Natural Secreted Nano Vesicles as a Source of Novel Biomass Products for Circular Economy

This BioFuture2025 project targets the nano- and micro vesicles that are called collectively here as extracellular vesicles (EVs). The EVs represent a new humoral, systemic layer that controls homeostasis. Since the EVs are around the size of viruses and they are present in almost every biological fluid in animal world and plant world, the EVs may function also as a novel bioaerosol class. The EVs transmit various types of relevant cellular biomolecules such as proteins, RNA/DNA and the metabolites. Due to these reasons the EVs may offer openings to target (biological) drugs, image tissues and organs in vivo and ways to develop even non-invasive surgery therapies at the end. The EVs can be expected to offer fundamental opportunities for disease diagnostics. Individual EVs may themselves serve as biological drugs when produced in mass quantities for medical practice. In summary the EVs offer important opportunities to develop significant bio economically valuable products. We have also studied nanocellulose based filter materials (potential material for WV collection) filtration efficiencies and ice nucleating particles (INP's).

Mammary epithelial cells produce nano- and micro sized membrane vesicles for secretion. We have analyzed bovine milk EV proteome and nucleic acid cargo to generate a biomolecule database for naturally secreted nanovesicles. We were able to observe several membrane proteins that are known to enrich to mammalian EVs. In RNA sequencing we identified several RNA biotypes, including various small and long non-coding RNAs, ribosomal RNAs, transfer RNAs and messenger RNAs. The major small RNA group identified was micro RNA (miRNA). miRNAs are post-transcriptional regulators of gene expression. We set up a bioinformatics pipeline for EV DNA/RNA analytics.

We have also developed functionalized nanocellulose with pH-responsive surface charge. This nanocellulose can be used in a novel method for rapid separation of extracellular vesicles from bovine milk and possibly from other biological fluids. The method is cost-effective, fast and gentle with no need for time-consuming conventional ultracentrifugation step. According to results from mass spectrometry, EVs separated by the new method have similar protein content than those obtained with traditional ultracentrifugation, and all the most common

EV-related proteins can also be detected. The feasibility of new method can likely be expanded to new biofluids.

We have tested nanocellulose-based aerogels prepared by freeze-drying technique as air filters. The filters consist of sponge-like, highly porous and super-light material. The filters show promising results in aerosol filtration in size range of 10-250 nm with the removal efficiencies of over 99,99%. In future these filters will be still improved and tested for aerial vesicles filtration.

We have already identified potential RCC EV markers induced in cells cultured under hypoxia among proteins and RNA species. We also started to characterize tumorigenic mechanisms of RCC-derived EVs using two target cell types: model mouse kidney cells and primary mouse hepatocytes, which represent typical site of RCC metastasis with an exceptionally poor prognosis. The experiment to study the involvement of potential candidates, already identified by Renca EVs analysis, in tumorigenesis are now running in the lab. We are generating Renca lacking caveolin-1 gene using Crispr/Cas9 genome editing approach. We also perform experiments to track labelled EV using both classical in vitro models, chick embryo model, and a novel kidney organoid co-culture assay developed by our group.

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