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Imide Derived Carbon Dots Exhibit Promising Antitumoral Properties on Multiple *In Vitro* Experimental Designs

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Abstract

Cancer is still a leading cause of death worldwide. Multiple and heterogeneous intrinsic molecular defects account for malignancy aggressiveness features. Distorted and inappropriate control of fundamental cell biology programs, such as cell survival, cell suicide, cell differentiation and cell tissular architectural integration stand at the core of tumor development. Such factors activate Epithelial-to-Mesenchymal Transition (EMT) that allows tumor cells to acquire different characteristics enabling them to invade and increase survival to different type of treatments. Nanomaterial-based technologies offer new promising approaches to disease diagnostics and therapeutics. We investigate the potential antitumoral properties of one of the newest classes of carbon based nanomaterials, Carbon Dots. Two types of previously physico-chemically characterized Carbon-Dots have been assessed using *in vitro* models. Cell proliferation, apoptosis and vimentin as a marker of EMT have been investigated in cell cult ures models: 2D, 3D, mono-culture or co-culture. Imide derived C-Dots affect cancer cell survival in a graded way, with minimal impact on normal cells, indicating promising antitumoral effects for cancer treatment. Moreover, the presence of the imide derived C-Dots down regulates the expression of EMT-associated marker Vimentin. Tested Carbon-Dots possess selective antitumoral properties which may be defined by precursor type.

Keywords: ***Anti-cancer agent; Carbon dots; EMT; Nanomedicine; In vitro evaluation***

Abbreviations: ***A375: Human malignant melanoma; A549 - Lung adenocarcinoma; C-Dots - NHF N-hydroxyphthalimide carbon dots; HT29 - Corectal adenocarcinoma; HMLE - human mammary epithelial cells; HUVEC - Human umbilical vein endothelial cells; L363 - Human Myeloma; MDA-MB 231 - human breast adenocarcinoma; NHF - N-Hydroxyphthalimide; NHS - N-Hydroxysuccinimide; Pa-vSMC - Pulmonary artery smooth muscle cells; RPMI8226 - Plasmocytoma, Myeloma; U87 - Glioblastoma astrocytoma-human brain; 4T1 - Mouse breast cancer***

Introduction

Carbon Dots (C-Dots) are a new class of nanostructured materials, which mainly consists of a carbonaceous core and surface located in various functional groups. As a result of their particular structure, C-dots present several remarkable features like

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excitation dependent tunable photoluminescence, facile preparation starting from a wide range of potential precursors, low toxicity, dispersibility in different solvents. Due to their unique features, they were thoroughly considered for applications ranging from optoelectronics and sensors to the biomedical field in recent 5 years [1].

Very recent studies report the use of C-Dots as efficient agents for drug delivery and antitumoral activity [2, 3]. The present paper assesses the potential antitumoral properties of two C-Dots types synthesized from imide precursors. One C-Dot type is prepared through thermal decomposition of N-hydroxyphthalimide (NHF) precursor in a controlled partial pyrolytic process as described in a previous paper [4]. The second type of C-Dots was prepared from N-hydroxysuccinimide (NHS) according to a previously described method [5] and tested in parallel with the first type. Tested C-Dots have been previously physic-chemically characterized [4,5] and the batches of C-Dots prepared for this study were morpho-structurally validated by FT-IR, TEM, and DLS techniques. The NHF precursor has been proved to have antitumoral activity [6] while NHS [7], routinely used as reagent for peptide synthesis has no reports in this respect.

Metastasis, meaning cancer cells that escape from the primary tumor, disseminate and engraf in distant organs, are responsible for 90% of cancer-related deaths. The Epithelial-to-Mesenchymal Transition is a complex molecular program where non-motile, polarized epithelial cells degrade their cell-cell junctions and convert to mesenchymal-like types, which facilitate cell invasion and metastasis, resistance to chemotherapy, resistance to radiation-induced DNA damage and increased cell survival [8-11]. Defects in programmed cell death pathways including apoptosis play important roles in tumor pathogenesis, allowing neoplastic cells to survive. Apoptosis is a regulatory system for balancing the homeostasis situation during the growth, development, and differentiation among multi-cellular organisms. Caspases are a family of cysteine proteases that play key roles in programmed cell death and are the essential elements in the apoptosis process. It is known that apoptosis defects may allow epithelial cells to survive without attachment to extracellular matrix that later facilitates metastasis [12-15].

The effect of C-Dots prepared through pyrolysis of N-hydroxyphthalimide or N-hydroxysuccinimide was investigated in different cellular microenvironments with notable antitumoral effects. Assessment of the antitumoral activity included sequential evaluation of the proliferation, survival, invasiveness and tissue scale interactions of several representative malignant cell lines. As expected, C-Dots prepared from NHF showed antitumoral activity, but surprisingly, the C-Dots prepared from NHS also displayed antitumoral activity on certain cell lines, with minimal toxic effects on normal cells. The fact that the tested C-Dots do not overlap entirely their antitumoral properties indicates that developing new C-Dots types may lead to the identification of new active molecules suitable for cancer treatment.

Materials and Methods

N-Hydroxyphthalimide (NHF) (97%) and N-hydroxysuccinimide (NHS) (98%) were purchased from Sigma-Aldrich. High purity Milli-Q water was used for C-Dots synthesis. Both types of C-Dots used in this study were prepared through pyrolytic processing of N-Hydroxysuccinimide (NHS) and N-Hydroxyphthalimide (NHF), respectively according to the experimental path described in previous papers [4,5]. In each case, the resulted aqueous dispersion was centrifuged twice at 15000 rpm for 10 min. The resulted batches were morpho-structurally investigated using FT-IR, TEM, and DLS analysis, the results being in close agreement with the reported studies, as detailed in the Supplemental Information Section. MDA-MB-231, 4T1,

(American Type Culture Collection, Rockville, MD ATCC) were cultured as described by Gjerdrum et al. [16]. HMLE (ATCC) (a gift from Dr. J. Lorens) were maintained in MEBM media (Lonza) supplemented with MEGM bullet-kit (Lonza). A549 (ATCC) were cultured in F-12K Medium, supplemented with 100 U/mL of penicillin and 100 µg/mL of streptomycin and 5% bovine serum (Sigma-Aldrich). A375 (ATCC) were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 100 U/mL of penicillin and 100 µg/mL of streptomycin and 15% bovine serum (Sigma-Aldrich). RPMI 8226 and L363 (ATCC) were cultured in RPMI-1640 supplemented with100 U/mL of penicillin and 100 µg/mL of streptomycin and 10% bovine serum (Sigma-Aldrich). HT-29 (ATCC) were cultured in McCoy's 5a Medium supplemented with100 U/mL of penicillin and 100 µg/mL of streptomycin and 10% bovine serum (Sigma-Aldrich). U87 (ATCC) was cultured in Eagle's Minimum Essential Medium supplemented with 100 U/mL of penicillin and 100 µg/mL of streptomycin and 10% bovine serum (Sigma-Aldrich). Human umbilical vein endothelial cells (HUVEC) and pulmonary artery smooth muscle cells (Pa-vSMC), were purchased from Lonza (C2517A, CC2581). Mouse anti-human Vimentin (DAKO, Clone V9, M0725), Cell Titer 96® Non-Radioactive Cell Proliferation Assay (MTT) (Promega) and Cell Titer-Blue® Cell Viability Assay (Promega), Matrigel (Cornning), Caspase 3 (ab39401), Caspase3/7 (Apo-ONE Homogenous Caspase-3/7 Assay, G7790, Promega) have been used according to manufacturer's instructions.

Characterization methods/sample preparation

The FT-IR spectra were acquired in the 400-4000 cm^{-1} range with a Shimadzu IRAffinity 1S Spectrometer **(Supplementary FIG. 1)**. Aqueous dispersions of NHS and NHF Carbon Dots were freeze-dried, and the resulted powders were analyzed according to the KBr method. Dimensional analysis and size distribution (DLS) were carried out on a Beckman-Coulter Delsa Nano **(Supplementary FIG. 2).** Prior to investigation, the aqueous dispersion was additionally centrifuged for 10 min. at 15000 rpm. TEM micrographs **(Supplementary FIG. 3)** were recorded on a Zeiss Libra 200MC UHR-TEM working at 200 KV acceleration voltage. The samples were deposited on carbon grids from diluted Carbon Dots dispersion in isopropanol.

Cell proliferation and apoptosis activity

For cell viability, CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) (Promega) and CellTiter-Blue® Cell Viability Assay (Promega) was used. The tetrazolium dye is converted into an insoluble formazan by the action of nicotinamide adenine dinucleotide hydrogenase present in metabolically active cells. This cellular conversion into a purple-colored formazan is directly proportional to the number of viable cells. Cells were seeded into a 96-well flat-bottom tissue culture plate at a density of 2000 cells/well and allowed to adhere to the plate by incubating at 37°C under 5% CO_2 overnight. Following overnight cell attachment, the cells were incubated with the tested C-Dots at concentrations ranging between 1%-10% for 72 h. Control cells were treated with similar DMSO concentrations as equivalent to the amount of DMSO used in C-Dots suspension. After each 72 h treatment time period, 20 µL of 5 mg/mL MTT solution was added to each well and the plate was re-incubated for 4 h to facilitate catalysis by mitochondrial dehydrogenases. Next, 100 µL DMSO was added to each well to solubilize the formazan crystals. The absorbance of the resultant solutions was determined at 590 nm using multi-plate reader (FilterMax F5, Sunnyvale, CA, USA).

For apoptosis, assay cells were incubated for 72h with C-Dots and then subjected to Caspase 3 (Abcam) and measure absorbance using multiplate reader, or Caspase 3/7 assay (Promega) and the fluorescence was recorded via fluorescence microscopy using a TissueGnostic rig (Vienna, Austria) containing Axio Observer.Z1 Microscope.

3D matrigel assay

For 3D matrigel assays, 1000 cells were seeded in Ibidi plates, between 2 layers of Matrigel (Matrigel Matrix, Growth Factor Reduced-BD Biosciences). 12 h post-seeding 3D embedded cells were treated with 5% NHF or NHS C-Dots for 72 h and then the treatment was removed and cultured 14 days before microscopy analysis (Zeiss AxioObserver Z1 microscope; TissueGnostic rig).

Co-culture assay

Tumor cells were seeded together with Pa-vSMC and HUVEC, centrifuged briefly at 200 g to achieve an even distribution of cells and cultured in EGM-2 for 72 hrs, to allow network formation before treatment. The culture medium was changed every second day. Cell numbers and culture volume were as follows (per well): 96-well plates: 5 × 104 Pa-vSMC, 10 × 103 HUVEC, 10 × 103 tumor cells in 200 µl EGM-2. Brightfield pictures were acquired using Axio Observer.Z1 Microscope from TissueGnostic rig using the TissueFAXS 4.2 software.

Immunofluorescence (IF)

Immunofluorescence is an antigen-antibody reaction where the antibodies are labeled with a fluorescent dye and the antigen-antibody complex is visualized using fluorescent microscopy. The samples were fixed in 4% paraformaldehyde for 30 min, blocked with PBS, 5% normal goat serum, permeabilized with 0.1% Triton X-100 and incubated with anti-human Vimentin (DAKO, Clone V9, M0725) for 72 h at 4°C, then visualized with fluorescent secondary antibodies (Invitrogen) incubated overnight. Immunofluorescence analysis: Vimentin (green); nucleus: DAPI, (blue).

Statistical analysis

Graphpad Prism was used for statistical analysis with a t-test for multiple comparisons. Significance was established when $p<0.05$.

Results

In order to assess the role of NHS and NHF C-Dots in cancer progression, the ability of these nanostructures in affecting the viability in different cell types was investigated **(FIG. 1)**. Our previous experiments indicated that a 5% concentration (5%, 50 µg/ml) for 72 hours exposure provided the optimal antitumoral activity with minimal impact on normal cells. Overall, the experimental data showed that C-Dots induce different biological effects in various types of cells.

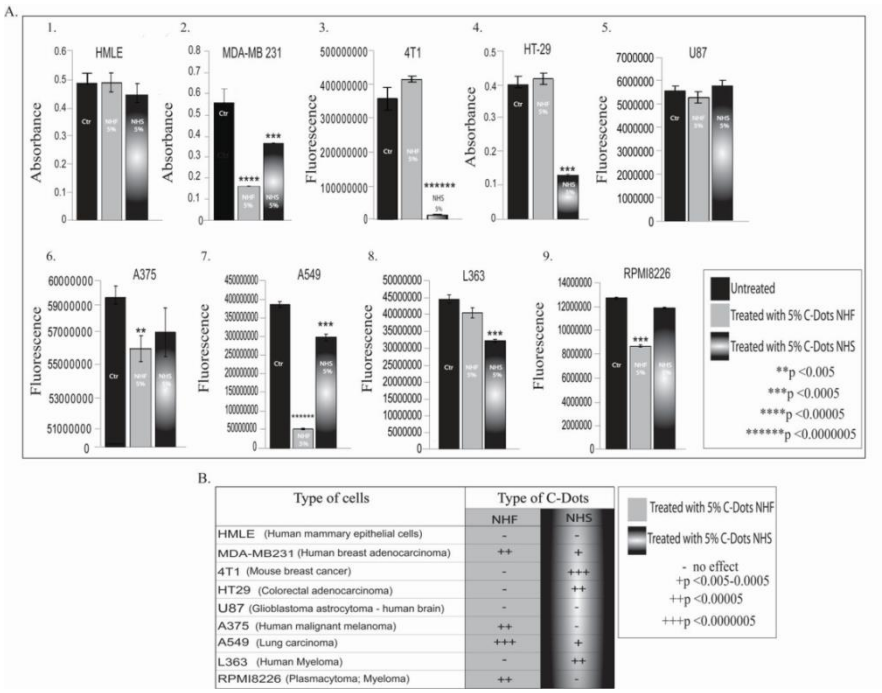

FIG. 1. **Effects of C-Dots from NHF and NHS in different cell lines. A. Viability level in different type of cells treated with C-Dots (NHF and NHS). 1; HMLE (Normal human mammary epithelial cells); 2: MDA-MB 231 (Human breast adenocarcinoma); 3: 4T1 (Mouse breast cancer); 4: HT29 (Colorectal adenocarcinoma); 5: U87 (Glioblastoma astrocytoma-human brain); 6: A375 (Human malignant melanoma); 7: A549 (Lung adenocarcinoma); 8: L363 (Human Myeloma); 9: RPMI8226 (Plasmocytoma, Myeloma); B: Scorization of significance with +++, ++, + and - represents: +++p<0.0000005, ++p<0.00005, +p<0.005-0.0005 and-no effect referenced to control cultures of native cells.**

The presence of C-Dots amputates cancer cell culture viability of certain tumor lines (like A549), but might only marginally affect other cells (like U87), this being an indicator for an important and discriminating biochemical impact in cancer pathogenesis, whereas the normal cells like mammary epithelial cells (HMLE) were not significantly affected by NHF and NHS C-Dots treatment at the same doses (**FIG. 1**).

Therefore, the effect of NHF and NHS derived C-Dots in apoptosis events was evaluated (**FIG. 2a-2c**). In normal breast epithelial cells (HMLE-**FIG. 2A**), no difference was observed between untreated and treated cells, while in malignant cells such as MDA-MB 231 the number of apoptotic events increased significantly (**FIG. 2b,2c)**.

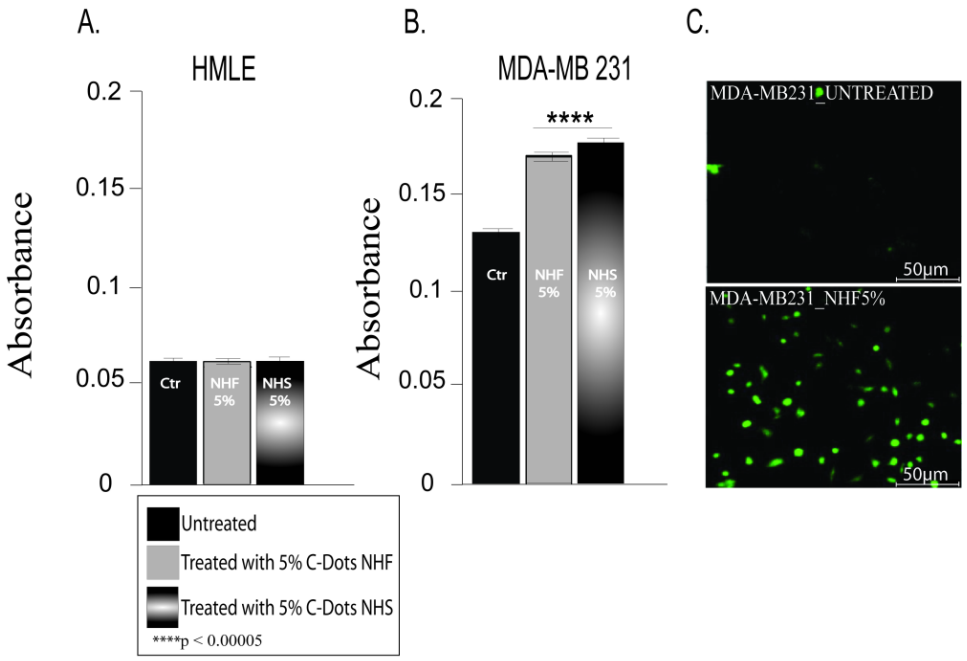

FIG. 2. **Apoptosis measurement on cells treated with 5% C-Dots (NHF and NHS). Caspase 3 measurement on A: Human mammary epithelial cells (HMLE); B: Human breast adenocarcinoma (MDA-MB231) treated. C: Apoptosis (Caspase 3/7) evidence by immunofluorescence microscopy in MDA-MB231 untreated (upper C) and treated with 5% NHF (lower C). Magnification 10x.**

Clinical evidence suggests that the regulators of EMT in cancer correlate with poor patient outcome [17-19]. Among the factors involved in EMT, Vimentin over-expression was found in several types of cancer [20] and represents the mesenchymal marker most commonly associated to EMT [21]. Therefore, the influence of NHF C-Dots on the expression of Vimentin in breast cancer was investigated, being analyzed the MDA-MB231 cells non-treated and treated with C-Dots 5%. The results suggest that the expression of Vimentin associated EMT program is reduced upon 5% NHF C-Dots in breast adenocarcinoma cell line (MDA-MB 231) **(FIG. 3B).**

To create a better similarity with the living organism in *in vitro* systems we used a standard 3D culture model which creates significant different microenvironmental niches [22,23] - i.e. spheroid's edge with nutrients supply versus spheroid core were hypoxia and starvation occurs. These microenvironmental niches affect cellular characteristics and behavior [24,25]. The effects of C-Dots NHS **(FIG. 4)** on HT-29 (colorectal cancer), 4T1 (mouse mammary breast cancer) and C-Dots NHF **(FIG. 5)** on HMLE (normal human mammary epithelial), MDA-MDB231 (human breast adenocarcinoma) and A375 (human melanoma), respectively, were evaluated in a 3D model.

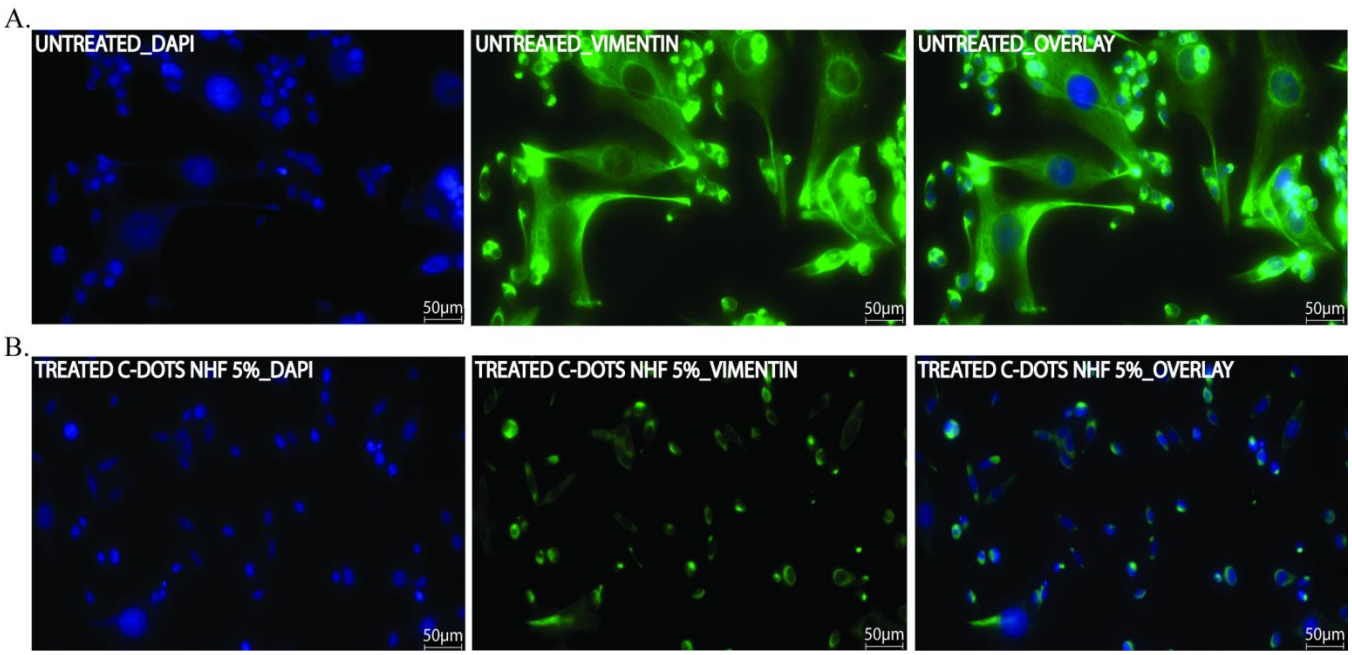

FIG. 3. **Effect of C-Dots NHF 5% on EMT associated genes. A. Vimetin expression in MDA-MB231 cells untreated cells. B. Vimetin expression in MDA-MB231 cells treated with C-Dots NHF 5%. Magnification 20x.**

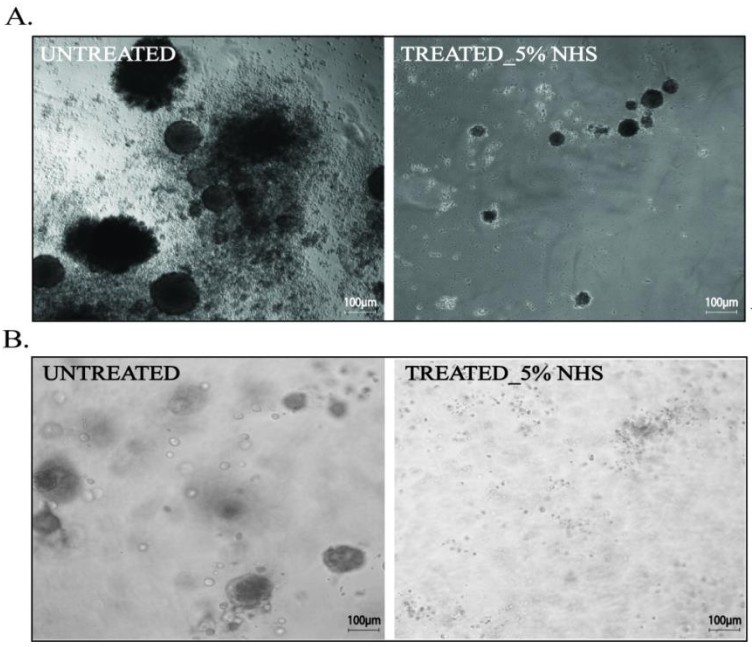

FIG. 4. **Effect of NHS C-Dots in 3D matrigel assay. A: Effect of C-Dots NHS in HT29 cells (colorectal adenocarcinoma); B: Effect of C-Dots NHS in 4T1 cells (mouse mammary breast cancer). Magnification 5X, N=3 matrigels/experiment.**

Normal breast epithelial cells became quiescent and self-organized into polarized, spheroidal acinar structures in 3D-Matrigel matrices **(FIG. 5-a,b)** and were not affected under C-Dots treatments **(FIG. 5b-a,b).** The malignant cells used in this set of experiments continue to proliferate, forming large disorganized colonies with invasive cell projections that reflect aggressive

tumors **(FIG. 5a-c,d,e,f).** Upon C-Dots treatments, the number of spheroids and their projection were significantly reduced **(FIG. 5b-c,d,e,f).**

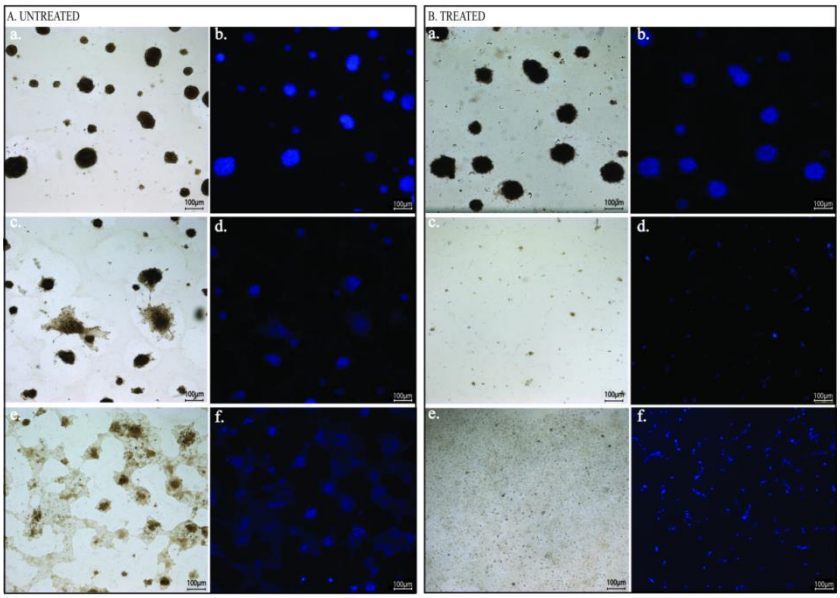

FIG. 5. **The effect of C-Dots NHF in 3D matrigel assay. A. The morphological aspect of untreated 3D culture, a-b: HMLE (Human Mammary Epithelial Cells); c-d: MDA-MB 231 (Human Breast Adenocarcinoma Cells); e-f A375 (Human malignant melanoma); B. Morphological aspect of treated 3D culture, a-b: HMLE (Human Mammary Epithelial Cells); c-d: MDA-MB 231 (Human Breast Adenocarcinoma Cells); e-f: A375 (Human malignant melanoma); Magnification 5x, N=3-3D matrigel/experiment.**

In tumors, the cancer cell interacts with other cell types like lymphocytes, fibroblasts, endothelial cell, macrophages etc. and form tumor microenvironments that influence tumor progression. As a consequence, the effect of C-Dots in co-culture conditions in both 2D **(FIG. 6)** and 3D **(FIG. 7)** system was investigated. co-culture allowed the evaluation of C-Dots's impact at the same time on both tested normal and cancer cells while they interact with each-other.

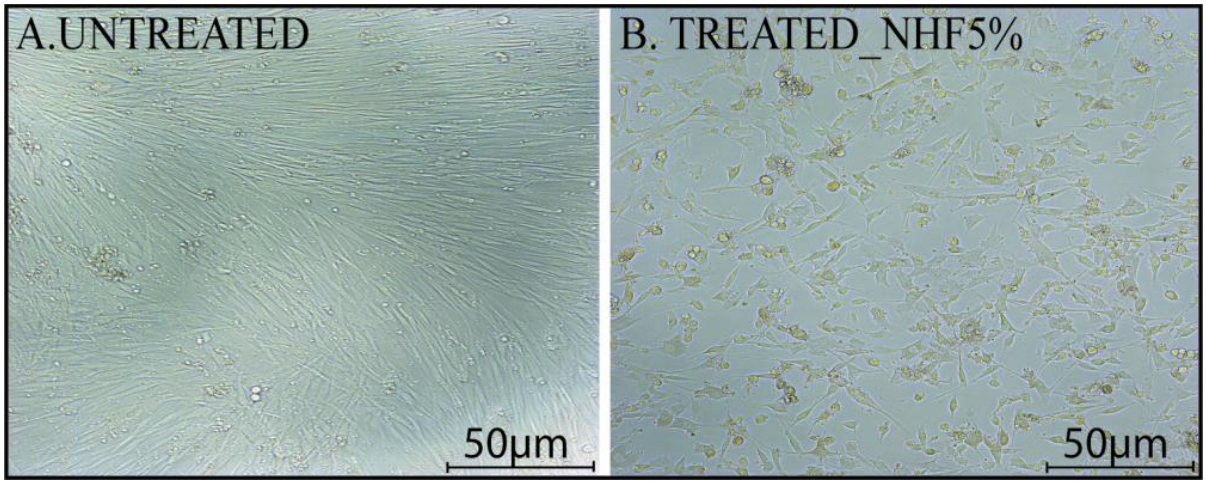

FIG. 6. **Morphological aspect of 2D co-culture (mammary tumor cells+endothelial cells+Pa-vSMC) A: Untreated culture; B: Treated with 5% NHF, Magnification 10x, N=3 wells/experiment.**

In 2D co-cultures, a disruption **(FIG. 6B)** of the fibroblastic basal layer of cells that act as a feeder layer **(Figure 6A)** for aggregates of endothelial cells and EMT prone cancer cells was noticed, occurrence of multiple cell debris (B) and less motility **(FIG. B).**

In a 3D co-culture system **(FIG. 7)** under NHF C-Dots treatment, the spheroids presented a net unfavorable structure, with obviously less and shorter expansion branches in both very aggressive cancer types.

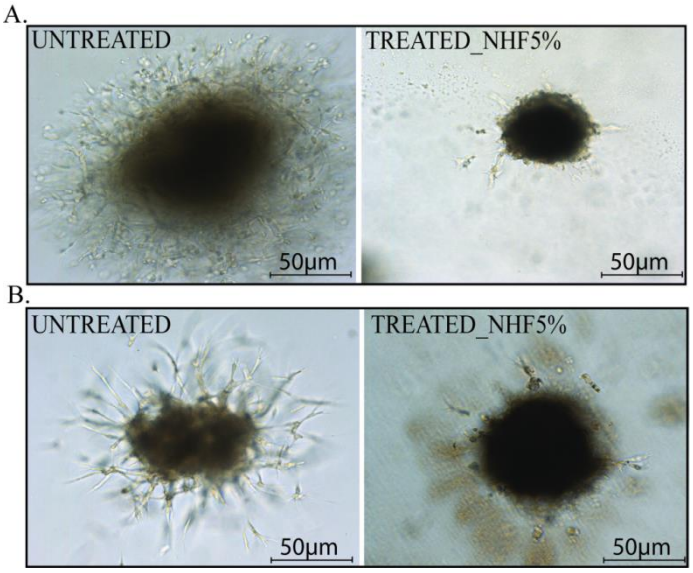

FIG. 7. **Morphological aspect of 3D co-culture (tumor cells+endothelial cells+Pa-vSMC) A: MDA-MB 231 (Human breast adenocarcinoma cell line); B: A375 (Human malignant melanoma), Magnification 10x, N=3D matrigel/experiment.**

These data suggest that C-Dots are affecting mesenchymal-like invasiveness of different metastatic cell lines and distort microenvironmental type interactions associated with tumor progression in tissular niches.

Discussion

Recent years showed an increased interest in the potential antitumoral effect of C-Dots prepared from various precursors [26]. In this line, the current results reveal that NHF and NHS-derived C-Dots have different biological effects in various types of cancer cells **(FIG. 1)**. The chemical structure of the precursor used to prepare the C-Dots influences the biological effects on different cell types. Some cancer cell lines, e.g. MDA-MDB231 and A549, are significantly affected by treatments with both C-Dots types, while other lines are affected by either NHF C-Dots (A375, RPMI8226) or NHS C-Dots (HT29, 4T1, L363). Using the same C-Dots concentration, neither type affects U87. Since the various cancer cell lines tested rely on both common and distinctive biochemical distortions determining their malignant properties, it is suggested that the heterogeneous anti-cancer effects of C-Dots are based on the selective biochemical interactions that these structures intercept. Meanwhile, at the optimal used dosage (5%, 50µg/ml) no significant effects of C-Dots on normal cell types were observed. Cancer development is ultimately the result of proliferation and cell death. The next set of experimental settings will investigate if

tested imide C-Dots may influence apoptosis (a major form of programmed cell death) in addition to proliferation by assessing caspase3/7. The current results (**FIG. 2B**) prove that NHF and NHS derived C-Dots treatment increases the expression of the apoptotic marker caspase3/7 in tested neoplasic cell line, but they show no effect on its expression in the normal cell line.

Reversible transition from epithelial to mesenchymal phenotype called EMT is a complex process involved in normal development, wound healing, tumor progression and cancer cell dissemination. The presented results **(FIG. 3)** show that the expression of Vimentin associated EMT program is reduced on 5% NHF derived C-Dots. One also has to mention that the morphology of mesenchimal cell-like (MDA-MB231) has been changed from an elongated shape to a spherical one **(FIG. 3B)**.

The 3D cultures data **(FIG. 4, 5 and 7)** further support the antitumoral effects of the investigated Carbon Dots. The spheroid tumors developed under this treatment are considerably lagged in their growth, with striking less cellular protrusions and less invasive expansions in the extracellular matrix. Moreover, co-culture experiments suggest that the Carbon Dots impact on cancer development might also include targets in the stromal cell compartments of the growing tumors. Recently, we have shown that the same NHF C-Dots tested while entrapped in gels as a method of delivery to prevent aggregation significantly reduced mitochondrial activity of two breast cancer cell lines [27].

Conclusion

Tested C-Dots derived from N-hydroxyphthalimide and N-hydroxysuccinimide are biochemically active by intervening in essential cellular processes such as survival, apoptosis, tissular growth. However, these nanostructures have different effects on the 2D vs 3D growth pattern of certain tumor cell lines. The chemical distinctiveness between the two precursors of the carbon dots are possible causes of variable biological effects. The dissimilar number of carbon atoms of the two precursors induce peculiar pyrolysis dot core with different densities of imidic residues. Thus, in addition, the carbon core and the imidic residues create a nanocarrier platform that could be coupled with various therapeutic active molecules. All these results suggest the potential therapeutic effect of C-Dots as a promising adjuvant in cancer treatment.

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REFERENCES

1. Li H, Kang ZH, Liu Y, et al. Carbon nanodots: synthesis, properties and applications. J Mater Chem. 2012;22(26):24230-53.
2. Tuerhong M, XU Y, YIN XB. Review on carbon dots and their applications. Chinese J Anal Chem. 2017;45(1):139-50.
3. Molaei MJ. Carbon quantum dots and their biomedical and therapeutic applications: a review. RSC Adv. 2019;9:6460-81.
4. Stan CS, Horlescu P, Ursu LE, et al. Facile preparation of highly luminescent composites by polymer embedding of carbon dots derived from N-hydroxyphthalimide. J Mater Sci. 2017;41:185-96.
5. Stan CS, Albu C, Coroaba A, et al. One step synthesis of fluorescent Carbon Dots through pyrolysis of N-hydroxysuccinimide. J Mater Chem. 2015;3:789-95.
6. Wang M, Zhou A, An T, et al. N-Hydroxyphthalimide exhibits antitumor activity by suppressing mTOR signaling pathway in BT-20 and LoVo cells. J Exp Clin Cancer Res. 2016;35:41.
7. https://pubchem.ncbi.nlm.nih.gov/compound/N-Hydroxysuccinimide#section=GHS-Classification
8. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7(2):131-42.
9. Wang Y, Shang Y. Epigenetic control of epithelial-to-mesenchymal transition and cancer metastasis. Exp Cell Res. 2013;319(2):160-9.
10. Buck E, Eyzaguirre A, Barr S, et al. Loss of homotypic cell adhesion by epithelial-mesenchymal transition or mutation limits sensitivity to epidermal growth factor receptor inhibition. Mol Cancer Ther. 2007;6(2):532-41.
11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
12. Frisch SM, ScreatonRA. Anoikis mechanisms. Curr Opin Cell Biol. 2001;13(5):555-62.
13. Dawar DS, Kumar S, Shalini S, et al. Old, new and emerging functions of caspases. Cell Death Differ. 2015;22(4):526-39.
14. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol. 2008;9(3):231-41.
15. Rodrigue-Gervais IG, Saleh M. Caspases and immunity in a deadly grip. Trends Immunol. 2013;34(2):41-9.
16. Gjerdrum C, Tiron CE, Høiby T, et al. Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival. PNAS. 2010;107(3):1124-9.
17. Tadokoro A, Kanaji N, Liu D, et al. Vimentin regulates invasiveness and is a poor prognostic marker in non-small cell lung cancer. Anticancer Res. 2016;36(4):1545-52.
18. Tanaka K, Tokunaga E, Inoue Y, et al. Impact of expression of vimentin and axl in breast cancer. Clin Breast Cancer. 2016;16(6):520-6.
19. Vuoriluoto K, Haugen H, Kiviluoto S, et al. Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. Oncogene. 2011;30(12):1436-48.
20. Satelli A, Li S. Vimentin as a potential molecular target in cancer therapy Or Vimentin, an overview and its potential as a molecular target for cancer therapy. Cell Mol Life Sci. 2011;68(18):3033-46.
21. Kokkinos M, Wafai R, Wong MK, et al. Vimentin and epithelial-mesenchymal transition in human breast cancer-observations *in vitro* and in vivo. Cells Tissues Organs. 2007;185(1-3):191-203.
22. Brown Y, Hua S, Tanwar PS. Extracellular matrix-mediated regulation of cancer stem cells and chemoresistance. Int J Biochem Cell Biol. 2019;109:90-104.

23. Socovich AM, Naba A. The cancer matrisome: From comprehensive characterization to biomarker discovery. Semin Cell Dev Biol. 2019;89:157-66.
24. Pérez-Velázquez J, Gevertz JL, Karolak A, et al. Microenvironmental niches and sanctuaries: a route to acquired resistance. Adv Exp Med Biol. 2016;936:149-64.
25. Wang X, Mooradian AD, Erdmann-Gilmore P, et al. Breast tumors educate stromal tissue with individualized but coordinated proteomic signatures. Sci Signal. 2017;10(491):8065.
26. Vasimalai N, Vilas-Boas V, Gallo J, et al. Green synthesis of fluorescent carbon dots from spices for *in vitro* imaging and tumor cell growth inhibition. Beilstein J Nanotechnol. 2018;9:530-44.
27. Savin C-L, Tiron CE, Carasevici E, et al. Entrapment of N-hydroxyphthalimide carbon dots in different topical gel formulations: new composites with anticancer activity. Pharmaceutics. 2019;11(7):303.