Preparation, characterization and anticancer activity of β-cyclodextrin nanosponges formulated with two chemotherapeutic drugs

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Cyclodextrin (CD)-based polymers and nanoparticles offer significant opportunities in drug delivery due to their high drug loading capacity, low cytotoxicity, and ease of surface functionalization for specific cell targeting. In our laboratory, β-cyclodextrin (β-CD)-based nanosponges (CNs) were synthesized using two different crosslinkers: citric acid and epichlorohydrin, by varying the β-CD/crosslinker ratios. After purification, the epichlorohydrin-crosslinked CNs were further modified with 2,3-Epoxypropyl trimethyl ammonium chloride to generate positively charged CNs. The resulting library of CNs exhibited extremely small particle sizes ranging from 50 to 30 nm (DLS, SEM and AFM) and carried a surface charge between −20 mV and +20 mV.

CNs, which possess both hydrophobic and hydrophilic domains, were used to co-encapsulate different anticancer drugs: N,N-bis (5-ethyl-2-hydroxybenzyl) methylamine (EMD), Dasatinib and Doxorubicin, that act through different mechanisms on cancer cells. EMD promotes apoptosis through c-Myc suppression, Das is a multi-kinase inhibitor that primarily targets the BCR-ABL1 oncoprotein and SRC-family kinases while Doxorubicin damages DNA and stops cancer cells growth. CNs were encapsulated with single drugs and combinations of them. Successful drug encapsulation was confirmed and quantified by UV, fluorescence and ^{1}HNMR spectroscopy. By NOESY NMR spectroscopy, Fluorescence Spectroscopy and Molecular Dynamic we were able to differentiate drugs encapsulated in the hydrophobic β-CD pocket or in the hydrophilic regions between β-CDs.

In vitro drug release studies were conducted at different pH to simulate physiological and endosomal environments. Cellular uptake and cytotoxicity studies of drug-loaded CNs were performed with human lung cancer cells (A549), human breast cancer cells (MCF-7), and normal human lung fibroblast cells (WI-38). Results demonstrated synergistic cytotoxic effects against cancer cells when EMD and Doxo were co-encapsulated, enabling the use of lower drug doses compared to single-drug treatments, with potential decrease on drug side effects. Furthermore, the CNs loaded with two drugs exhibited higher cytotoxicity toward cancer cells than toward normal cells. These findings suggest that β-CD-based nanosponges are promising nanocarriers for the co-delivery of anticancer agents, enhancing therapeutic outcomes through improved efficacy and cell specificity.

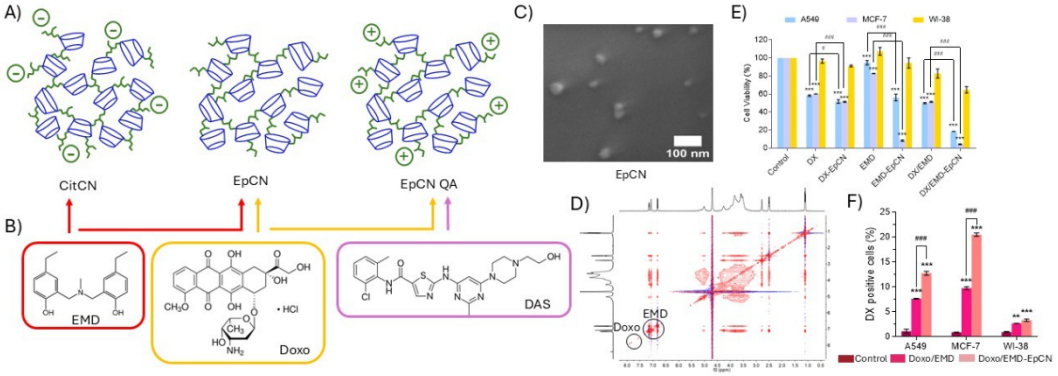

Figure. A) CNs prepared, B) drugs encapsulated, C) EpCN SEM, D) Doxo/EMD-EpCN NOESY NMR, E) cell viability and F) cellular uptake.

References:

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