

BENJAMIN T. LANHAM, Dept. of Chemistry, Shepherd University, Shepherdstown, WV, 25443, and AUDRY FERNANDEZ GOMEZ and DAVID J. KLINKE II Department of Chemical and Biomedical Engineering, West Virginia University, Morgantown, WV, 26506. Characterizing the Production of Extracellular Vesicles over Time by LLC1, B16F0, and WISP1 KO B16F0 Cell Lines

An emerging mode of intercellular communication is through the release of extracellular vesicles, which can transfer proteins and RNA between cells. Characterizing the role that extracellular vesicles play is accomplished using vesicles isolated from *in vitro* cell culture. However extracellular vesicles isolated from cell-conditioned media can be contaminated with cellular debris produced during apoptosis. The purpose of this experiment was to determine the rate by which specific cell lines produce extracellular vesicles and how long before cell viability limits vesicle production. Here, we collected samples from media conditioned by three different cancer cell lines: Lewis Lung Carcinoma (LLC1), melanoma (B16F0), and modified B16F0 (WISP1 KO B16F0). Cell-conditioned media samples were obtained every 12 hours for 72 hours. To characterize the size distribution of the collected vesicles, each sample was analyzed on a NanoSight NS300. Results indicate that extracellular vesicles are primarily produced during the first twenty-four hours without interferences of cell debris. The cells cultured in PBS produced the most extracellular vesicles particles within the size range of exosomes. Interestingly, the WISP1 KO B16F0 cell line produced few vesicles, many of which were larger than 200nm after 48-hours. A possible reason is the WISP1 KO B16F0 cell line lacks WISP1, a gene that is suspected of promoting epithelial-mesenchymal transition and suppressing apoptosis. In summary, cell lines should be used to produce vesicles for at most 36 hours, but optimally for 24 hours. As for WISP1, more research is needed to identify the role that WISP1 plays on extracellular vesicle production.

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