

VCSQUS VES4US: Extracellular vesicles from a natural source for







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Nicolas Touzet (1), Daniele Romancino (2), Giorgia Adamo (2), Mauro Manno (3), Antonella Cusimano (2), Sabrina Picciotto (2), Samuele Raccosta (3), Vincenzo Martorana (3), Rosina Noto (3), Rita Carrotta (3), Elia Di Schiavi (4), Giovanna L. Liguori (5), Annamaria Kisslinger (6), Katharina Landfester (7), Blanca Rodriguez (8), Svenja Morsbach (7), Darja Božič⁽⁹⁾, Ales Iglic ⁽⁹⁾, Veronika Iglic ⁽⁹⁾, Laura Corcuera ⁽⁸⁾, Paolo Arosio ⁽¹⁰⁾, Gabriella Pocsfalvi ⁽⁴⁾ and Antonella Bongiovanni ⁽²⁾ (1) Institute of Technology Sligo – ITSligo, (2) Institute of Biomedicine and Molecular Immunology (IBIM) - National Research Council of Italy (CNR), (3) Institute of Biophysics - CNR, Italy

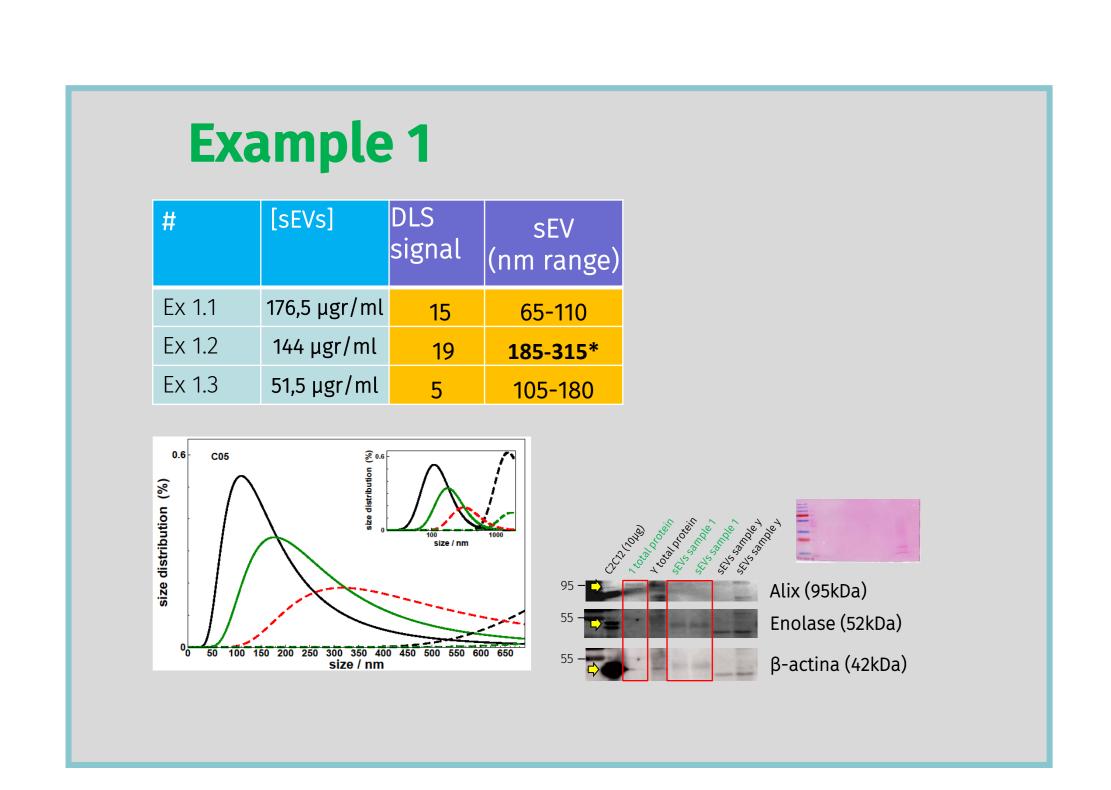
(4) Institute of Bioscience and BioResosrces (IBBR) – CNR, Italy, (5) Institute of Genetics and Biophysics - CNR, Italy, (7) Max-Planck Institute for Polymer Research, (8) Zabala Innovation Consulting, (9) University of Ljubljana, (10) ETH Zurich Institute for Chemical and Bioengineering

VES4US is a new European project funded by the Horizon 2020-Future and Emerging Technology (FET) Open program, which aims to develop an innovative platform for the efficient production of extracellular vesicles (EVs) from a renewable biosource, enabling their exploitation as tailor-made products in the fields of nanomedicine, cosmetics and nutraceutics (https://ves4us.eu). The project will run for the next three years (2018-2021) with 6 organizations from 6 European countries.

A core aspect of the project is to focus on an identified natural source to constitute a cost-effective and sustainable source of EVs.

In the ongoing first phase, the focus will be the selection of the natural source and the optimization of culture condition at preindustrial scale. Then, the isolation and physiochemical characterization of the extracellular vesicles will be realized.

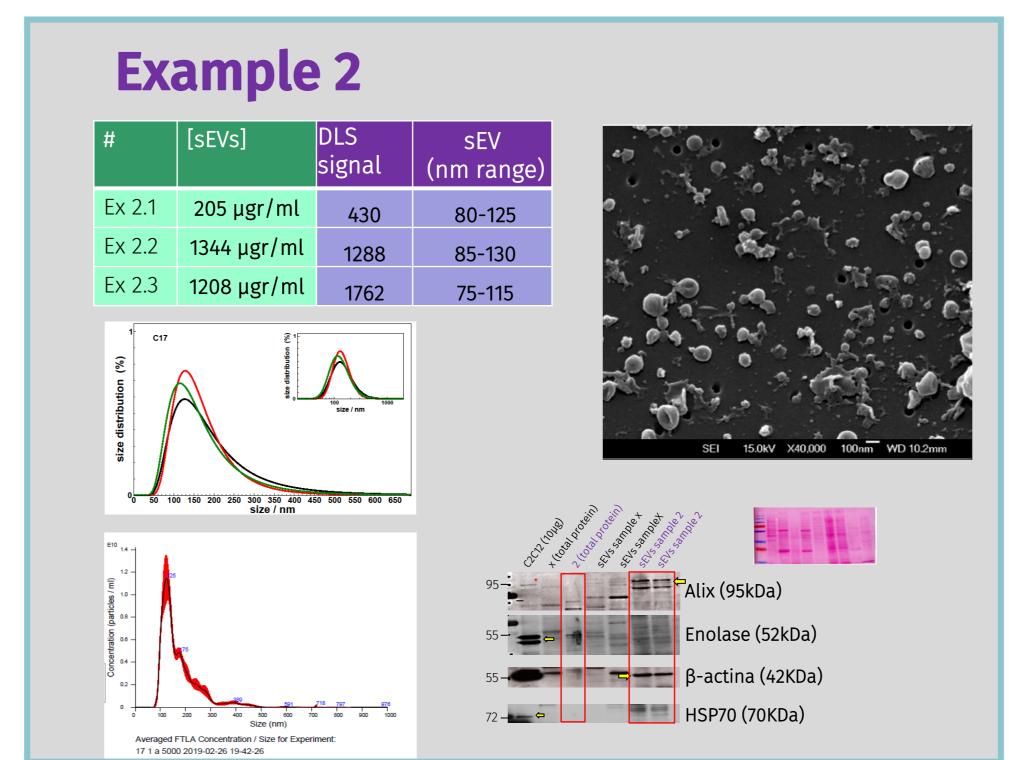
In the second phase, the functionalization and load of the EVs will be approached. Finally, the biological activity of the EVs will be explored both in vitro and in vivo.



MATERIAL & METHODS

- differential standard gold ultracentrifugation protocol has been applied. EVs (small EVs, sEVs) have been recovered by 118 kg UC and analyzed;
- > Particle number has been measured by light scattering technologies, such as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS);
- > Total EV protein amount has been measured by colorimetric assays microbicinchonic acid (BCA);
- > ImmunoBlot analyses were performed to identify and establish markers of natural source EVs;
- > Imaging analyses were produced by microscopy scanning electron (SEM) techniques;
- In the decision matrix, each criterion is given a weight (1 = lowest to 3 = highest) based on its importance in the final decision, and each proposal is assessed against each criterion (0 = lowest to 3 = highest).

The sum of the weighted assessments gives the final score for each source.



Criteria		Source				A		В		С		D				E		F		G		Н	
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'settling' for separation by gravity/centrifugation				ion 2		1	2	1	2	1	2	1	2	0	0	1	2	0	0	1	2	0	C
lesser contamination risk				2		2	4	2	4	2	4	2	4	2	4	2	4	2	4	2	4	1	2
sequenced genome				1		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	C
[sEV proteins+n°particles]				2		1	2	1	2	2	4	2	4	1	2	0	0	3	6	2	4	0	С
DLS signal quality				1		0	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1	0	C
size distribution (DLS+NTA)				3		1	3	3	9	2	6	2	6	1	3	1	3	2	6	3	9	2	6
EV Marker 1 (Alix/PDCD6IP)				11		0	0	1	1	2	2	3	3	1	1	2	2	2	2	1	1	3	3
EV Marker 2 (enolase)				1		0	0	2	2	1	1	1	1	0	0	1	1	1	1	2	2	2	2
EV Marker 3 (others)				1 1		0	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	0	C
sensitivity to NP40/SDS 2					2		2		2		2	-	2		2		2		2	_	2		
		SEM		2		Х	۷	2	4	1	2	1	2	Х	2	Х	2	2	4	3	6	2	4
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3		3	2	6	-	2	6	3	9	3		9	3	9	1	3	2	6	1		3	0	0
2		2	1	2		2	4	1	2	1	2	2	0	0	1	2	3	6	1		2	0	0
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					1										1				1				

Legend: W = weight of criterion (1=low; 2=medium; 3=high impact); A = Assessment of option (1=low; 2=medium; 3=high adherence to criteria); Result = W x A

settling' for separation by gravity/centrifugation: bad=0 good=1

sequenced genome: 0=no sequenced; 1=sequenced

[EV proteins]: 0=0; 1=0.1-0.2; 2=0.2-0.5; 3=>0.500 mg/ml size distribution: 1=low signal/wide distribution; 2=medium signal/distribution; 3=good signal/nice distribut

protein marker: 0=0; 1=low level; 2= high level

OBJECTIVES – First Phase

- 1. Objective 1. The isolation of EVs from different natural sources and the design of the EV-source selection grid
- 2. Objective 2. The selection of the best EV-producing natural source strain/s

RESULTS

According to the ISEV Minimal information for studies of extracellular vesicles (MISEV2018) we are selecting the best of 19 EV-producing natural source strain/s.

For any EV samples we have information about the protein concentration (BCA assay), the particles dimension and particles concentration (DLS, NTA analysis). Finally the electron microscopy analyses give us information about the shape of EV isolated and about the grade of purity of samples.

For each biosource, we have generated an 'EV Identity Card', containing all EV features. The EV identity Cards allowed us to finalize a decision grid, the EV **Evaluation Grid.**

The resulting score, for each biosource, allowed to elaborate a ranking of the best EV-producing natural source/s.

CONCLUSIONS

- > We have enough information to select the source eligible for the next phase.
- > Safe, efficient and specific nanodelivery systems are essential to current therapeutic medicine, cosmetic and nutraceutics sectors. The ability to optimize the bioavailability, stability, and targeted cellular uptake of a bioactive mitigating while toxicity, molecule immunogenicity off-target/side and effects is of the utmost priority. VES4US aims at creating a fundamentally new bioprocessing approach to generate and functionalize EVs from a renewable biological source.