Title: Development of ^{177}Lu-labeled Cellulose Nanocrystals for Drug Delivery and Theranostic Applications in Metastatic Melanoma

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Introduction: Nanocrystalline cellulose (CNC) is a renewable and abundant nanoscale biopolymer available from various natural resources such as plants, bacteria, and tunicates, and widely investigated as biomaterial scaffolds for the use in biomedical applications.[1] CNC has distinctive properties, including facile and inexpensive preparation process and diversity in the available chemical modifications of a reactive hydroxyl surface of CNC, which make it a promising scaffold for the development of targeted and controlled drug delivery systems (DDS). CNC-based biomaterials have demonstrated good biocompatibility and non-toxicity in both cell and animal models.[2,3] Moreover, cotton-derived CNC used in our studies have suitable dimensions for DDS development, typically the average width of CNC is 5-10 nm and length 100-300 nm. Lutetium-177 is a theranostic radioisotope widely used in the clinic as ^{177}Lu-labeled DOTA-peptide conjugates for targeted radiotherapy because of its favorable physical characteristics: a relatively long half-life ($t_{1/2}$=6.73 d) and emission of both beta and gamma radiation, allowing the imaging with single-photon emission computed tomography (SPECT) and therapy with the same isotope simultaneously. Recently, we have developed DOTA-functionalized multimodal CNC and reported the studies of their behavior *in vitro* and *in vivo*.[3] The biodistribution profiles of DOTA-CNC demonstrated a high but transient accumulation in the lung *in vivo*. Therefore, the developed DOTA-CNC can be further radiolabeled with ^{177}Lu in order to investigate the radiotherapeutic potential of ^{177}Lu-DOTA-CNC and the synergistic effects of ^{177}Lu-radiotherapy and B-Raf inhibitor vemurafenib in lung metastatic melanoma models harboring the V600E mutation both *in vitro* and *in vivo*. Herein, we aim to optimize the radiolabeling methods for ^{177}Lu-DOTA-CNC and investigate the stability of the ^{177}Lu-DOTA-CNC in physiological conditions (1× PBS, pH 7.4), 50 and 100% human plasma, and challenges with iron (potentially displacing ^{177}Lu in the chelate cavity *in vivo*) and EDTA (transchelation) *in vitro*.

Methods: DOTA-functionalized CNC was prepared as described in our previous study.[3] The radiolabeling conditions was performed with the initial specific activity of 100 MBq/mg CNC in 0.2 M ammonium acetate buffer (pH 4) at the CNC concentration of 1 mg/ml, 100 ˚C for 1 h and radiolabeled product was subsequently purified by several washing steps with 50 mM EDTA and 1x PBS. The *in vitro* stability test of ^{177}Lu-DOTA-CNC was carried out in 1× PBS (pH 7.4), 50 and 100% human plasma, 0.2 mM $FeCl_3$, and 2 mM EDTA at the concentration of 100 µg/ml, 37 ˚C over 5 days of incubation. Radio-TLC chromatogram (eluent: 0.1 M citrate buffer, pH 5) and autoradiography were used to determine radiochemical purity and the release of ^{177}Lu from CNC in stability test (^{177}Lu-R_f = 0.9-1.0).

Results: ^{177}Lu-DOTA-CNC was successfully radiolabeled and characterized, showing 77% decay- corrected radiochemical yield, 99.8% radiochemical purity, and 78 MBq/mg CNC specific activity. The radiolabeled CNC demonstrated good stability under stimulated physiological conditions and under the Fe and EDTA challenges by maintaining the percent of ^{177}Lu intact in the CNC above 99% over a period of 5 days, prompting use in preclinical studies in a mouse model of metastatic melanoma to the lung generated by an intravenous injection of YUMM1.G1 melanoma cells in C57Bl/6J mice.

Conclusion: The radiolabeling conditions and physiological *in vitro* stability including iron and EDTA challenges for ^{177}Lu-DOTA-CNC were optimized and tested. The developed theranostic ^{177}Lu-DOTA-CNC herein can be used for the further investigation of the CNC nanoprobe behavior *in vivo*, and radiotherapeutic response of ^{177}Lu in the lung metastatic melanoma animal model. In addition, the potential synergistic effects between vemurafenib chemotherapy and radiotherapy will be an ultimate outcome in the CNC-based DDS development.

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