A renewable and scalable bioprocess: extracellular vesicles from microalgae

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CellTech-Hub

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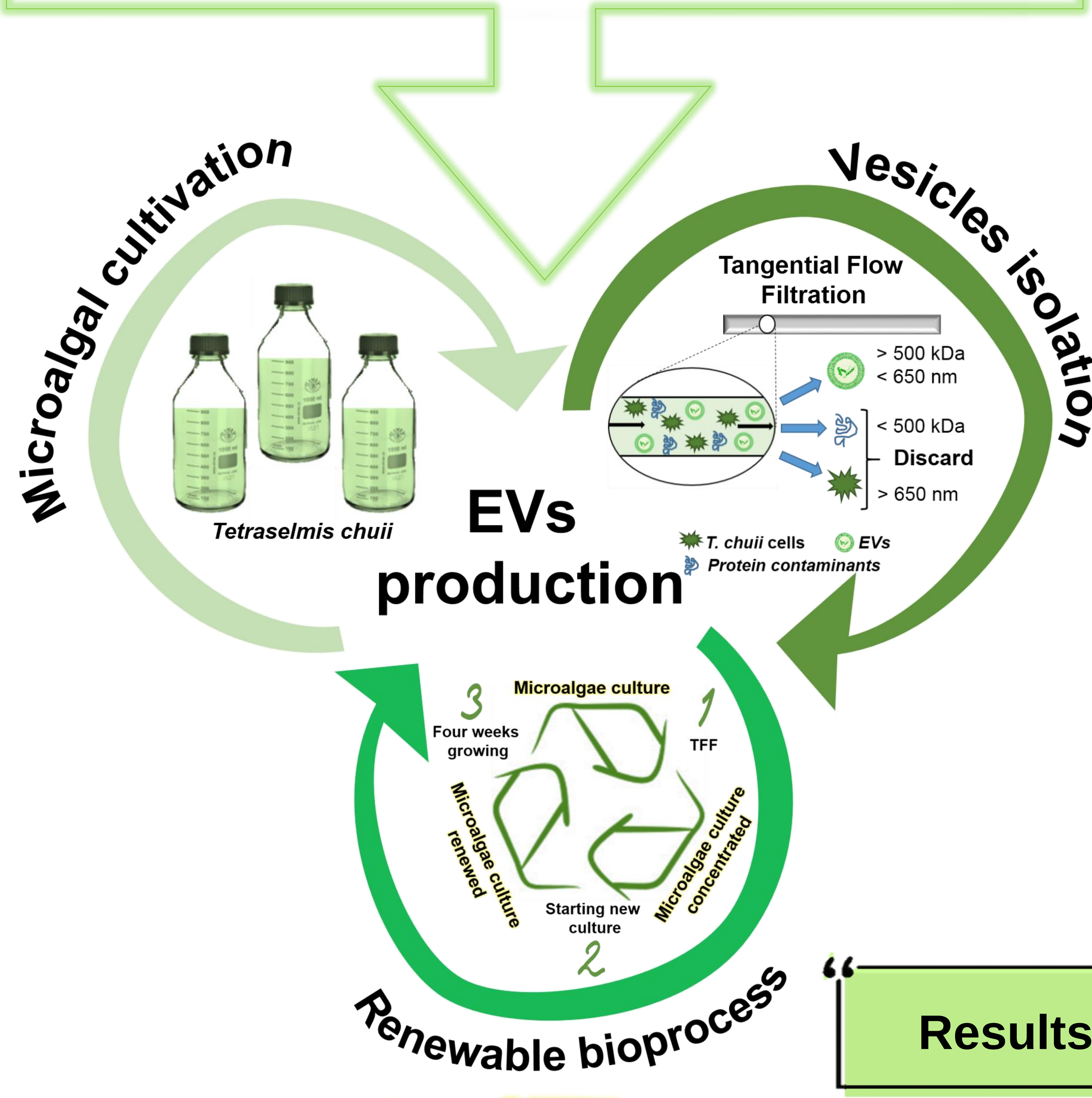
Outline

- *Microalgae: EVs natural source*
- *Nanoalgosomes: small EVs from T. chuii*
- *EVs production and isolation from a fully renewable source*
- *Application in theranostics, cosmetics and nutraceutics*

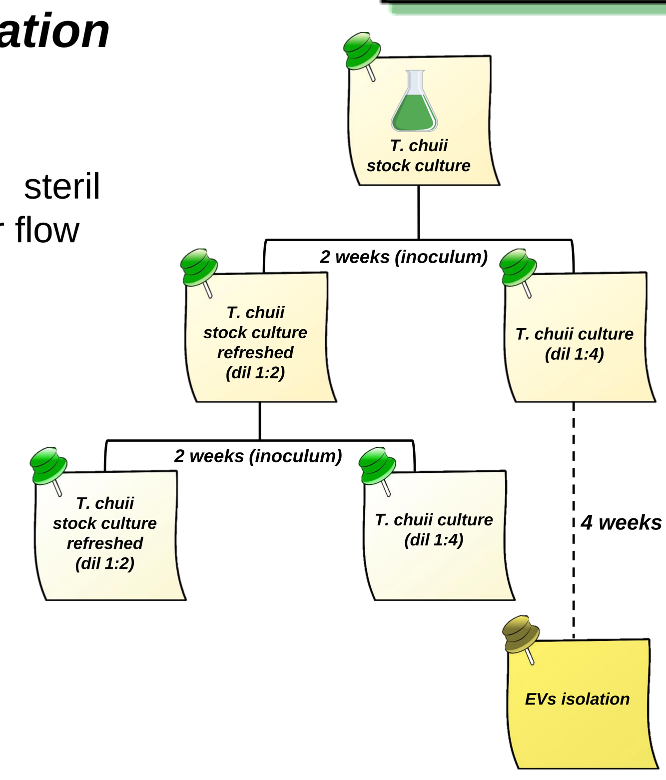


Methods

Microalgal cultivation

- Microalgae strain *Tetraselmis chuii*
- Cultivation in bioreactors, in steril conditions and under continuous air flow
- Vesicles isolation after 4 weeks

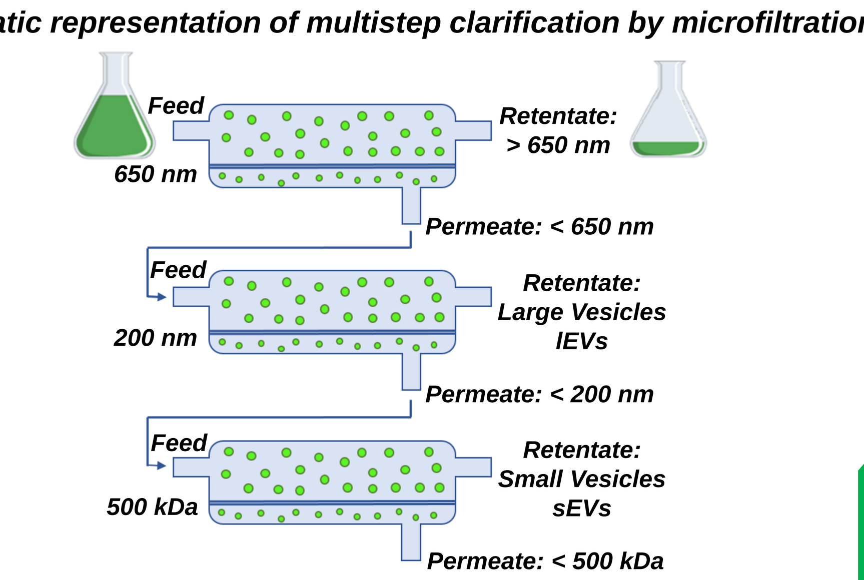
Tetraselmis chuii
(Image from http://cfb.unh.edu)



Vesicles isolation

Tangential Flow Filtration

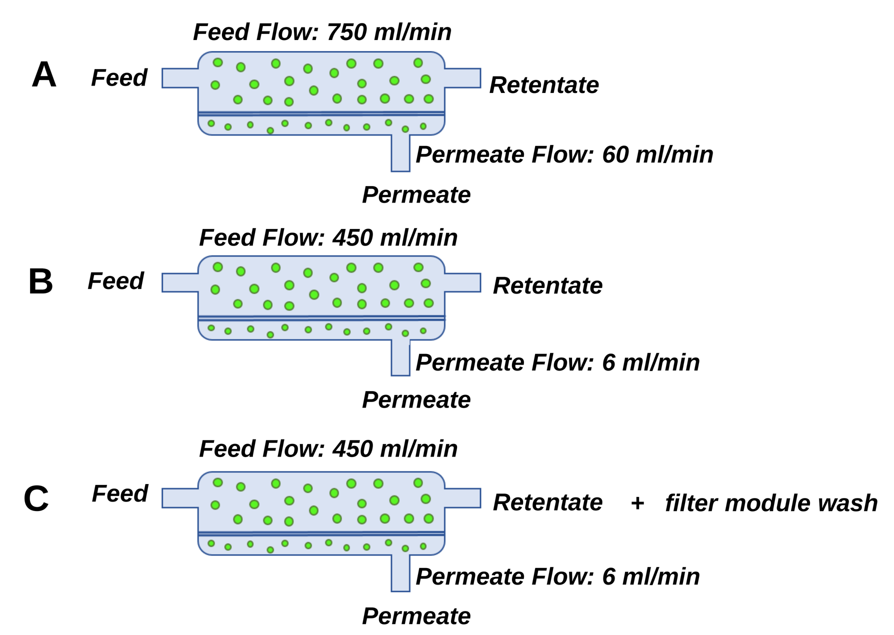
Schematic representation of multistep clarification by microfiltration



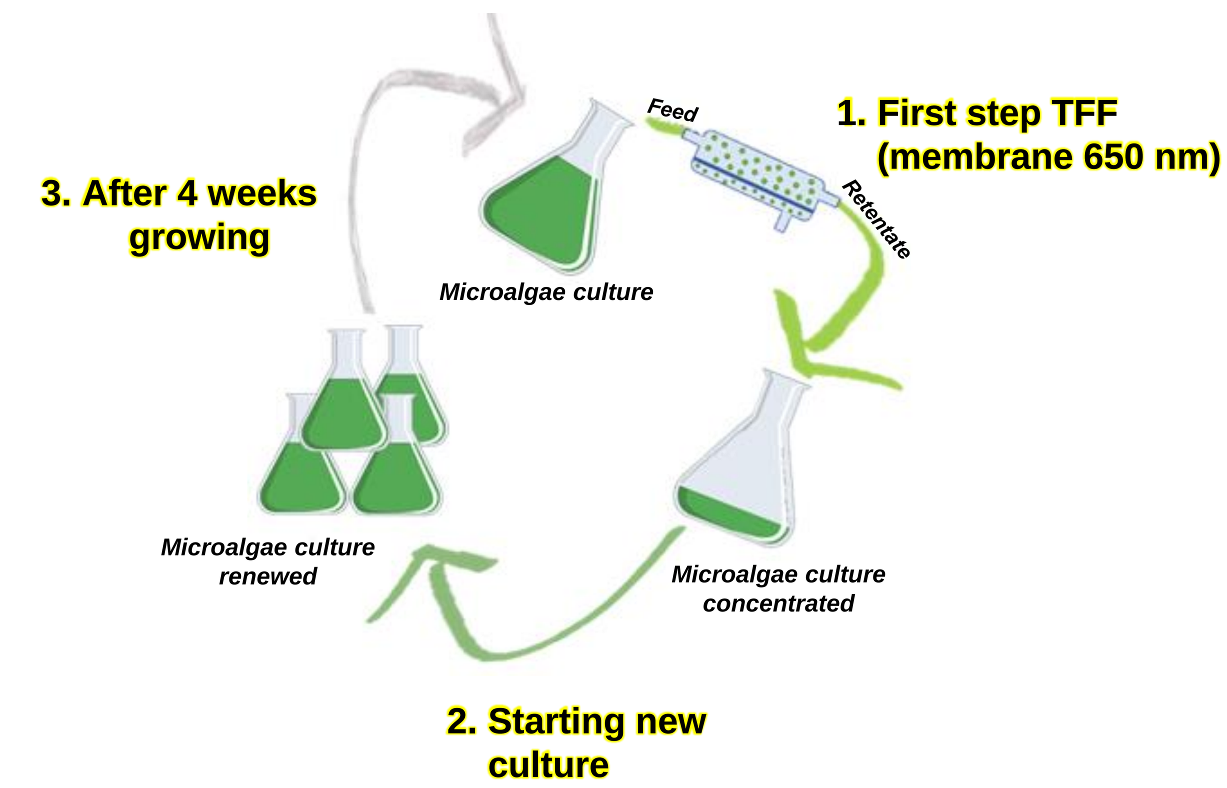
Large and small vesicles have been concentrated and diafiltrated through a smaller 500 kDa membrane reaching enriched samples of EVs in PBS.

Optimization of TFF parameters

Different conditions have been investigated in order to increase the yield of isolation process.



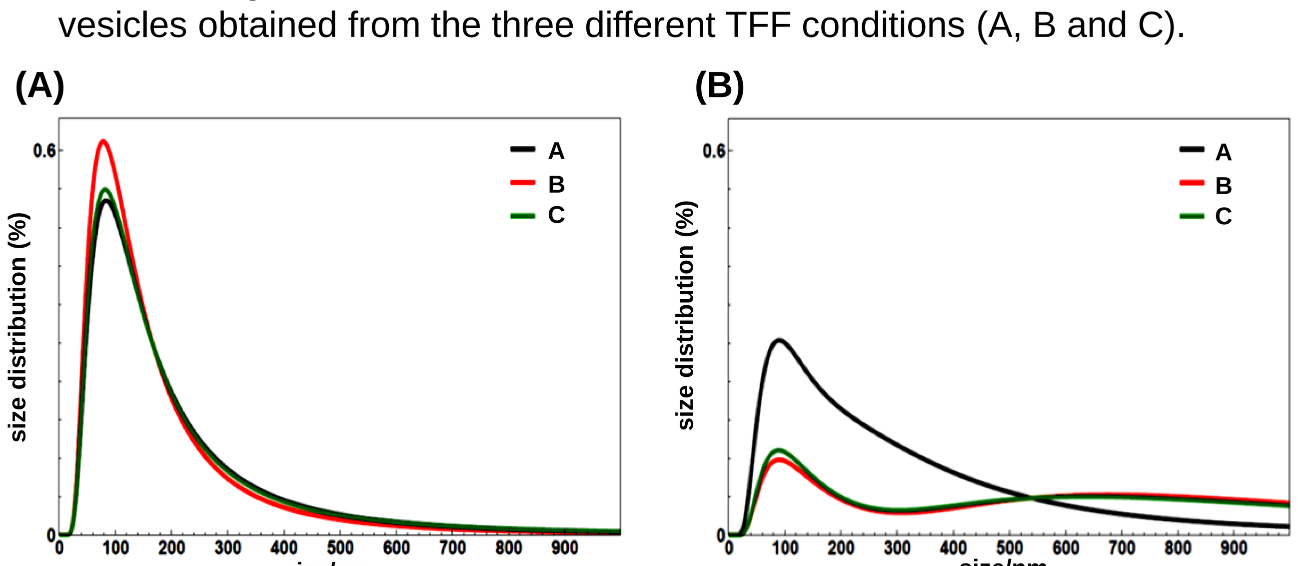
EVs production is a Renewable Bioprocess



Results

Optimization of TFF parameters

DLS analysis. Size distribution of the A) small vesicles and B) large vesicles obtained from the three different TFF conditions (A, B and C).

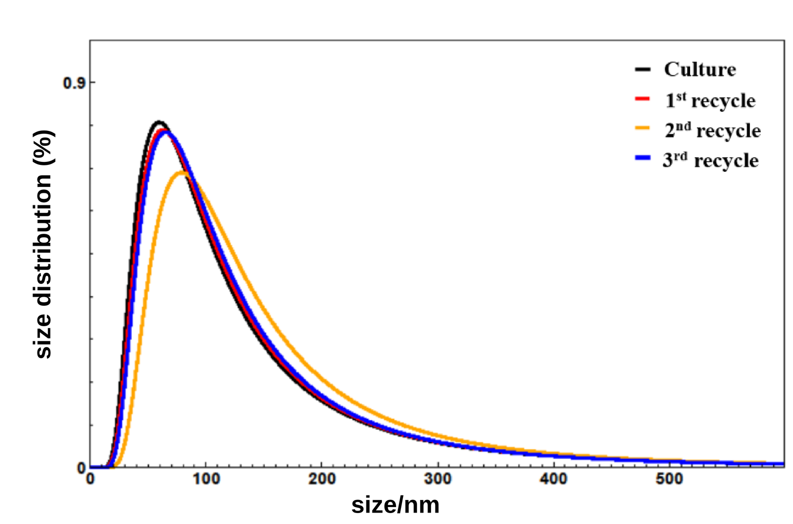


	Small vesicles (sEVs)			Large vesicles (lEVs)		
	A	B	C	A	B	C
z-average hydrodynamic diameter (nm)	85 ± 5	85 ± 5	85 ± 5	120 ± 15	360 ± 15	340 ± 15
Rayleigh ratio (10^{-6} cm^{-1})	605 ± 5	1000 ± 5	1250 ± 5	790 ± 5	320 ± 5	300 ± 5

	Small vesicles (sEVs)		
	A	B	C
Protein content (µg/mg)	0.05 ± 0.007	0.029 ± 0.007	0.042 ± 0.004
Particles number (p/mg)	3.2 x 10^8	7.63 x 10^8	8.11 x 10^8

EVs production is a Renewable bioprocess

DLS analysis. Size distribution of small vesicles isolated from *T. chuii* cultures (culture, 1st, 2nd and 3rd Recycle).



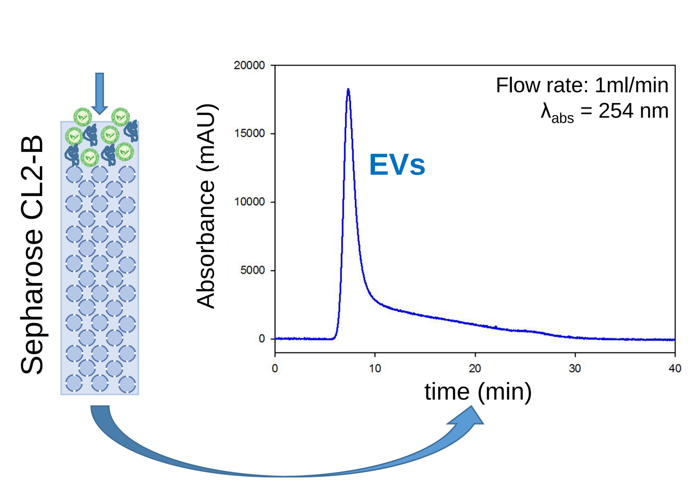
	Small vesicles (sEVs)			
	Culture	1st recycle	2nd recycle	3rd recycle
z-average hydrodynamic diameter (nm)	85 ± 5	85 ± 5	100 ± 5	90 ± 5
Rayleigh ratio (10^{-6} cm^{-1})	7255 ± 5	6010 ± 5	7645 ± 5	5085 ± 5

	Small vesicles (sEVs)			
	Culture	1st recycle	2nd recycle	3rd recycle
Protein content (µg/mg)	0.375 ± 0.004	0.383 ± 0.006	0.425 ±0.007	0.435 ± 0.004
Particles number (p/mg)	5.15 x 10^8	5.00 x 10^9	5.4 x 10^9	1.14 x 10^{10}

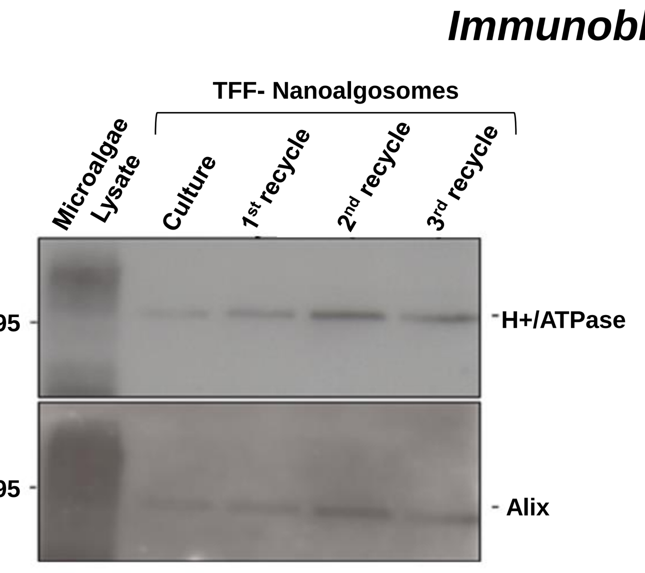
EVs characterization. Z-average hydrodynamic diameter and Rayleigh ratio have been determined by light scattering measurements. Protein content and particle number have been determined by means of BCA assay and NTA; respectively. Results are expressed as the ratio between µg of protein or particles number and mg of dry biomass.

Quality Check

Size Exclusion Chromatography



Immunoblot analysis

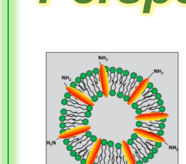


Representative immunoblot analysis of H+/ATPase and Alix in *T. chuii* cells lysate (Microalgae lysate; 10 µg) and nanoalgosomes isolated by TFF from *T. chuii* cultures (culture, 1st, 2nd and 3rd Recycle; 5 µg).

Conclusions

- *Improvement of TFF isolation method*
- *Higher yield and purity*
- *Nanoalgosomes production is a scalable and renewable process*

Perspective



FUNCTIONALISATION of EVs and cargo enrichment

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BOW VES4US