Milk Whey Extracellular Vesicles as a Drug Delivery System for Neuroblastoma Therapy

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Extracellular vesicles (EVs) are membrane-enclosed nanoparticles that are naturally released by cells and play a pivotal role in intercellular communication, rendering them promising candidates for the diagnosis of various diseases, for drug delivery, and their efficacy in regenerative medicine has been demonstrated. The current literature suggests that, irrespective of their provenance, EVs are currently isolated by sucrose gradient centrifugation, immunoaffinity chromatography, or differential ultracentrifugation (UC), the latter of which is regarded as a benchmark. These techniques have been demonstrated to yield high recovery rates; however, their implementation on an industrial scale is impeded by the high costs and limited scalability.

This work presents an economical and scalable membrane filtration process for the isolation of EVs from whey derived from the production of Parmesan cheese. A series of experiments were conducted to ascertain the optimal operating conditions, with particular attention devoted to the membrane type and the filtration modes (i.e., concentration and diafiltration). The isolated vesicles were then subjected to physical and biochemical analysis. An optimized membrane ultrafiltration (UF) process can lead to a reduction of impurities up to 99.8%, allowing the continuous production of a purer stream compared to UF (although less concentrated), containing up to $4 \cdot 10^{11}$ EVs/mL with a mean hydrodynamic size around 180 nm and a surface charge of -11.8 mV. This process was employed to engineer a drug delivery system (DDS) for neuroblastoma (NB) therapy, with curcumin serving as the model drug. The efficacy of the process was evaluated by cell viability and internalization studies on SK-N-AS NB cells. The curcumin-loaded EVs demonstrated higher cellular uptake and greater inhibition of SK-N-AS cell proliferation compared to free curcumin, with a lower IC50 (16.34 µM vs. 31.14 µM). This scalable and efficient method for producing Cur-loaded EVs from bovine milk whey demonstrates considerable promise for therapeutic applications in cancer treatment.

Keywords: *Extracellular vesicles; Tangential flow filtration; Drug delivery system; Neuroblastoma therapy.*