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ANTICANCER ACTIVITY OF CARBON NANOMATERIALS-CAMPTOTHECIN COMPLEXES

R. Garriga[1], T. Herrero-Continente[2], M. Palos[3], V. L. Cebolla[4], J. Osada[2], E. Muñoz[4], M. J. Rodríguez-Yoldi[3]

[1]Departamento de Química Física, Universidad de Zaragoza, Spain, [2]Departamento de Bioquímica y Biología Molecular, Universidad de Zaragoza, Spain, [3]Departamento de Farmacología y Fisiología, Universidad de Zaragoza, Spain, [4]Instituto de Carboquímica ICB-CSIC, Spain

MOTIVATION

Carbon nanomaterials are promising as drug nanocarriers suitable for medical treatments, due to their large surface area and chemical stability that allows efficient loading of drugs via both covalent and non-covalent interactions. The enhanced permeability and retention (EPR) effect enables these nanomaterials to transport chemotherapeutic agents preferentially to tumor sites as compared to healthy tissues, thereby reducing toxic side effects. Much research activity has been devoted to perform experiments either by systemic administration and localized drug delivery strategies.

Camptothecin (CPT) is a potent anticancer agent with topoisomerase I inhibiting activity whose practical use in viable cancer therapeutic systems is greatly hampered due to its low solubility in biological media. The need to formulate water-soluble salts of CPT led to chemical modifications of the molecule with loss of anti-tumor activity. CPT is a more potent anticancer agent than other well-known anticancer drugs such as doxorubicin (DOX), so developing new drug delivery nanocarriers for CPT would be of high interest. In this work, the potential of carbon nanotubes (CNT), graphene oxide (GO), reduced graphene oxide (RGO) and carbon nanodiamonds (ND) as nanocarriers for CPT and DOX drug delivery were compared under the same experimental conditions. *In vitro* studies were performed on human epithelial colorectal adenocarcinoma (Caco-2) cells.

CARBON NANOMATERIALS

TEM

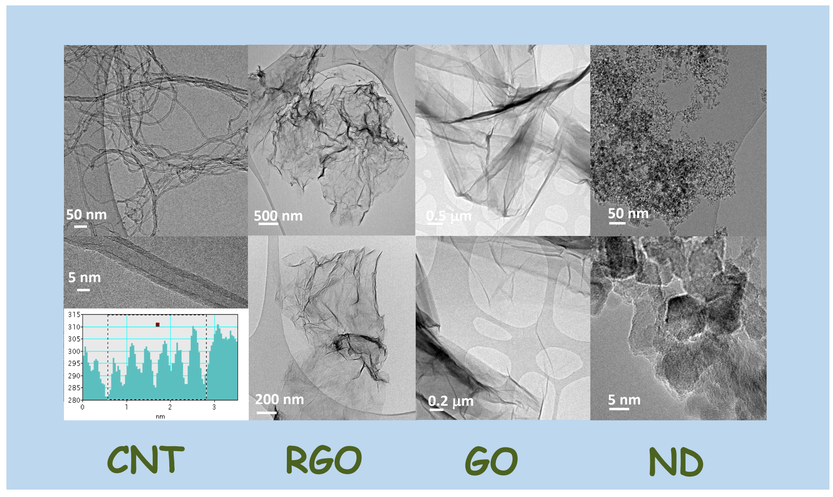


Figure 1. Transmission electron microscopy (TEM) characterization of the carbon nanomaterials tested here. The CNT used here are relatively short MWCNT (up to 1 micron in length) and ~10 nm in diameter, comprising around six concentric nanotubes. TEM micrographs of two-dimensional, graphene derivatives GO and RGO reveal that most flakes are up to 1 micron in length as well as their high exfoliation degree. Finally, Figure 1 shows aggregates comprising ND of about 5 nm in diameter.

XPS

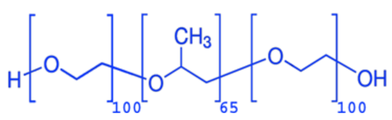
Table 1. Surface chemical analysis (at. %) of the carbon nanomaterials, obtained from XPS spectra.

At.%	CNT	RGO	GO	ND [1]
C	94.7	82.0	50.8	80.5
O	5.3	18.0	49.2	16.5

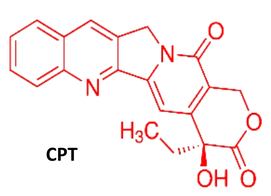
[1] For ND, at. % N is 3.0, calculated from the N1s peak in XPS spectra.

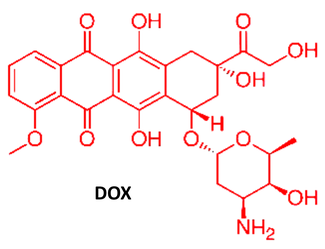
No significant transition metal contamination was observed in XPS spectra. XPS spectra of CNT and RGO are quite similar and correspond to C sp^2-based nanomaterials materials, with a low O:C ratio and, therefore, are highly hydrophobic. In contrast, GO has a significant high oxygen content (49.2 at.%), as it contains abundant oxygen-containing functional groups, which provide enhanced hydrophilicity. Finally, although O content in ND is not as high as in GO, ND are known to disperse easily in polar solvents, which is due to the hydrophilic functional groups on the outer shell.

Pluronic F-127 (15 µg·mL^{-1}) was used here to assist the dispersion of carbon nanomaterials in cell culture media.

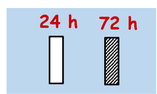


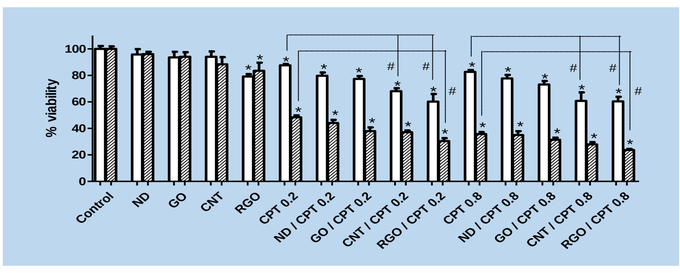
ANTICANCER DRUGS



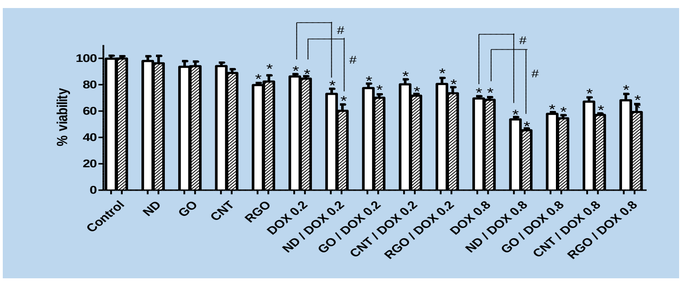


Cell viability assays





(a)



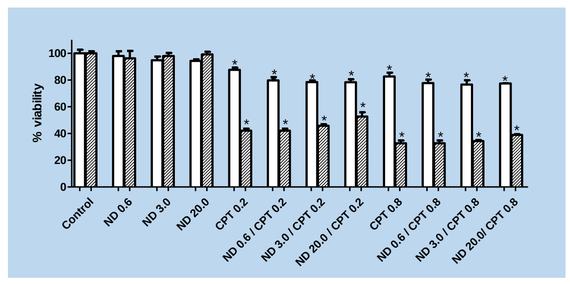
(b)

Figure 2. Cell viability assays after 24 h (white) and 72 h (striped) of incubation of Caco-2 cells with ND, GO, CNT and RGO at 0.6 µg·mL^{-1} concentration, free drug CPT (a) and DOX (b) at both 0.2 and 0.8 µg·mL^{-1} concentrations, and CPT- (a) and DOX- (b) loaded carbon nanomaterials. (* and # represent significance at $p<0.05$ when compared to untreated control cells and free drug-treated cells, respectively).

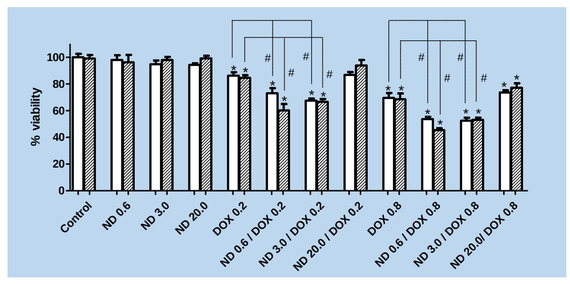
3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was performed for assessing cell metabolic activity. In brief, mitochondrial dehydrogenases of viable cells reduce the yellowish water-soluble MTT to water-insoluble formazan crystals, which are later resolubilized by replacement of the medium with DMSO, obtaining a purple colored solution.

The highest improvement in CPT anticancer activity compared to the free drug was observed for RGO and CNT nanocarriers, as shown in Figure 2. On the contrary, when it comes to DOX, ND showed the highest efficiency.

Figure 3. Cell viability assays after 24 h (white) and 72 h (striped) of incubation of Caco-2 cells with ND at different concentrations (0.6, 3.0 and 20.0 µg·mL^{-1}), and CPT (a) and DOX (b).



(a)

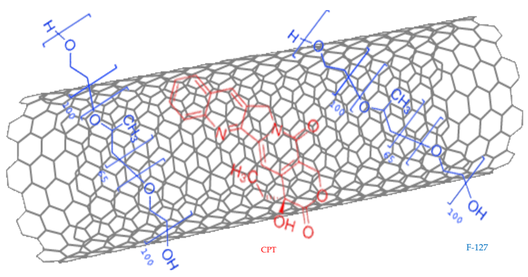


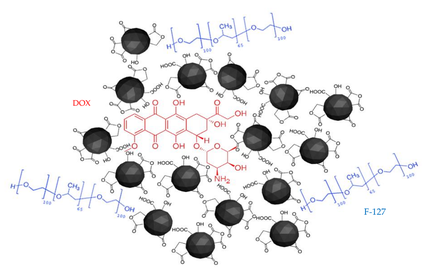
(b)

ND concentration was increased up to 20.0 µg·mL^{-1} , as shown in Figure 3. Results show that the efficiency was worse than those at 0.6 µg·mL^{-1}, both for CPT and DOX, probably due to ND aggregation forming higher size clusters, which offer less surface area for drug loading and more difficulty to enter the cells.

CONCLUSIONS

Due to their sp^2 carbon structure and inherent hydrophobic nature, RGO and CNT are capable of establishing π-π interactions with the aromatic rings of CPT, leading to high CPT loading efficiency. Thus, the highest improvement in CPT anticancer activity compared to the free drug was observed for RGO and CNT nanocarriers. In contrast, ND form clusters, wherein drugs can be loaded by interaction with their surface functional groups, and therefore ND will be much more efficient in loading hydrophilic drugs, such as DOX, which readily attach to their functional groups on their surface, rather than hydrophobic drugs, such as CPT.





CONTACT PERSON

Dr. Rosa Garriga
Email: rosa@unizar.es

Facultad de Ciencias Universidad Zaragoza

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