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Effects of Cell Culture Conditions on Extracellular Vesicle Production in Human Embryonic Kidney Cells

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Extracellular vesicles (EVs) have potential as a novel drug delivery system and EV-based therapeutics are typically produced *in vitro*. Fetal bovine serum (FBS), a commonly used nutrient source for cells, is a well-known contaminant in the production of therapeutic EVs. These contaminants can reduce the efficacy and reproducibility of EV production. Establishing optimal *in vitro* culture conditions, including nutrient sources and seeding density, are essential for the advancement of the EV therapeutic field.

PURPOSE: To determine if more extensive downstream processing of FBS reduces contaminants without the removal of beneficial nutrients. Additionally, we sought to determine the optimal cell seeding density and cell passage number for EV production. **METHODS:** Human embryonic kidney cells (HEK293 cells) were cultured to isolate EV enriched media. The cells were grown to confluency in regular FBS media, washed 2x with phosphate buffered saline (PBS), then placed in vesicle-free culture media for 48h. We tested a high seeding density (100k cells/cm²) and a low seeding density (20k cells/cm²). We hypothesized that a lower seeding density would produce a higher yield of EVs. In a subsequent experiment we tested 6 different cell culture supernatants. Ultracentrifuge (Vesicle-Free) and a proprietary blend FBS (Exo-Free). Both groups were then further processed with Polyethylene Glycol (PEG) and ultracentrifuge spin column (100 kDa). This gave us a total of 6 groups Exo-Free, Exo-Free-PEG, Exo-Free-100kda, Vesicle-Free, Vesicle-Free-PEG, and Vesicle-Free-100kda. Data was quantified using Nanoparticle tracking analysis (NTA), cell viability and cell count. **RESULTS:** Combining NTA and cell counting we found there was 3x more EVs ($P < 0.0001$) in the lower seeding density group. The lower seeding density group also had significantly smaller EV ($p = 0.0067$). We did not observe any differences between the various FBS formulations, cell viability or passage number. **CONCLUSION:** A lower seeding density yielded significantly more EVs. Various FBS formulations do not affect cell viability and passage number does not affect EVs.

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