Development of a multimodality molecular imaging probe for the *in vitro* and *in vivo* evaluation of a cellulose nanocrystal drug delivery system

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Background: One of nuclear molecular imaging methodologies, single-photon emission computed tomography (SPECT), is an important technology of noninvasive imaging widely available and used in the clinic for obtaining sensitive and quantitative information on tissue biochemistry and function. ^{111}In is one of the radioactive isotopes for SPECT with its relatively long physical half-life (2.80 days) perfectly matched for tracking nanoscale drug delivery systems *in vivo* within the time frame of their biological interactions such as tumor accumulation, mononuclear phagocyte system (MPS) uptake, elimination and passage through the gastrointestinal (GI) tract. By the addition of a fluorescent label, a multimodality probe is generated, allowing the use of the same imaging probe also in cells and tissues to observe events that occur beyond the spatial resolution of SPECT. Cellulose attracts attention in the form of cellulose nanocrystal (CNC) as potential nanocarrier in drug delivery applications. [1,2] CNC has a reactive surface covered with numerous active hydroxyl groups that allow the incorporation of a wide range of functional groups for chemical modification and attachment of labels and therapeutic payloads. Herein, we aim to prepare a CNC-installed DOTA precursor for labeling with ^{111}In and the far-red fluorescent dye Cy5 in order to study cytotoxicity, biocompatibility and behavior of CNC *in vitro* and *in vivo*.

Methods: DOTA hydrazide precursor was synthesized *via* methylester and hydrazinolysis reactions of 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-tert-butyl acetate-10-acetic acid (**Figure 1a**). The DOTA hydrazide precursor and Cyanine5 hydrazide were selectively bonded with the aldehyde on the reducing end of CNC and hydroxyl activated CDI in dried DMSO at 40 – 60 °C under argon atmosphere. The labeled CNC were characterized by zeta potential and electron microscopy size measurements. Moreover, the cytotoxicity and cellular uptake were investigated in murine RAW 264.7 macrophages. The cells were incubated 24 hours with unmodified CNC, DOTA_CNC, and Cy5_CNC at 5, 25, and 100 µg/ml concentrations for cytotoxicity and Cy5_CNC at 100 µg/ml for cellular uptake assays. A LIVE/DEAD® cytotoxicity assay (Molecular Probes) was used for the determination of cell viability. Both cell viability and Cy5-labeled CNC uptake were studied with confocal fluorescence microscopy. Experiments to optimize the ^{111}In-labeled DOTA-Cy5_CNC construct in CD-1 mice with SPECT/CT and *ex vivo* radioactivity measurements are underway.

Result: The ^{1}H and ^{13}C NMR, and mass spectrometry revealed the success of the DOTA hydrazide synthesis. The zeta potential was not significantly altered in presence of CNC surface modification in comparison to the unmodified CNC and the average size of CNC was 5 nm in diameter and 200 nm in length. Both unmodified and modified CNC showed good biocompatibility in RAW 264.7 cells after 24 hours of incubation together with an interaction between the CNC nanoparticles and macrophages *in vitro* warranting *in vivo* evaluation.

Conclusions: The multimodal CNC developed herein can be used for the evaluation of the suitability of the material for the construction of molecular imaging probes and drug delivery systems both in vitro and in vivo with SPECT and fluorescence imaging, a key step in advancing the biomedical applications of nanocrystalline cellulose.

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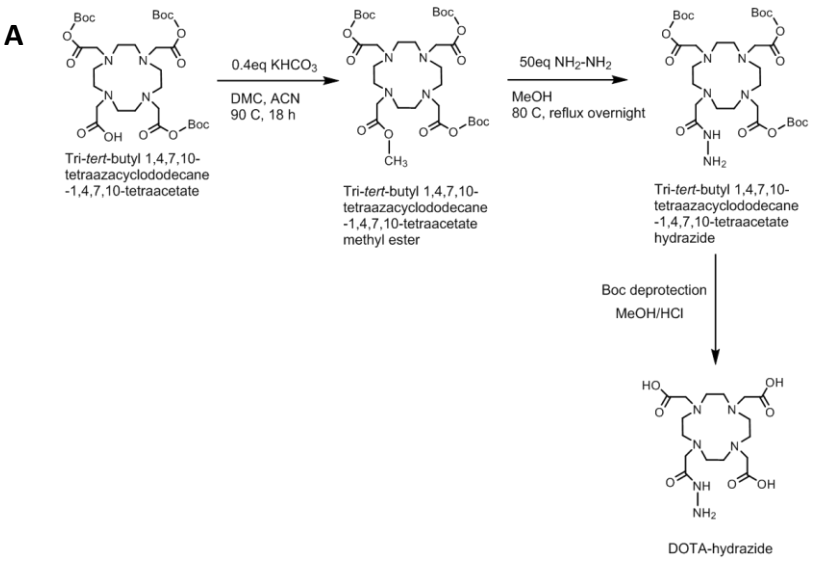

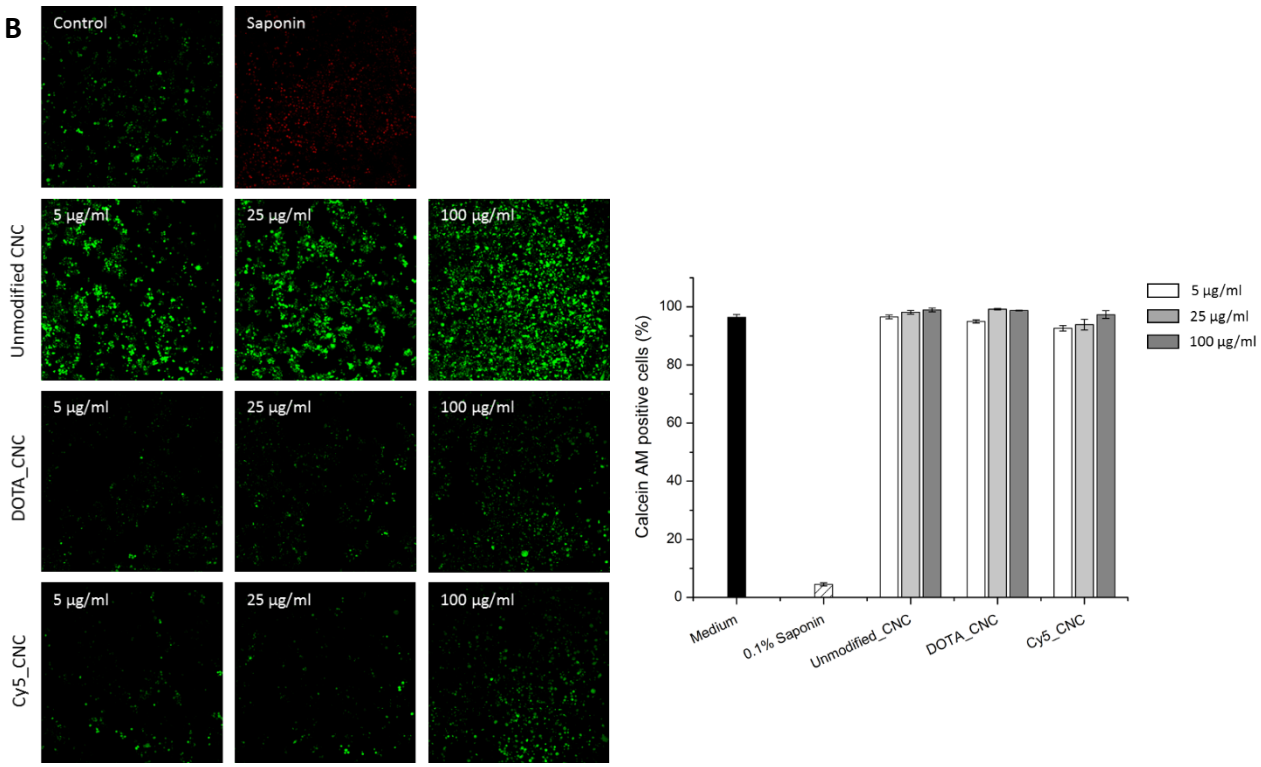

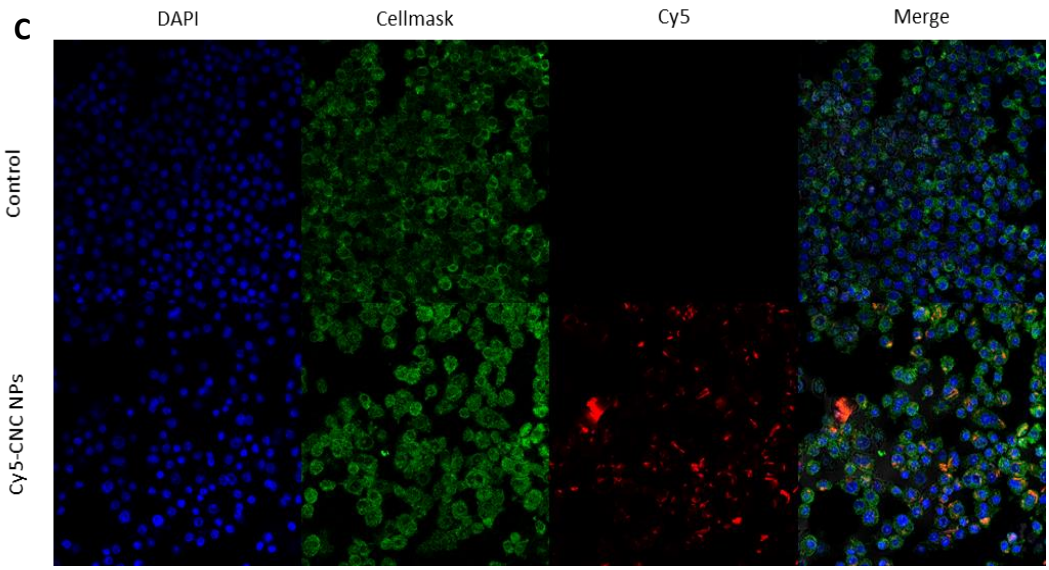

Figure 1. (A) The synthesis scheme of DOTA hydrazide **(B)** The cytotoxicity test in RAW 264.7 macrophage cells of unmodified-CNC, DOTA-CNC, and Cy5-CNC at 5, 25, and 100 µg/ml for 24 hours **(C)** The cellular uptake of Cy5-CNC at 100 µg/ml, 24 hours in RAW 264.7 macrophage cells

References:

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Detailed references (short versions will be shown in the abstract)

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