Characterization of human milk exosomes by Infrared Spectroscopy and LC-HRMS

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OBJECTIVES

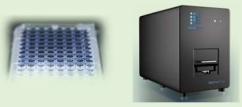
Exosomes are nanosized vesicles containing specific cargos of DNA, RNA, proteins, metabolites, and intracellular and membrane lipids. Lipid analysis of exosomes is a relatively unexplored field of research, so the main objective of this study was to:

- Determine the feasibility of Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) spectroscopy for the routine control of isolated exosomes and the rapid characterization of their lipid profiles.

STUDY DESIGN

- Three centrifugations (3000 g, 10 min, 4 °C)
- Filtration with a 40 µm *cell strainer*
- Two ultracentrifugations (10000 rpm, 1 h, 4 °C)
- Filtration with a 45 µm *cell strainer*
- Two ultracentrifugations (30000 rpm, 2 h, 4 °C)
- Pellet reconstitution in 22 mL phosphate buffered saline (PBS)
- Ultracentrifugation (30000 rpm, 2 h, 4 °C)
- Exosome pellet reconstitution in 200 µL PBS

EXOSOME ANALYSIS



QUALITY CONTROL

- ✓ Bicinchoninic acid (BCA) assay to determine the amount of retrieved exosomes
- ✓ ExoView platform (NanoView Biosciences, MA, USA) to determine the exosome size distribution and quantification of vesicles

[1]Ten-Doménech, et al., Comparing Targeted vs. Untargeted MS2 Data-Dependent Acquisition for Peak Annotation in LC–MS Metabolomics. *Metabolites* **2020**, *10*, 126.

ATR-FTIR

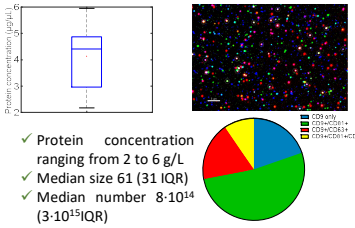
- ✓ 2 µL dried HM exosomes were used for IR spectra acquisition
- ✓ Range: 4000 to 400 cm^{-1}
- ✓ Scans: 32
- ✓ Resolution: 4 cm^{-1}

UPLC-QqTOF-MS

- ✓ Untargeted HM and HM exosomes analysis by ultra-high performance liquid chromatography quadrupole-time-of-flight mass spectrometry (UPLC–qTOF-MS) operating in positive and negative ionization mode (ESI+ and ESI-)
- ✓ Column: Acquity BEH C18 (100 mm x 2.1 mm, 1.7µm)
- ✓ Mobile phase: (A) 5:1:4 IPA:CH_3OH:H_2O 5mM CH_3COONH_4 0.1%HCOOH; (B) 99:1 IPA:H_2O 5mM CH_3COONH_4 0.1%HCOOH
- ✓ Automated MSMS-based annotation of metabolites[1]

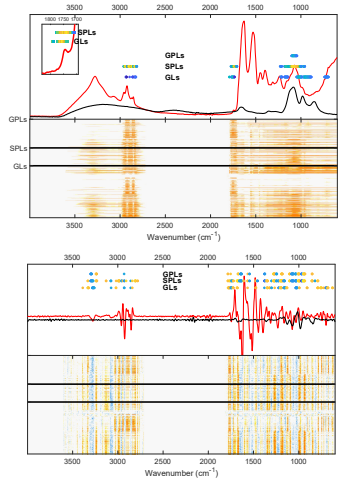
RESULTS

QUALITY CONTROL



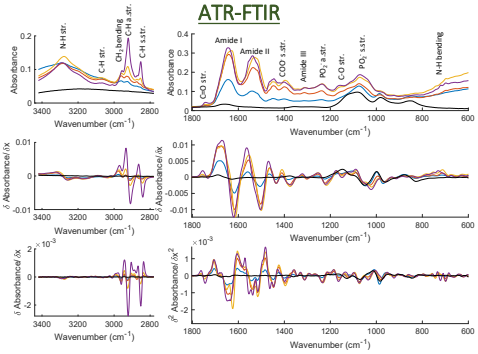
- ✓ Protein concentration ranging from 2 to 6 g/L
- ✓ Median size 61 (31 IQR)
- ✓ Median number $8 \cdot 10^{14}$ ($3 \cdot 10^{15}$IQR)

QUALITATIVE ANALYSIS

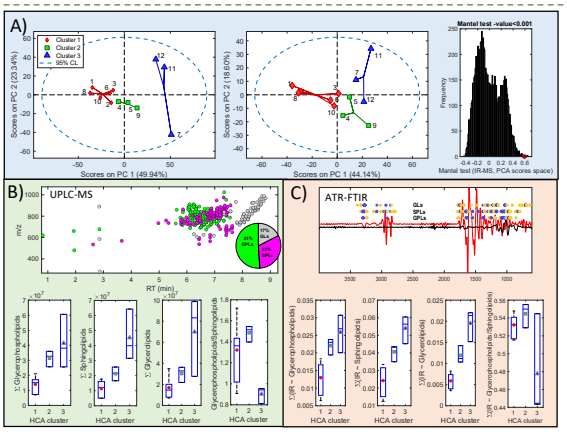


Statistical heterospectroscopy was employed to assess the correlation among IR bands and LC-MS intensities of the annotated features showing significant associations

ATR-FTIR

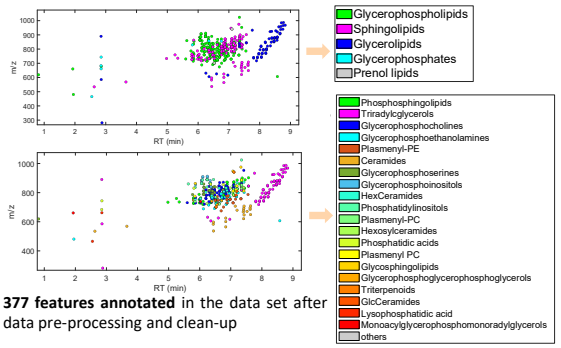


IR spectra (top) of dry residues of exosomes and the first (middle) and second derivative (bottom). Black line: PBS blank



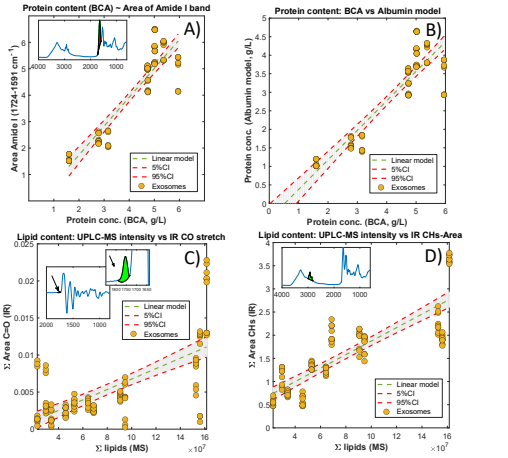
A) Principal component analysis was used to reveal samples with similar lipidomic profiles, as they were clustered mainly along PC1 ($p < 0.05$). **B)** and **C)** Sum of intensities of lipids annotated in the three selected clusters for LC-MS and second derivative IR spectra, respectively

UPLC-QqTOF-MS



377 features annotated in the data set after data pre-processing and clean-up

QUANTITATIVE ANALYSIS



Quantitative analysis using **A)** the area of the Amide I band and the results of the BCA assay; **B)** the same band as in A) of an albumin external calibration and the results of the BCA assay; **C)** and **D)** the sum of LC-MS intensities of annotated lipids as surrogate of the total lipid content and the area of the CHs and C=O streching bands, respectively

CONCLUSIONS

- ✓ A **correlation** between the **lipidomic profile** of HM exosomes and their **ATR-FTIR spectra** has been obtained
- ✓ The latter technique can be used for a **rapid evaluation** of the **composition** of HM exosomes (both, qualitative and quantitative)
- ✓ A **new tool** for a direct and fast **quality control** of the exosome isolation procedure

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