School of Nanomedicine 2022
Rome, 8th-10th June 2022

Università degli Studi di Messina

ORANGE PEEL DERIVED NANOBIOCHAR FOR TARGETED CANCER THERAPY

C. Celesti [1,2*], C. Espro[2], S. V. Giofré,[3] S. Scalese[4], A. Di Pietro[5], D. Iannazzo[2]

[1] *Department DIMED, University of Messina, Via Consolare Valeria, I-98125 Messina, Italy*
[2] *Department of Engineering, University of Messina, Contrada Di Dio, I-98166, Messina, Italy*
[3] *Department CHIBIOFARAM, University of Messina, Via D'Alcontres, I-98166, Messina, Italy*
[4] *Institute for Microelectronics and Microsystems, CNR-IMM, 8a Strada, I-95121, Catania, Italy*
[5] *Department BIOMORF, University of Messina, Via Consolare Valeria, I-98125 Messina, Italy*
**e-mail: ccelesti@unime.it*

Background

Cancer represents a major public health problem worldwide, leading to ever increasing deaths, globally. Graphene based nanomaterials (GBMs) have opened up extraordinary avenues in cancer therapy, allowing the development of cancer targeted drug delivery systems (DDSs) able to improve the efficacy of conventional anticancer drugs [1,2]. New green routes for GBMs synthesis have been recently exploited, starting from renewable resources, such as lignocellulosic biomass waste, affording the nanobiochar (NBC) [3].

Synthesis

NBC was synthesized by hydrothermal carbonization (HTC) starting from orange peel waste, in a stainless steel autoclave at 240° C for 1 h. The biochar was treated with NaOH, filtered through a 0.1µm membrane and purified by dialysis bags. NBC was characterized and then covalently linked with biotin, folic acid, hyaluronic acid, riboflavin by means of a PEG linker.

The synthetic strategy towards the synthesis of TLs conjugated NBC involved the reaction of the carboxyl group present in the graphene surface with the bidentate PEG linker protected with the tert-butyloxycarbonyl protecting (BOC) group, at a single amine functionality. The subsequent BOC-deprotection afforded the amino-functionalized nanosystem which was then conjugated with the TLs possessing a free carboxyl functionality. For riboflavin, the preventive coupling reaction with succinic anhydride was performed to introduce the carboxyl group needed for the subsequent coupling reaction (Fig.2).

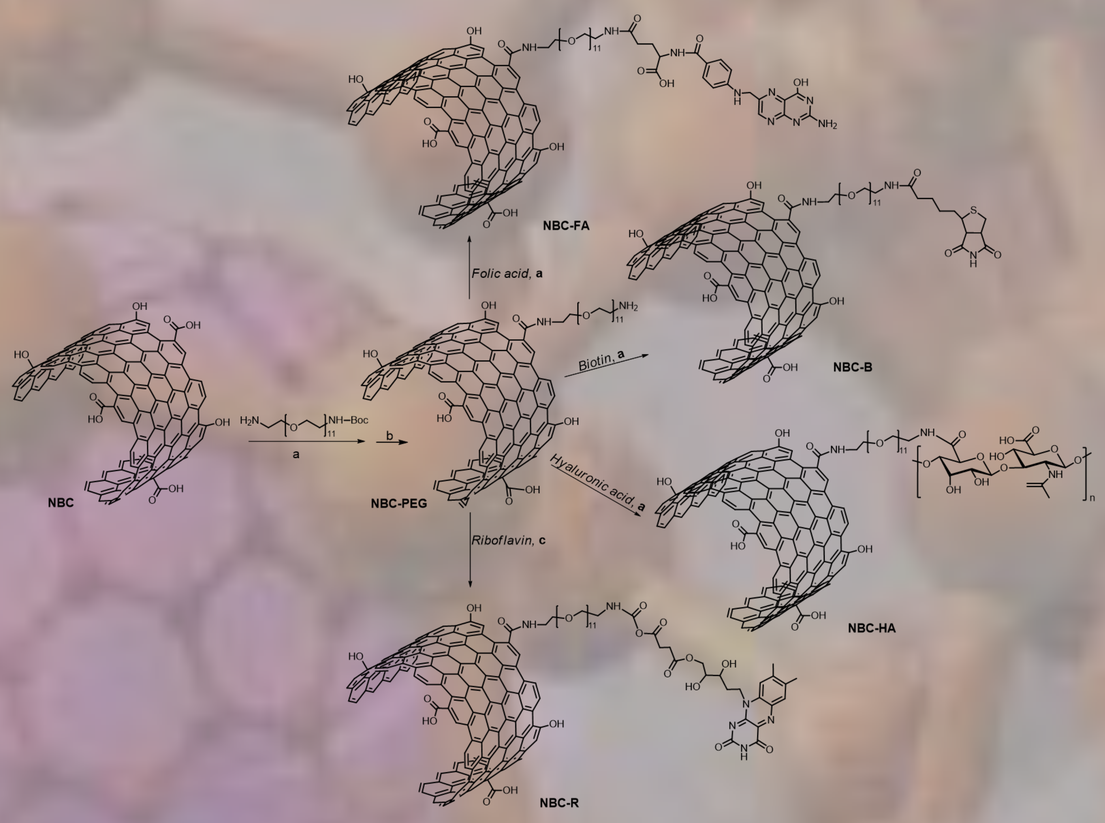

Figure 2. Synthesis of NBC-TL samples. *Reagents and conditions*: (a) DMF, EDC·HCl, ETA, HOBt, DMAP, r. t., 5 d; (b) HCl 4M, dioxane, r. t., 1h; (c) DMF, EDC·HCl, ETA, DMAP, HOBt, succinic anhydride, r.t., 5 d.

The nanosystems endowed with best uptake ability (systems conjugated with biotin and riboflavin) have been loaded with the dihydrofurofuranone DHF [4] and evaluated for their anticancer activity in A549 cell lines. The loading of DHF was performed in basic buffer solution at pH 7.4 in order to allow the effective π–π stacking and hydrogen bond interaction with the graphene (Fig. 3).

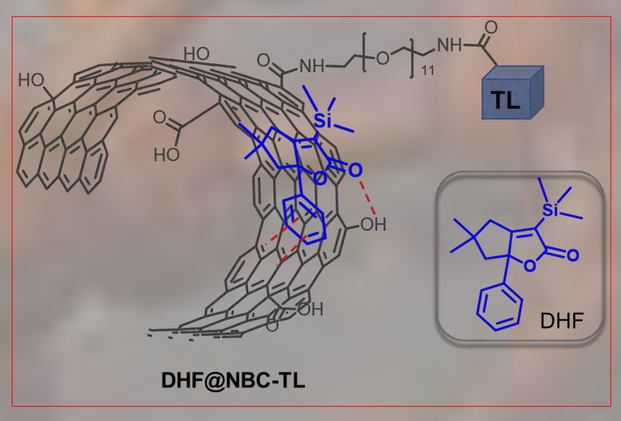

Figure. 3 DHF@NBC-TL: diagram illustrating the interaction of DHF with NBC-TL

Conclusions

The high water dispersibility of the NBC-based systems has shown the potential to overcome some critical limitations of the currently used anticancer drugs. The ability to selectively deliver anticancer agents to tumor cell was tested on the TL conjugated nanocarriers which showed the best uptake ability towards the selected cancer cell lines, namely NBC-B and NBC-R. Cytotoxicity evaluation showed the low cytotoxicity of NBC and the ability of the systems to enhance the interactions between the anticancer drug and tumor cells, causing a cytotoxic effect with higher potency than that exerted by the drug alone.

References

1. D. Iannazzo, C. Celesti, C. Espro, Recent Advances on Graphene Quantum Dots as Multifunctional Nanoplatforms for Cancer Treatment, *Biotechnol. J.*, **2021**, 16, 1900422
2. C. Espro, A. Satira, F. Mauriello, et al., Orange peels-derived hydrochar for chemical sensing applications. *Sensors & Actuators: B. Chemical*, **2021**, 341, 130016.
3. R. Mancuso, I. Ziccarelli, A. Chimento, et al., Catalytic Double Cyclization Process for Antitumor Agents against Breast Cancer Cell Lines. *iScience*, **2018**, 3, 279–288.

Aim of the work

In this study, we evalua[illegible] ompared the cancer targeting ability of NBC conjugated with four TLs namely, riboflavin (vi[illegible] B[illegible] (vitamin B7), folic acid (vitamin B9) and hyaluronic acid, widely used in studies rel[illegible]d therapy, by investigating the cell uptake of TL-conjugated NBC in cancer cell lines (Fig. 1).

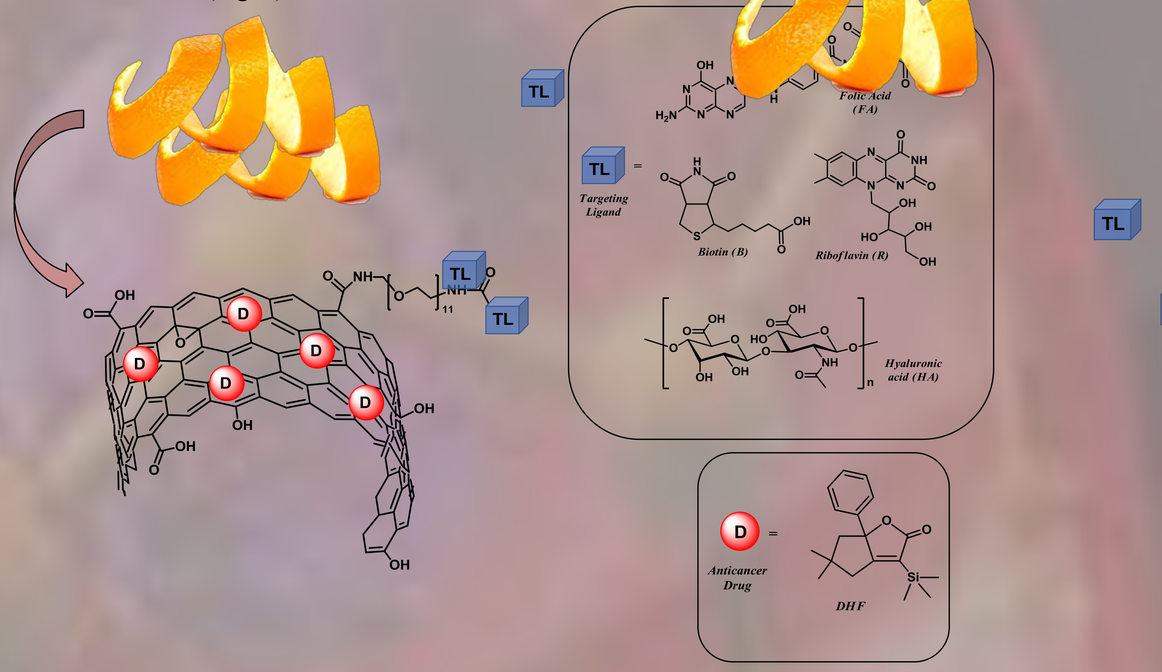

Figure 1. NBC-based DDS for anticancer therapy.

Characterization

The chemical structure of the synthesized nanomaterial was investigated by Raman, XRD and FTIR measurements while the optical properties have been evaluated by PL analysis (Fig. 4). Raman spectra of NBC show the distinctive D-band at 1352 cm^{-1} and the G-band at 1580 cm^{-1} normally encountered for sp^2 carbon nanomaterials. The XRD diffraction spectrum of NBC shows a broad (002) diffraction peak centered at 2θ = 18.5° related to the interlayer graphene spacing. The FTIR spectrum of NBC clearly demonstrates the presence of oxygenated groups on the nanomaterial surface. The small dimension of NBC was also demonstrated by evaluating the optical properties of the synthesized nanomaterials. PL measurements confirm the emission properties of the nanomaterial since a strong emission peak at 560 nm was recorded after exciting the NBC water dispersion at the excitation wavelength of 360 nm. The representative TEM image reports the presence of homogeneously distributed nanostructures, arranged in concentric manner around a hollow centre.

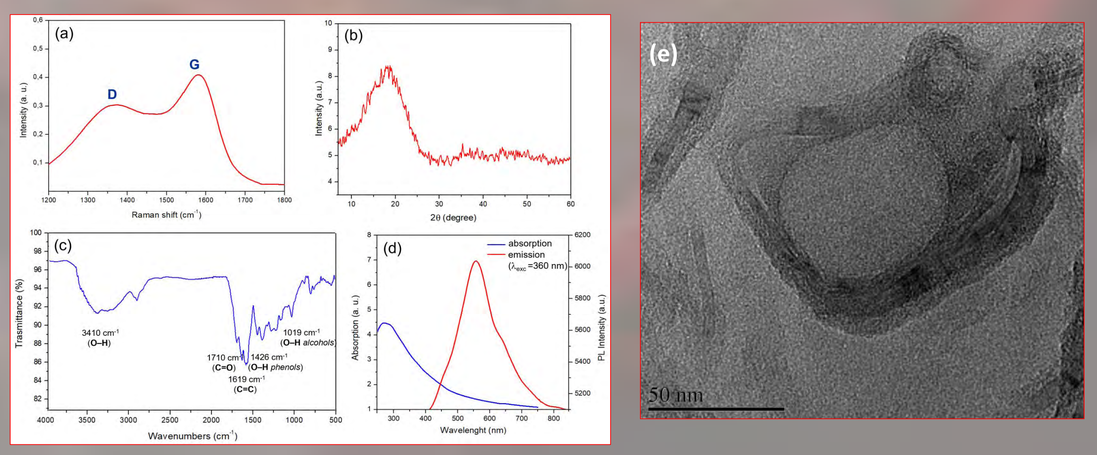

Figure 4. (a) Raman spectrum of NBC at the recorded at λ= 532 nm; (b) XRD spectrum of NBC; (c) ATR-FTIR spectrum of NBC; (d) optical properties of NBC dispersion in deionized water: UV–vis absorption spectrum (blue line) and PL spectrum at the λexc of 360 nm (red line); (e) Representative TEM image of NBC.

After the NBC conjugation with the TLs, the nanosystems have been characterized by FTIR, TGA, PL and DLS measurements then and loaded with DHF. The presence of a stable interaction of DHF was investigated by FTIR spectroscopy and by PL measurements. The FTIR spectra of the drug conjugated samples DHF@NBC-B and DHF@NBC-R show the presence of the characteristic peaks present in the drug. (Fig.4a) The presence of a stable interaction between DHF and the nanosystem was also proved by PL spectra which show the decrease in the fluorescence intensity due to the quenching phenomena together with the redshifts of conjugated samples, (Fig. 4b)

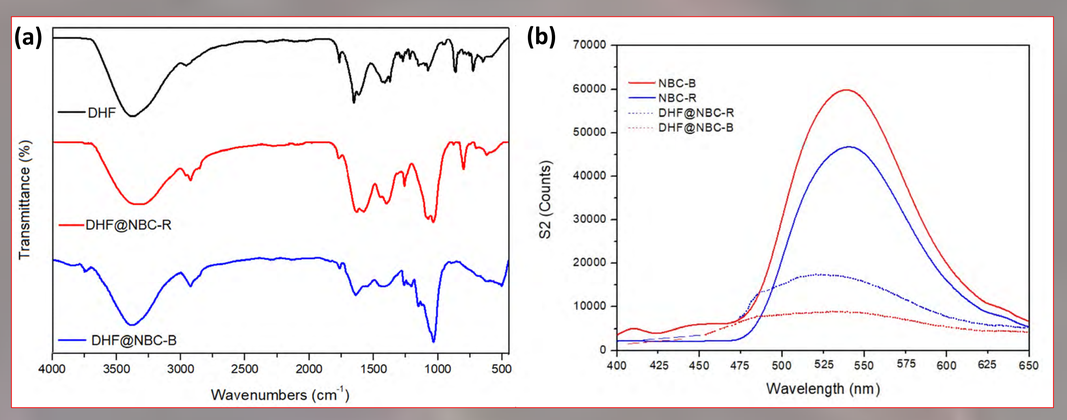

Figure 5. (a) FTIR spectra of DHF and of the drug conjugated samples DHF@NBC-B and DHF@NBC-R; (b) PL spectra of NBC-B, NBC-R, and of the drug conjugated samples DHF@NBC-B and DHF@NBC-R.