In vitro Cytocompatibility Study of Nanocellulose

Lu Huang [1], Yuan Lu [2], Soydan Ozcan [2], Wei He [1, 3]

1. Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN 37996
2. Materials Science and Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831
3. Department of Mechanical, Aerospace and Biomedical Engineering, The University of Tennessee, Knoxville, TN 37996

Statement of Purpose: Nanocelluloses (NCs) hold promising potential as green materials derived from nature, owing to their advantages in large availability and favorable mechanical properties [1]. Recently, NCs are also making inroads into the biomedical field, highlighted by their use in bionanocomposites for tissue engineering related research. Given the degradable nature of these nanocomposites, it is thus inevitable that the embedded NCs, which are non-biodegradable, will be released as the bulk matrix degrades, making biocompatibility of NCs an essential issue. Although reports can be found studying the biocompatibility of such bionanocomposites as a whole [2], few investigated the effects of NCs alone on living cells. This critical knowledge gap hence drives the present research.

Methods: NC crystals (0.94 wt% sulfur) were kindly provided by U.S. Department of Agriculture-Forest Product Laboratory. Cytotoxicity of the NCs was measured by WST-1 assay using two different murine derived cell lines, including RAW264.7 macrophages and NIH3T3 fibroblasts. The dosage dependence of NC cytotoxicity was studied at 0, 10, 100, and 200 μg/ml after 16 h incubation with cells. The time dependence of cytotoxicity was evaluated at the sublethal dosage at day 1, 3, and 5. Cell viability after exposure to NCs was further characterized using Live/Dead staining. SEM was adopted to observe cell morphological changes. Potential cell membrane damages induced by NC exposure were quantified with LDH assay. Moreover, the release of a proinflammatory cytokine, TNF-α, from the cells after NC treatment was measured using ELISA assay.

Results: The morphology of the as-received NCs was revealed by SEM (Fig. 1). Zeta potential measurement shows the NCs are negatively charged in water, with an average surface charge of -42.5 mV demonstrate the morphology of as-received NCs. The cytotoxicity assay using WST-1 reagent shows that more than 95% of the cell viability was maintained after exposure to up to 200 μg/ml NCs (Fig. 2), with respect to the non-treated group (0 μg/ml). No statistically significant differences were found amongst different groups, indicating the good cytocompatibility of NCs within the dosage range tested. In addition, the high intensity of green fluorescence in Fig. 3 revealed high viability of macrophages after 16 h exposure to NCs at all testing concentrations, confirming the WST-1 assay results. Positive red staining of dead cells was observed in all samples, and the 100 and 200 μg/ml NC treated groups showed a slightly higher number of dead cells. Similar morphology was observed for cells after NC exposure to those untreated ones under SEM, while interactions with the precipitated NCs were visualized for cells treated with 100 and 200 μg/ml NCs (data not shown). Cell behavior after NC exposure will be further discussed biomolecularly.

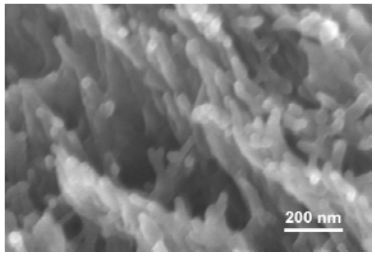

Figure 1 SEM image presenting the morphology of NC crystals

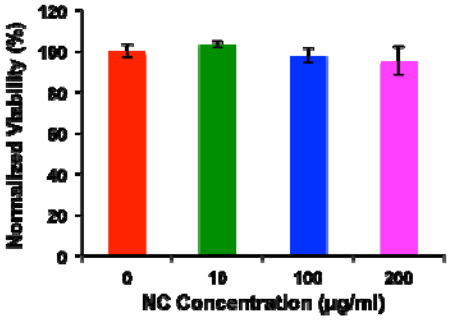

Figure 2. Cell viability of RAW264.7 macrophages evaluated by WST-1 assay after exposure to NCs at different concentrations for 16 h (Mean ± SEM).

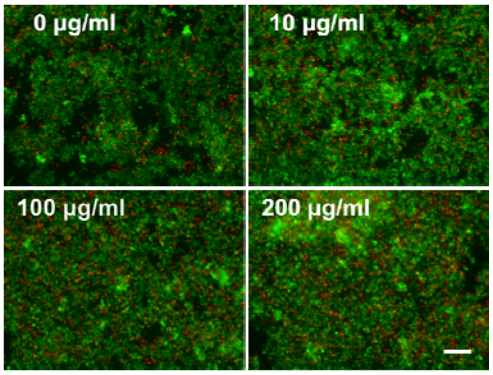

Figure 3. Live/Dead staining of RAW264.7 macrophages after 16 h exposure to NCs at different concentrations. (scale: 100 μm)

Conclusions: Our study shows that NCs are cytocompatible with murine derived cell lines at concentrations up to 200 μg/ml. The effect of NC surface functionality on its cytocompatibility is under further investigation.

Acknowledgement: We appreciate the funding support from The Science Alliance at UTK. Our gratitude also goes to the USDA Forest Service Forest Products Laboratory for providing the cellulose nanomaterials as well as information on the properties of the cellulosic nanomaterials

References:

[1] Klemm D, et al. Angew Chem. 2011;50:5438-5466.
[2] Bhattacharya M, et al. J. Control. Release 2012;164:291-298.