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Extracellular vesicles: current knowledge, challenges and clinical perspectives

M1 - Development of Drugs and Health Products

TU 08 – Biotechnology

2024-2025

sameh.obeid@universite-paris-saclay.fr

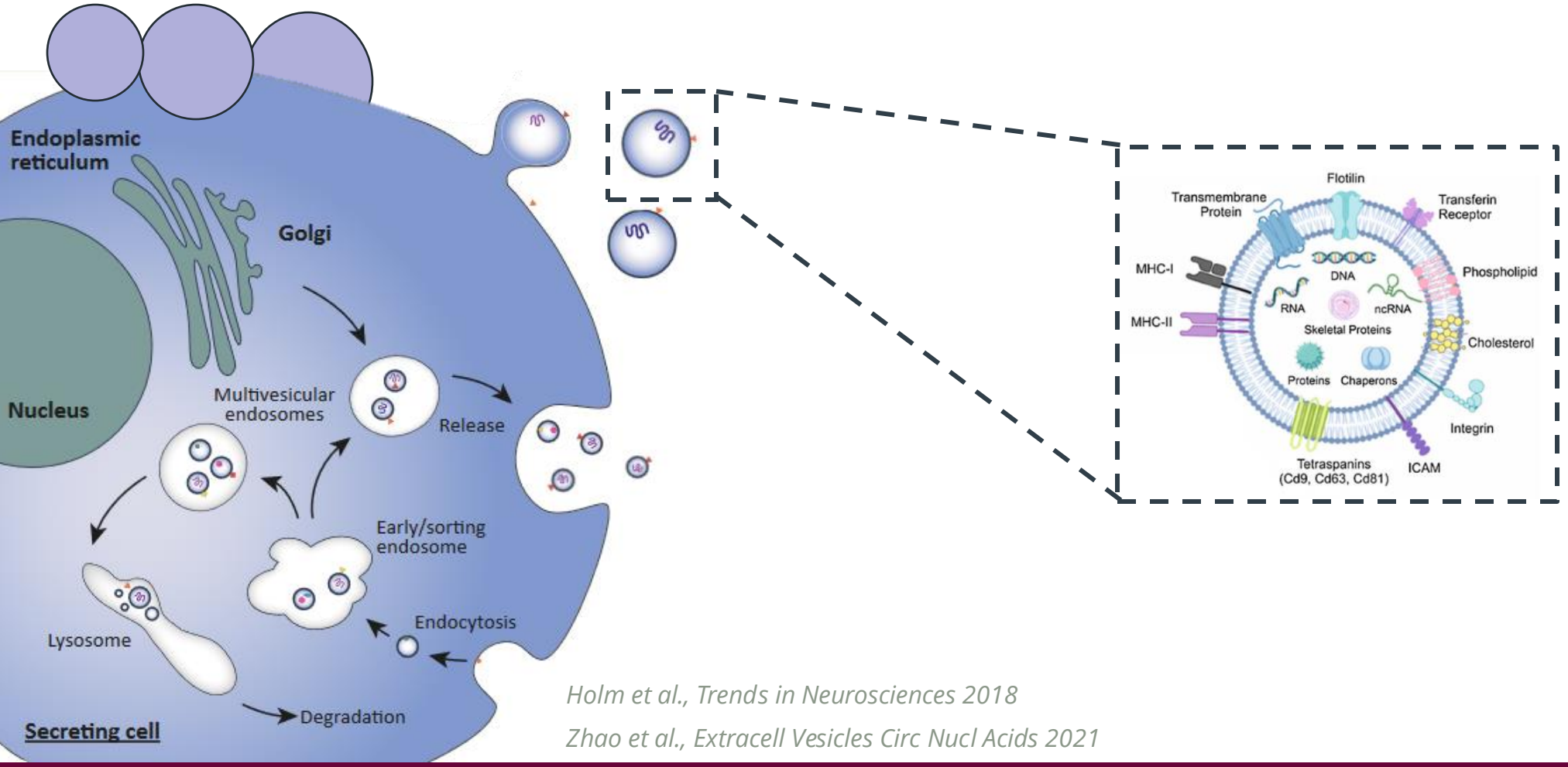
Outlines

- Extracellular Vesicles (EVs): biology and functions
- Biomolecular composition of EVs
- Clinical potential of EVs
- EVs as potential biomarkers of diseases
- Therapeutic potential of EVs
- EV-based therapy Vs cell-based therapy
- Sacale-up and manufacturing of EVs
- EV isolation and characterization

Extracellular Vesicles (EVs)

Extracellular Vesicles (EVs)

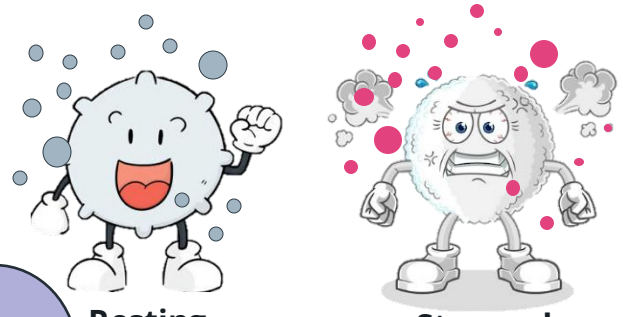
- Membranous **cell-derived nanoparticles**



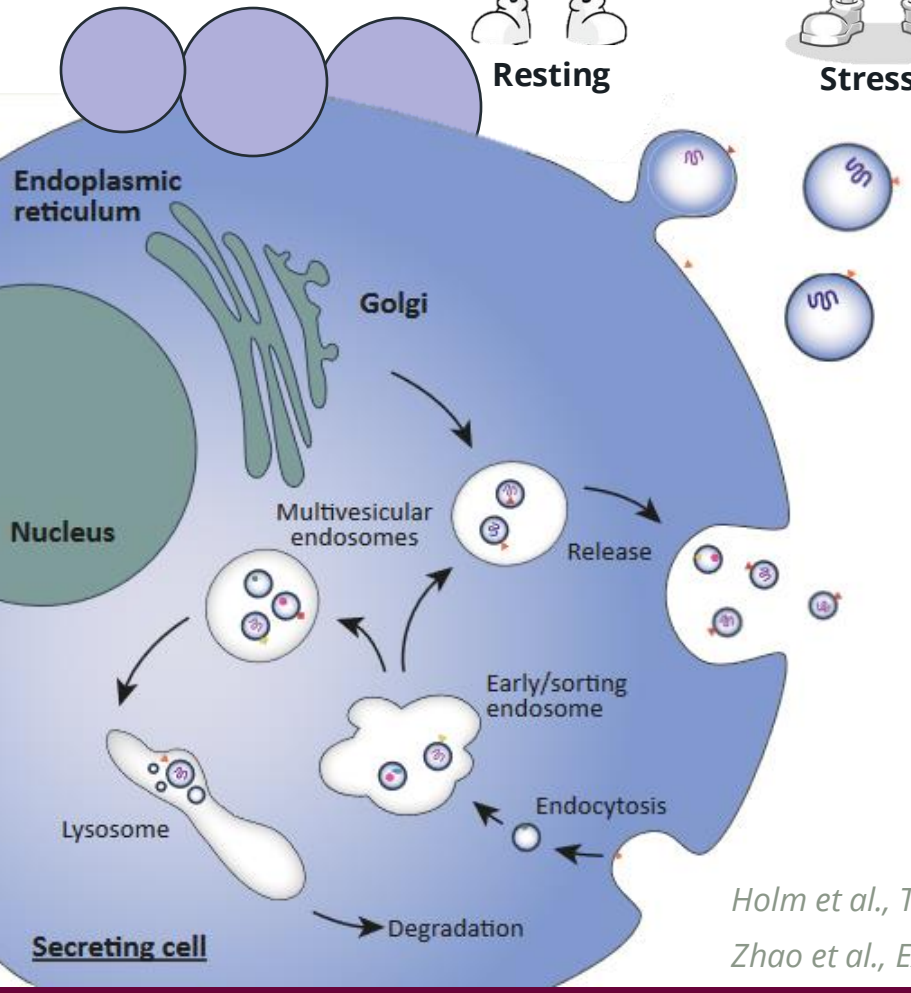
Holm et al., Trends in Neurosciences 2018

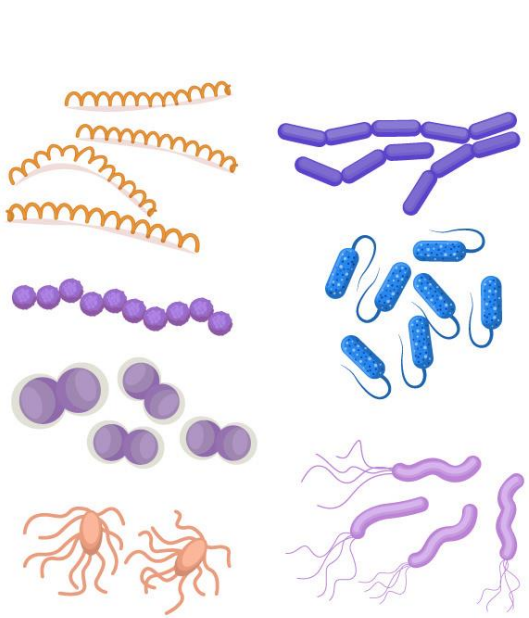
Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

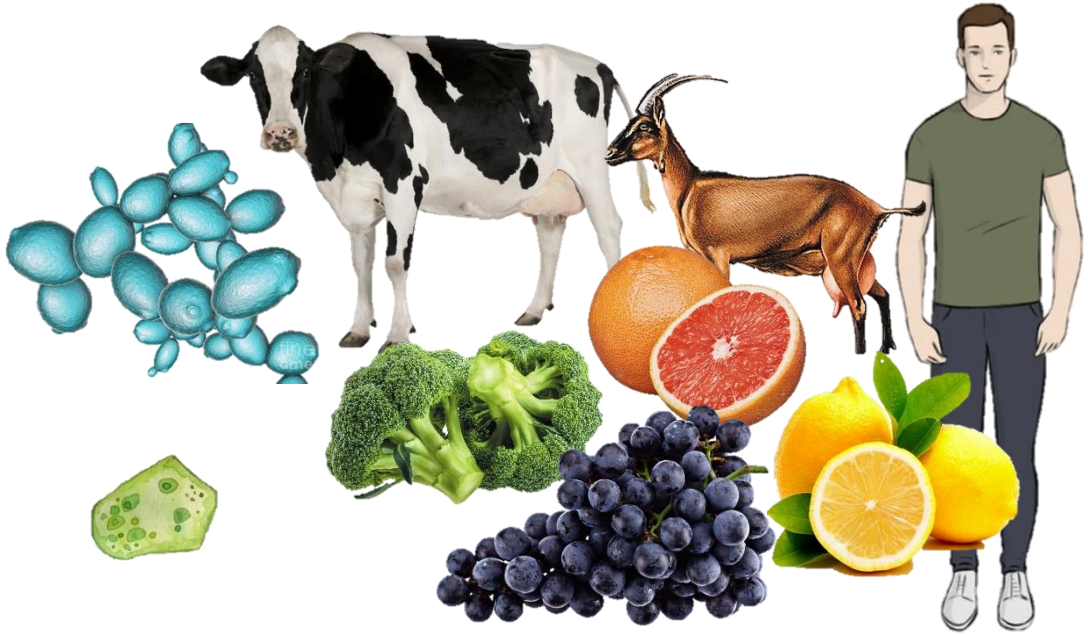
Extracellular Vesicles (EVs)



- Membranous **cell-derived nanoparticles**
- **Secreted by all cells** (eukaryotes et prokaryotes)





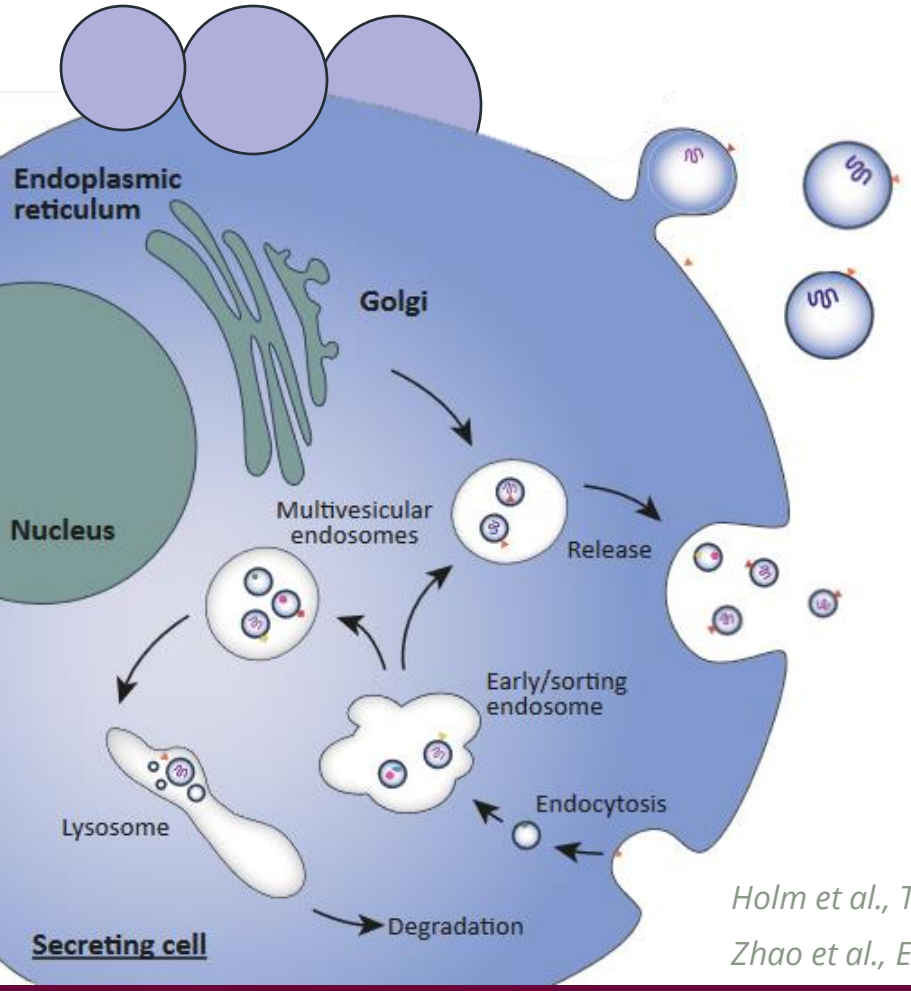


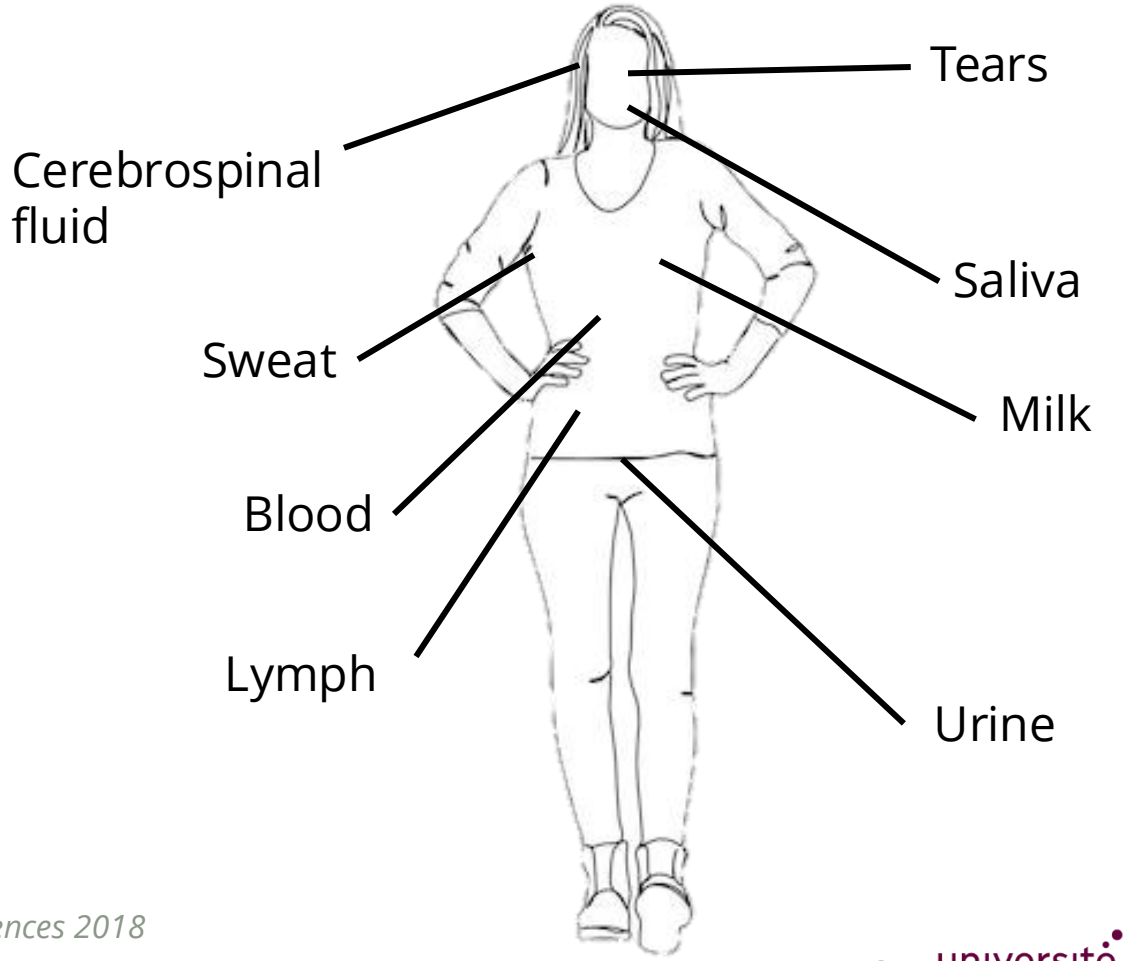
Holm et al., Trends in Neurosciences 2018

Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

Extracellular Vesicles (EVs)

- EVs are present in all **body fluids**



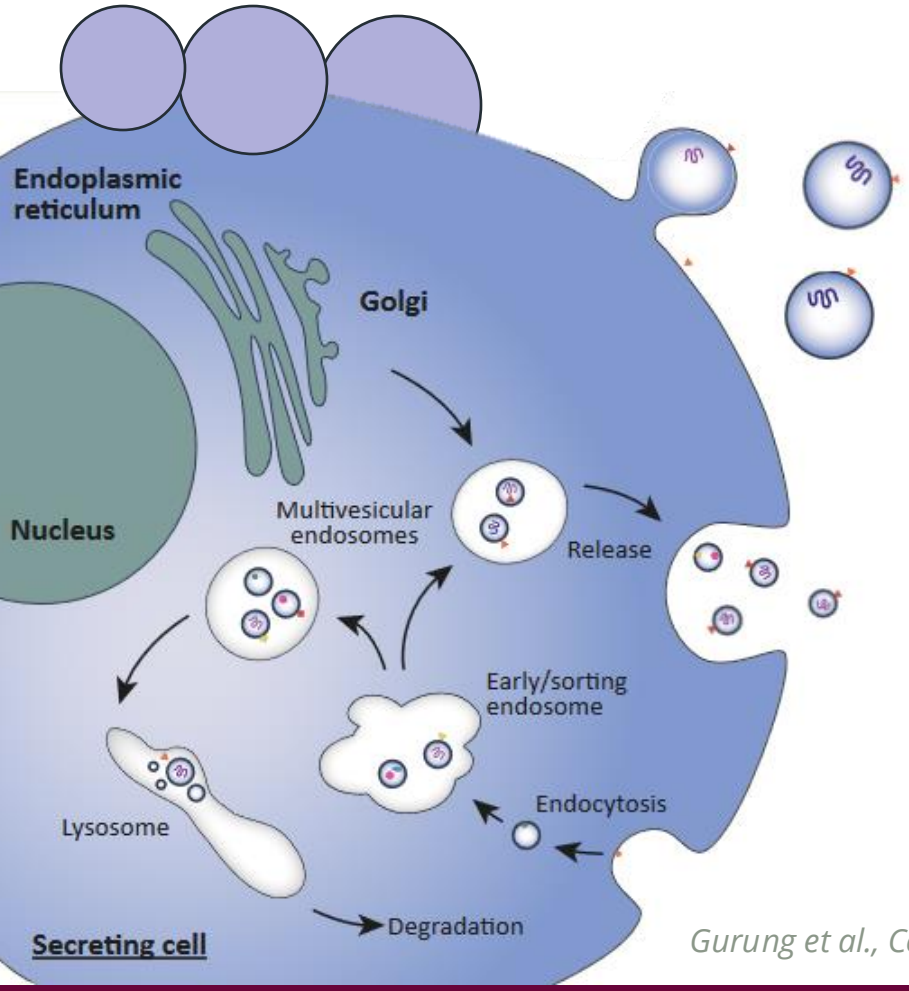


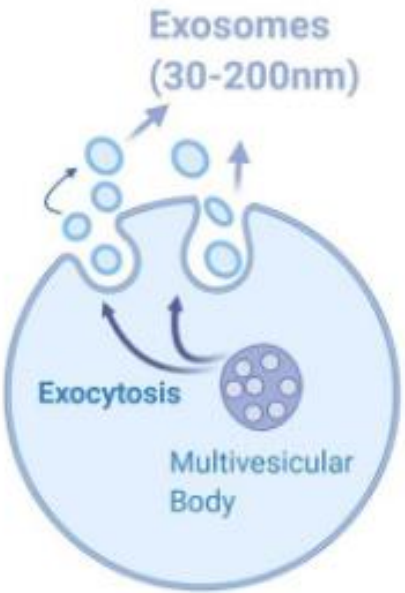
Holm et al., Trends in Neurosciences 2018

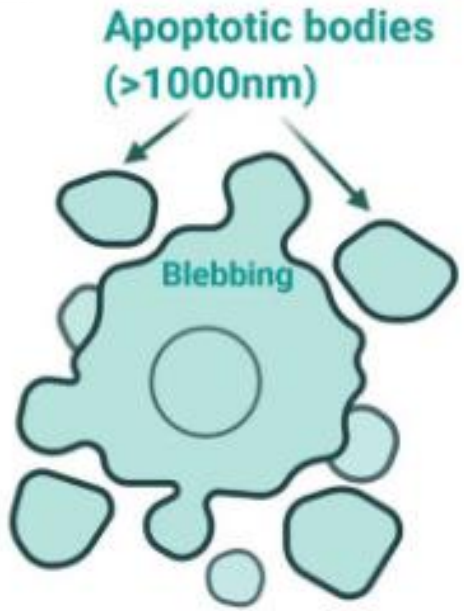
Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

Extracellular Vesicles (EVs): Classification

- Different mode of biogenesis => various subtypes
- Three main groups based on their size and biogenesis



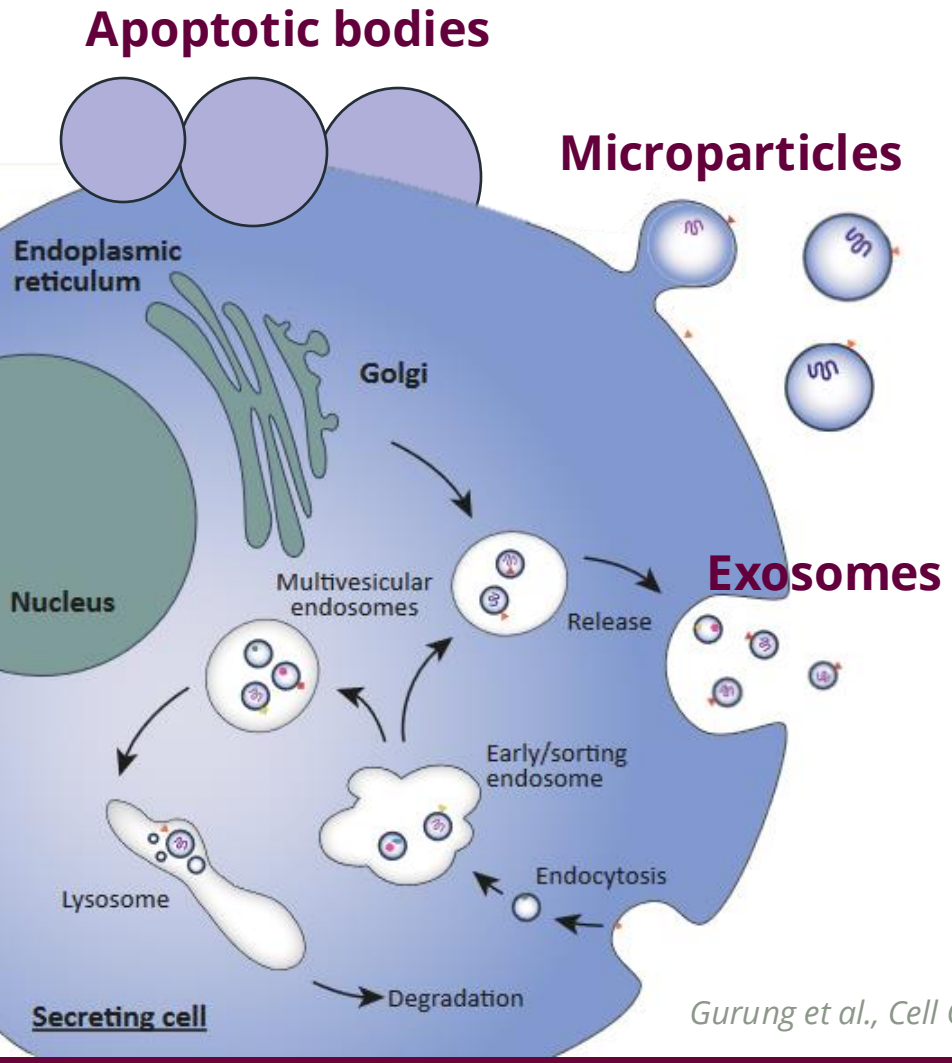




Gurung et al., Cell Communication and Signaling 2021

Extracellular Vesicles (EVs): Classification

- Different mode of biogenesis => various subtypes

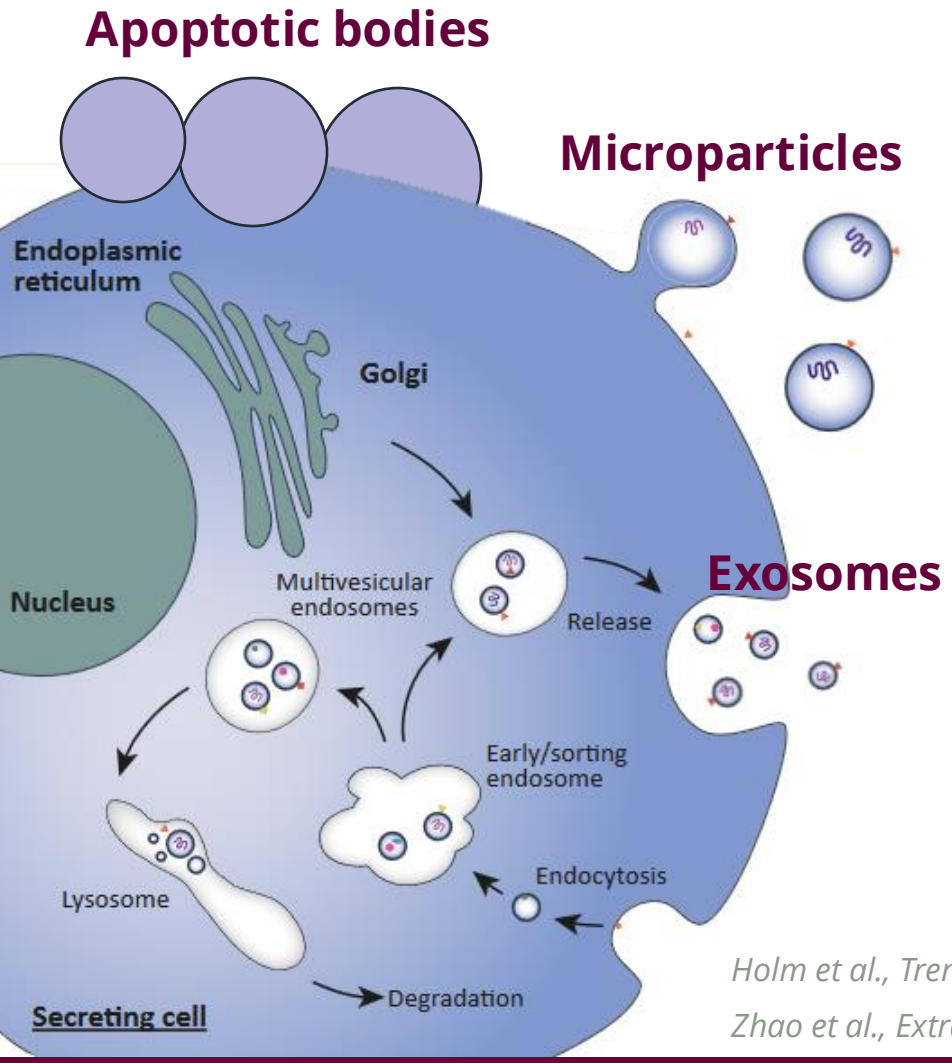


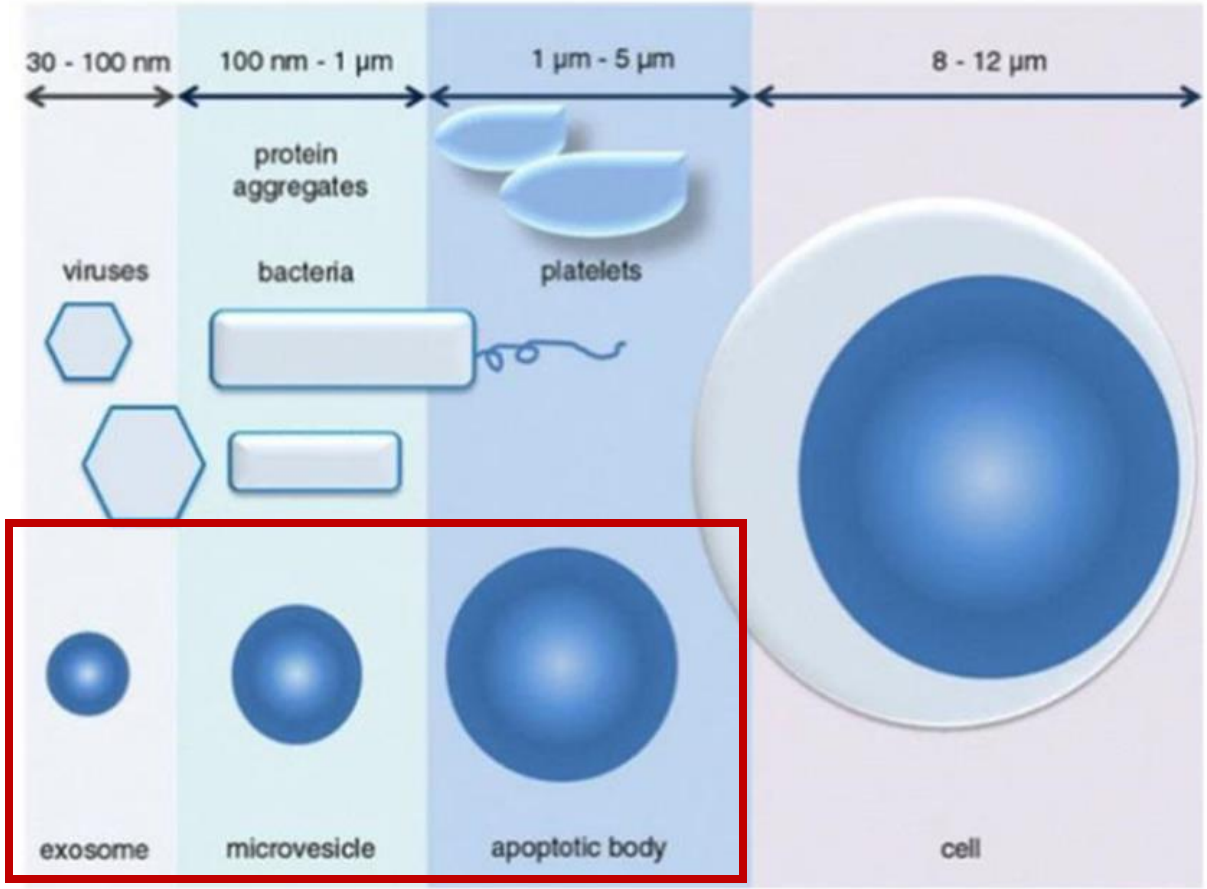
	Exo	MPs	Apop. bodies
Size	30-200 nm	100-1000 nm	500-5000 nm
Origin	Endocytic	Plasma membrane	Blebbing
Shape	Spheroid	Irregular	Variable
Composition	Protein, nucleic acids, lipids & metabolites	Protein, nucleic acids, lipids & metabolites	DNA fragments, histone, chromatine remnants, degraded proteins
Typical markers	Tetraspanins, ESCRT proteins (Alix, TSG101), Rabs, HSP	Integrins, slectins, CD40 ligand, flotilin-2, phosphatydilserine	Annexin-V, phosphatidyl-serine

Gurung et al., Cell Communication and Signaling 2021

Extracellular Vesicles (EVs): Classification

- Nanometric size de 30-5000 nm



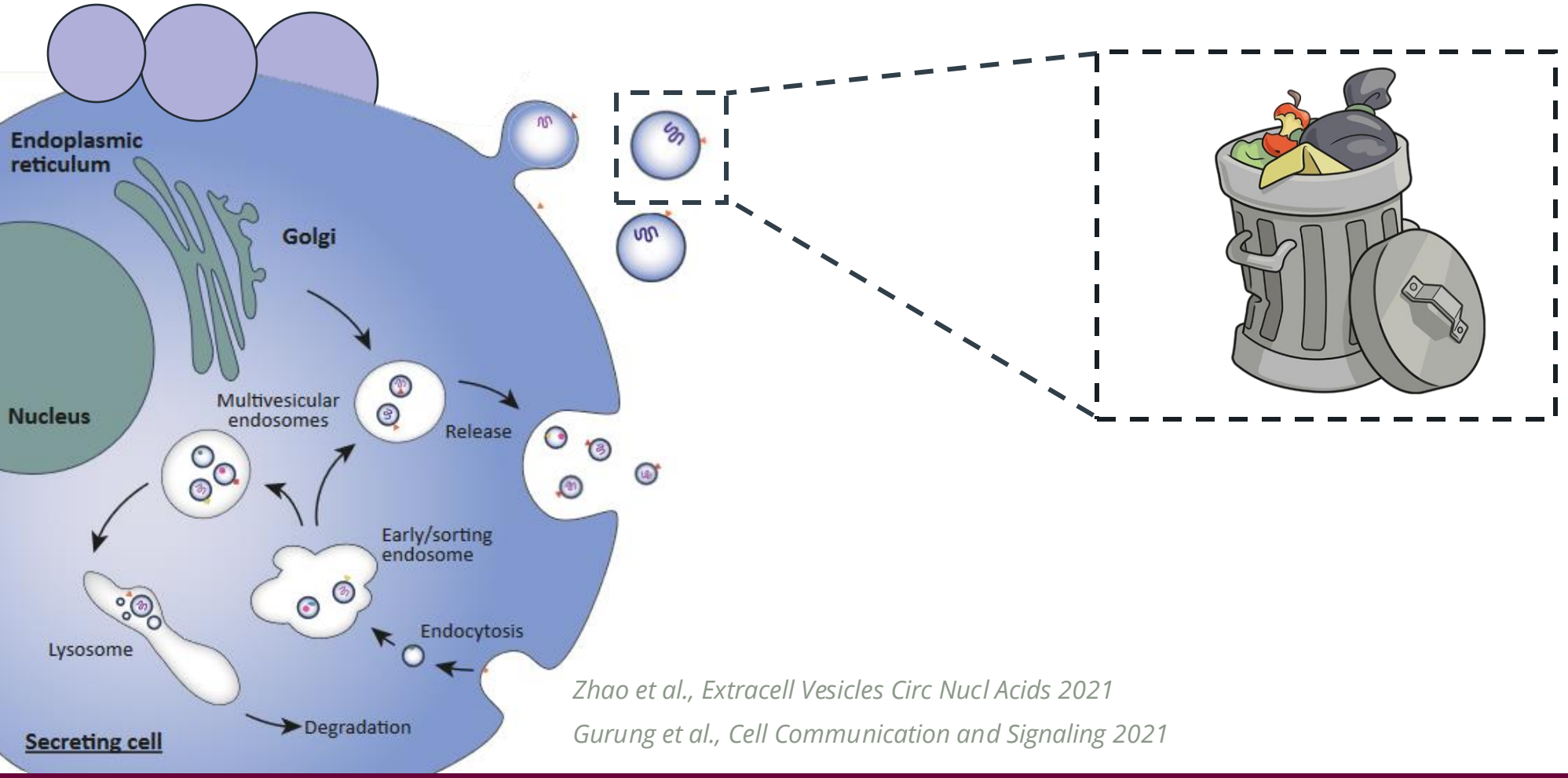


Holm et al., Trends in Neurosciences 2018

Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

Extracellular Vesicles (EVs): Role

- EVs **were described as waste carriers** of harmful molecules

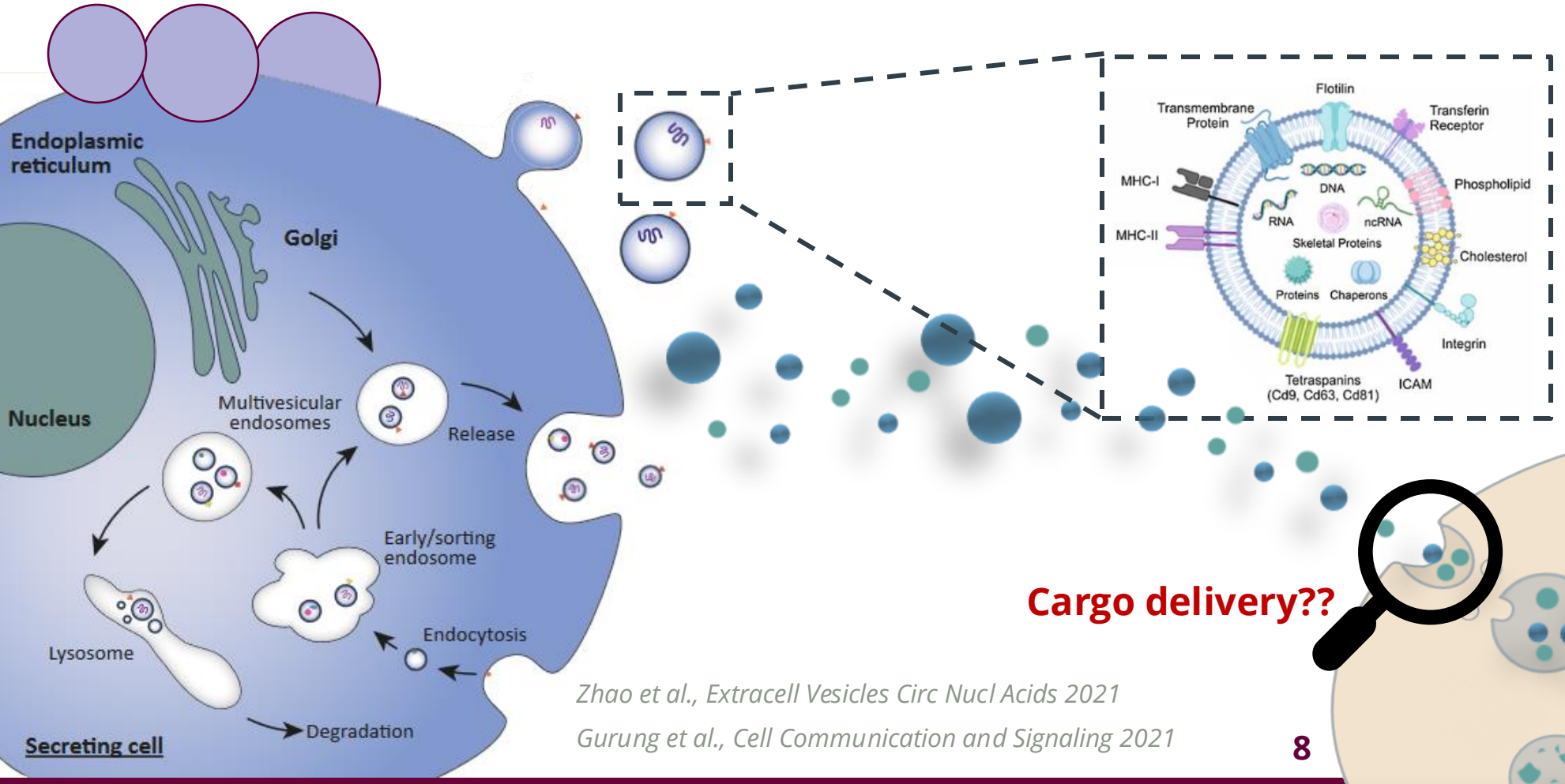


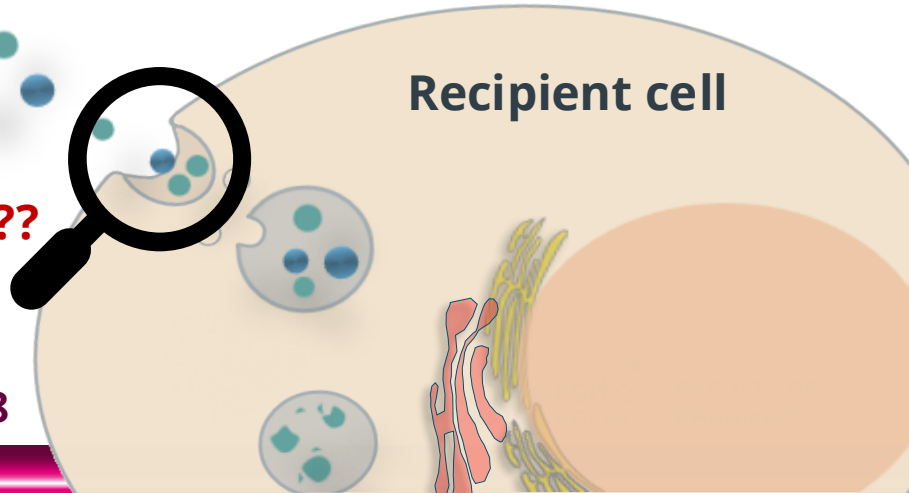
Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

Gurung et al., Cell Communication and Signaling 2021

Extracellular Vesicles (EVs): Role

- EVs play crucial role in short and long-distance intercellular communication



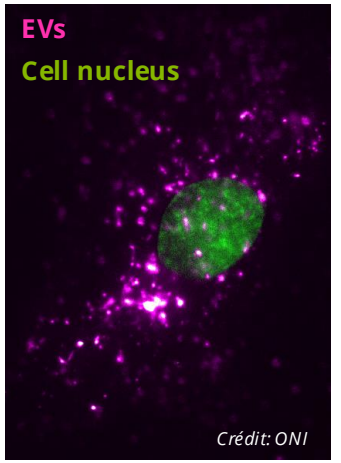


Cargo delivery??

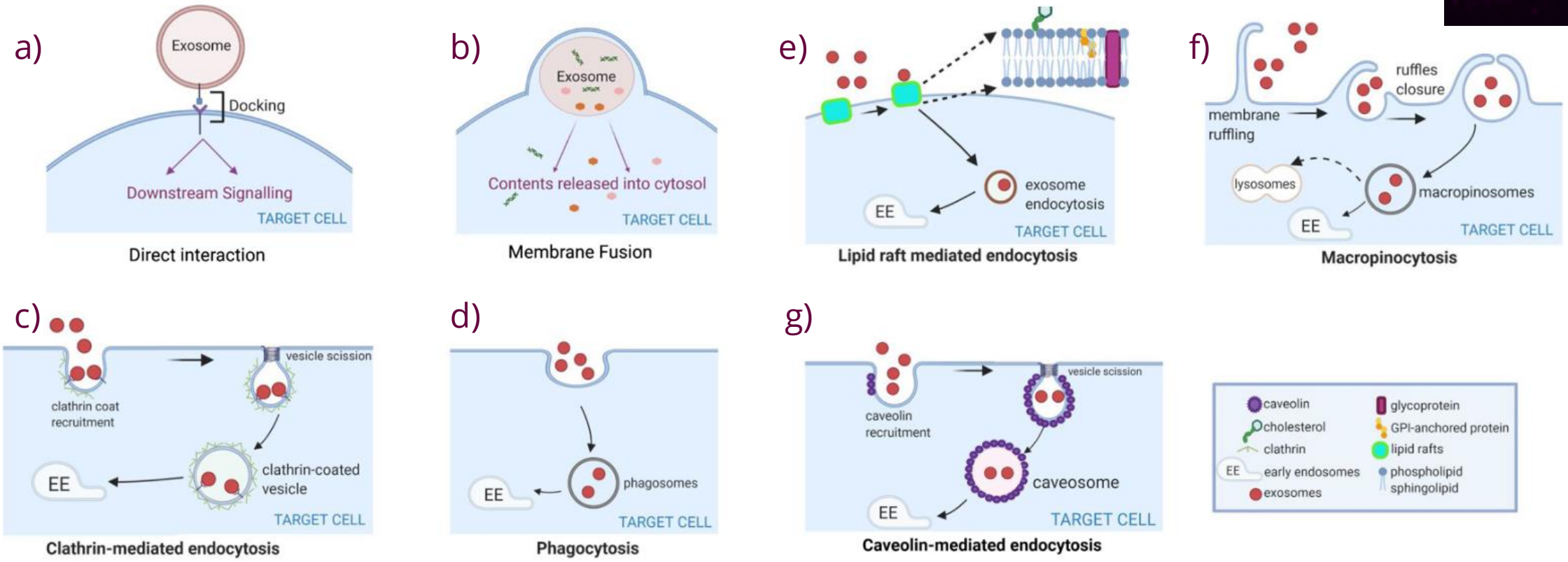
Zhao et al., Extracell Vesicles Circ Nucl Acids 2021

Gurung et al., Cell Communication and Signaling 2021

Extracellular Vesicles (EVs): Cargo delivery



- Recipient cells appear to take up EVs by a variety of pathways



Gurung et al., Cell Communication and Signaling. 2020

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Composition of EVs

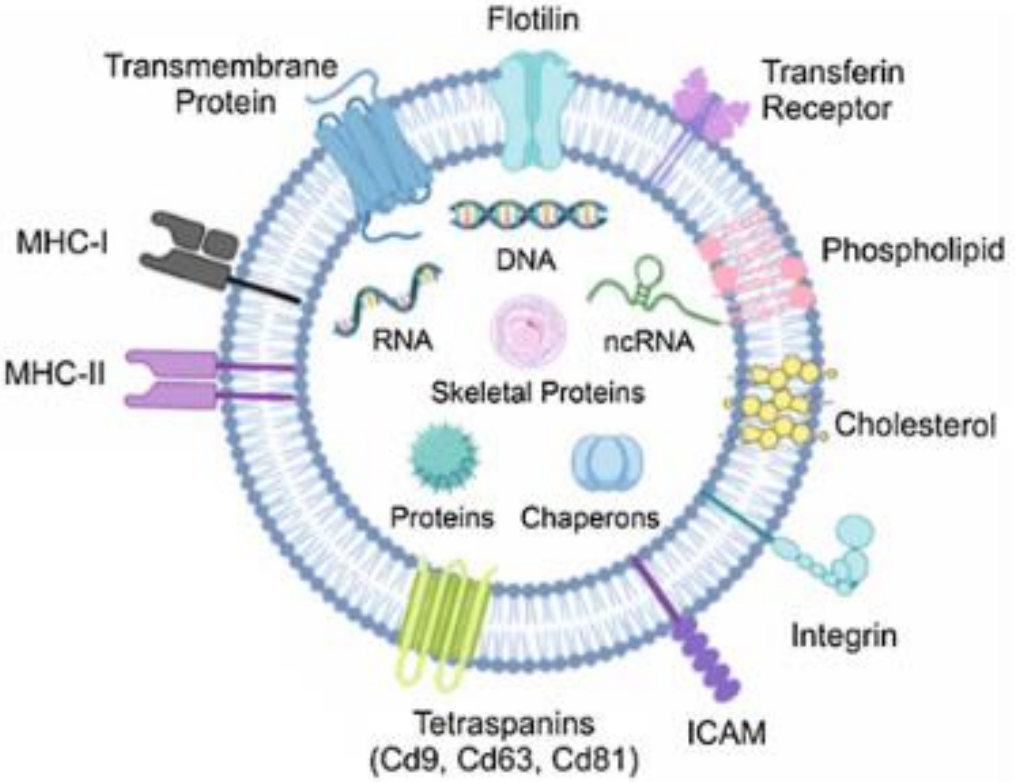
Composition of EVs

- Compositions depend on their **cell origin**, the secretion **stimulus** and their **mode of biogenesis.**
- Databases about EV compositions

ExoCarta

Vesiclepedia

EVpedia



Gangadaran et al., Pharmaceutics. 2020

Gézsi et al., Exp Mol Med 2019

De Sousa et al., Nanomed Nanobiotechnol 2023

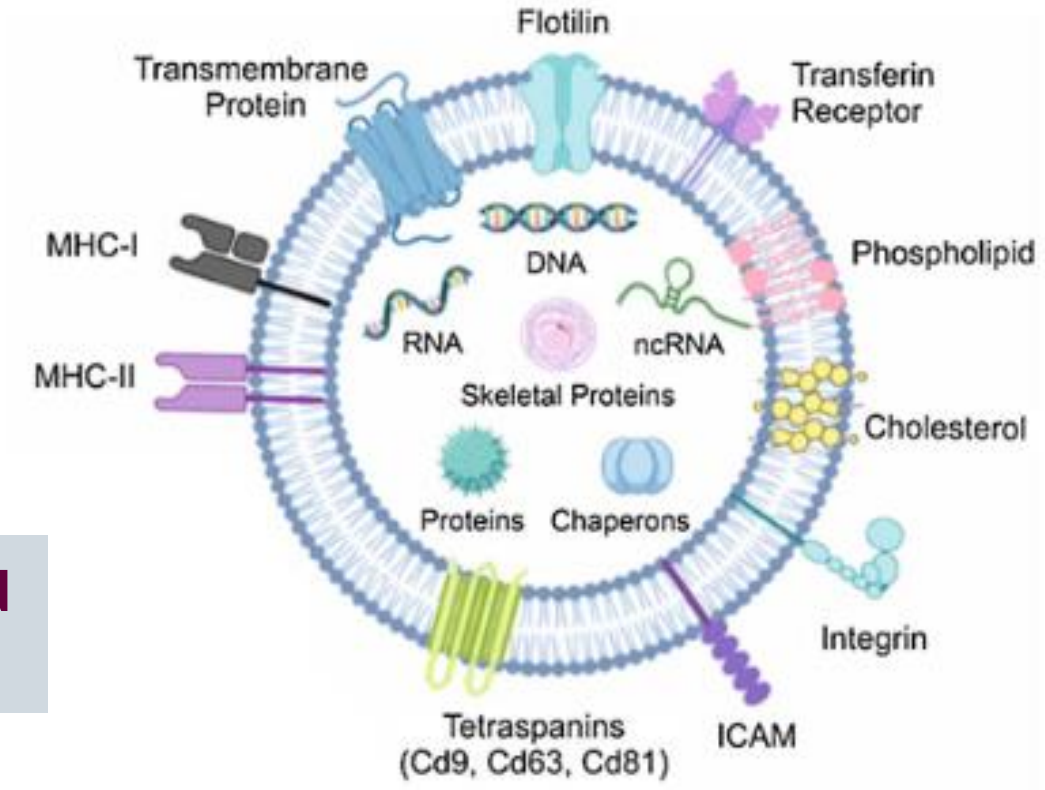
Composition of EVs: Lipids

- **Lipid content of EVs:**

➢ Lipid membrane bilayer

➢ Enriched in elements of lipid rafts:

cholesterol, ceramide, sphingomyelin, phosphatidylcholine and phosphatidylserine



Participate in membrane fusion between EVs and recipient cells

Gangadaran et al., Pharmaceutics. 2020

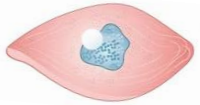
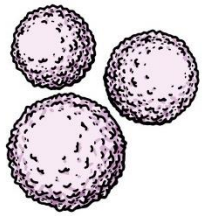
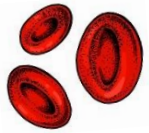
Gézsi et al., Exp Mol Med 2019

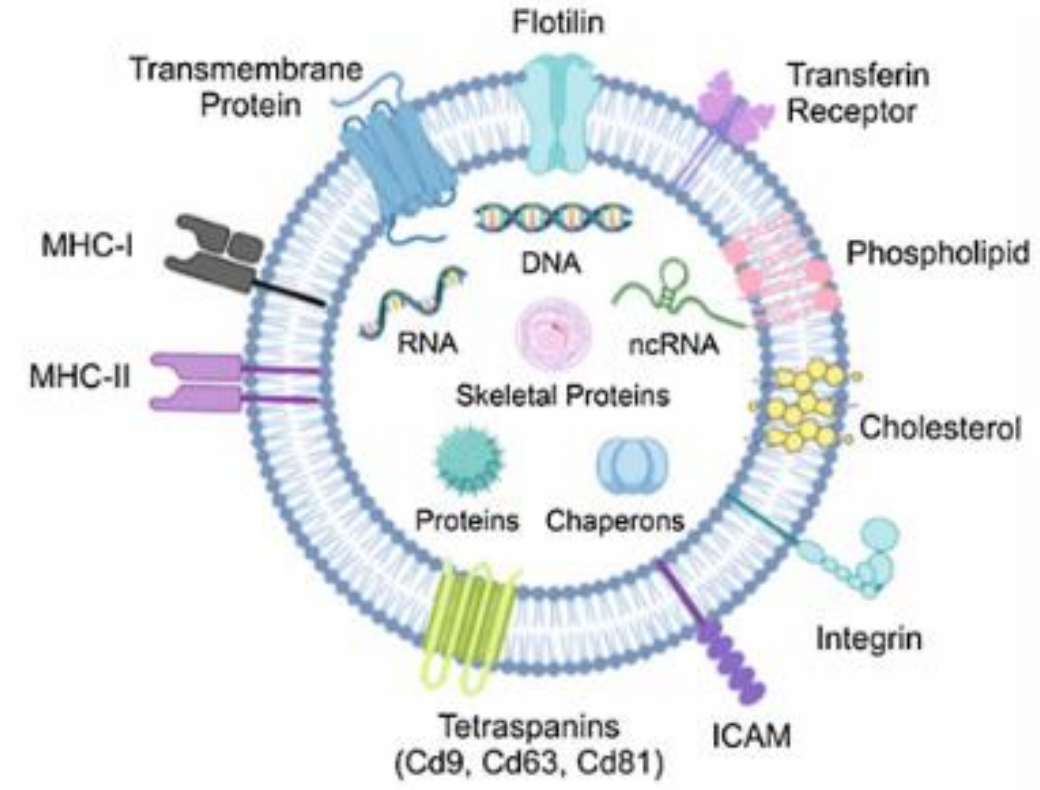
Composition of EVs: Proteins

- **<u>Protein content of EVs</u>:**

➢ Main components

➢ Show a cell origin signature

	Cell origin	Specific markers
	Platelets	CD41, CD31, CD42a/b, CD61, CD62P
	Endothelial cells	CD144, CD31, CD34, CD62E, CD51, CD105
	Lymphocytes	CD4, CD8, CD45, CD3, CD66b
	Monocytes	CD14
	Red blood cells	CD235a



Gangadaran et al., Pharmaceutics. 2020

Gézsi et al., Exp Mol Med 2019

Composition of EVs: Proteins

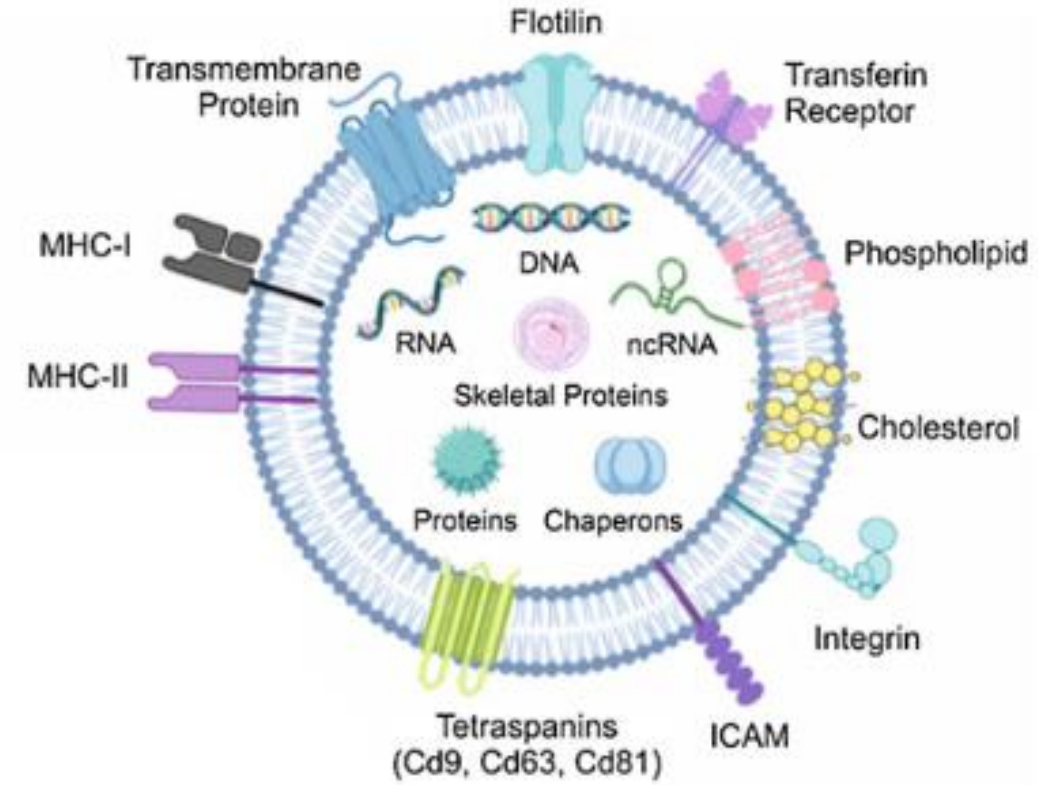
- **Protein content of EVs:**

➢ Main components

➢ Show a cell origin signature

➢ **Exosomes**: endosomal proteins (Rab GTPase, SNAREs..), tetraspanins & MHC-II

➢ **Microparticles**: diverse proteins (glycoproteins, integrins, receptors..)



EV targeting, uptake and signaling

Gangadaran et al., Pharmaceutics. 2020

Gézsi et al., Exp Mol Med 2019

Composition of EVs: Nucleic acids

- **Nucleic acids:**

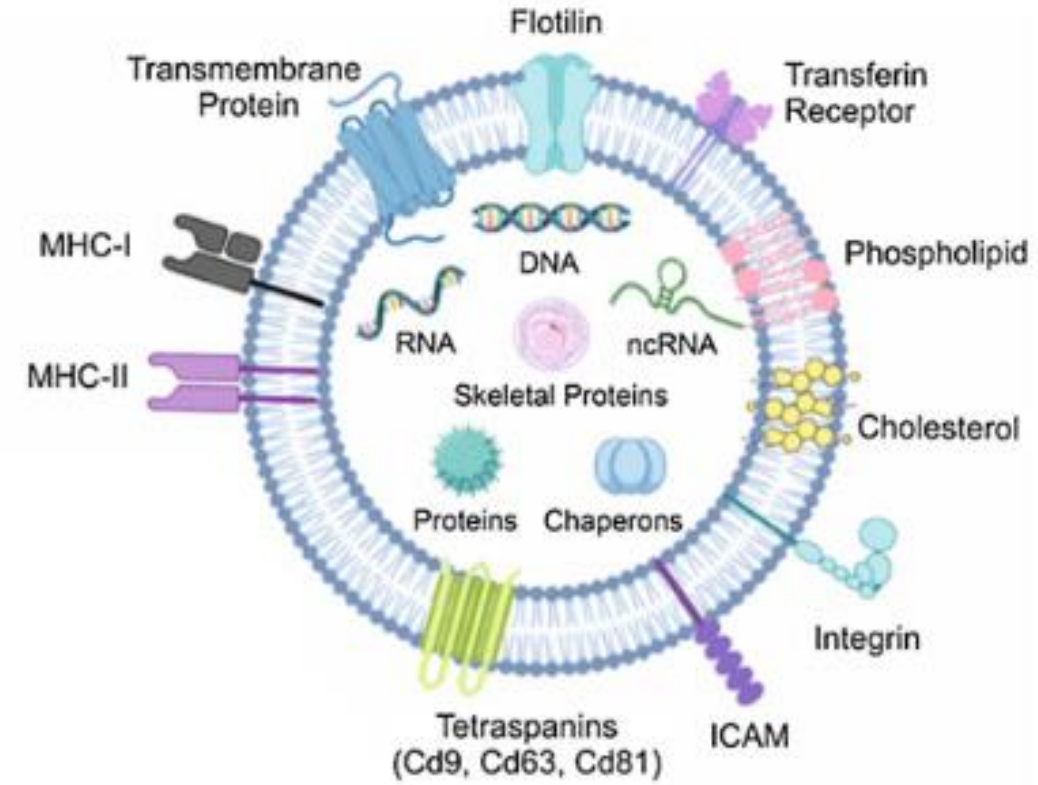
➢ **ARN**: ARNm, miro-ARN, rRNA, lncRNAs, snRNAs…

➢ **ADN**: ssADN, dsADN, ADN mitochondrial

➢ Show a cell origin signature

(ex. MSC-derived EVs are enriched in miR-210 et miR-126)

Recipient cell signaling



Gangadaran et al., Pharmaceutics. 2020

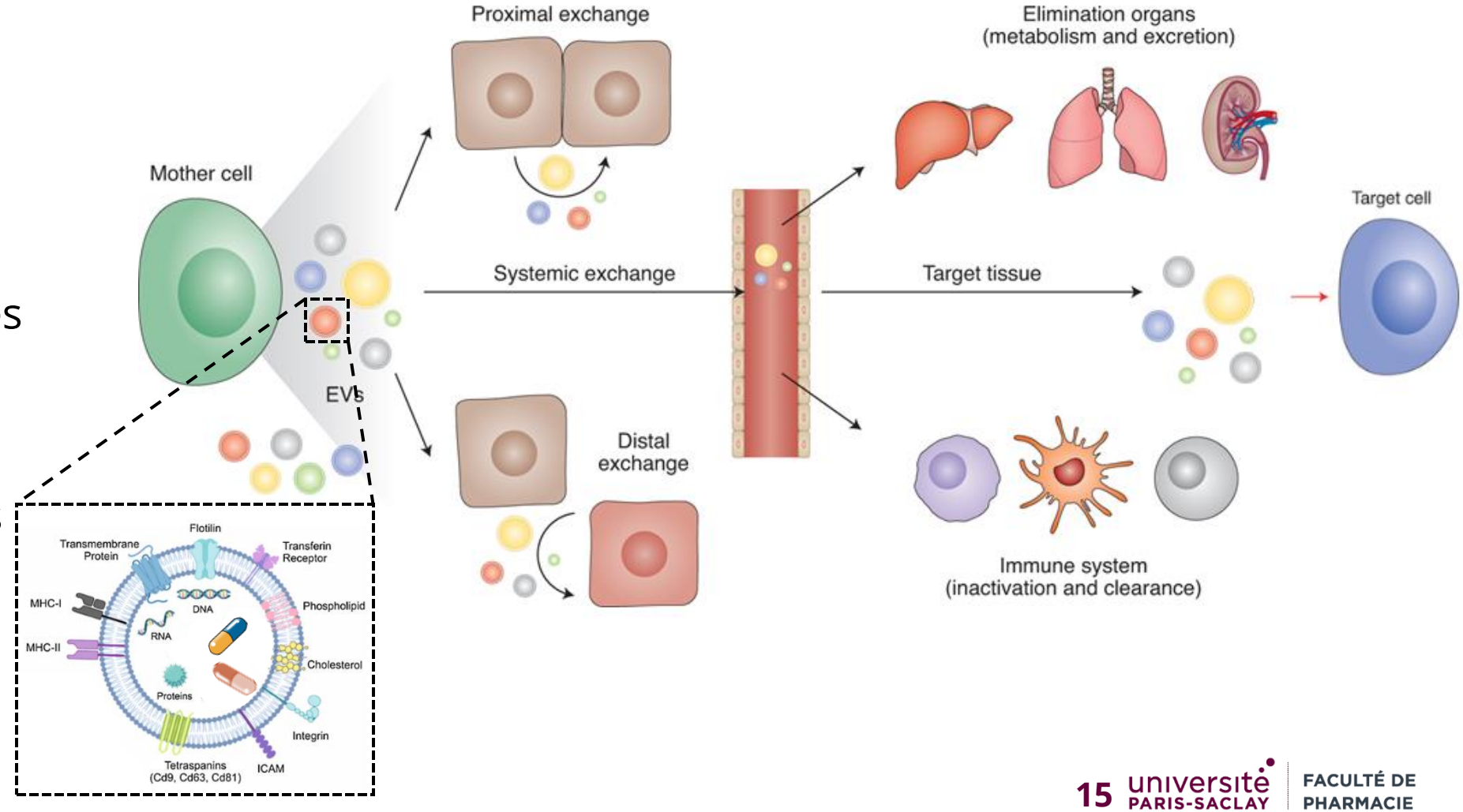
Gézsi et al., Exp Mol Med 2019

Clinical potential of EVs

Clinical potential of EVs

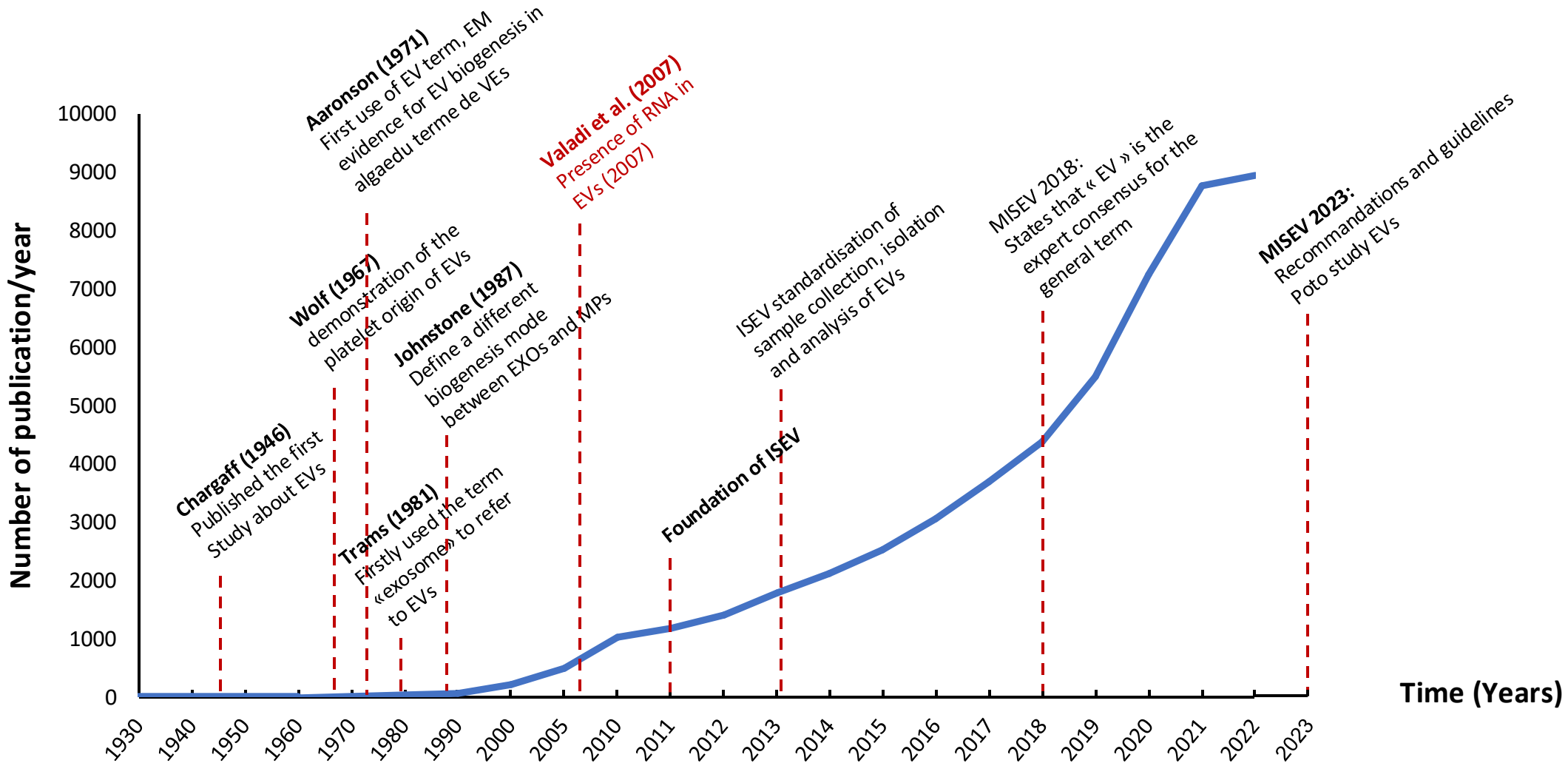
How to exploit EVs?

- Biomarkers of diseases
- Biotherapeutics
- Nanovecteur of therapeutic molecules



Herrmann et al., Nat. Nanotechnol. 2021

Clinical potential of EVs: Growing interest in EVs



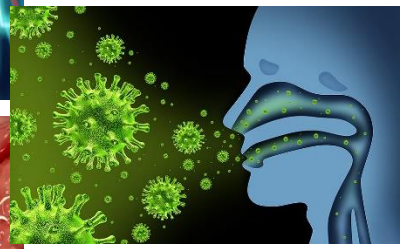
Adapted from Bazzan et al., Int. J. Mol. Sci. 2021

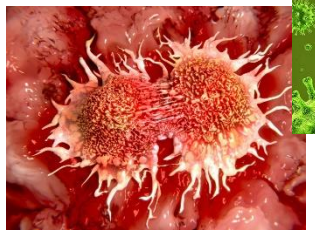
EVs as potential biomarkers of diseases

EVs as potential biomarkers of diseases

Clinical condition	Cell origin	EVs level
Healthy individuals		
Smokers	Endothelial cells	↑
Pregnancy	All	↑
Physical activities	Endothelial cells	↓
	Platelets	↑
Cardiovascular diseases		
Hypertension	Endothelial cells & platelets	↑
Acute Coronary Syndrome	Endothelial cells & platelets	↑
Atherosclerosis	Macrophages, red blood cells, muscles & platelets	↑
Venous thrombosis	Endothelial cells & platelets	↑
Scott syndrome	Platelets	↓
Cancer		
Gastric cancer	Platelets	↑
Lung cancer	Monocytes & Platelets	↑
Colorectal cancer	Cancer epithelial cells	↑
Melanoma	Melanoma cells	↑
Infectious diseases		
Sepsis	Monocytes	↑
HIV	T lymphocytes	↑
Malaria	Platelets	↑

EVs level in various physio(patho)logical conditions





EVs as potential biomarkers of diseases: cancer

List of tumor diseases for which EVs have been proved useful for the detection and identification of clinically relevant biomarkers.

Pathology	Source	EV type	Cargo	Biomarkers	Application	Detection method	References
Breast Cancer	Plasma	EVs	Protein	Del-1, Fibronectin	Distinguish BC from benign breast tumors and noncancerous diseases	ELISA	Moon et al., 2016a,b
	Serum	EXOs	Protein	Survivin 2B	Discriminates early stage patients from high stage patients and controls	Western Blot	Khan et al., 2014
	Plasma	MVs	Protein	EGFR	Association with in situ and stage I	Western Blot	Galindo-Hernandez et al., 2013
	Serum	MVs	Protein	EMMPRIN	Differences between BC patients and healthy controls	Flow cytometry	Menck et al., 2015
	Plasma	MVs	Protein	FAK	FAK present in BC patients, mainly in stage III	Western Blot	Galindo-Hernandez et al., 2013
	Serum	EXOs	RNA	*GSTP1*	Chemoresistance marker	RT-PCR	Yang et al., 2017
Prostate Cancer	Urine	EXOs	RNA	*ERG*, *PCA3*, and *SPDEF*	Distinguish high-grade (GS7) from low-grade (GS6) cancer and benign disease	RT-PCR	McKiernan et al., 2016
	Plasma and serum	EXOs	Protein	Survivin	Discriminates PC patients from BPH and healthy controls	Western Blot	Khan et al., 2012
	Plasma	EXOs	RNA	*PTEN*	Distinguish between PC patients and healthy controls	RT-PCR	Gabriel et al., 2013
Colorectal Cancer	Serum	EXOs	RNA	KRAS	Matches mutations in EXOs and tissue with sensitivity 73,5%; specificity 100%	PCR and gene sequencing	Hao et al., 2017
	Serum	EXOs	RNA	*BRAF*	Matched mutations in EXOs and tissue with sensitivity 75%; specificity 100%	PCR and gene sequencing	Hao et al., 2017
	Plasma	EXOs	Protein	GPC1	Discriminates CRC patients from controls	Flow cytometry	Li et al., 2017
Lung Cancer	Plasma	EXOs	Protein	EGFR	Discriminates NSCL patients from healthy controls	ELISA	Yamashita et al., 2013
	Plasma	EXOs	RNA	*EML4-ALK*	EML4-ALK rearrangements detection	qPCR	Brinkmann et al., 2015
	Urine	EXOs	Protein	LRG1	LRG1 higher levels in NSCLC patients	Western Blot	Li et al., 2011
Pancreatic Cancer	Plasma	EXOs	DNA	*KRAS*	KRAS mutations in metastatic PDAC patients	Droplet digital PCR	Allenson et al., 2017
	Serum	EXOs	Protein	GPC1	Increased levels of GPC1-positive EVs in 100% of patients with PDAC	Flow cytometry	Melo et al., 2014
Brain tumors	Serum	EXOs	RNA	EGFR VIII	Detection of EGFR VIII	RT-PCR	Venkata Manda et al., 2017
	Serum	EVs	DNA	IDH1	Detection of $IDH1^{G395A}$	Cold-PCR and gene sequencing	Garcia-Romero et al., 2017
Melanoma	Serum	EXOs	Protein	S100, MIA	Relation with patient survival	ELISA	Alegre et al., 2016
	Plasma	EXOs	Protein	TYRP, VLA-4, HSP70, HSP90 MET	Relation with metastatic patients survival	Western Blot	Peinado et al., 2012
Ovarian Cancer	Ascytic fluid	EXOs	Protein	EpCam, CD24	Relation with treatment response	Nano-plasmonic exosome	Im et al., 2014

Garcia-Romero et al., Mol Aspects Med. 2018

EVs as potential biomarkers of diseases: non tumoral

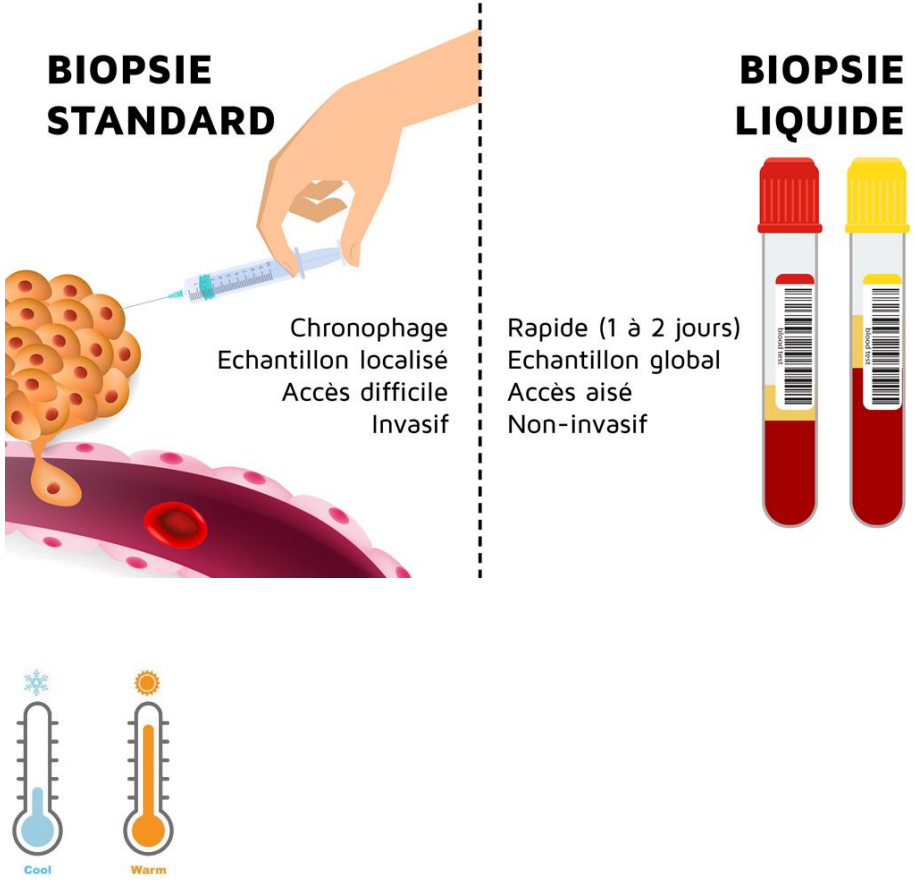
List of non-tumoral pathologies for which EVs have been proved useful for the detection and identification of clinically relevant biomarkers.

Pathology	Source	EV type	Donor cells	Biomarker	Function	Detection method	References
Coronary artery disease, type 2 diabetes	Plasma	MVs	Endothelial cells	CD144, CD31, CD62E	Leukocyte adhesion, inflammation	Leukocyte adhesion, inflammation	Flow cytometry Bernal-Mizrachi et al., 2003; Koga et al., 2005
Risk of cardiovascular events	Plasma	MVs	Lymphocytes; Smooth muscle cells	CD45, CD3; SMA-α	Inflammation; thrombus formation	Flow cytometry	Chiva-Blanch et al., 2016
Type 2 diabetes	Plasma	MVs	Platelets	Fibrinogen, Tisuue factor, P-selectin	Thrombosis, inflammation, vascular dysfunction	Flow cytometry	Zhang et al., 2014
Coronary artery diseae	Plasma	MVs	Endothelial cells; Platelets	miR-126; miR-199a	Cardioprotective	RT-PCR	Jansen et al., 2014
Atherosclerosis	Plasma	MVs	Leukocytes	CD11b, CD66	Plaque instabilty	Flow cytometry	Sarlon-Bartoli et al., 2013
Cardiac surgery	Plasma	EXOs	Cardiomyocytes	miR-1, miR133a, miR-24, miR-210, miR-133b	Biomarkers of myocardial damage	RT-PCR	Emanueli et al., 2016
Acute coronary syndrome	Serum	EXOs	Cardiomyocytes	miR-208a	Early diagnosis and prognosis of the disease	RT-PCR	Bi et al., 2015
Atherosclerosis	Aorta	EXOs	Smooth muscle cells; Endothelial cells	EXOs	Intercellular communication	Transmission electron microscopy	Perrotta and Aquila, 2016
Venous thromboembolism	Plasma	MVs	Endothelial cells	CD31, E-selectin	Thrombosis, vascular dysfunction	Flow cytometry	Chirinos et al., 2005
	Plasma	MVs	Platelets	CD41	Coagulation, thrombosis	Flow cytometry	Bucciarelli et al., 2012
Venous thromboembolism in cancer patients	Plasma	MVs	Platelets, endothelial cells	Tissue factor, CD146, CD61	Coagulation, inflammation	Flow cytometry	Campello et al., 2011
Venous thromboembolism in GBM patients	Plasma	MVs	Glial cells	Tissue factor, GFAP	Coagulation, thrombosis	Flow cytometry	Sartori et al., 2013
Systemic lupus erythematosus	Plasma	MVs	Endothelial cells	CD31, Annexin V	Endothelial damage and dysfunction	Flow cytometry	Parker et al., 2014
	Urine	EXOs	Nephron cells	miR-146a	Renal inflammation, fibrosis	RT-PCR	Perez-Hernandez et al., 2015
Lupus nephritis	Urine	EXOs	Epithelial cells	miR-29c	Renal fibrosis reduction	RT-PCR	Sole et al., 2015
Rheumatoid arthritis	Plasma	MVs	Platelets	CD61	Inflammation, thrombosis	Flow cytometry	Knijff-Dutmer et al., 2002
	Synovial fluid	MVs	Platelets	CD41	Inflammation	Flow cytometry	Boilard et al., 2010
	Synovial fluid	MVs	Leukocytes	CD66b, CD14	Coagulation	Flow cytometr	Berckmans et al., 2002
	Plasma, urine	MVs	T cells, B cells, monocytes, platelets, endothelial cells	CD3, CD19, CD14, CD41, CD62E	Inflammation	Flow cytometry	Viñuela-Berni et al., 2015
Preeclampsia	Plasma	MVs	Platelets, leukocytes	CD61, CD62-P, CD45, tissue factor	Coagulation, inflammation	Flow cytometry	Campello et al., 2015
Pregnancy	Plasma	MVs	Platelets, endothelial cels, leukocytes	CD61, CD62P, CD62E, CD45, CD142	Coagulation, inflammation	Flow cytometry	Radu et al., 2015
Tuberculosis	Blood, urine	EXOs	Infected macrophages	LAMP1, MHC-II, Hsp70	Stimuate proinflammatory response	Flow cytometry	Bhatnagar et al., 2012; Kruh-Garcia et al., 2012
Alcoholic hepatitis	Plasma	EXOs	Liver cells, heart cells	miRNA-192, miRNA-30a	Liver injury, Inflammation	RT-PCR	Momen-Heravi et al., 2015
Chronic obstructive pulmonary disease	Plasma	MVs	Pulmonary capilaries	CD144, CD31, CD62-E	Endothelial damage	Flow cytometry	Takahashi et al., 2012

Garcia-Romero et al., Mol Aspects Med. 2018

Advantages of EVs as source of disease biomarkers

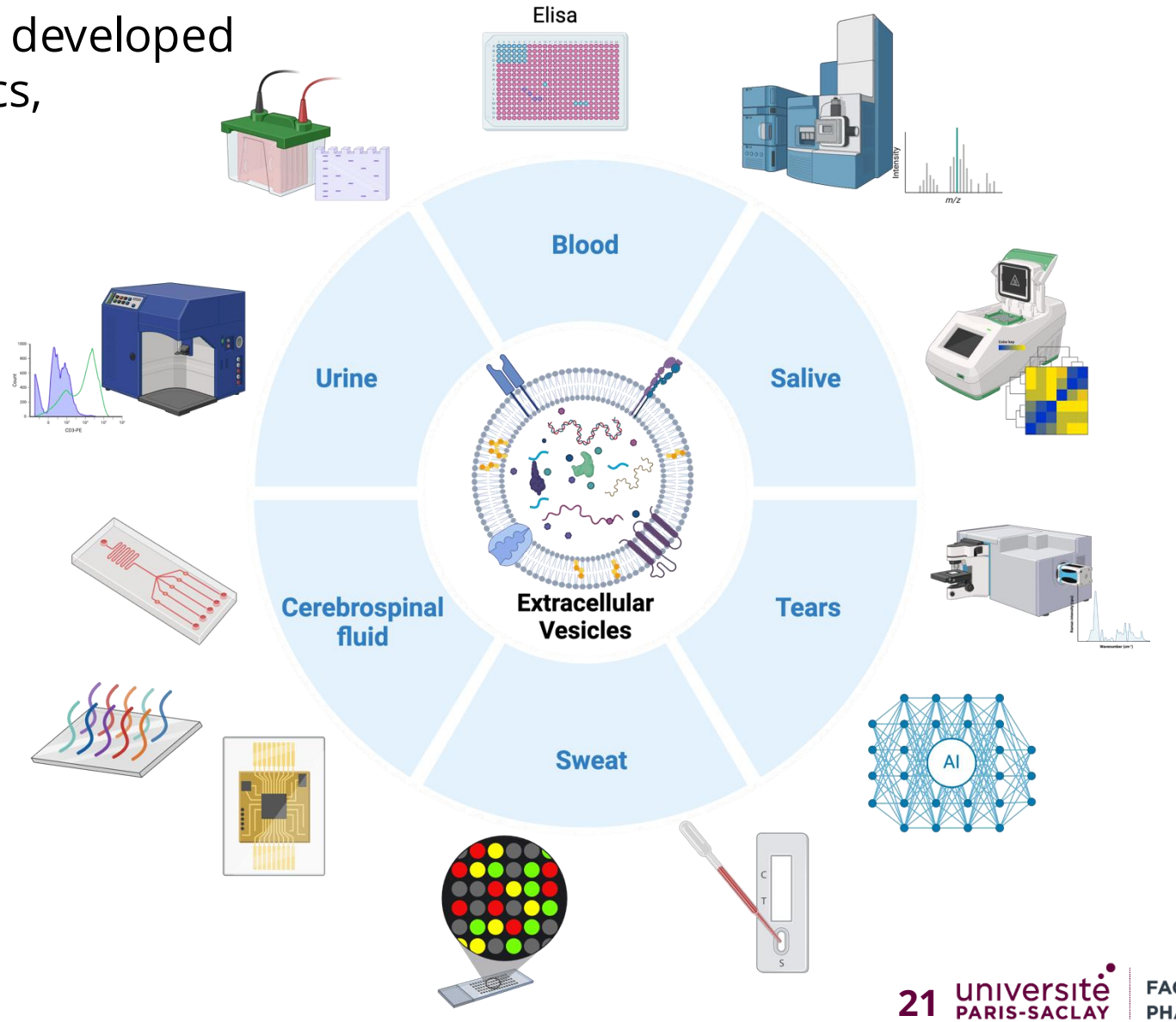
- Accessible via liquid biopsy => non-invasive monitoring
- Protection of biomarkers (*in vivo*, storage)
- Cell-specific signature
- Carry multiple biomarkers
- Crossing biological barriers



Huang and Lai, Ann Transl Med 2019
Aubertin et al., Med Sci 2021

Advantages of EVs as source of disease biomarkers

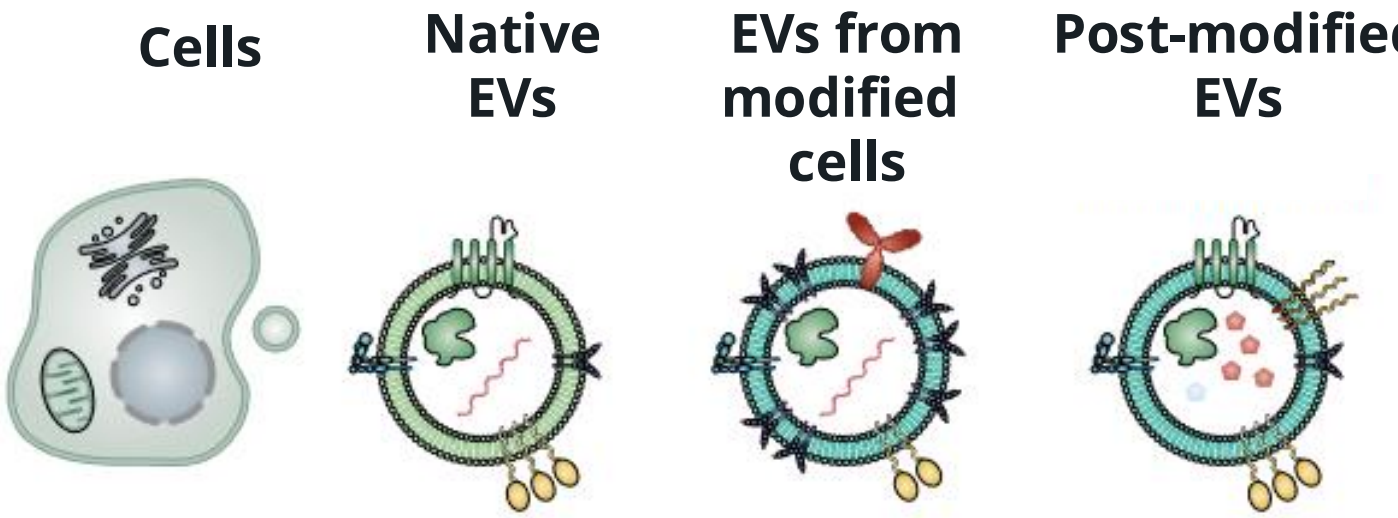
- Various approaches have been developed to explore EV biomarkers (omics, microfluidic…)



Therapeutic potential of EVs

Therapeutic potential of EVs

- **Three groups of therapeutic EVs based on the origin of their constituents**
  1. **Native EVs**
  2. **Evs from modified cells**
  3. **EVs modified after secretion**

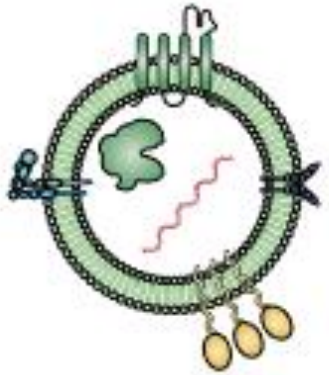


Herrmann et al., Nat. Nanotechnol. 2021

Therapeutic potential of native EVs

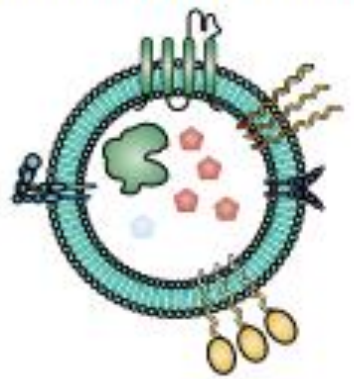
Cells

Native EVs



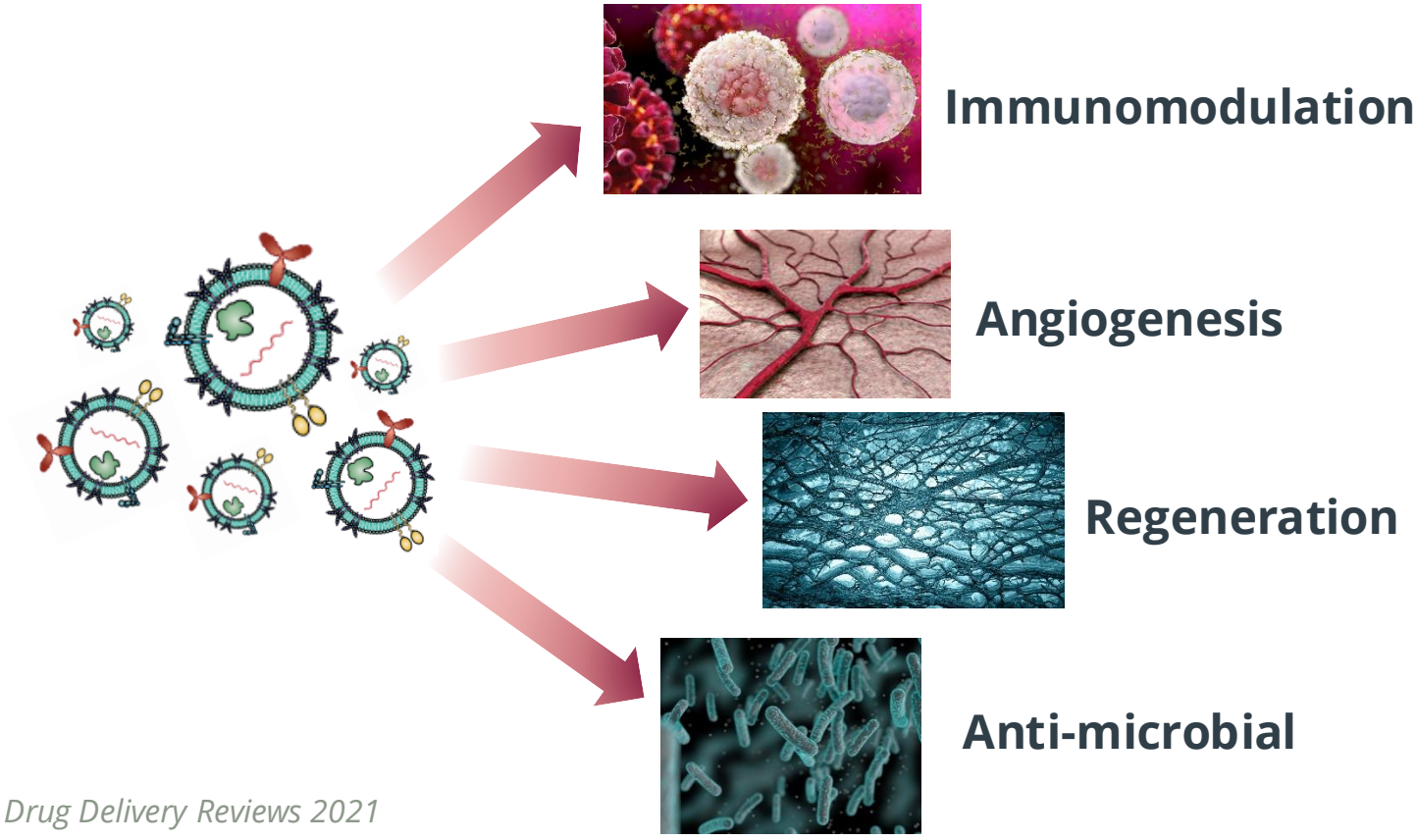
EVs from modified cells

Post-modified EVs



Therapeutic potential of native EVs

- **EVs from various sources have shown therapeutic effects**
  - **MSC, HSC, iPSC, macrophages, platelets…**
  - **MSC from bone marrow, adipose tissue or blood are used preferentially**



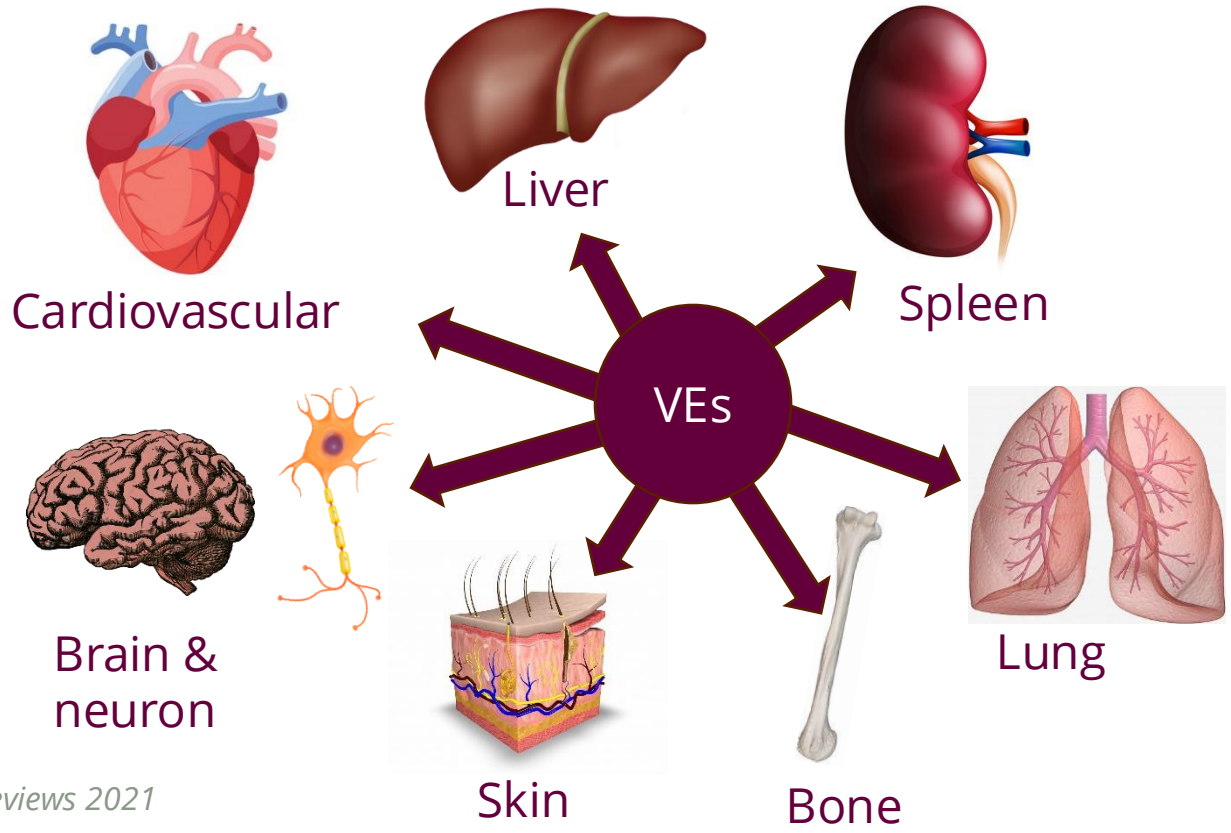
Functional effects mediated by EV's proteins, mRNA & miRNA

Nagelkerke et al., Advanced Drug Delivery Reviews 2021

Therapeutic potential of native EVs

- **EVs from various sources have shown therapeutic effects**
  - **MSC, HSC, iPSC, macrophages, platelets…**
  - **MSC from bone marrow, adipose tissue or blood are used preferentially**

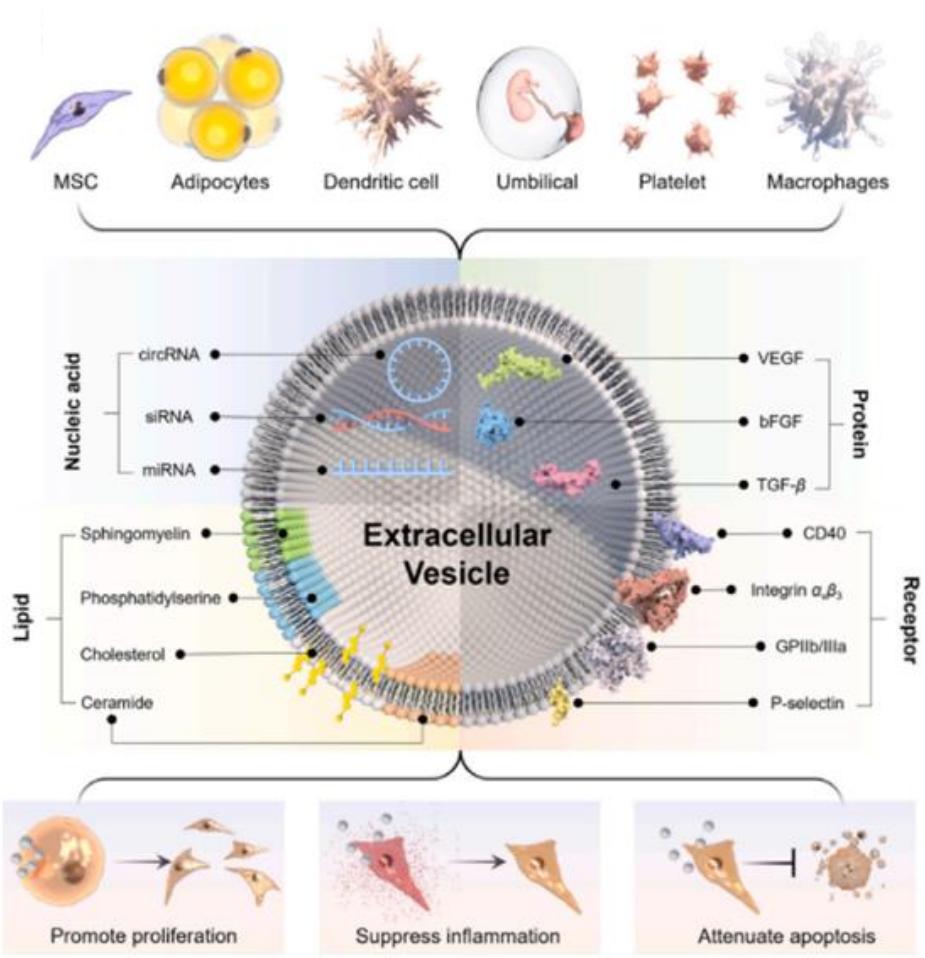
The use of EVs in the **repair & regeneration** of various organs and tissue types



Nagelkerke et al., Advanced Drug Delivery Reviews 2021

Therapeutic potential of native EVs

- EV regenerative activities mediated by proteins, mRNA and miRNA transfer



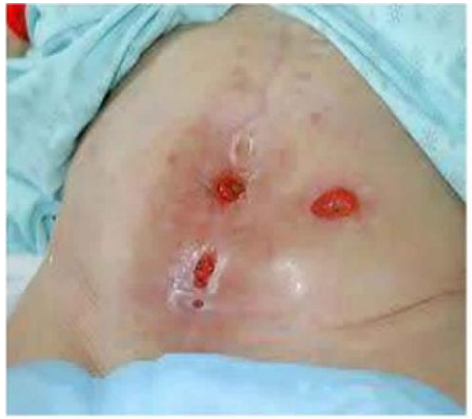
Ju et al., Materials Today Bio 2023

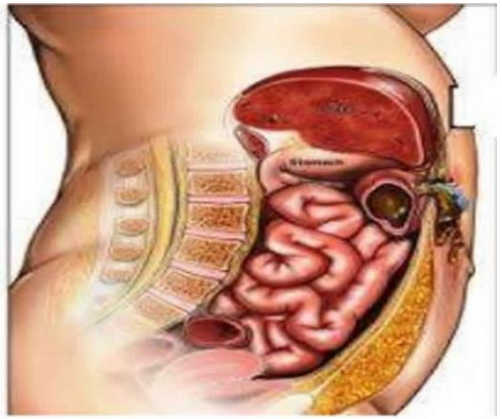
Examples…

Treatment of digestive fistula

- Abnormal connections between the digestive tract and another organ in the body (e.g. skin)
- Result from infection, inflammation, trauma or surgery

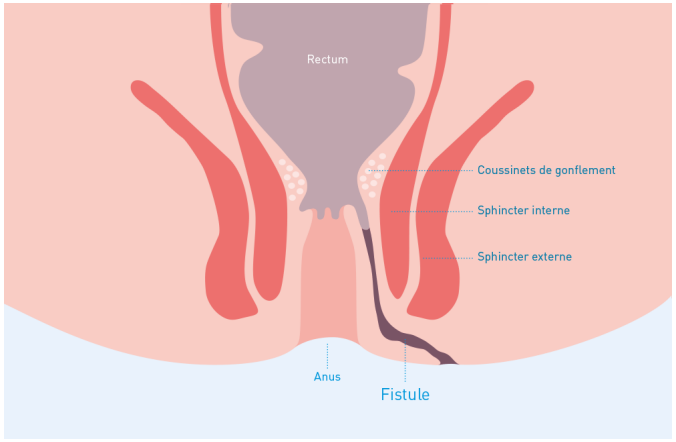
Enterocutaneous fistula



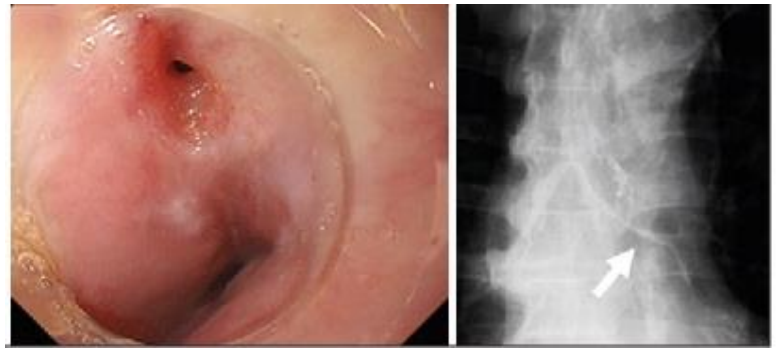


Credit: Health jade

Anal fistula



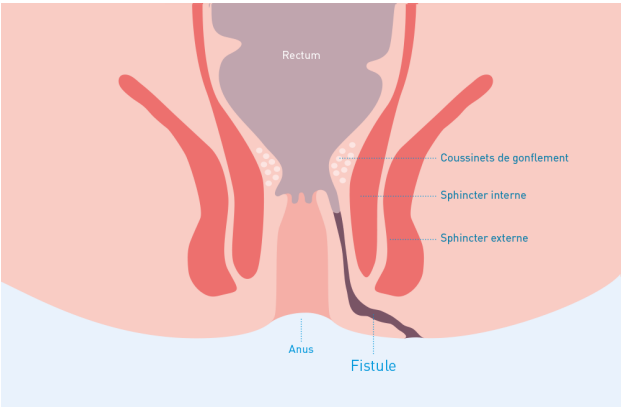
Esophageal fistula



Matsui et al., Ann Gastroenterol. 2017

Treatment of digestive fistula: Adipose MSC

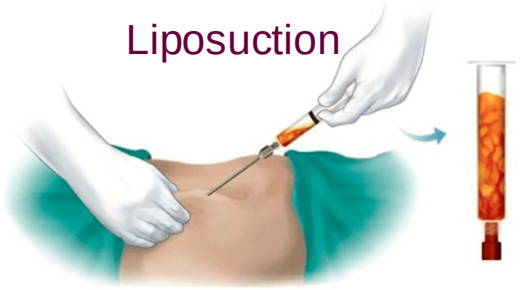
- **ALOFISEL® , Darvadstrocel**
  - ➢ Expanded human **allogeneic mesenchymal adult stem cells** extracted from adipose tissue
  - ➢ Treatment of complex anal fistulas in adults with Crohn's disease
  - ➢ Not reimbursed



Donor

Extraction of MSC

Liposuction



MSC preparation

Enzymatic digestion, ultracentrifugation, cell culture…

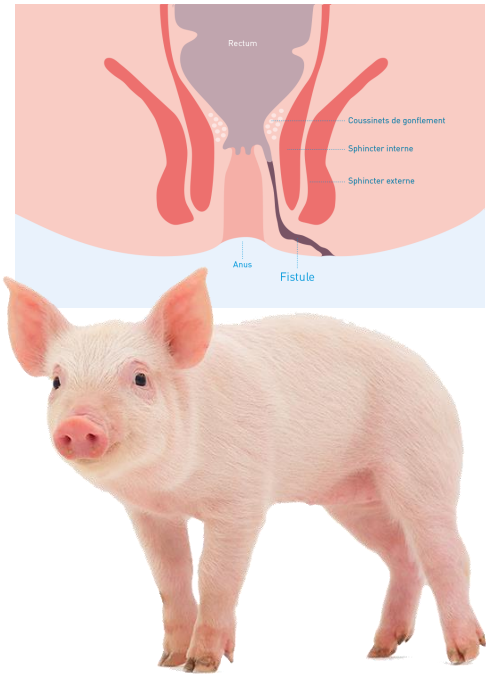
Cryopreservation

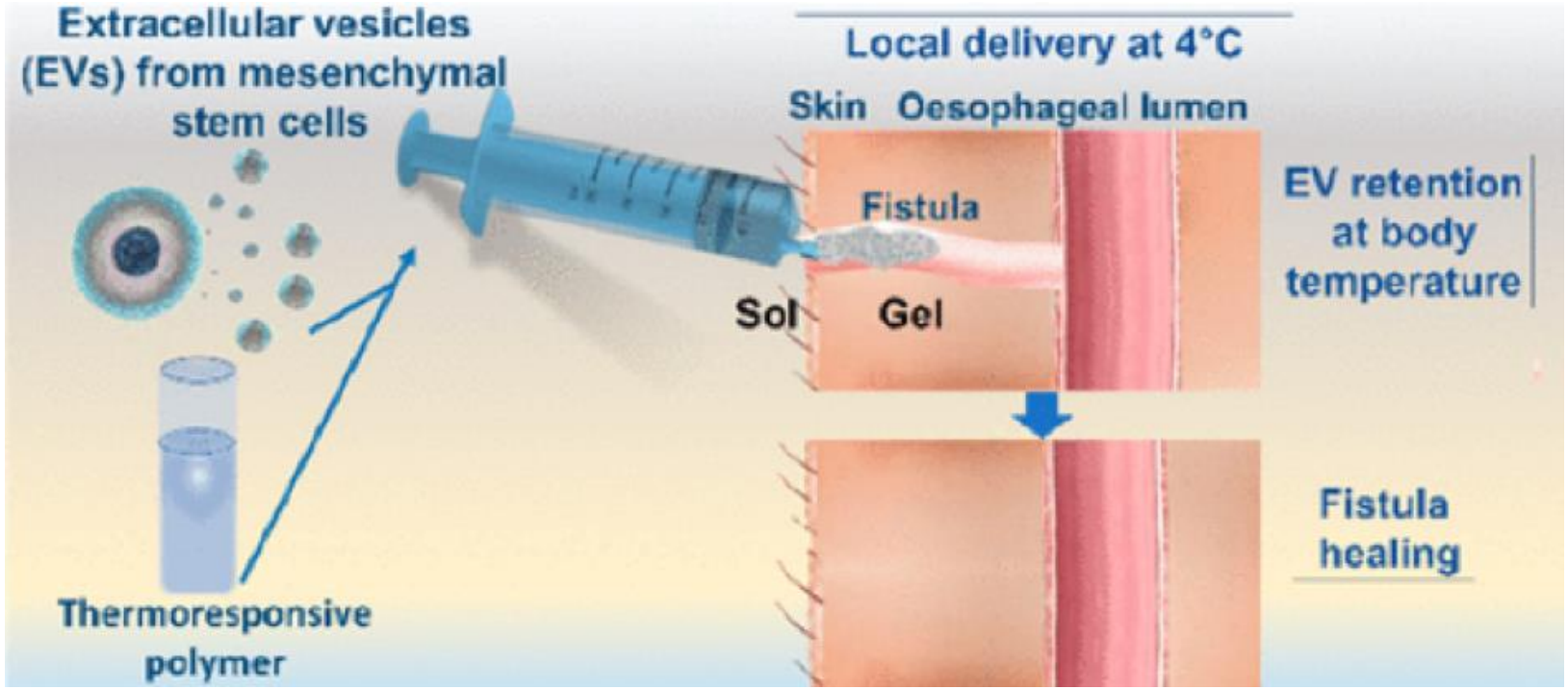
Administration

5 million cells/ml

Treatment of digestive fistula: EVs of adipose MSC

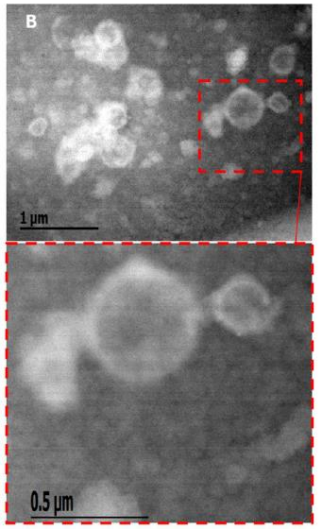
- EVs from adipose MSC to treat
- Treat of **Esophageal Fistula** in porcine model
- **Thermoresponsive Gel (PF-127) Embedded with MSC-EVs**

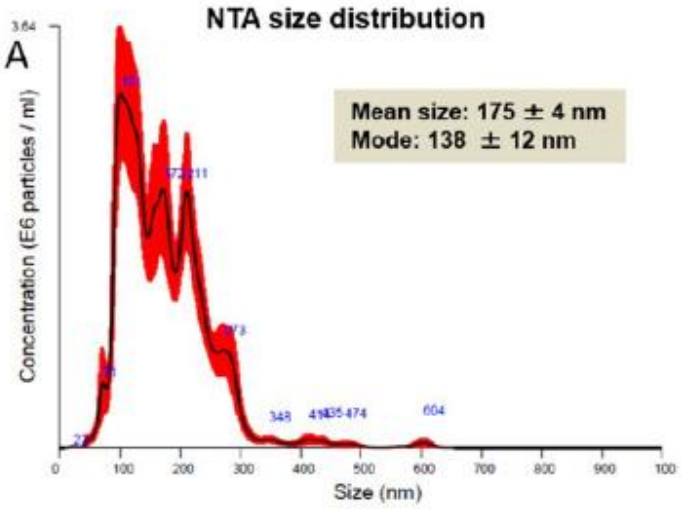


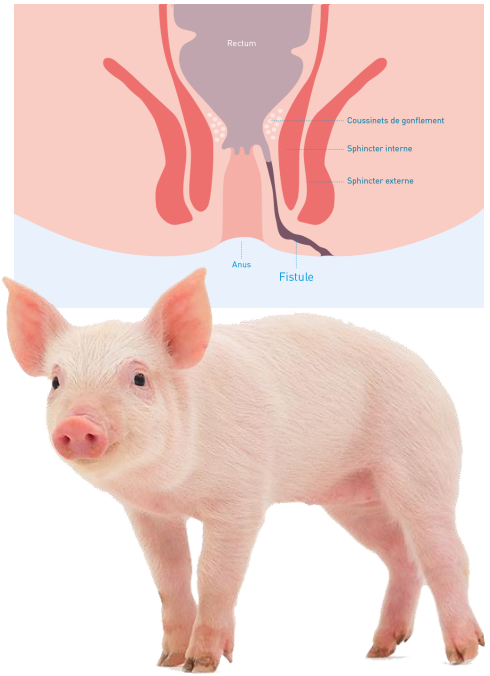


Silva et al., ACS Nano 2018

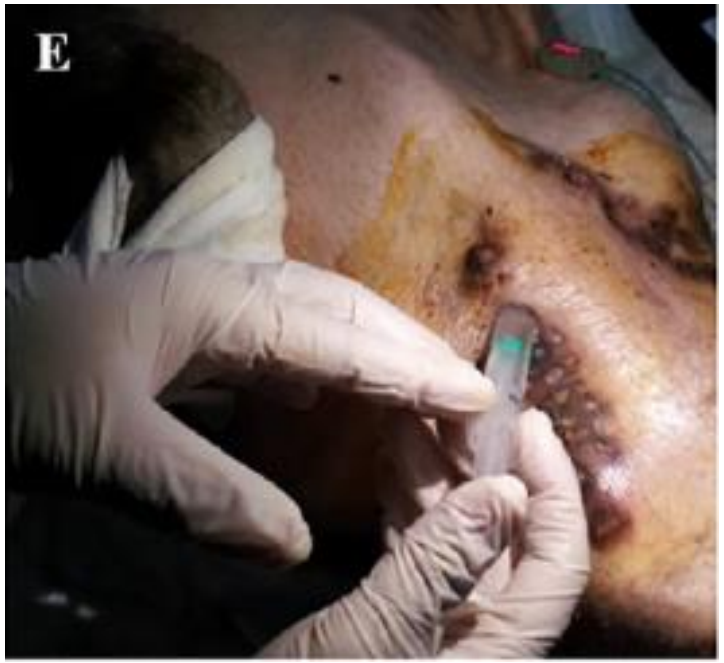
Treatment of digestive fistula: EVs of adipose MSC







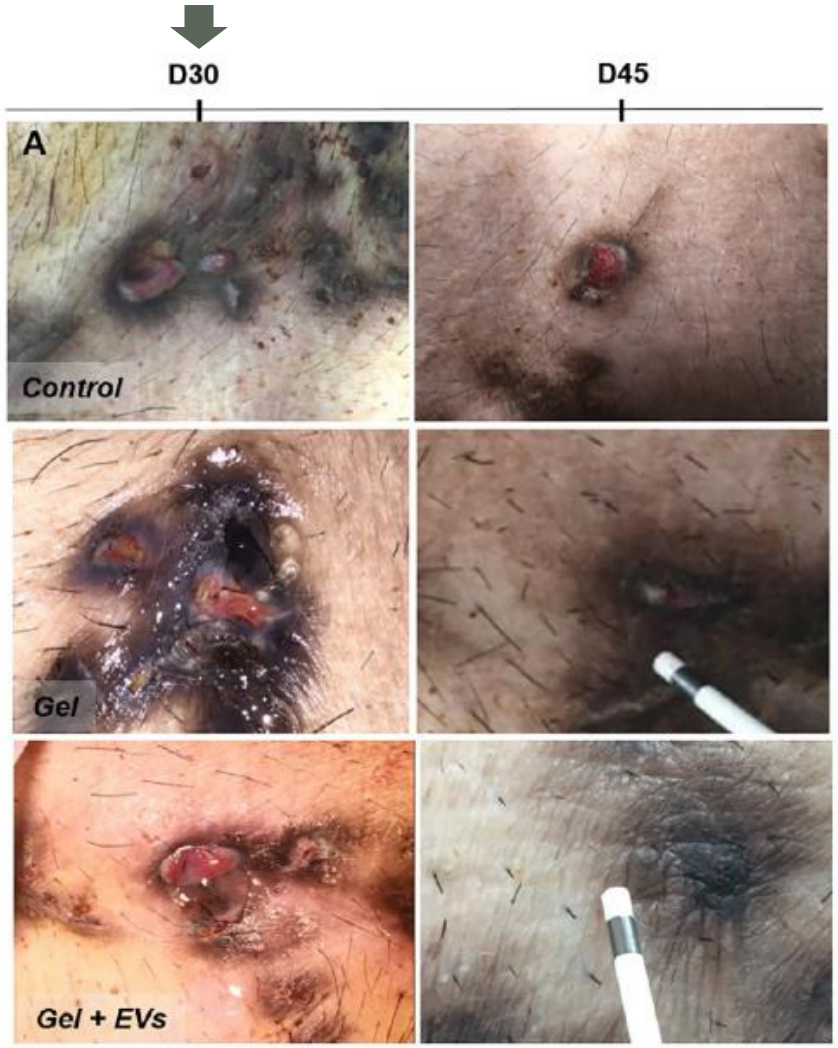
Injection of gel PF-127 embedded with MSC-EVs, at 4°C, through the external fistula orifice

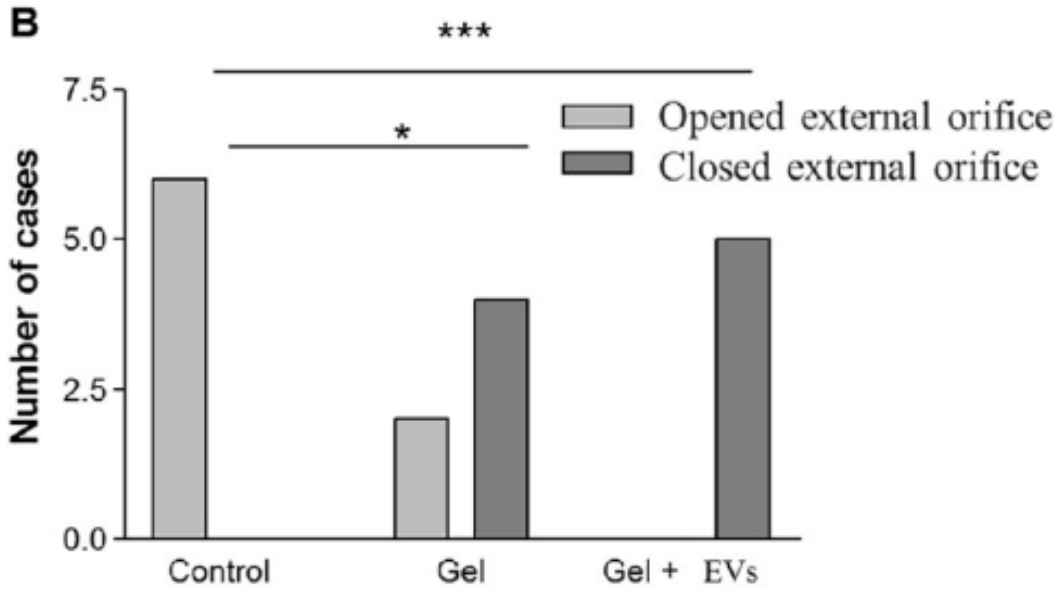


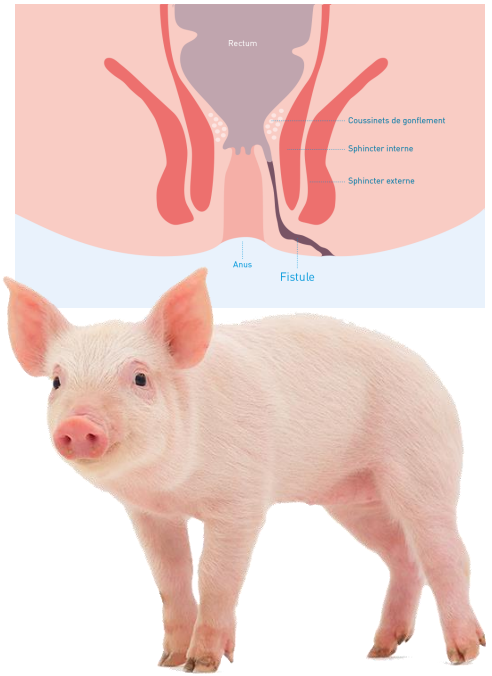
Silva et al., ACS Nano 2018

Treatment of digestive fistula: EVs of adipose MSC

Treatment





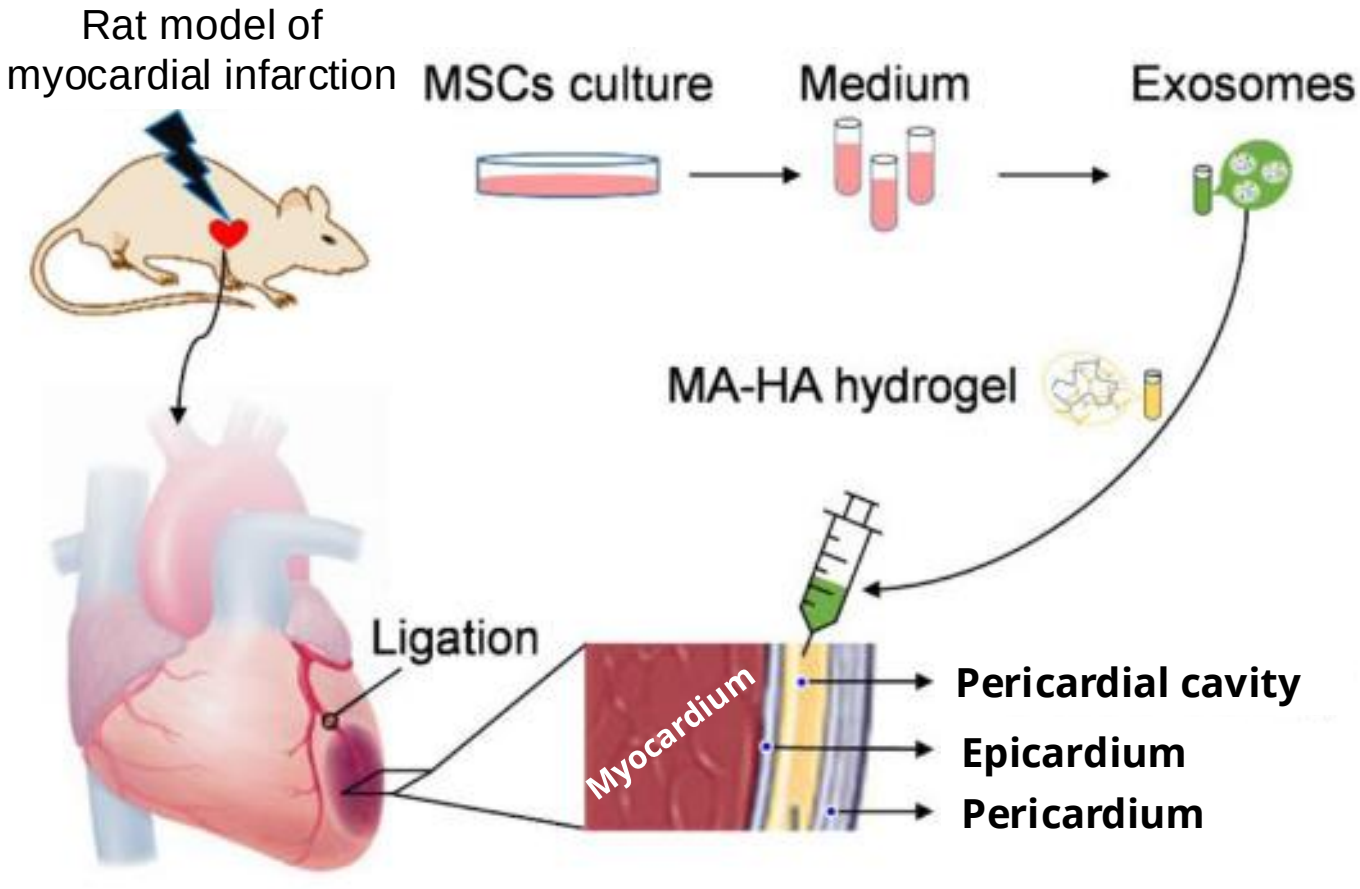


100 % healing after treatment with gel+EVs

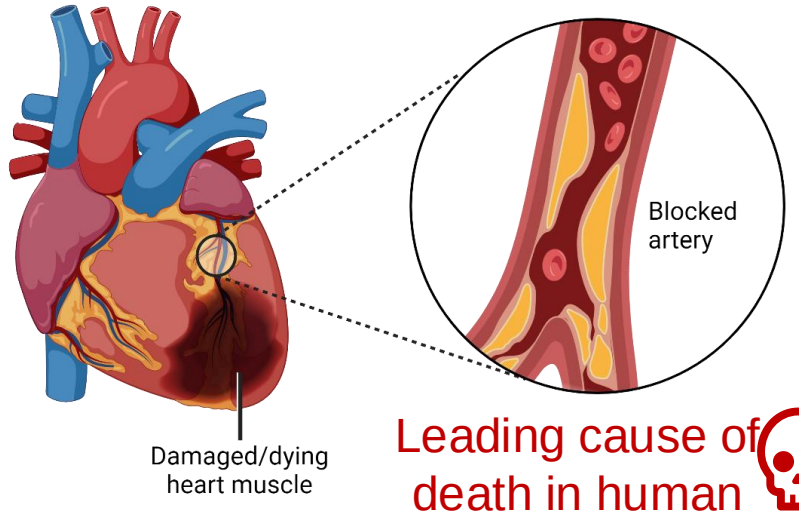
- EVs may represent advantageous alternative to cell therapy (Alofisel) ???

Silva et al., ACS Nano 2018

CSM-derived EVs: treatment of ischemic heart diseases



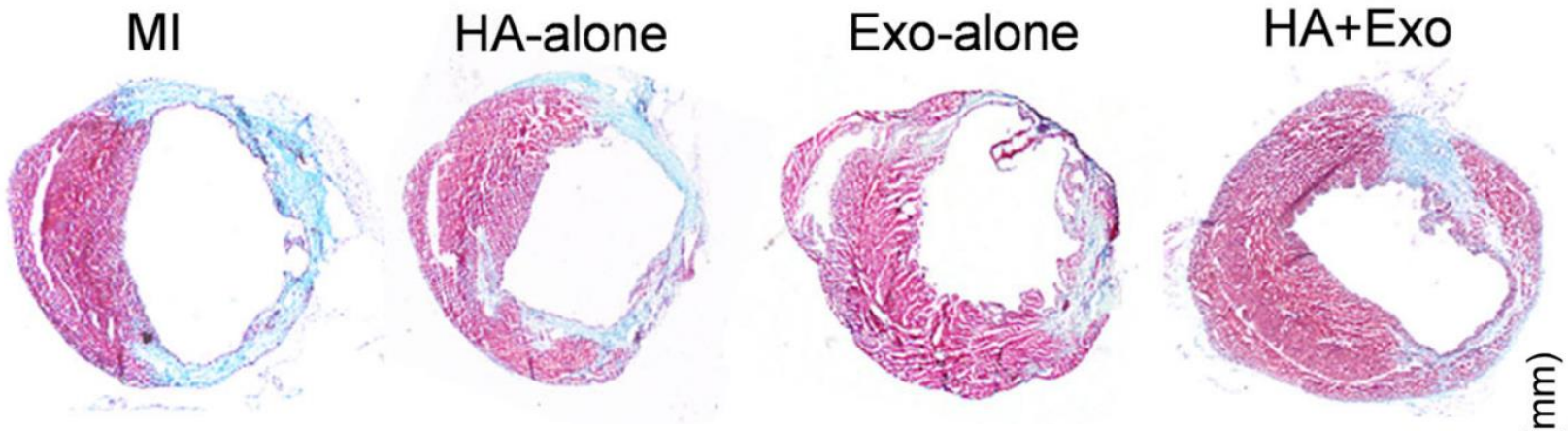
Myocardial infraction



Leading cause of death in human

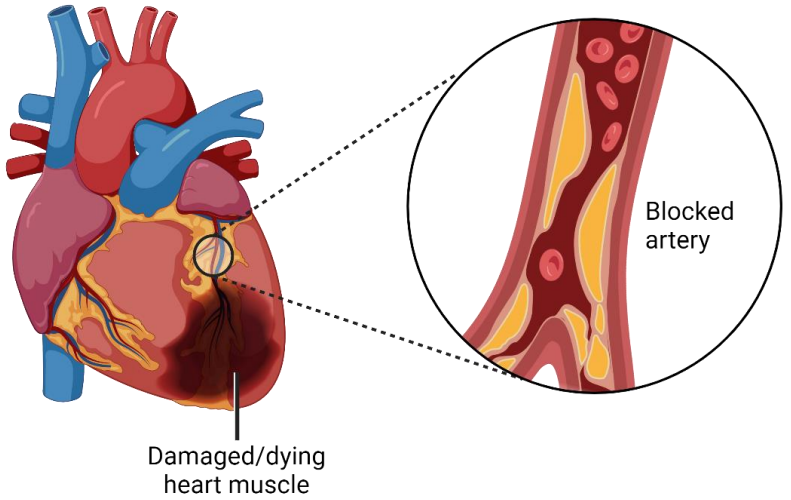
Zhu et al., Nature Communications 2021

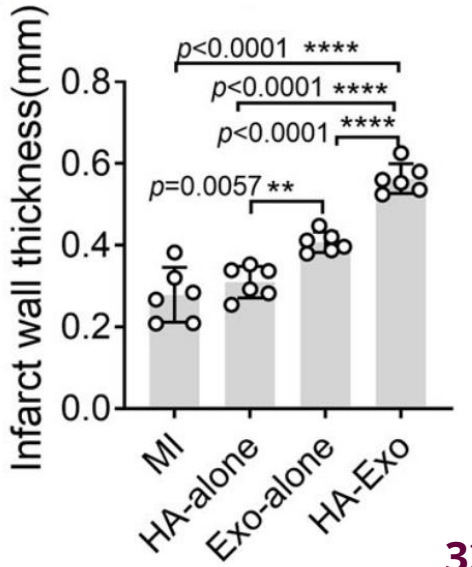
CSM-derived EVs: treatment of ischemic heart diseases



Repair of the myocardial infraction

Myocardial infraction



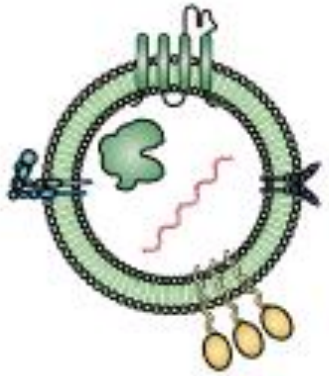


Zhu et al., Nature Communications 2021

Therapeutic potential of modified EVs

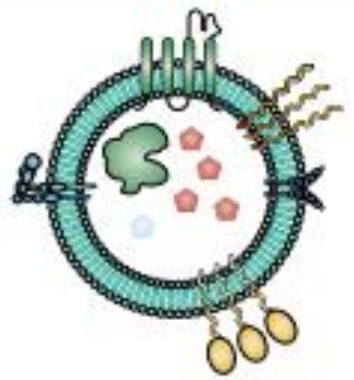
Cellules

Native EVs



EVs from modified cells

Post-modified EVs

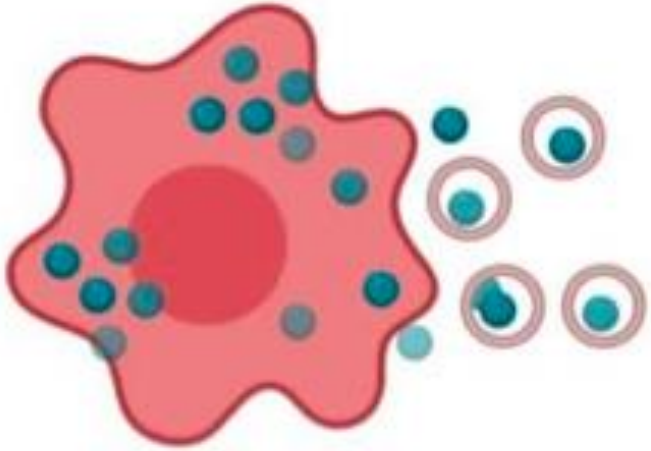


Gangadaran et al., Pharmaceutics. 2020

Therapeutic potential of EVs from modified cells

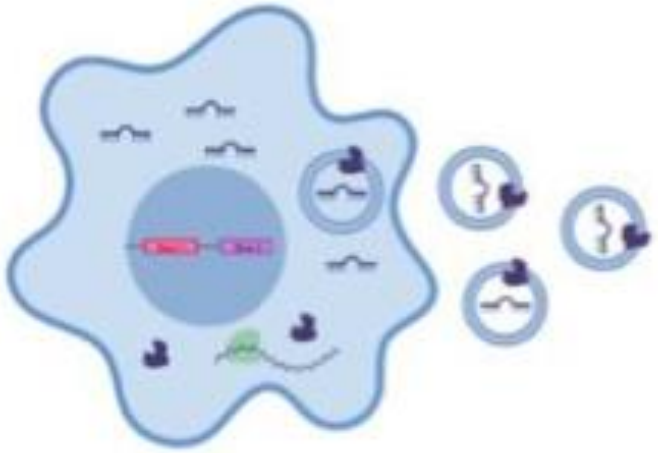
- Therapeutic agents are expressed, overexpressed or loaded in secreting cells prior to EV production

Drug Treatment of Parental Cells



- Mainly anti-cancer drugs

Gene Engineering of Parental Cells



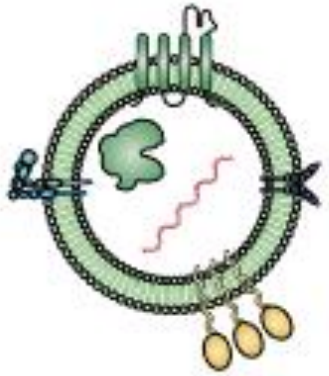
- Transfection with miRNA
- Transfection with target/therapeutic protein-coded plasmids

Gangadaran et al., Pharmaceutics. 2020

Therapeutic potential of post-modified EVs

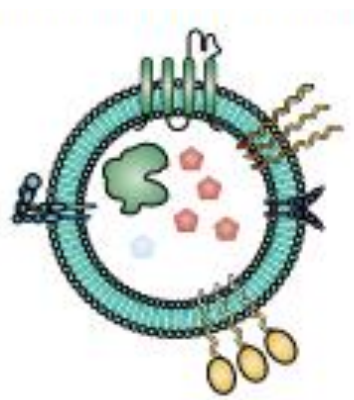
Cellules

Native EVs



EVs from modified cells

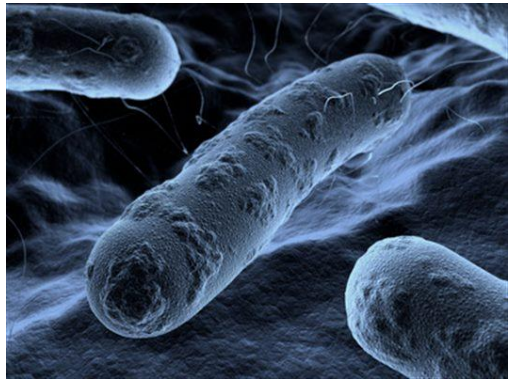
Post-modified EVs



Gangadaran et al., Pharmaceutics. 2020

Therapeutic potential of post-modified EVs

- Drug loading into EVs
- Loading efficacy depends on **physico-chemical properties of the therapeutic agent** (size, hydrophilicity…) and on the **loading method**
- **Cancer**: most active field in the developemtn of drug loaded EVs
- Variety of EV cell sources : human, plant & bacteria



Bactéries

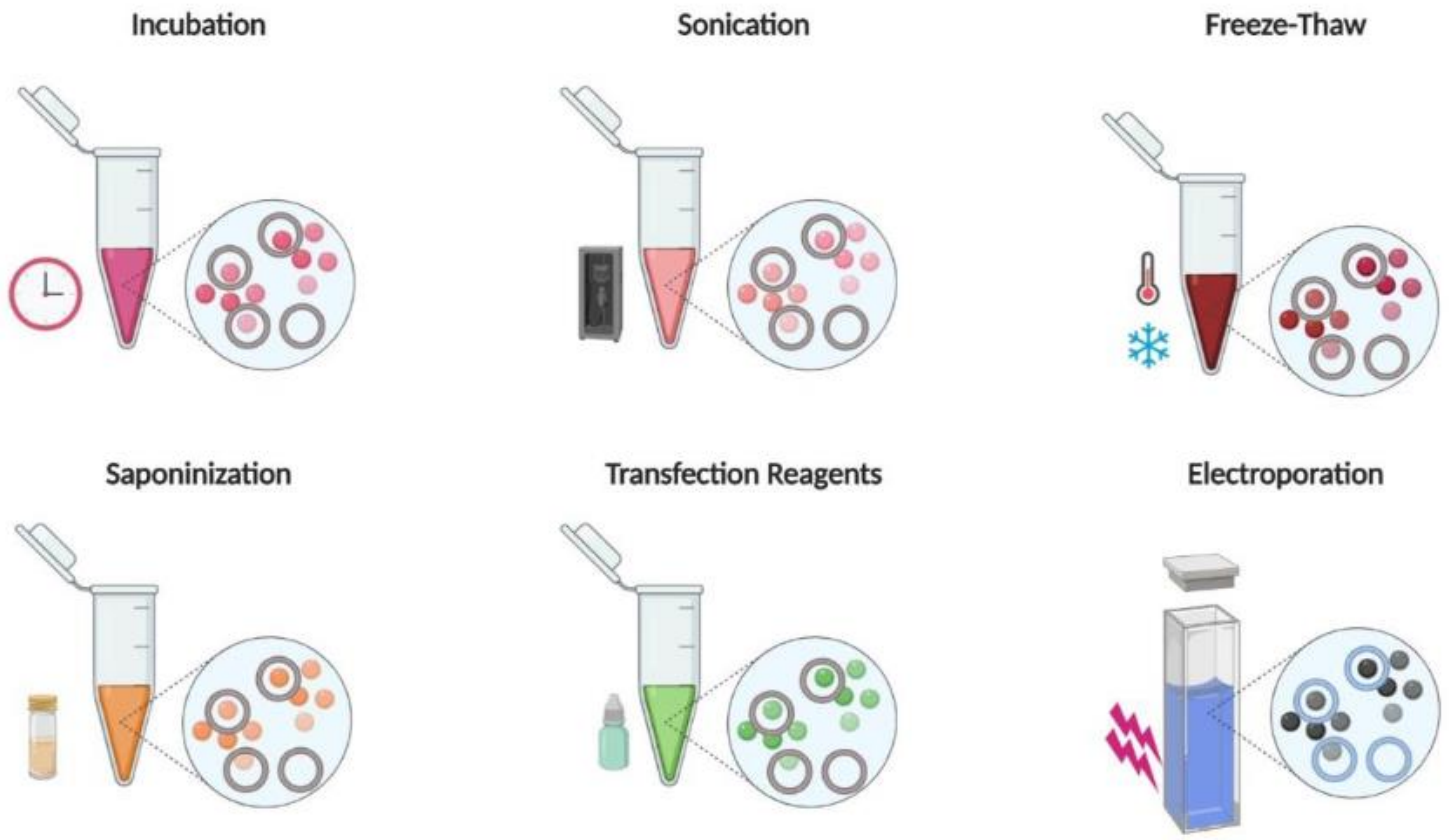
Microalgue

Lait bovin

Raisin

Therapeutic potential of post-modified EVs

- Drug loading into EVs
  - ➢ Different methods for loading therapeutics into EVs



Gangadaran et al., Pharmaceutics. 2020

Therapeutic potential of post-modified EVs

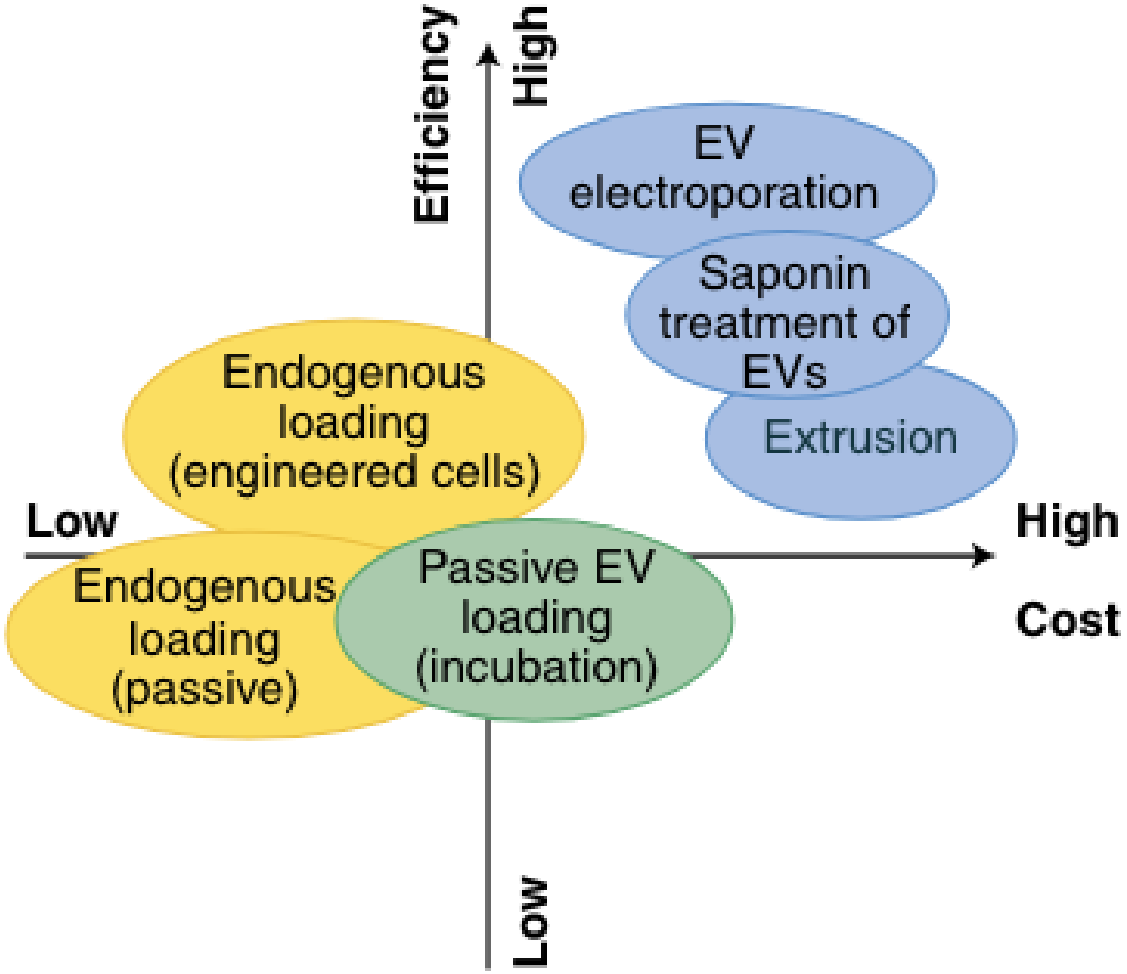
➢ Drug loading into EVs

Méthode	Avantages	Inconvénients
Incubation	Simple, no additional equipment required, Not affect the EVs (size and morphology)	Low loading capacity
Sonication	High loading capacity, able to load anticancer drugs, siRNAs and proteins	Not suitable for hydrophobic drugs
Electroporation	Able to load large molecules (nucleic acids) and anticancer drugs, moderate loading capacity	Deformation the EVs, siRNA aggregation, low loading capacity compared to sonication or saponin
Freeze-Thaw	Simple, moderate of loading capacity, membrane fusion is possible: Generation of Hybrid EVMs from EVs and liposomes	Low loading efficiency compared to extrusion and sonication, aggregation of EVs
Transfection reagents	Simple, able to load nucleic acids	Expensive, transfection reagent may associate with siRNA and delivered into recipient cells, mechanism of action not well-known
membrane permeabilizer (saponin)	Simple, higher drug-loading capacity	Saponin is a toxic agent, requires additional washing (affect the integrity of EVs)

Gangadaran et al., Pharmaceutics. 2020

Therapeutic potential of post-modified EVs

- Drug loading into EVs



Herrmann et al., Nat. Nanotechnol. 2021

Ongoing clinical trials with EVs

No.	Status	Condition	Type of EV[b]	Location	NCT number
Stem-cell-derived EVs					
1	Recruiting; phase 1/2	Drug-resistant infections	MSC-/progenitor-cell-derived exosomes	Shanghai, China	NCT04544215
2	Unknown status	Diabetes mellitus type 1	MSC-derived exosomes	Sahel, Egypt	NCT02138331
3	Enrolling by invitation; phase 1/2	SARS-CoV-2 pneumonia	MSC-derived exosomes	Samara, Russia	NCT04491240
4	Completed; phase 1	SARS-CoV-2 pneumonia	MSC-derived exosomes	Shanghai, China	NCT04276987
5	Enrolling by invitation; phase 2	SARS-CoV-2 pneumonia	MSC-derived exosomes	Samara, Russia	NCT04602442
6	Recruiting; phase 1/2	Dry eye	Umbilical MSC-derived exosomes	Guangzhou, China	NCT04213248
7	Recruiting; early phase 1	Macular holes	MSC-derived exosomes	Tianjin, China	NCT03437759
8	Recruiting; phase 1	Safety and tolerance studies	MSC-derived exosomes	Shanghai, China	NCT04313647
9	Completed; phase 1/2	Cerebrovascular disorders	Mesenchymal-stromal-cell-derived exosomes	Tehran, Iran	NCT03384433
10	Recruiting; early phase 1	Periodontitis	Adipose-derived stem-cell-derived exosomes	Cairo, Egypt	NCT04270006

Herrmann et al., Nat. Nanotechnol. 2021

Allogenic and autologous EVs					
11	Recruiting; phase 1/2	Alzheimer's disease	Allogenic adipose MSC-derived exosomes	Shanghai, China	NCT04388982
12	Not yet recruiting; phase 1/2	Acute respiratory distress syndrome	Allogeneic human MSC-derived exosomes	Ruijin, China	NCT04602104
13	Not yet recruiting; phase 1	Dystrophic epidermolysis bullosa	Allogeneic MSC-derived EVs	Aegle Therapeutics	NCT04173650
14	Enrolling by invitation; early phase 1	Ulcer	Autologous exosome-rich plasma	Kumamoto, Japan	NCT02565264
Other cells or EV sources					
15	Active; phase 1	SARS-CoV-2 pneumonia	T-cell-derived exosomes	Kayseri, Turkey	NCT04389385
16	Not yet recruiting; phase 2	SARS-CoV-2 pneumonia, acute respiratory distress syndrome	Bone-marrow-derived EVs	Direct Biologics	NCT04493242
17	Active; phase 1	Head and neck cancer, oral mucositis	Grape exosomes and fentanyl patch	Louisville, USA	NCT01668849
Drug-loaded EVs					
18	Recruiting; phase 1	Metastatic pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma	Mesenchymal-stromal-cell-derived exosomes loaded with siRNA against KrasG12D	Houston, USA	NCT03608631
19	Active; phase 1	Colon cancer	Plant exosomes loaded with curcumin	Louisville, USA	NCT01294072
20	Completed; phase 2	Non-small-cell lung cancer	Dendritic-cell-derived exosomes loaded with antigen	Villejuif, France	NCT01159288

Herrmann et al., Nat. Nanotechnol. 2021

Scale-up and manufacturing

Mass production of therapeutic EVs

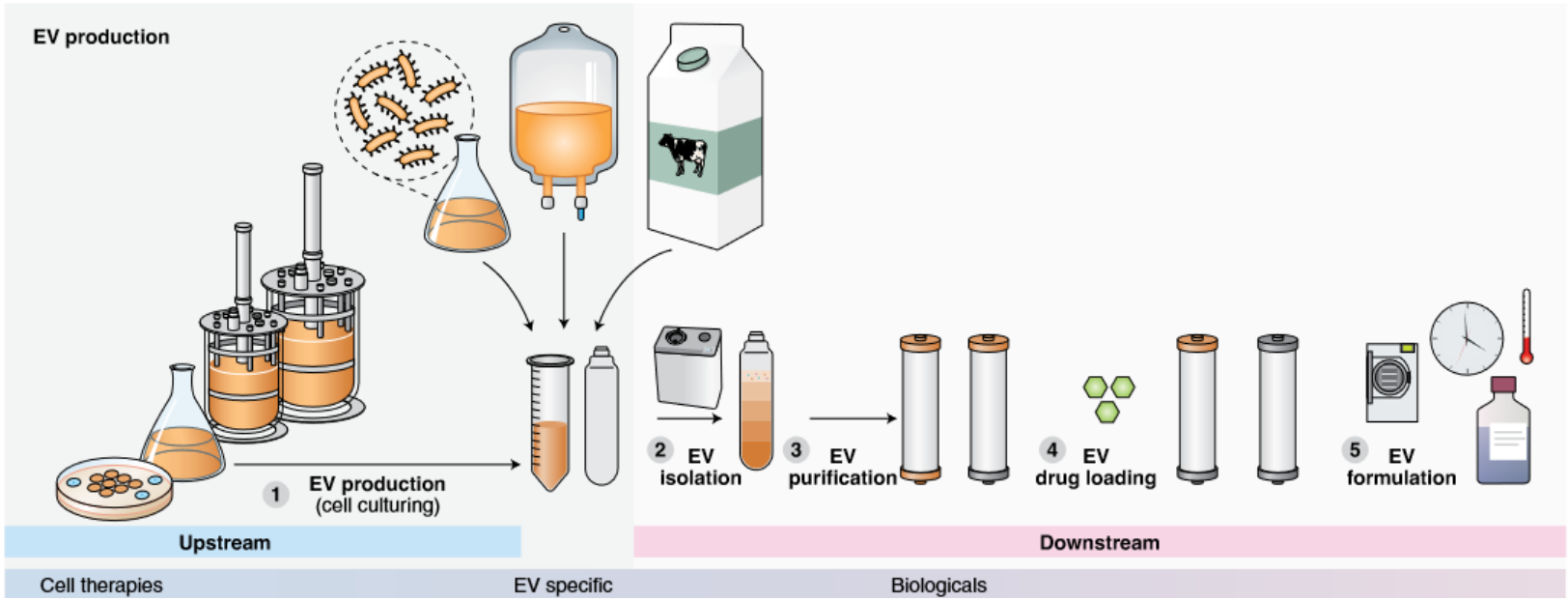
- Should benefit from the existing fields of bologics, liposomes and cell-based therapie.

Upstream process

Cell culture, drug loading

Downstream process

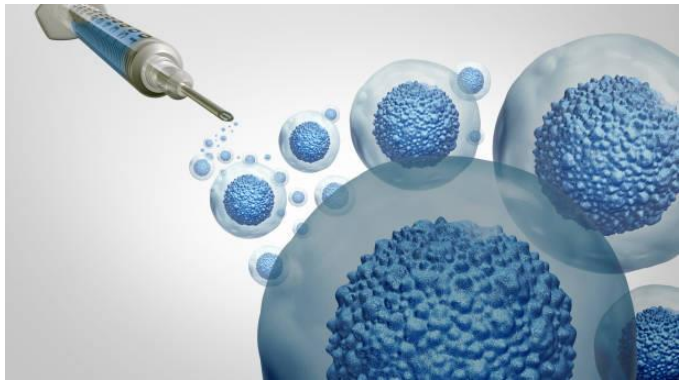
Isolation/purification of EVs, CQ, EV engineering, formulation, storage



Herrmann et al., Nat. Nanotechnol. 2021

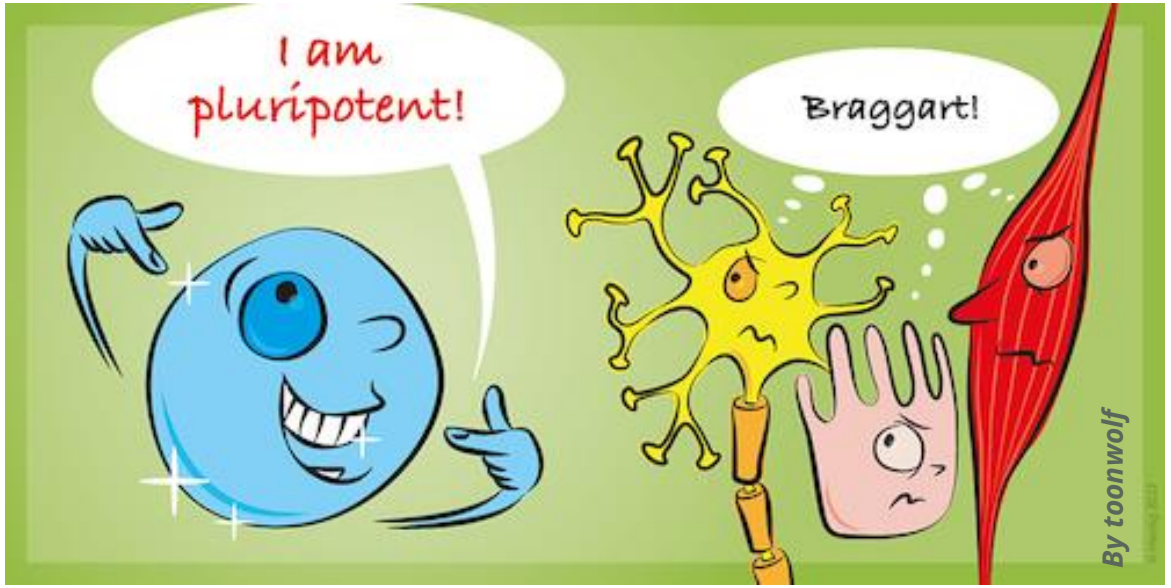
EV-based therapy Vs Cell-based therapy

Cell-based therapy



- Treatment of human diseases by **administration of living cells with in-vivo therapeutic effect**

- **To do what?**
  - Regenerative medicine
  - Immunotherapy
  - Cancer



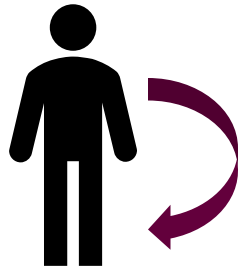
Cell-based therapy

- Classification based on the **origin of the cells**....
  - **Autologous**: use the person's own cells
  - **Allogenic**: cells come from a donor

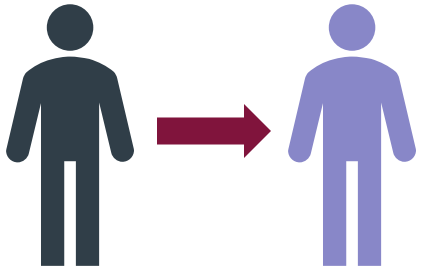
....and their **differentiation level**

- **Stem cells**
- **Differentiated/specialized cells**

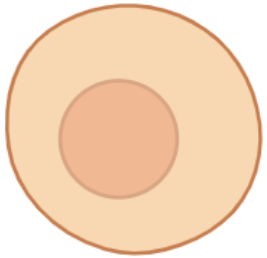
Autologous



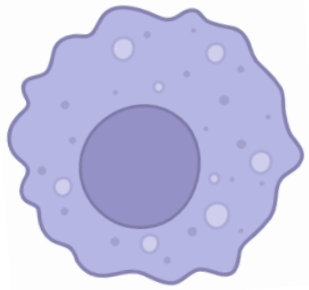
Allogenic



Stem cell



Differentiated cell

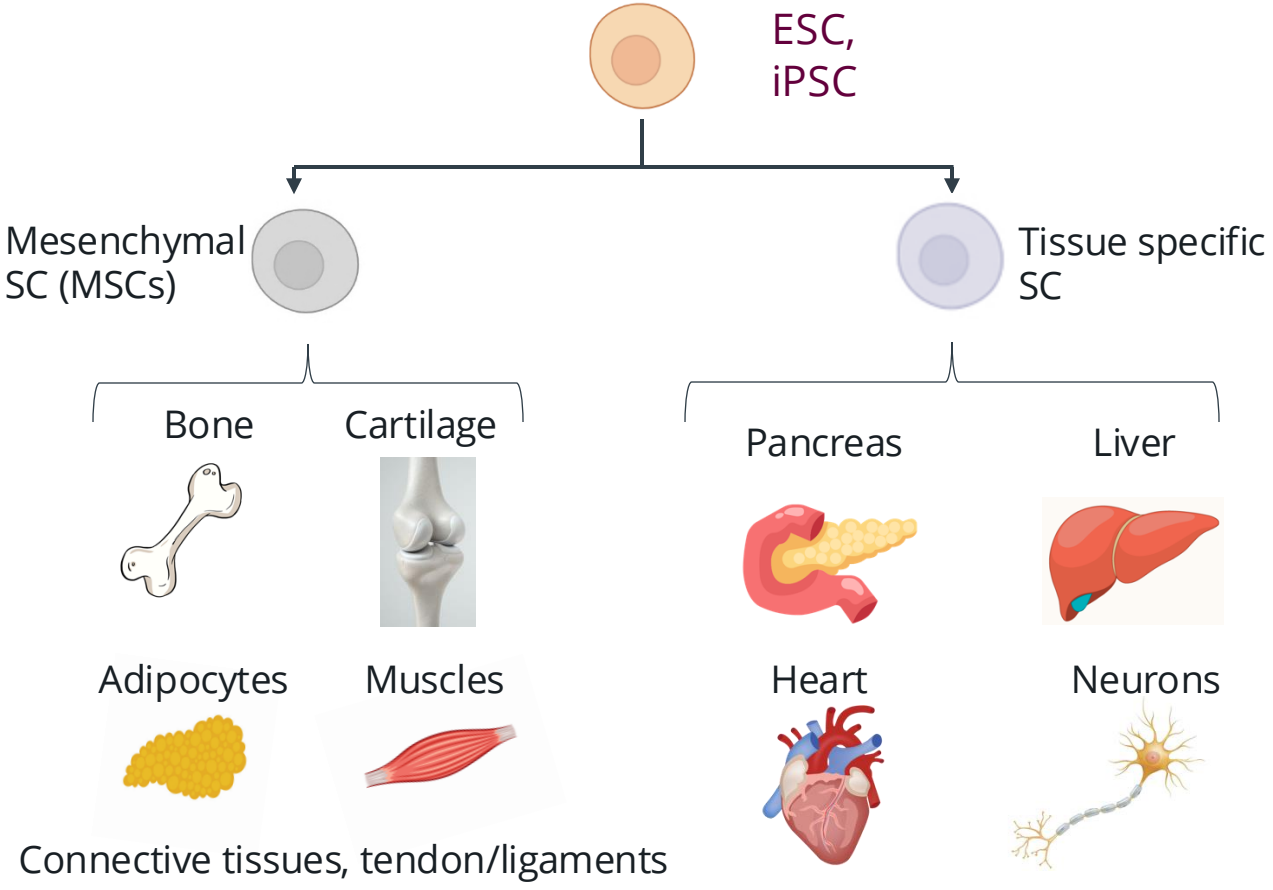


Course of Pr N. Chaput

Cell-based therapy

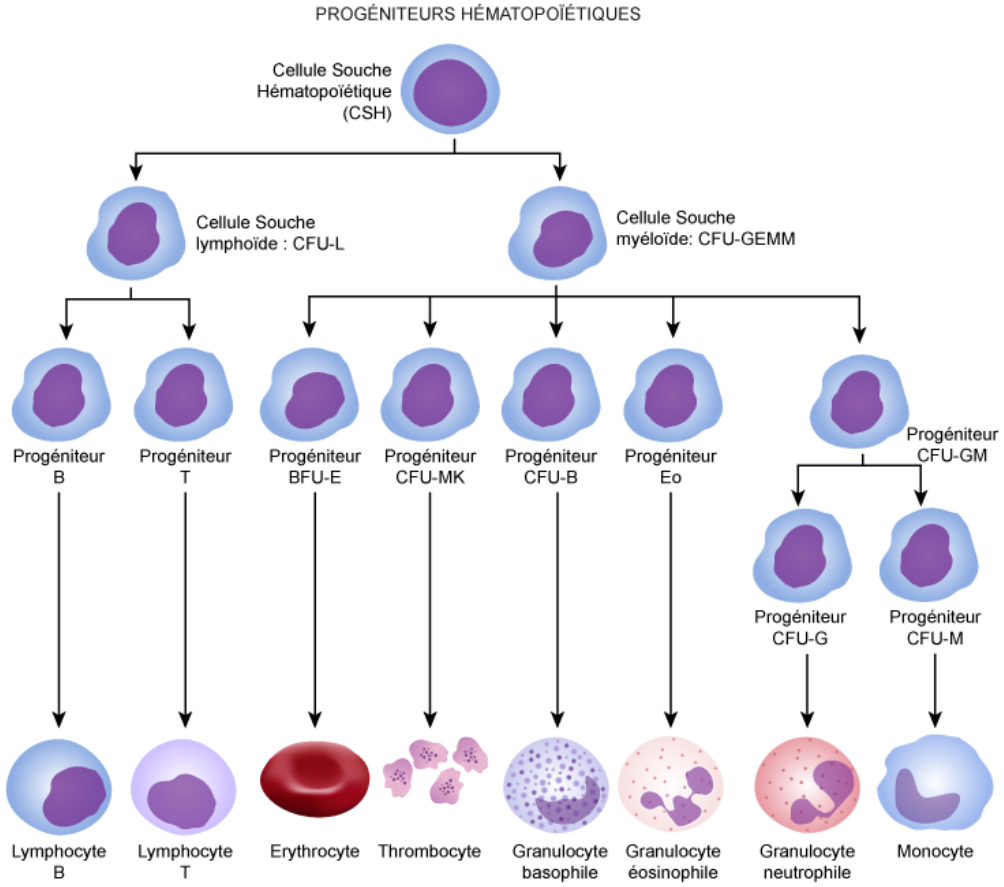
Pluripotent stem cells

Capable to give rise to all cells of the tissues of the body

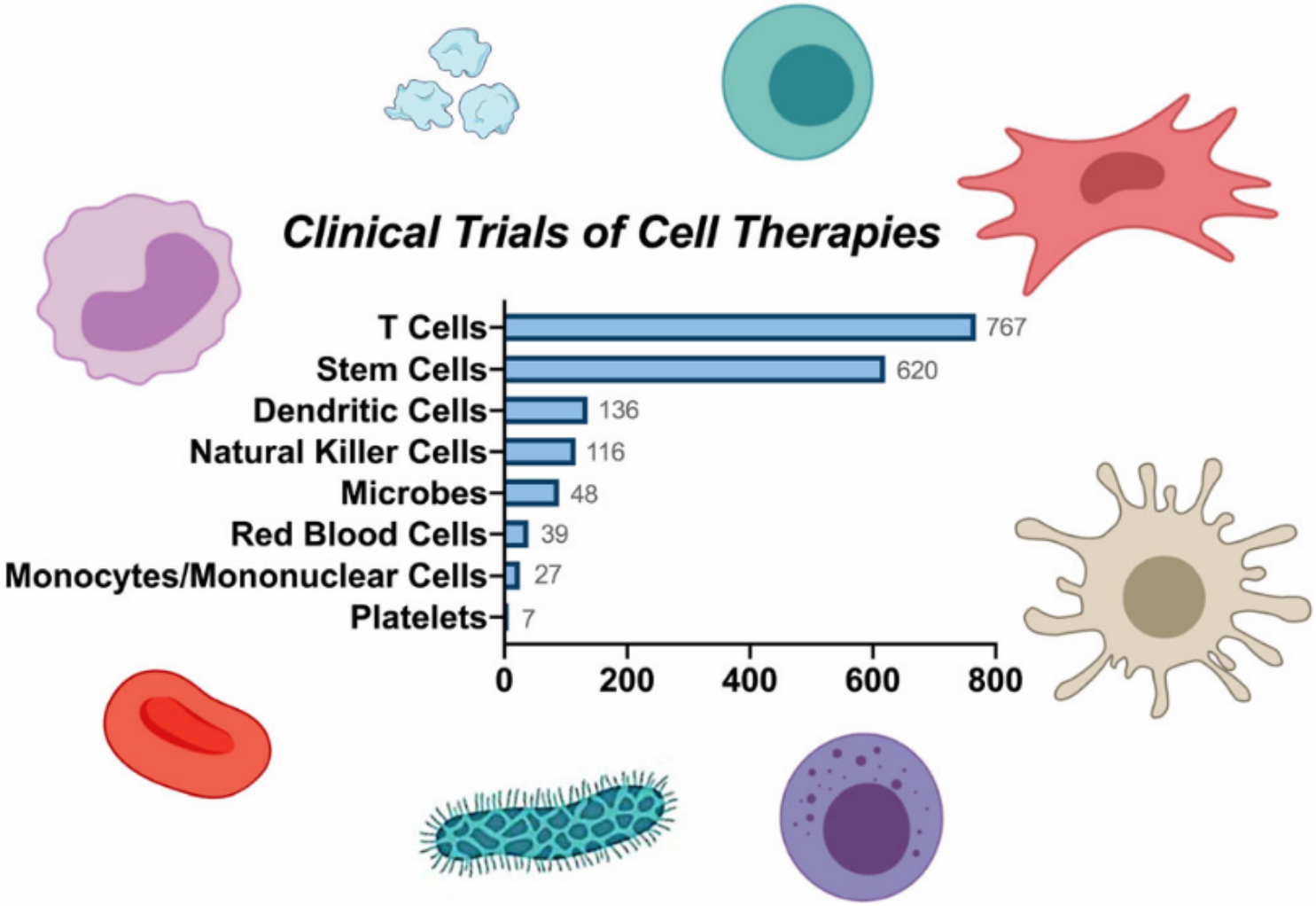


Multipotent stem cells

Capable to give rise to specific types of cells

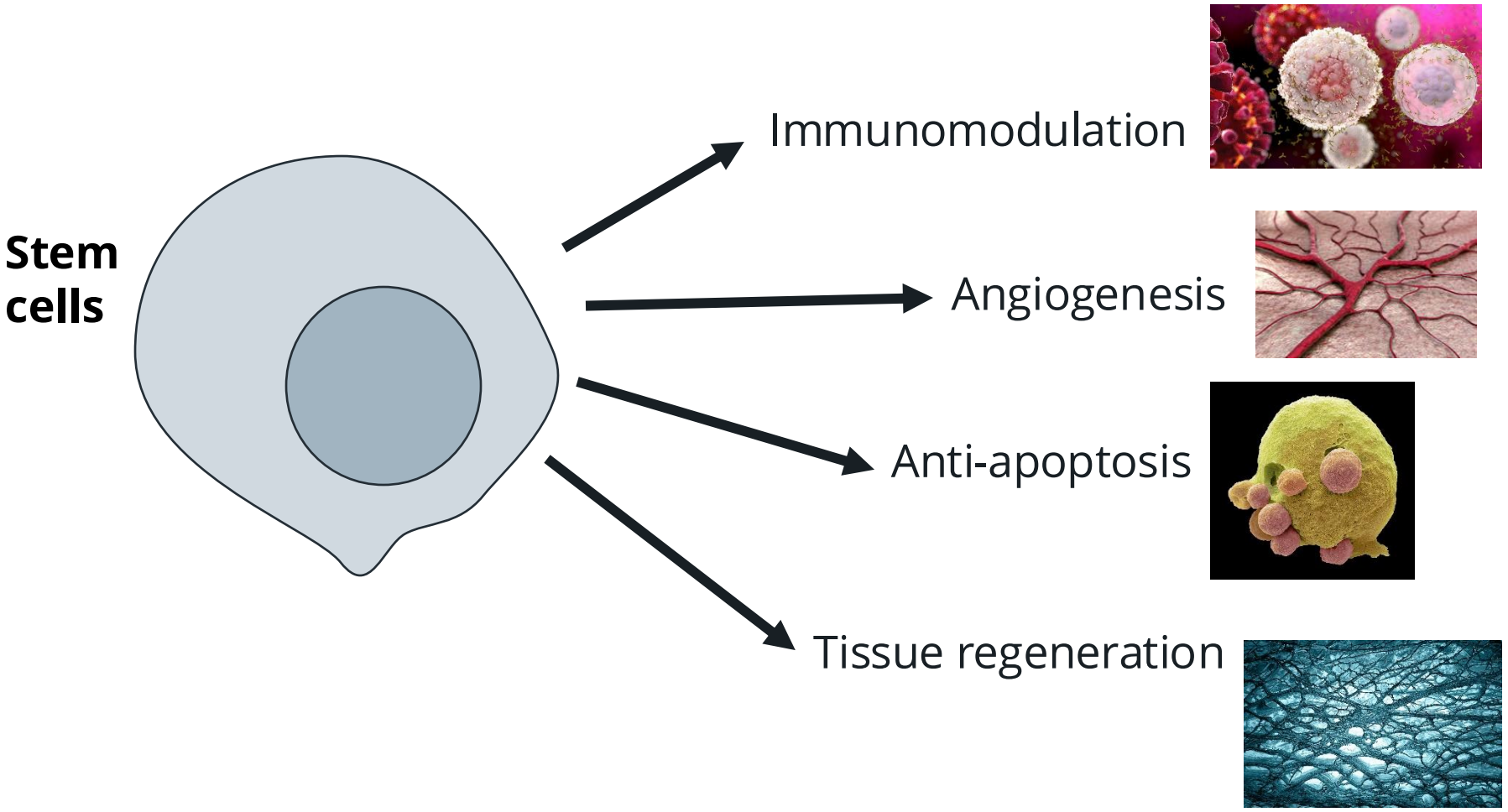


Cell-based therapy



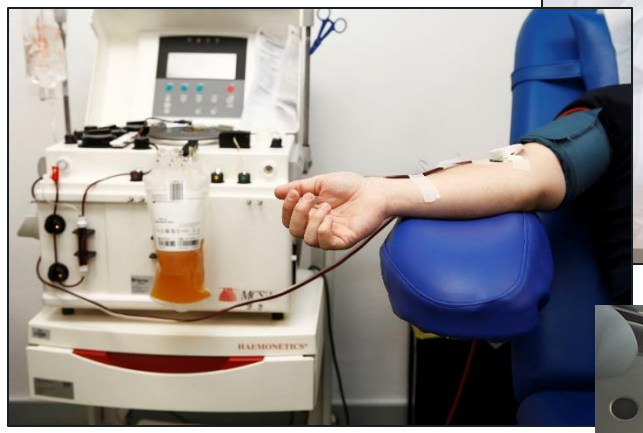
Wang et al., Bioeng Transl Med. 2021

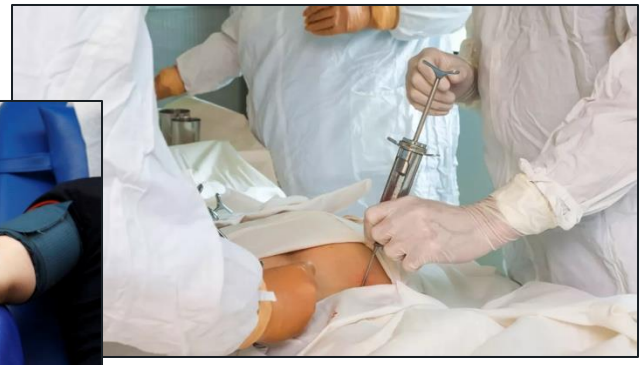
Cell-based therapy

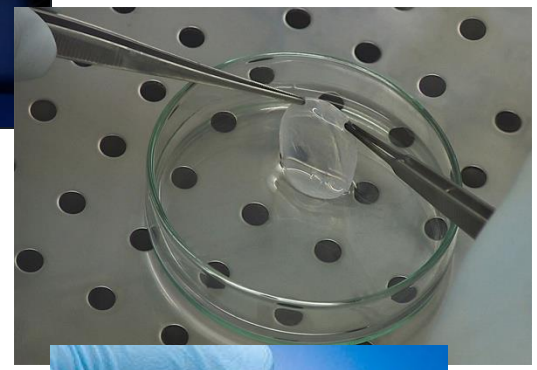


Challenges in cell-based therapy

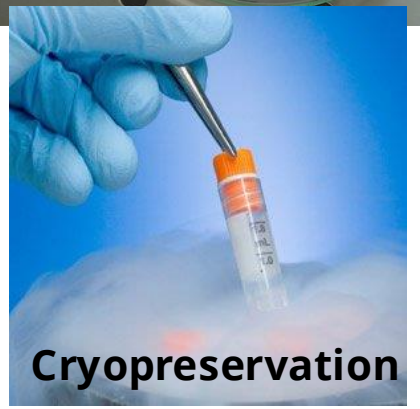
- Ethical issues
- Availability
- Collection
- Cellular senescence in culture
- Stability after administration
- Tumorigenicity
- Immunogenicity
- Risk of microvasculature occlusion
- Logistical challenges (cryopreservation, transport…)
- Cost







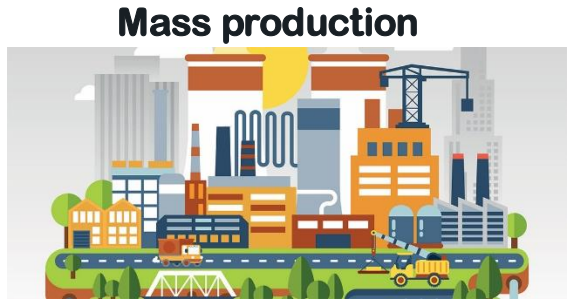
Blum & Benvenisty, Adv Cancer Res. 2008

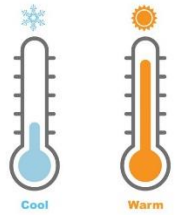


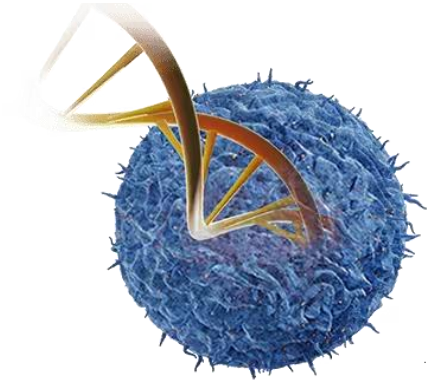
Huang and Lai, Ann Transl Med 2019
Aubertin et al., Med Sci 2021

Advantages of EVs-based therapy

- Logistics (availability, storage, stability)
- Tissue penetration/crossing biological barriers
- No replication/differentiation
- Minimal risk of microvasculature occlusion
- Low immunogenicity
- Possibility of engineering
- Cost



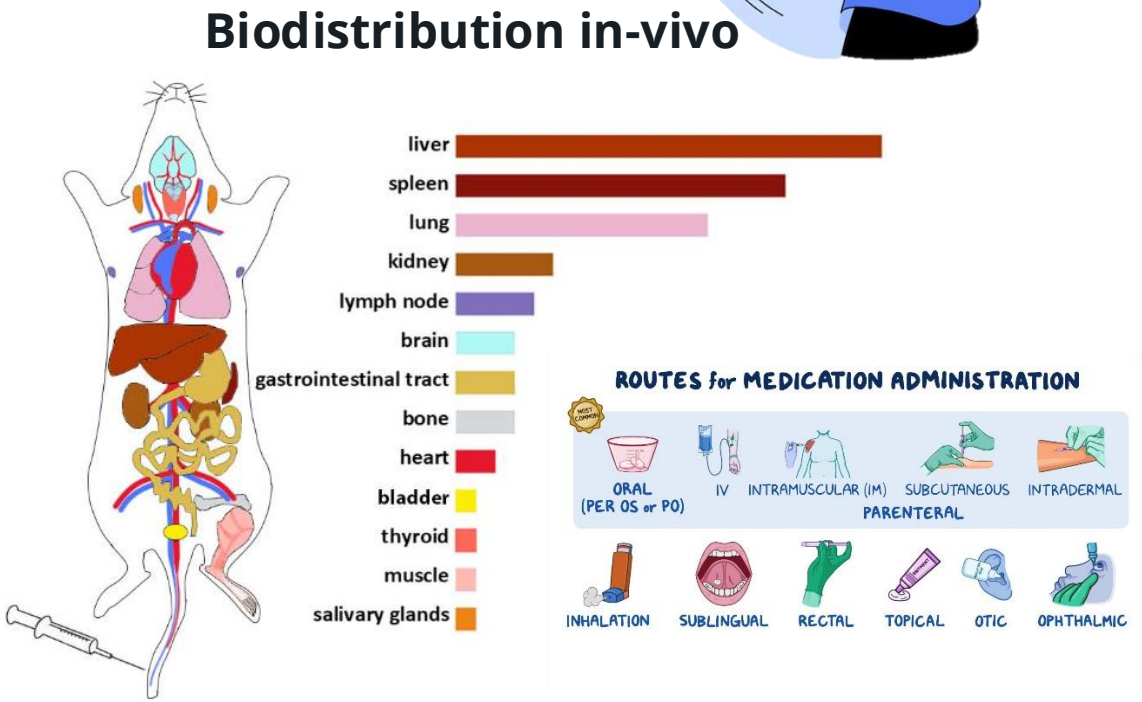




Huang and Lai, Ann Transl Med 2019
Aubertin et al., Med Sci 2021

Challenges in clinical translation of EV-based therapy

- Mass production (cell culture conditions, yield, reproducibility…)
- Isolation of EVs
- Characterization of VEs
- Choice of administration route
- Optimal dosage
- Regulatory frameworks

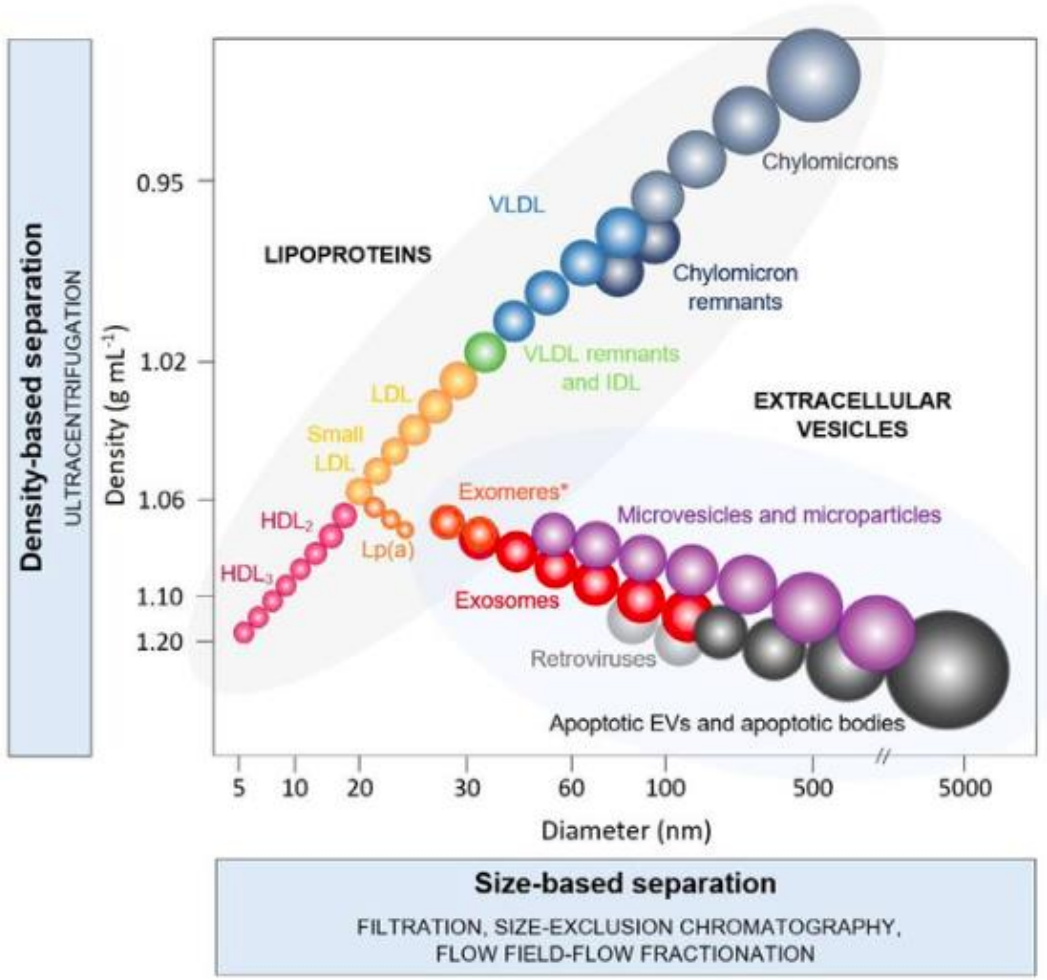


Huang and Lai, Ann Transl Med 2019
Aubertin et al., Med Sci 2021

EV isolation and characterization

Challenges in EV isolation and characterization

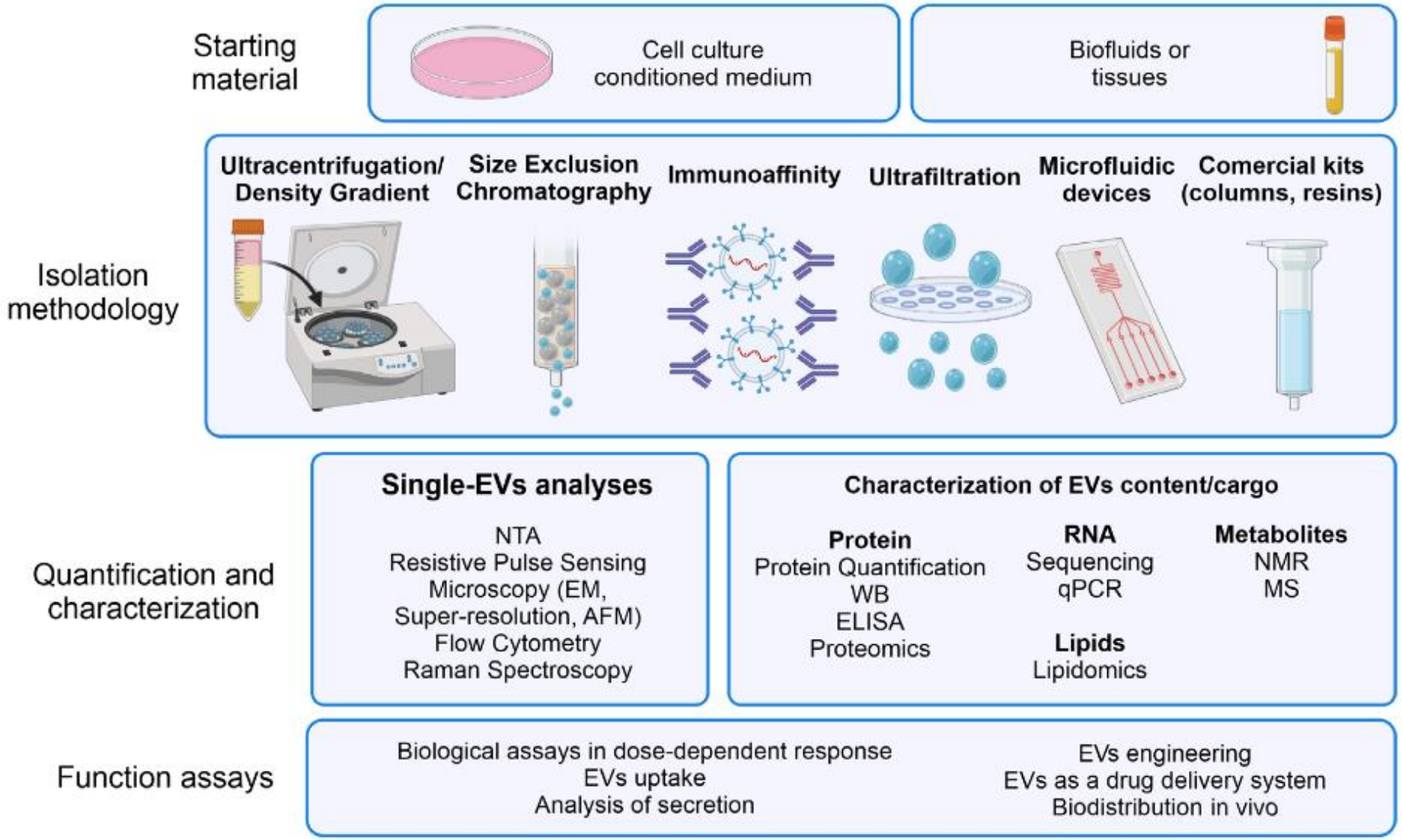
- EV isolation and characterization is hampered by:
  - ➢ Their high heterogeneity (size and composition)
  - ➢ Their nanometric size
  - ➢ The presence of contaminants with overlapping physico-chemical properties (viruses, lipoproteins, protein aggregates)



Liangsupree et al., J Chromatogr A. 2021

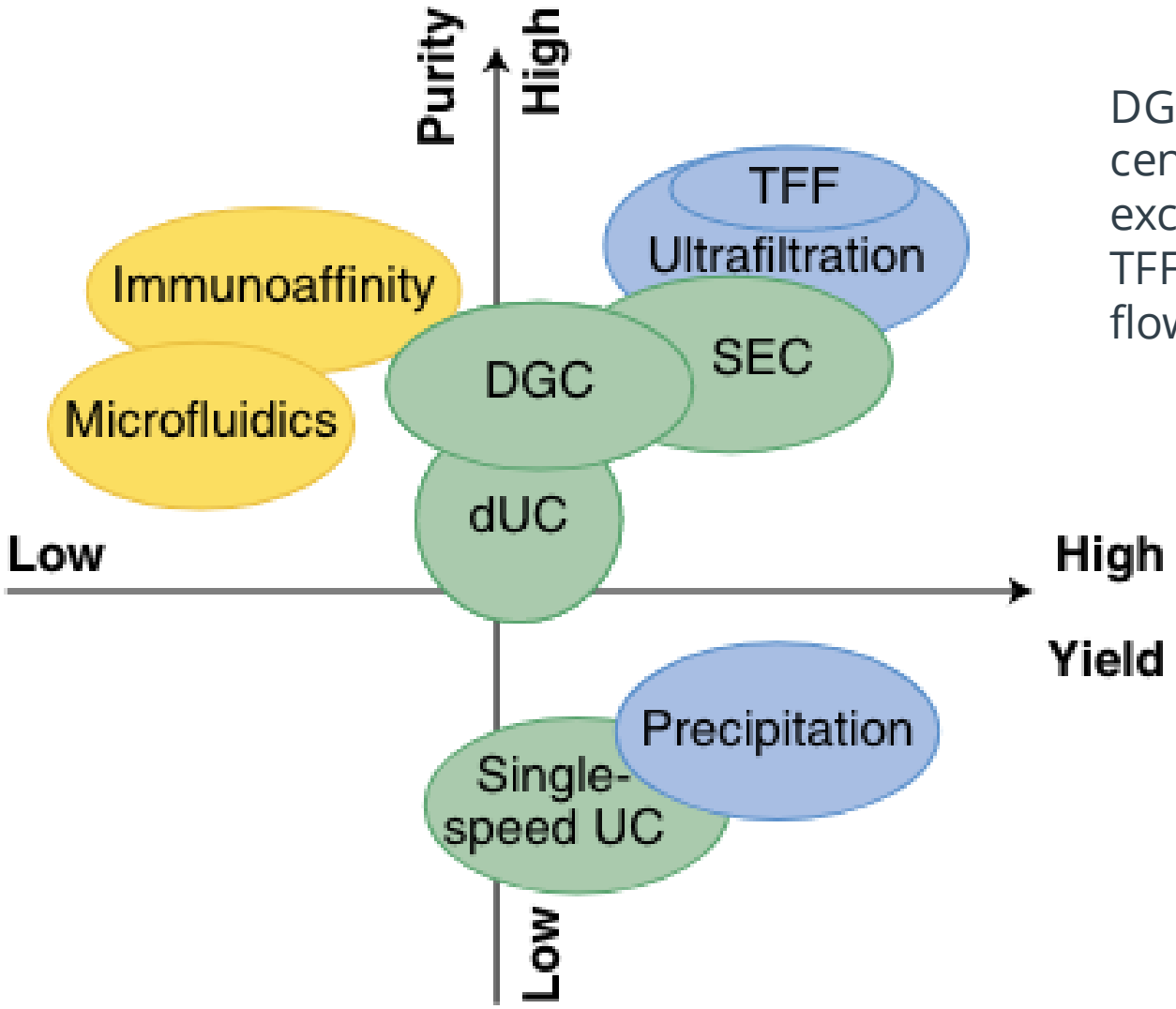
EV isolation and characterization

Schematic representation of the workflow for isolation and characterization of EVs



De Sousa et al., Wiley Interdiscip Rev Nanomed Nanobiotechnol 2023

EV isolation methods



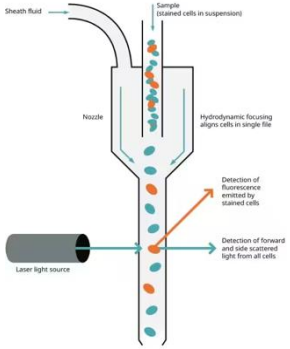
DGC: density gradient centrifugation; SEC, size exclusion chromatography; TFF, tangential flow (cross-flow) filtration.

Herrmann et al., Nat. Nanotechnol. 2021

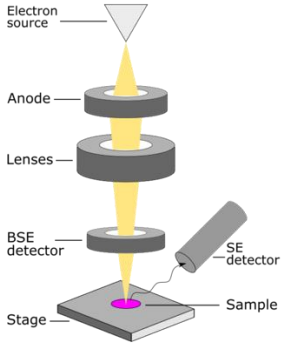
EV characterization techniques

- Physicochemical characterization of EVs is crucial to understand their functional roles
- Variety of techniques are used to detect/characterize EVs

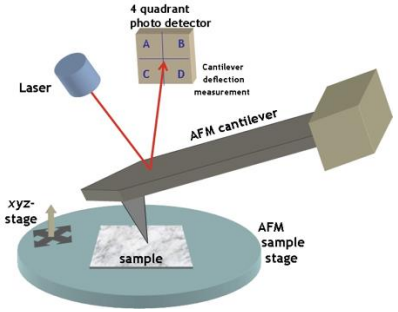
Flow cytometry



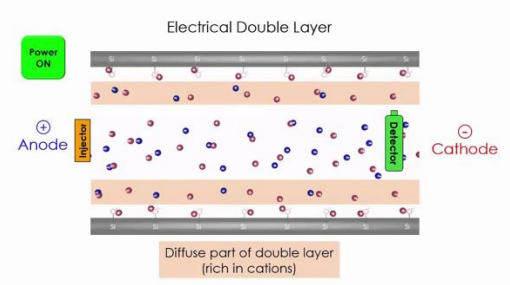
Electron microscopy



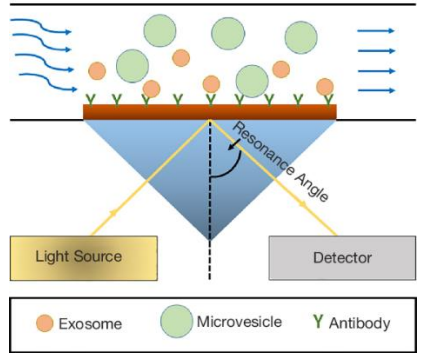
AFM



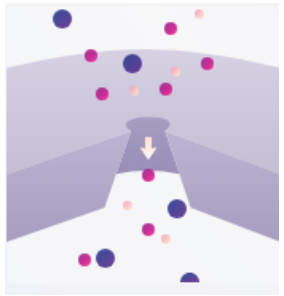
CE



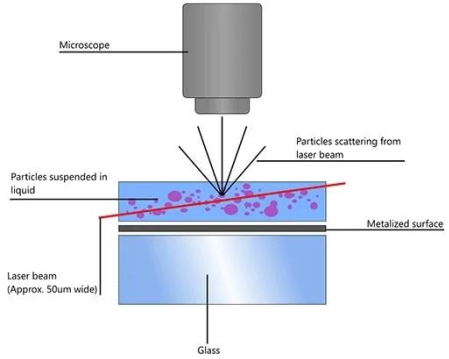
SPRi



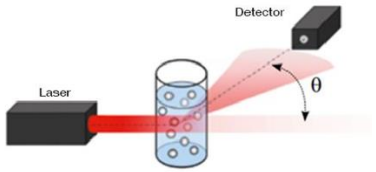
TRPS



NTA



DLS



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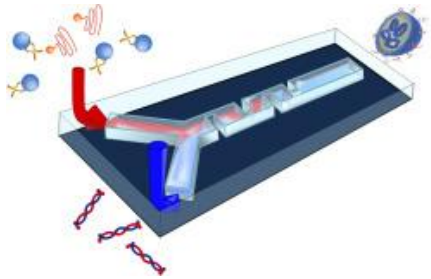
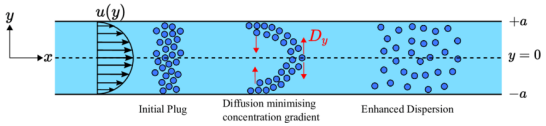


TDA

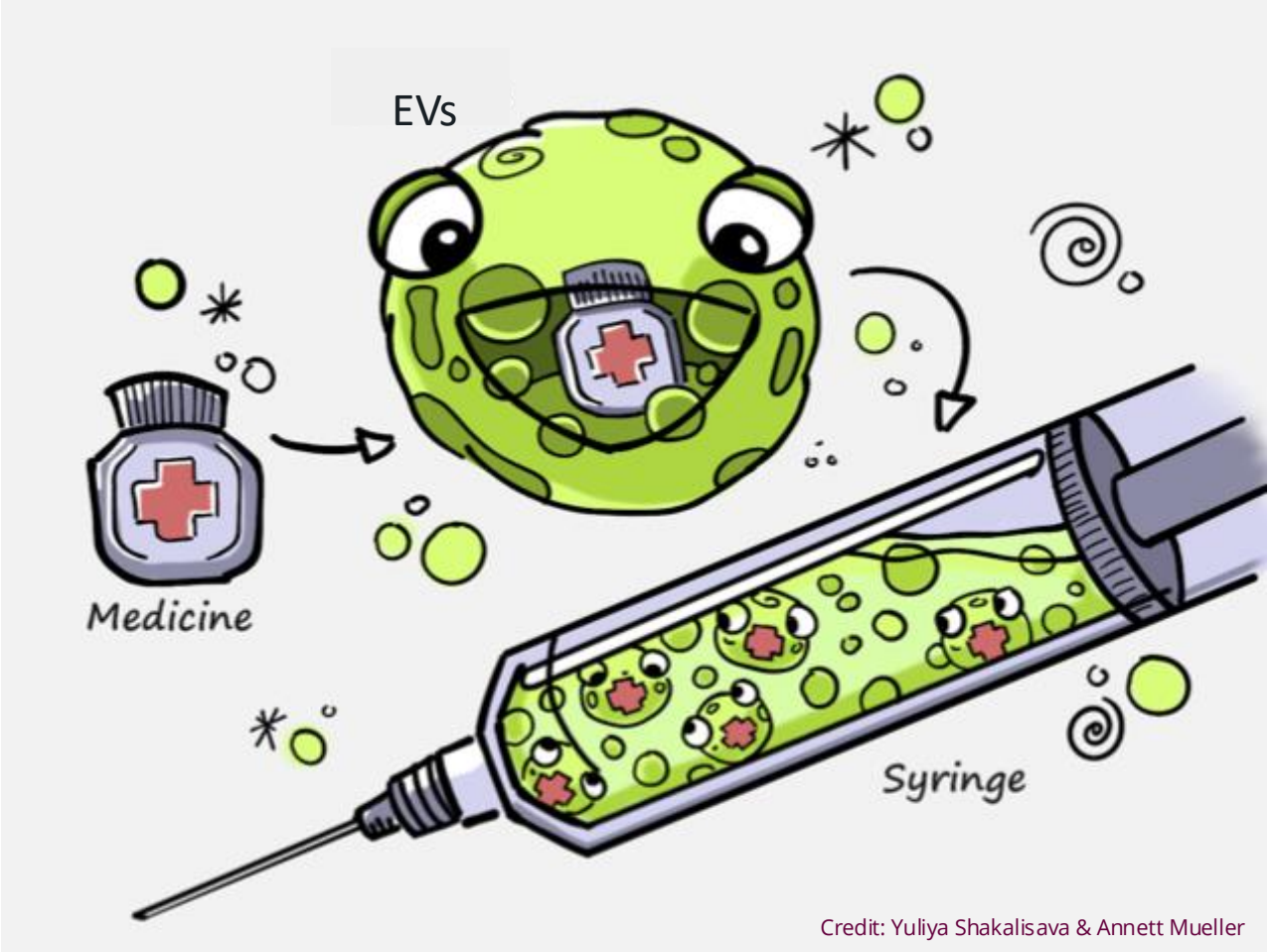


EV characterization techniques

Method	Information Acquired	Advantages/Limitations
Electron microscopy (EM)	EV dimension and morphology	- Direct assessment of morphology and size; small sample amount - Time consuming; size and morphology modifications due to sample preparation
Atomic force microscopy (AFM)	EV three-dimensional topography	- No sample fixation and staining; small sample amount - Size and morphology modifications due to sample dehydration on mica surface
Dynamic light scattering (DLS)	EV size distribution	- Fast; no sample preparation; sample preservation for downstream analysis - Inaccurate with polydispersed and size heterogeneous samples
Nanoparticle tracking analysis (NTA)	EV concentration and size distribution	- Fast; no sample preparation; sample preservation for downstream analysis - Inaccurate with size heterogeneous samples and particle aggregates
Tunable resistive pulse sensing (TRPS)	EV concentration, size distribution and surface charge	- Fast; no sample preparation - Difficulties with unknown and heterogeneous size distribution samples (difficult to select the correct nanopore setup); detection of non-vesicular material within size range
Flow cytometry	EV marker characterization, absolute counting	- Quantitative and qualitative (using specific antibodies) characterization of EVs - Detection limit (>100 nm, flow cytometer dependent); swarming effect (identification of multiple vesicles as a single event); detection of protein/antibody aggregates
ELISA/Western Blot	EV protein quantification	- Standard immunological methods; specific characterization of EV protein markers - Time consuming; possible detection of non-EV proteins; non-specific information on EV concentration/size/distribution

Each technique has its advantages and limitations

Chiriacò et al., Sensors 2018



Credit: Yuliya Shakalisava & Annett Mueller

End of 1st part