Bovine Milk-derived Extracellular Vesicles as new drug delivery system for bioactive compounds

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Recently, extracellular vesicles (EVs) have received great attention given the many possible uses, especially as a new drug delivery system. EVs can also be found in raw cow milk (MEVs), representing a low-cost source of extremely biocompatible EVs. The first aim of this work focused on optimizing the method of EVs isolation from raw cow milk, in particular with regard to the removal of contaminants such as casein micelles, which are similar to EVs in size and represent a major obstacle to isolation efficiency. Three different approaches for casein removal were compared, including chymosin treatment, isoelectric precipitation, and differential centrifugation, all combined with size-exclusion chromatography for EVs isolation. To assess the quality of the isolation method, Western blots, high-resolution microscopy, and dynamic light scattering were used, and thin-layer chromatography was performed to profile the lipid composition of MEVs. According to our data, the enzymatic removal of casein combined with SEC provided the best results in terms of higher purity, unaltered EVs quality, and homogeneous size. Once the appropriate isolation method was chosen, the use of MEVs as delivery system for bioactive compounds, i.e., curcumin (CurMEVs), were also studied. Specifically, different times for passive loading were tested and, by exploiting the intrinsic optical and fluorescence properties of curcumin, the loading efficiency, stability, and solubility were determined. CurMEV's morphology, structural integrity, and loading efficiency were also evaluated under different storage conditions and pH values over time. Maximum loading efficiency of curcumin was measured after 3 hours of incubation with MEVs. Loaded curcumin showed greater stability and solubility than free curcumin, and remain intact even in condition of variable temperatures and pH values. Finally, by recreating an intestinal barrier model *in vitro*, we investigated the feasibility of using CurMEVs through oral administration by testing their toxicity and transepithelial crossing ability.