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LABION LABORATORY OF NANOMEDICINE AND CLINICAL BIOPHOTONICS

Raman spectroscopy for the biochemical characterization of human salivary extracellular vesicles as a valuable source of brain biomarkers

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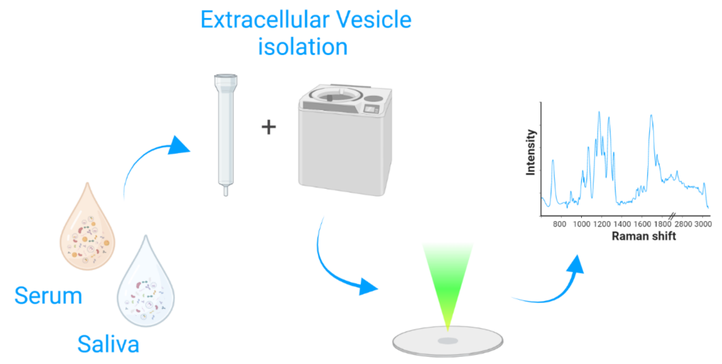
Saliva is an interesting, complex and easily available liquid biopsy.

Its use in diagnostics is fast increasing as well as the identification of specific salivary biomarkers for several disorders, spanning from neurodegenerative to cancer diseases.

Extracellular Vesicles (EVs) are known to be present in saliva, although their limited concentration has limited their use in clinics despite the remarkable potentialities.



What's the biochemical difference between SERUM and SALIVA EVs?

Aim of the study

In the present study, we compare the biochemical profile of salivary (SEVs) and blood-derived vesicles (serum-EVs) to investigate the use of saliva as a valuable source of EVs that could be studied as brain biomarkers in an easily accessible biofluid.

Methods

5 healthy volunteer women
(25-45 years old – non smokers)

serum → Size Exclusion Chromatography (SEC, Izon) Fractions from 7 to 11

saliva → Amicon 3K filter → Size Exclusion Chromatography (SEC, Izon) Fractions from 7 to 11

Size Exclusion Chromatography (SEC, Izon) Fractions from 7 to 11 → NTA; CONAN

Ultracentrifugation
(L7-65; Rotor SW60; Beckman Coulter)
100k g x 70 min

RAMAN SPECTROSCOPY[1,2]

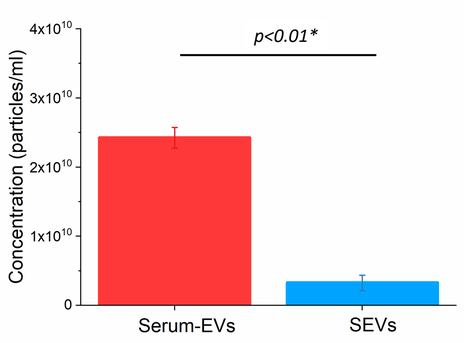
LabRAM Aramis, Horiba Jobin-Yvon

Drop volume	5-15 µl
Laser source	532 nm
Objective	50x
Spectral range	500–1800 cm^{-1} 2700–3200 cm^{-1}
Acquisition time	30s x 2 accumulations
Diffraction grating	1800 grooves/mm
Entrance slit	400 µm
Hole	600 µm
Number of spectra	20 spectra per drop, both at the edges and in the center of the drop area
Post-processing	Polynomial baseline subtraction and unit vector normalization

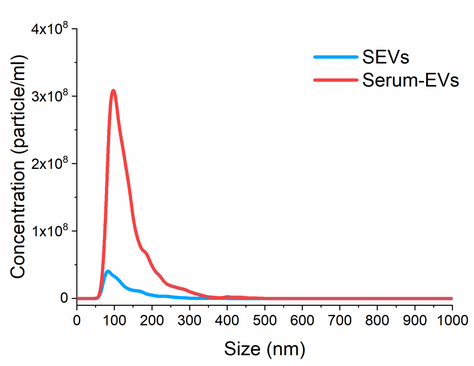
[1] Gualerzi A et al. Sci Rep 2017
[2] Gualerzi A et al. JEV 2019

Results

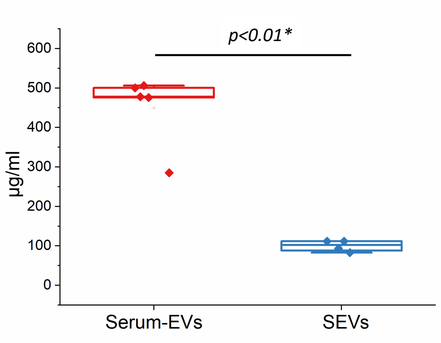
Particle concentration (NTA)



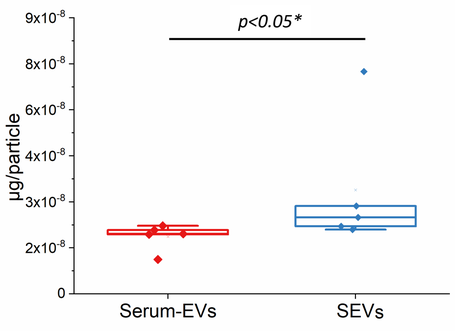
Size distribution (NTA)



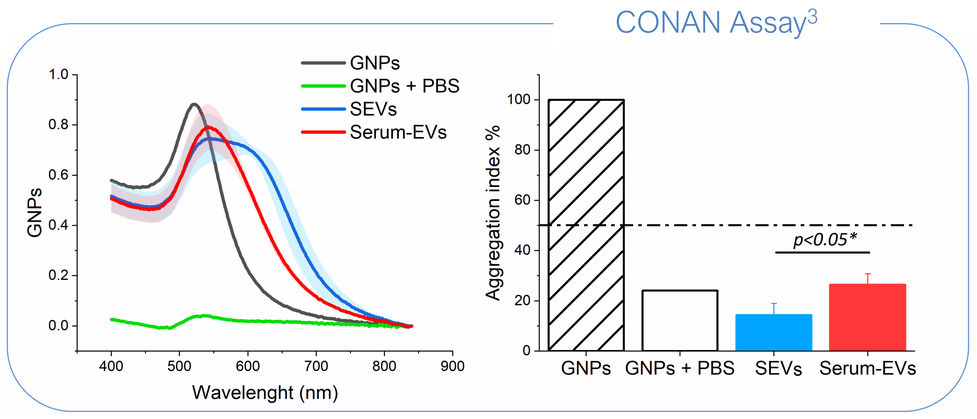
Protein concentration (BCA assay)



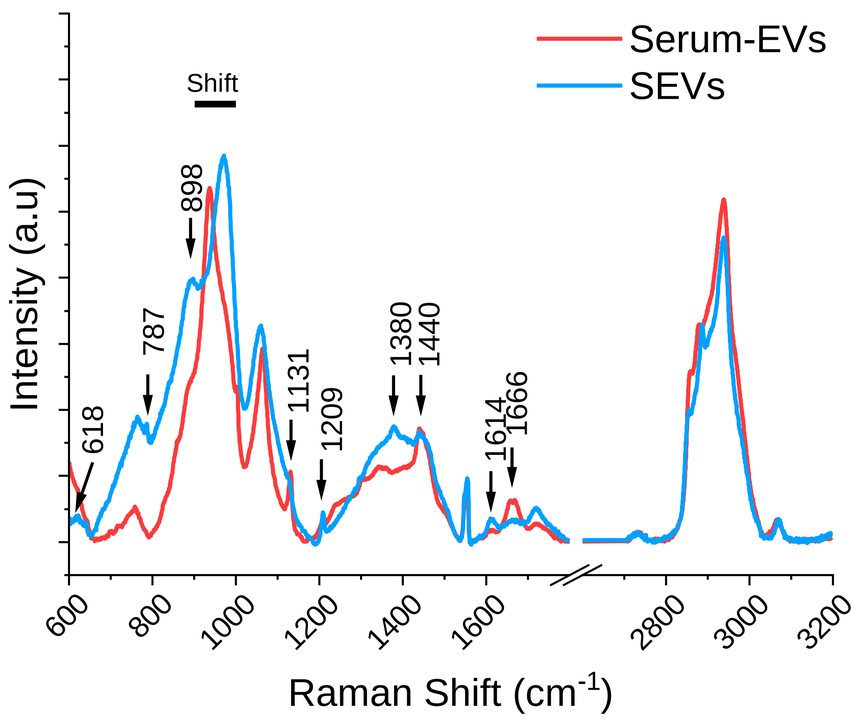
Purity of EV preparation



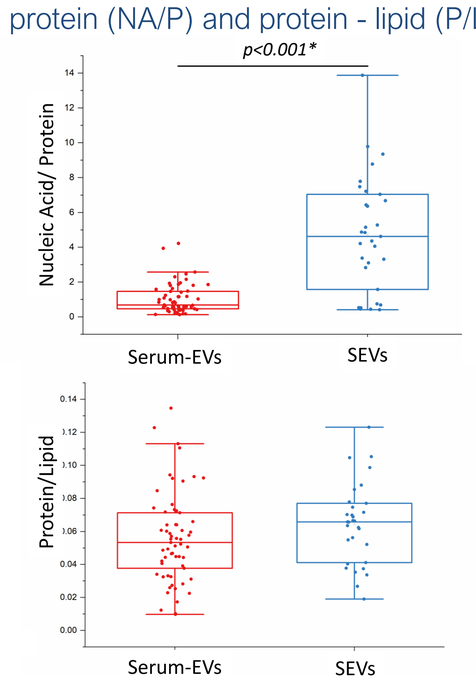
CONAN Assay[3]



[3] Maiolo D et al. Anal Chem 2015



Spectroscopic ratios of nucleic acid - protein (NA/P) and protein - lipid (P/L)



*Mann Whitney test

Conclusions

Advantages of the Raman analysis of salivary EVs:

- comprehensive biochemical profiling despite low yield
- limited interference of non-EV factors (i.e. proteins, lipoproteins)
- non invasive, straightforward and sensitive procedure

Raman spectroscopy can represent a turning point in the application of salivary EVs in clinics when non-EV factors might hinder biomarker detection

	serum EV	saliva EV	notes
EV concentration (NTA data)	○○	○	Potential co-isolated *lipoproteins* in serum EVs
Purity (CONAN)	○	○○	Potential co-isolated proteins and *protein corona* in serum EVs
Protein content (Raman Amide region)	○○	○	Potential co-isolated proteins and *protein corona* in serum EVs
Nucleic acid content (NA/P spectral ratio)	○	○○	Consistent cargo of nucleic acids biomarkers in saliva EVs

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