltage: 15 volt) composed of 96 LEDs in a 12 × 8 arrangement. In addition, LED-array and the standard 96-multiwell plate have been both placed into a case. MTS assay was performed 24h after irradiation in order to evaluate cell viability. This is a metabolic colorimetric assay for assessing cell metabolic activity.

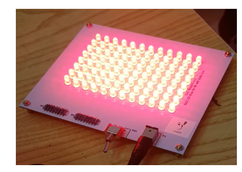

Figure 3: RED-LED array

Preliminary results and Conclusions

The so-obtained C-dot - squaraine complexes are completely soluble in water, with no formation of any kind of aggregate. Furthermore, photoluminescence tests show a 6 fold increase in fluorescence emission for complexes in H_2O compared to the emission of the same concentration of only squaraine in the same solvent, while also maintaining the typical profile of emission of squaraines in organic solvents (Figure 4). Finally, the *in vitro* PDT test not only show a photodynamic activity of the complexes when irradiated, but also the absence of toxicity for the non-irradiated treated cells (Figure 5 and 6).
These preliminary results investigate only one type of the above mentioned synthetized C-dots; further studies will cover the entire C-dots library.

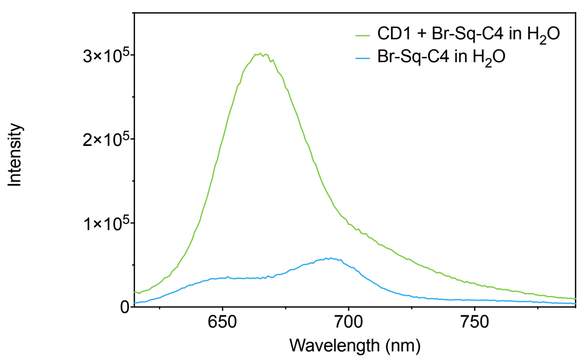

Figure 4: Fluorescence spectra of CD1+Br-Sq-C4 in H_2O (green) and Br-Sq-C4 in H_2O (Blue)

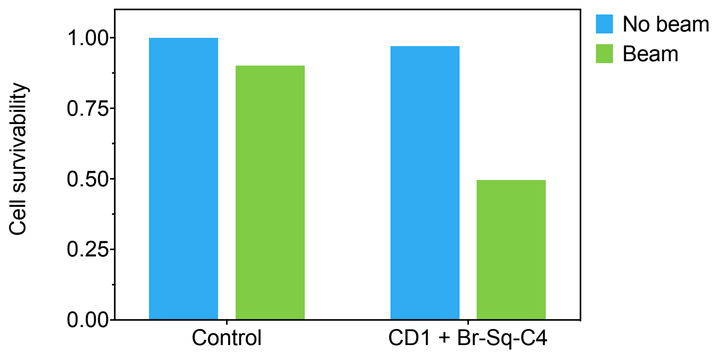

Figure 5: *in vitro* PDT test results for non-irradiated (blue) and irradiated (green) MCF-7 cell lines treated or not with C-dot – squaraine complexes.

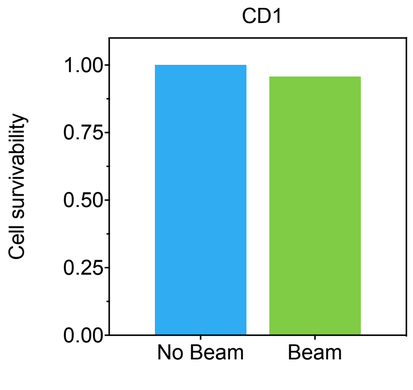

Figure 6: *in vitro* PDT test results for MCF-7 cell lines treated with CD1 sample.

References

[1] Kou J. *et* al., "Porphyrin photosensitizers in photodynamic therapy and its applications" *Oncotarget* 2017;8 (46): 81591–81603.

[2] L. Serpe *et al.*, "Squaraines bearing halogenated moieties as anticancer photosensitizers: Synthesis, characterization and biological evaluation," *Eur. J. Med. Chem.*, vol. 113, pp. 187–197, 2016.

[3] Y. Yuan *et al.*, "Doxorubicin-loaded environmentally friendly carbon dots as a novel drug delivery system for nucleus targeted cancer therapy" *Colloids Surfaces B Biointerfaces*, vol. 159, pp. 349–359, 2017.

Contact:
cosminstefan.butnarasu@unito.it

Nanomedicine 2019 2-3 May 2019
MedChem
Università degli Studi di Milano Bicocca