ACTIVATABLE MR PRODRUG FOR TARGATED DELIVERY AND TREATMENT OF CANCER

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ABSTRACT

In this study, a new multimodal theranostic tool is reported utilizing nanoceria delivery system conjugated with the ICAM1 antibody and a magnetic resonance (MR) probe as a prodrug with both MR and cytotoxic properties. The prodrug was synthesized from doxorubicin and phenyl-amine modified DTPA chelated with gadolinium utilizing dithiobis(succinimidyl propionate) (DSP) as a crosslinker. Nanoceria was synthesized from cerium oxide and polyacrylic acid using a water-based alkali precipitation technique. Doxorubicin and the synthesized prodrug were encapsulated separately within the nanoceria polymer matrix using a solvent diffusion method. The drug/prodrug-encapsulated nanoceria's carboxylated surface was functionalized with the ICAM1 antibody utilizing EDC/NHS chemistries and the resulting formulations were purified and characterized by DLS, zeta potential, UV/Vis, and MR. The efficacy of this platform was measured by treating MDA-MB-231 breast cancer (TNBC) cells and MCF-7 cells with the drug/prodrug-loaded, ICAM1-conjugated nanoceria and analyzing the results of the treatment. Results were evaluated by cytotoxicity assays (MTT), fluorescence microscopy, reactive oxygen species determination, and comet assays. In all, the results show the nanoceria platform is target-specific to TNBC, and the encapsulated prodrug is able to be activated releasing doxorubicin and initiating apoptosis in an in vivo breast cancer model.

INTRODUCTION

The need for new, multimodal therapies is of huge diagnostic and therapeutic importance to the field of oncology. When a patient is diagnosed with cancer, the identification and time to treatment has a significant impact on prognosis due to the aggressiveness and treatability of some cancers. Specifically, triple negative breast cancer (TNBC) poses a unique problem due to its aggressiveness. In addition, the lack of hormone epidermal growth factor receptor 2 (HER-2), estrogen receptors, and progesterone receptors make it more difficult to treat since common hormone therapies and drugs that target HER-2, estrogen, and progesterone are not effective. At this time, radiotherapy, chemotherapy, and high intensity focused ultrasound have been used extensively, successfully suppressing tumors and extending the lives of patients. However, a single method of treatment is often not able to completely eliminate cancer or prevent metastasis due to multidrug resistance in long-term therapies. In the interest of developing a multimodal therapy for cancer treatment, we continued to investigate the capabilities of polyacrylic acid (PAA)-coated nanoceria (PNC) as a multimodal theranostic tool for the detection and treatment of triple negative breast cancer (TNBC). We synthesized PNC nanoparticles and functionalized their surfaces with the ICAM1 antibody to specifically target MDA-MB-231 (TNBC) cells. To introduce multimodality, we synthesized a prodrug with both chemotherapeutic and magnetic resonance (MR) properties as shown in Figure 1. We coupled the chemotherapeutic drug doxorubicin with a phenyl-amine modified diethylenetriamine pentaacetate (DTPA) chelated with the T1 MR contrast agent gadolinium using dithiobis(succinimidyl propionate) (DSP) as the cross-linker. Using doxorubicin and gadolinium as a combination allows for simultaneous chemotherapy, MR imaging, and tumor tracking; and when coupled with targeted delivery it provides a valuable multimodal theranostic tool to diagnose and treat TNBC. In addition, this methodology can be applied to other cancer cells by changing the targeting ligand bound to the PNC nanoparticles. In all, these traits make our PNC nanoparticle-delivered prodrug a novel platform for a multimodal approach to monitor and treat cancer.

Synthesis of Activatable MR Prodrug and Scheme for the mechanism of activation of Prodrug

Mechanism of action of the proposed nanomedicine

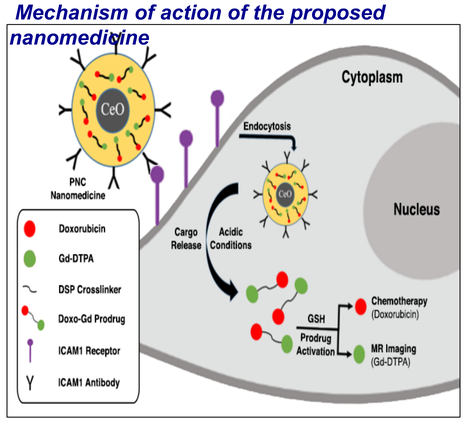
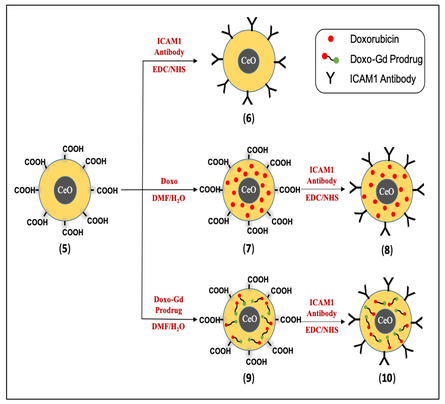
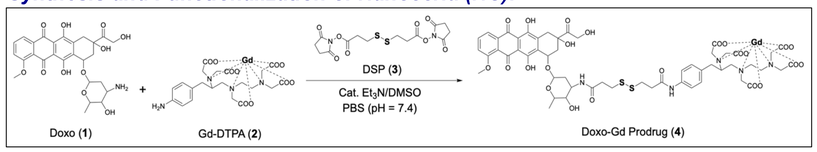


Figure 1: **(A)** The Schematic diagram for the proposed mechanism of nanomedicine formulated from ICAM1 functionalized nanoceria using a doxorubicin-gadolinium complex as a prodrug to treat TNBC.



Scheme 2: Stepwise synthesis of ICAM1-functionalized (6), drug (7)/prodrug (9)-loaded, and ICAM1-functionalized drug (8)/prodrug (10)-loaded Nanoceria.

Synthesis and Functionalization of Nanoceria (NC):



Scheme 1: Synthesis of activatable Doxo-Gd prodrug.

Characterization of the activatable probe:

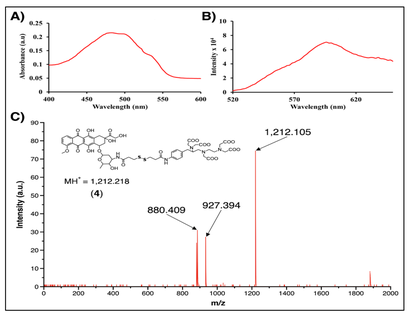


Figure 2: (A) Absorbance, (B) florescence, and (C) MALDI-TOF chromatogram showing molecular weight of our Doxo-Gd activatable prodrug (4)

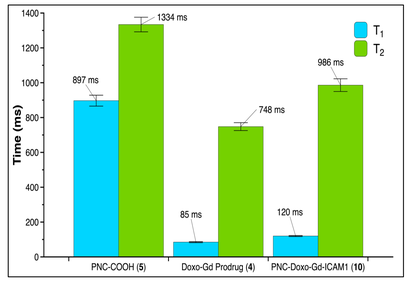


Figure S2: Comparative study of average magnetic resonance data over three measurements showing T1 and T2 values of PNC-COOH (5), the synthesized Doxo-Gd prodrug (4), and the ICAM1 conjugated PNC with our Doxo-Gd prodrug encapsulated (10).

Characterization of activatable NC:

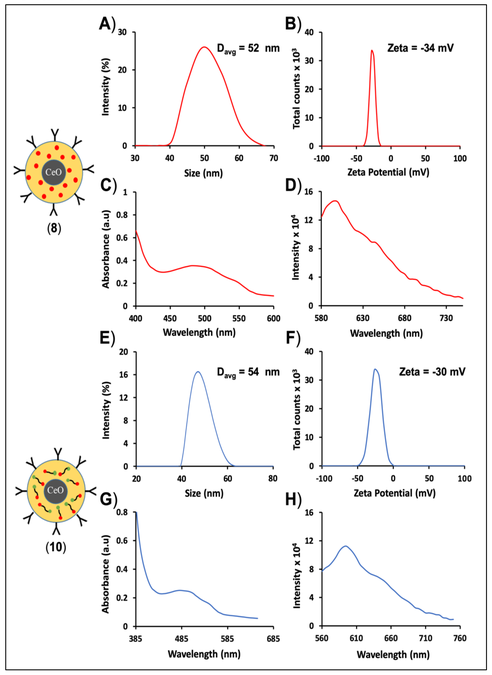


Figure 3: (A – D) Characterization studies of doxorubicin-loaded, ICAM1-functionalized Nanoceria (8) where (A), (B), (C), (D) represents size, zeta potential, UV absorbance and fluorescence, respectively. (E-H) Characterization studies of prodrug-loaded, ICAM1-functionalized Nanoceria (10) following the same order.

In-vitro Cell viability (MTT assay):

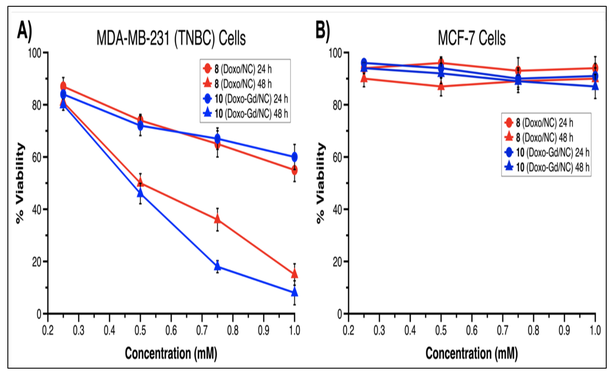


Figure 4: Cytotoxicity assay showing the effectiveness of our Nanoceria-based drug delivery system against the MDA-MB-231 cell line (TNBC) (A) and the MCF-7 breast cancer cell line (B). Experiments were done in triplicate and calculated against standard error.

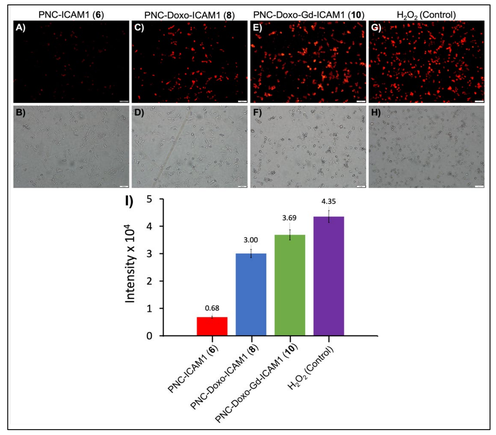


Figure 7: Determination (A – H) and quantification (I) of cytoplasmic ROS generation in TNBC cells (scale bar 500 μm). (A – B) Generation of ROS after treatment with PNC-ICAM1 (6), (C – D) generation of ROS after treatment with PNC-Doxo-ICAM1 (8), (E – F) generation of ROS after treatment with PNC-Doxo-Gd-ICAM1 (10), and (G – H) generation of ROS after treatment with H2O2, all labeled with DHE dye. (I) Each trial was performed in triplicate and the level of ROS generation was quantified directly from the corresponding fluorescence images using ImageJ software.

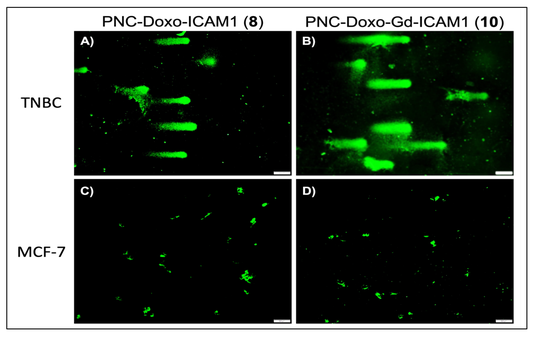


Figure 8: Comet assays performed on TNBC (A – B) and MCF-7 (C – D) cell lines after incubation with PNC formulations 8 (A and C), and 10 (B and D).

Cell Internalization studies:

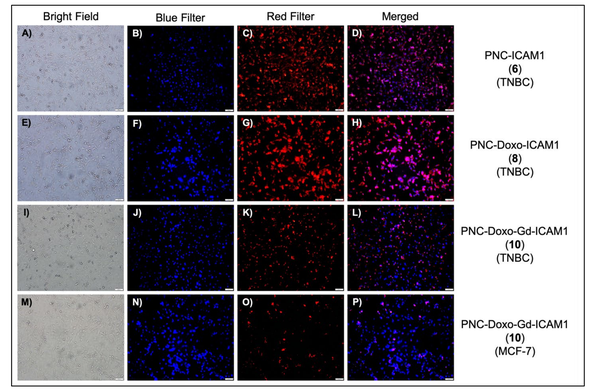


Figure 5: (A – D) Internalization of PNC-ICAM1 (6) in TNBC cells was observed after 24 h, due to ICAM1-receptor-mediated endocytosis (scale bar 500 μm). (E – H) Internalization of PNC-Doxo-ICAM1 (8) was also identified in TNBC cells after 24 h with cell morphology indicating cellular stress. (I – L) Internalization of PNC-Doxo-Gd-ICAM1 (10) was also detected in TNBC cells after 24 h with cell morphology pointing to cellular stress. Negligible internalization of PNC-Doxo-Gd-ICAM1 (10) was observed in MCF-7 cells demonstrating the effectiveness of the ICAM1 antibody as a ligand to target ICAM1-overexpressed cancer cells. Nuclei stained with DAPI (blue).

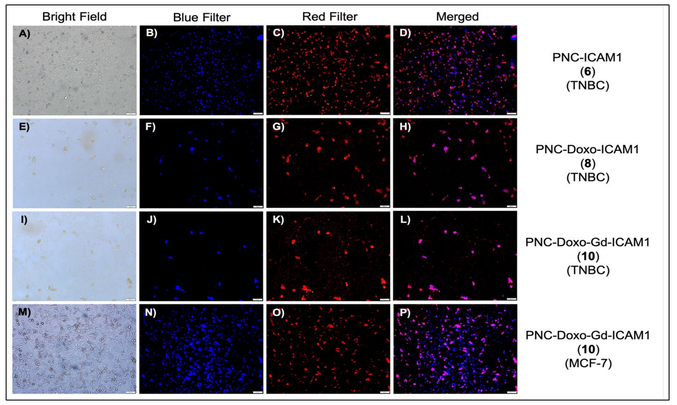


Figure 6: (A – D) Internalization of PNC-ICAM1 (6) in TNBC cells was observed after 48 h, due to ICAM1-receptor-mediated endocytosis (scale bar 500 μm). (E – H) Internalization of PNC-Doxo-ICAM1 (8) was also identified in TNBC cells after 48 h with cell morphology pointing to cell death. (I – L) Internalization of PNC-Doxo-Gd-ICAM1 (10) was also detected in TNBC cells after 48 h with cell morphology indicating cell death. Minimal internalization of PNC-Doxo-Gd-ICAM1 (10) was observed in MCF-7 cells (attributed to nonspecific internalization) demonstrating the effectiveness of the ICAM1 antibody as a ligand to target ICAM1-overexpressed cancer cells. Nuclei stained with DAPI (blue).

Conclusion

A doxorubicin/gadolinium-based prodrug was successfully synthesized by using a cleavable crosslinker to join doxorubicin and gadolinium chelated with an aminobenzyl derivative of DTPA. Quantitative internalization and cell viability studies proved our ICAM1-functionalized Nanoceria is selective to and successfully kills TNBC cells within 48 hours, which was visualized by fluorescence microscopy. ROS assays correlated with cell viability studies showing high levels of ROS where cell viability was the lowest. Finally, results from the comet assay further demonstrated that our prodrug (4) activates intracellularly by GSH mediated cleavage, releasing doxorubicin and generating DNA damage. Our results show the simultaneous selective delivery, chemotherapy, MR imaging and tumor tracking overcome the limitations of single modality treatments by providing valuable theranostic attributes for a physician in a single treatment, with the highest potential impact to the patient who could potentially experience less side effects by selective delivery and go through fewer injections due to the combination prodrug therapy.

References

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