Milk NanoVesicles for Oral Delivery of Nucleic Acids

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Oral delivery of nucleic acid therapies offers new therapeutic possibilities for management of local and systemic disease and enables rapid mass immunisation. However, presently there are no systems for oral delivery of nucleic acids. Synthetic systems suffer from poor stability in the gut and poor delivery efficiency. Extracellular vesicles (EVs) from bovine milk are highly capable of crossing the intestinal epithelium.[1] These are potentially interesting systems from a widely available source and there is clear evidence that milk EVs resist digestion and are absorbed systemically intact. Milk EVs therefore have significant potential as safe and inexpensive delivery systems. However, loading of EVs with macromolecules is currently a significant challenge as current drug loading methods are either inefficient or destructive (or both). We investigated the stability of milk EVs in intestinal fluids and their ability to permeate the intestinal epithelium, including in tissue-derived human intestinal organoids. We further loaded milk EVs with siRNA (as a model therapeutic) using different methods and show successful, functional delivery of this payload in cells and an animal model of inflammatory bowel disease (IBD).

Milk EVs were isolated using ultracentrifugation and characterised for expression of common exosomal proteins, as well as size (nanoparticle tracking analysis, dynamic light scattering), charge (zeta potential) and morphology (electron microscopy). Milk EVs were tested in fed-state and fasted-state simulated intestinal fluids using a fluorescent membrane probe. Epithelial permeability was tested in Caco-2 monolayers and human tissue biopsy-derived intestinal organoids, cultured as monolayers or with 'apical-out' polarity. EVs were loaded with siRNA through electroporation or by fusion with cargo loaded lipid nanoparticles. siRNA-loaded EVs were tested for downregulation of target proteins (GAPDH) or inflammatory response in cells and in a rat model of IBD.

Milk EVs showed superior stability in simulated intestinal fluids compared to liposomes and efficiently permeated Caco-2 monolayers. EVs clearly transported across 3D 'apical-out' and monolayer human intestinal epithelial organoids. Milk EVs loaded with siRNA induced gene silencing and anti-inflammatory siRNA-loaded EVs reduced inflammation in an IBD rat model.[2]

Overall, the work shows that milk EVs could act as natural and safe systems for oral delivery or nucleic acid therapies.

References

[1] Betker et al, Journal of Pharmaceutial Science, 108 (2019), 1496-1505
[2] Zhang et al, Journal of Nanobiotechnology, 21 (2023), 406