In Vitro Cellular Interactions of Multimodal Cellulose Nanocrystal and Lignin Nanoparticles for Drug Delivery Applications

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Background:

Nanoscale drug delivery systems (DDS) have an important role in the development of targeted therapies, especially in cancer treatment [1]. Cellulose nanocrystals (CNCs), which can be generated from native cellulose through acid hydrolysis, have attracted attention as a renewable source of nanoparticles (NPs) for drug delivery applications [2]. Lignin, another main component of lignocellulosic materials, is composed of three cinnamyl alcohol structural units and forms spherical NPs, which are amenable for DDS development [3]. A reactive surface of both CNC and lignin NPs covered with numerous hydroxyl groups allow a wide range of chemical modifications on their surface, as well as forming a stable well-dispersed colloidal suspension in aqueous media. We have prepared CNC and lignin NPs conjugated to a fluorescent dye and the radiometal chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) for labeling with ^{111}In, a diagnostic radionuclide for single-photon emission computed tomography (SPECT) for the biological evaluation of CNC and lignin DDS using nuclear imaging and fluorescence microscopy. Here, we report the cellular biocompatibility and interactions of our CNC and lignin constructed in murine RAW 264.7 macrophages as the first step of their evaluation for *in vivo* applications.

Methods:

CNC-installed Cy5 and DOTA were prepared by the selective conjugation of Cy5/DOTA hydrazide to the aldehyde on CNC reducing end in dried DMSO at 60 °C under argon. Lignin-installed Cy5 and DOTA were produced in one pot reaction by activating the hydroxyl groups with CDI overnight before coupling with Cy5 and DOTA amine in dried DMSO at 60 °C under argon. The labeled CNC/lignin were characterized by DLS, zeta potential, and electron microscopy. The cell viability and cellular uptake were studied in RAW 264.7 macrophages. The cells were incubated 6, 24 and 72 h with unmodified CNC/lignin, DOTA CNC/lignin, and Cy5 CNC/lignin at 5, 25, 100, 250, 500 and 1000 µg/ml for cell viability and Cy5 CNC/lignin at 100 µg/ml for cellular uptake assays. A CellTiter-Glo® assay was used for the viability determination. The cellular uptake was investigated with confocal fluorescence microscopy. The elemental analysis and *in vitro* stability studies of the NPs are underway.

Results:

The zeta-potential in both NPs was not significantly altered by the surface modification in comparison to the unmodified materials. The hydrodynamic diameter of the lignin NPs was about 173 nm (PDI 0.105). However, due to a strong light scattering of CNC, the size determination of CNC by DLS is not feasible. Therefore, transmission electron microscopy (TEM) revealed the average size of CNC was 5 nm in diameter and 200 nm in length corresponding to the dimensions of the unmodified material. Both unmodified and modified CNC/lignin NPs revealed good biocompatibility in RAW 264.7 cells at low concentration range (5-100 µg/ml) as shown in figure 1. The half maximal inhibitory concentration (IC_{50}) of CNC and lignin NPs were about 1000 and 850 µg/ml at 6 h, 370 and 250 µg/ml at 24 h, and 122 and 77 µg/ml at 72 h, respectively. Moreover, figure 2 shows interaction and internalization of CNC/lignin NPs to RAW 264.7, without morphological changes indicating an inflammatory response *in vitro* at 24 h warranting *in vivo* evaluation in the future.

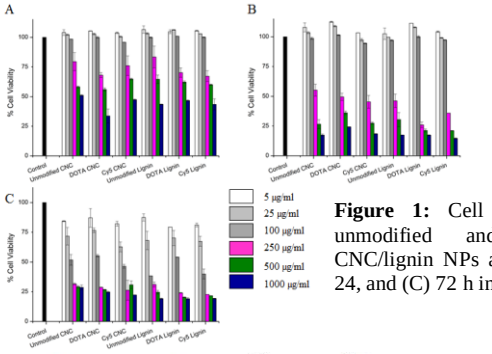

Figure 1: Cell viability of unmodified and modified CNC/lignin NPs at (A) 6, (B) 24, and (C) 72 h incubation

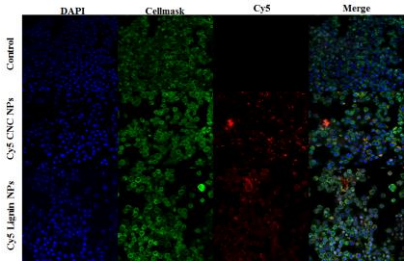

Figure 2: The cellular uptake of Cy5-CNC/lignin NPs at 100 µg/ml for 24 hours in RAW 264.7 macrophages

Conclusions:

The multimodal cellulose nanocrystal and lignin nanoparticles developed herein display good *in vitro* biocompatibility in the models used, prompting their use as a tool for the investigation of the materials as molecular imaging probes both *in vitro* and *in vivo*, a key step in advancing the biomedical applications of cellulose nanocrystal and lignin DDSs.

Acknowledgement:

Financial support from the Academy of Finland (decision no. 278056 and 298481) and the University of Helsinki is gratefully acknowledged.

References:

[1] Ding, H. et al. *Theranostics* **2012**, *2*, 1040-1053.
[2] Lin, N. et al. *Nanoscale* **2014**, *6*, 5384.
[3] Figueiredo, P. et al. *Nanomedicine* **2017**, 0219.