Máster en Biología Sanitaria

Vesículas extracelulares en la fisiología y fisiopatología vascular

Rafael Ramirez
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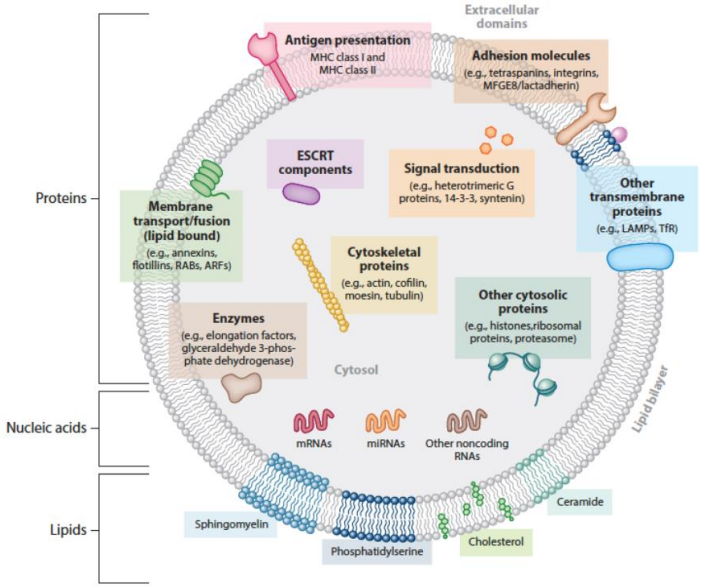
¿ Que son las vesículas extracelulares ?

Cell-Derived Vesicles



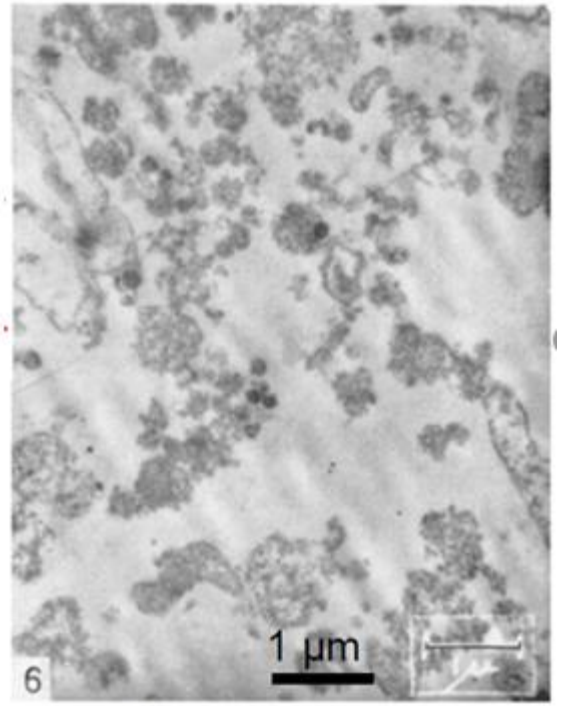
- **EVs are spherical particles enclosed by a phospholipid bilayer.**
- **Both Eukaryotic and Prokaryotic cells release vesicles.**
- **Contain several types of function molecules, such as proteins, mRNAs, and miRNAs….**

https://doi: 10.1146/annurev-cellbio-101512-122326

Un poco de historia….

- The preliminary studies of cell-derived vesicles were addressing “cell-free plasma contains a subcellular factor that promotes clotting of blood” (Chargaff and West, 1946).

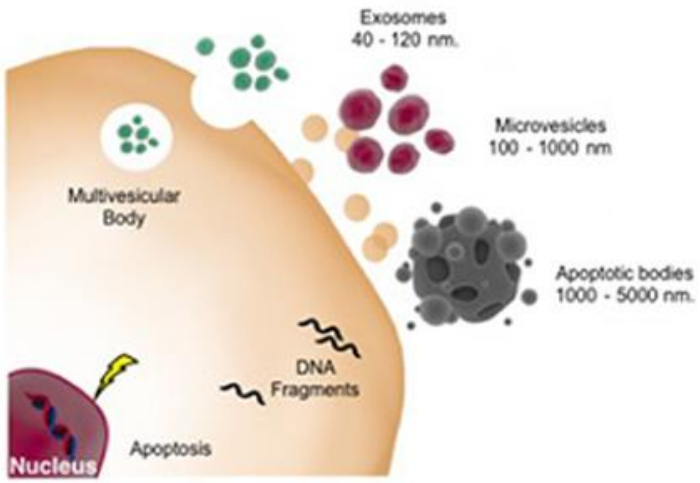
- More than 20 years later, in 1967, this subcellular fraction was identified by electron microscopy and was shown to consist of small vesicles, originating from platelets and termed platelet dust"



Platelet dust from serum
"... for the most part, the material consists of agglutinates of small particles"

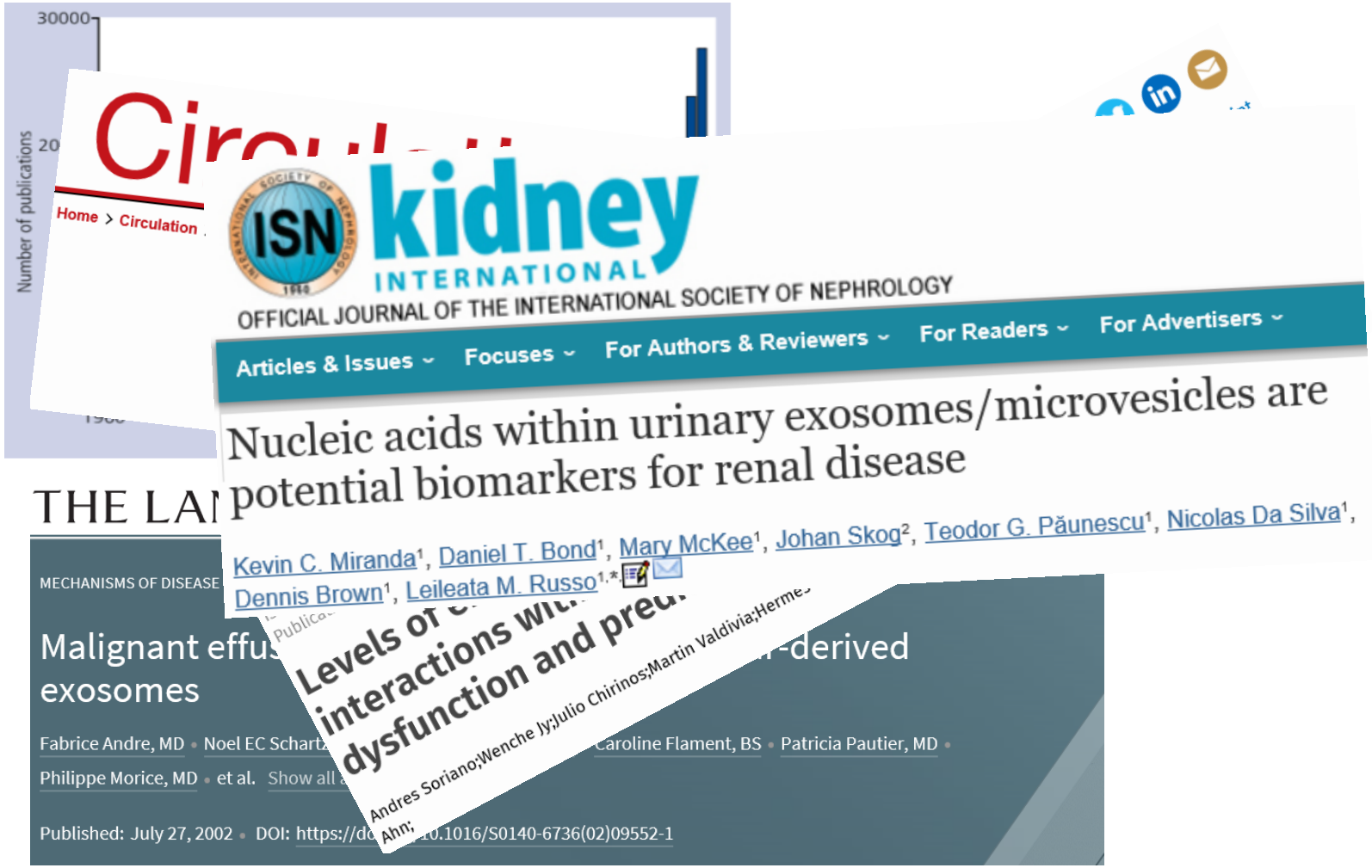
Peter Wolf *Brit. J. Haemat. 1967, 13:269-288*

Un poco de historia….

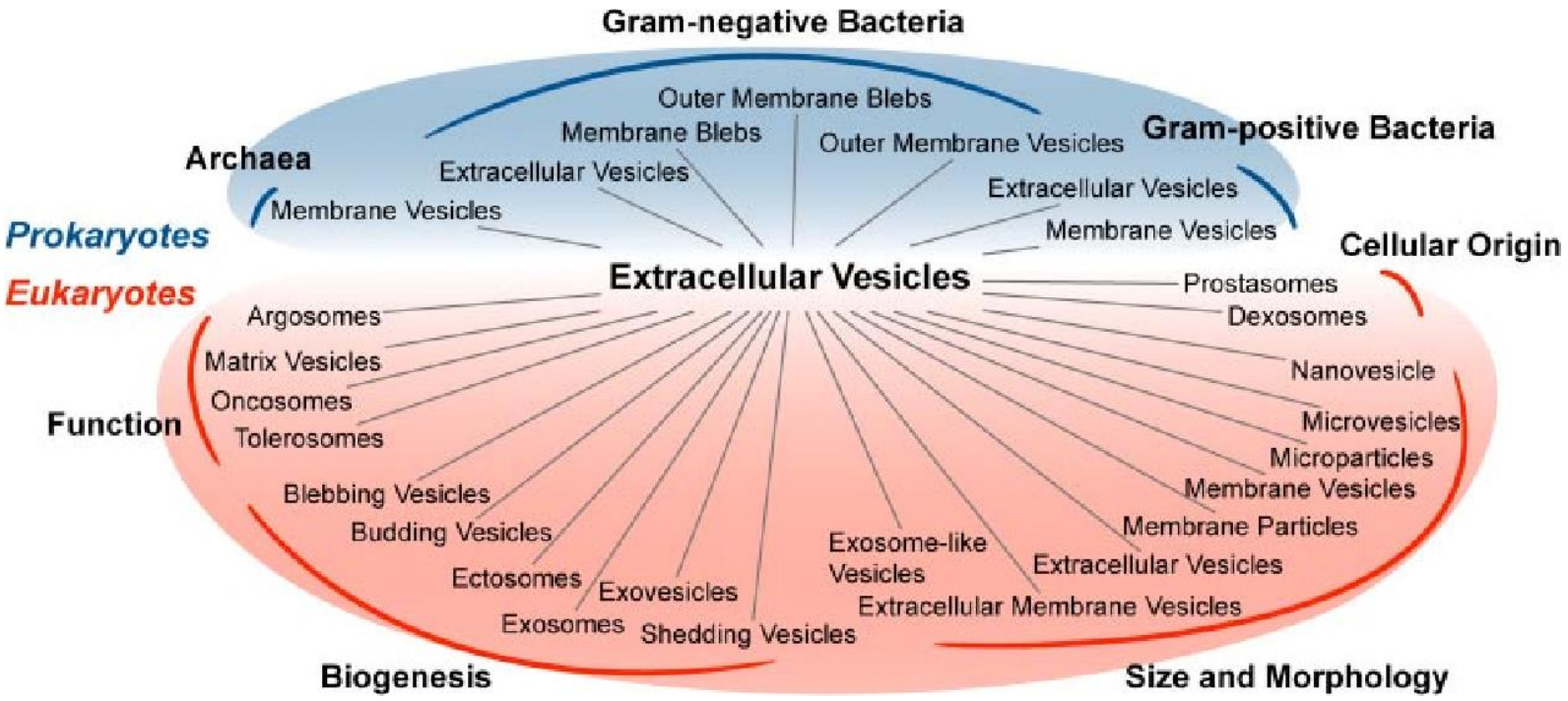


- **One decade later "numerous microvesicles" ranging in diameter from 30 to 60 nm were decribed in the FCS (Dalton, 1975).**
- **In 1987, the term "Exosomes" was introduced by Johnstone when vesicles were isolated from conditioned culture medium of sheep reticulocytes.**
- **In these studies the authors concluded that exosome shedding leads to loss of some plasma membrane functions due to the elimination of redundant membrane proteins**

Un poco de historia….



¿ Como se definen: microvesículas, exosomas… ?



https://doi.org/10.1002/mas.21420|

¿ Como se definen: microvesículas, exosomas… ?

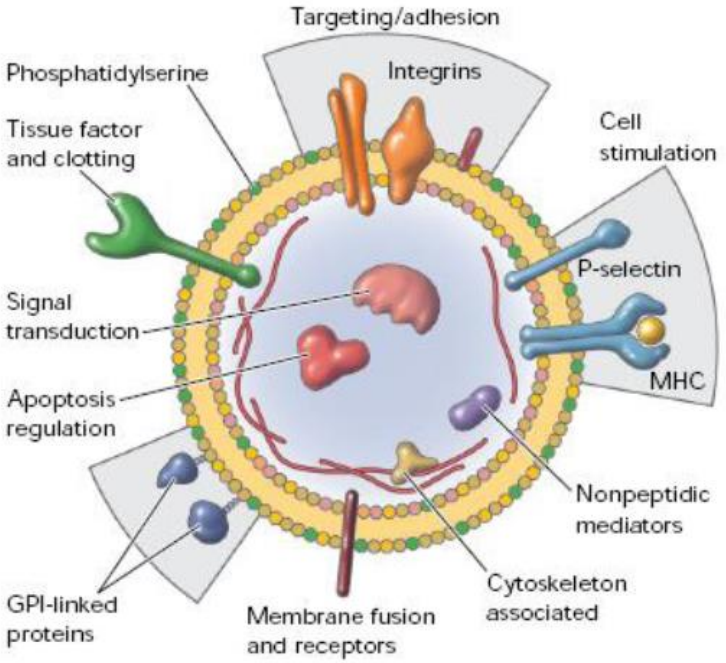
ISEV
INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES

Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines

"To prevent any confusion about the nomenclature of the cell release vesicles, the ISEV community suggests to use the term "extracellular vesicle (EV)" unless the subcellular origin of the vesicle is demonstrated"

¿ Qué son las vesículas extracelulares ?

- Cells release membrane vesicles in the extracelular milieu upon activation
- Evs are found in body fluids: plasma, urine, CSF ..., and in cell culture supernatants
- Evs present surface receptors and/or contain elements (RNAs, miRNAs ..) allowing to identify their cell of origin

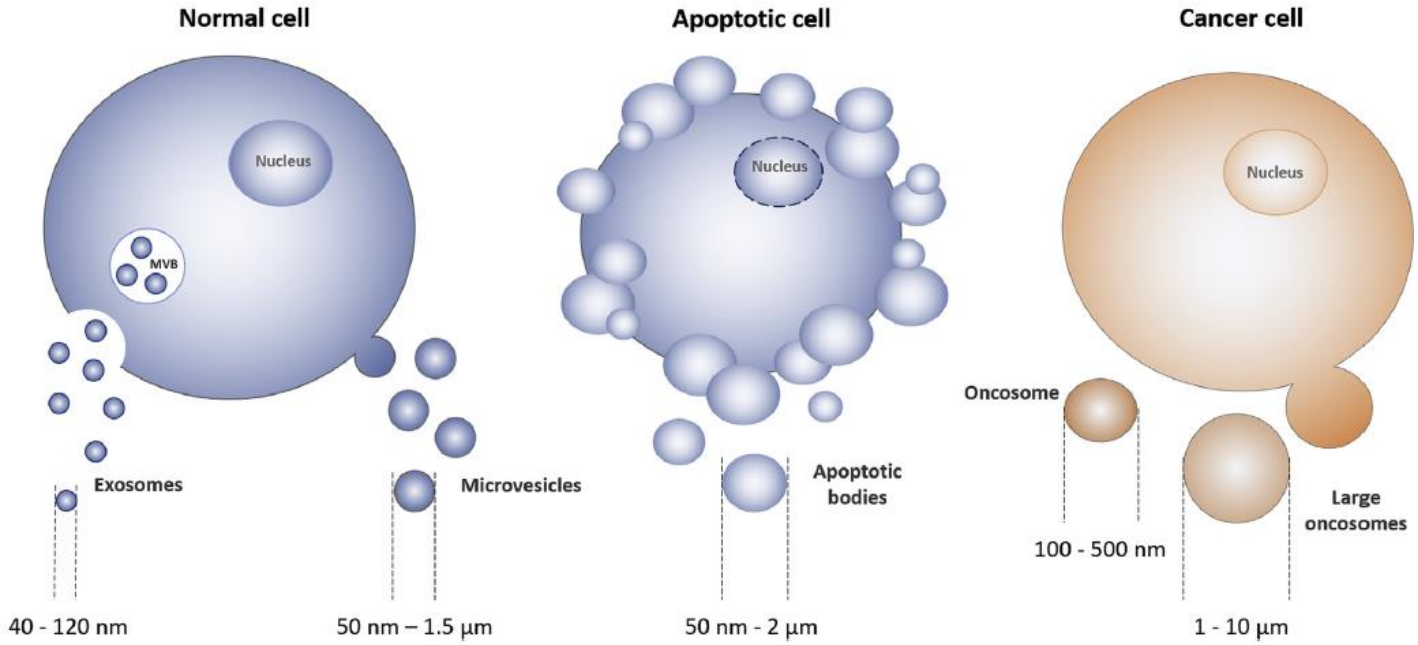


https://doi: 10.1152/physiol.00029.

¿ Qué son las vesículas extracelulares ?

Vesicular objects

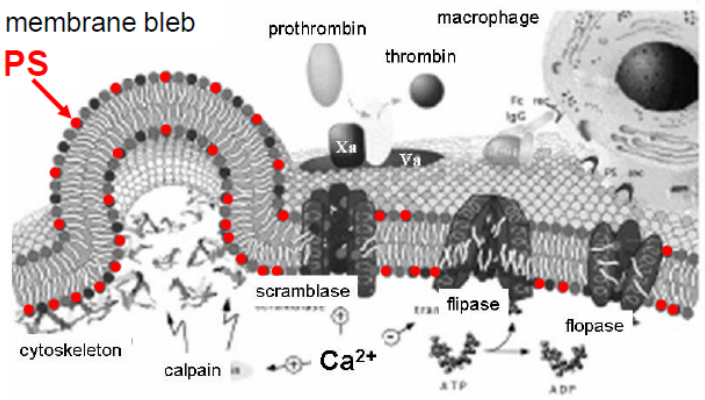
Non-vesicular objects



Modified from: https://doi.org/10.1016/j.mam.2017.12.003

¿ Como se producen ?

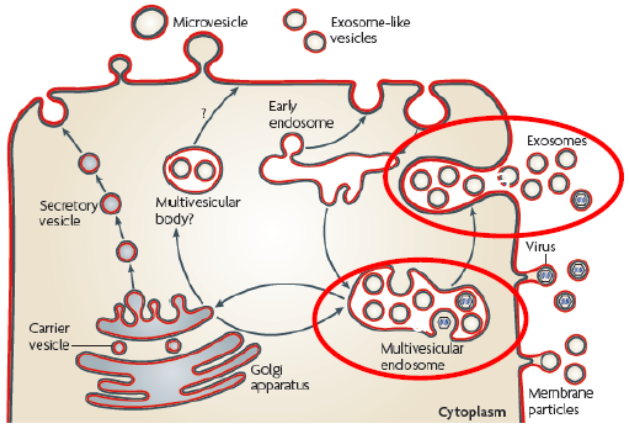
Mechanisms of formation of extracelular vesicles



Zwaal & Schroit 1997, *Blood* 89 (4):1121-1132

Microparticles, Microvesicles
(100 nm - 1 µm) form at the cell plasma membrane:

- entrance of Ca^{2+} & increase of $[Ca^{2+}_i]$
- Ca^{2+}-dependent regulation of enzymes
- loss of membrane phospholipid asymmetry
- exposure of phosphatidylserine (**PS**) on the outer membrane leaflet
- blebbing and shedding of microparticles

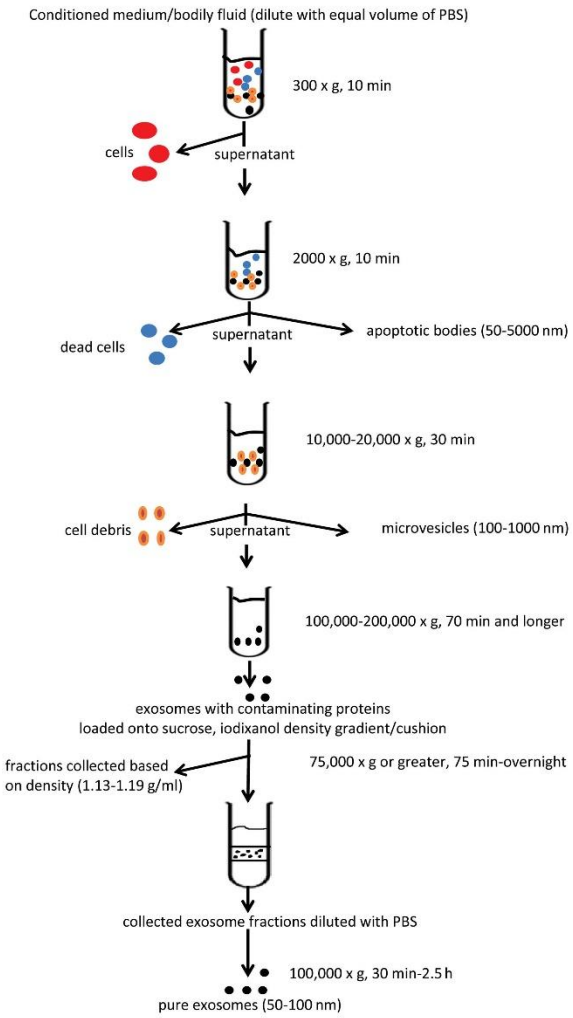


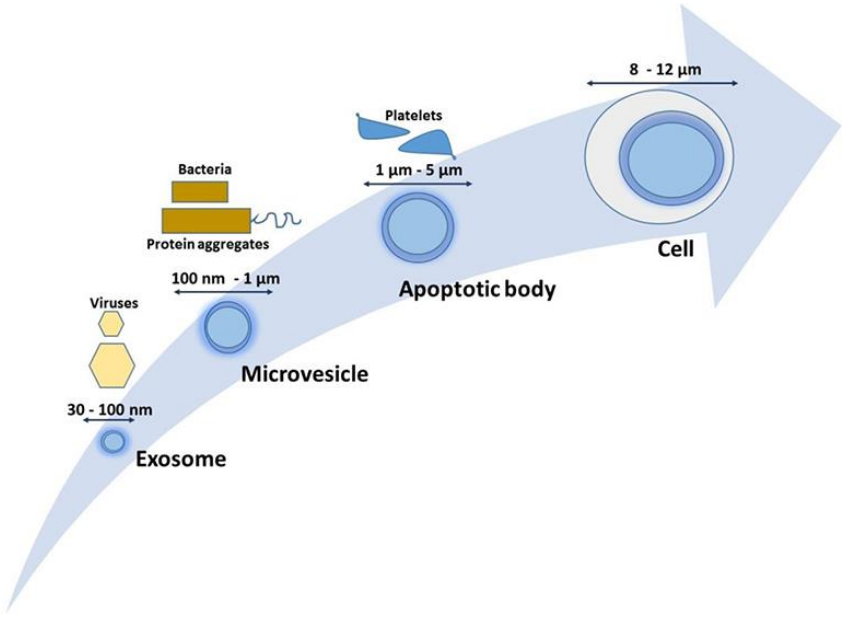
Théry et al. *Nat. Rev. Immunology* 2009, 9:581-593

Exosomes (50 - 100 nm)

- form in multi-vesicular bodies (MVBs)
- are released in the extracellular milieu after fusion of MVBs with the cell plasma membrane
- rich in tetraspanins (CD63, CD81, CD9)

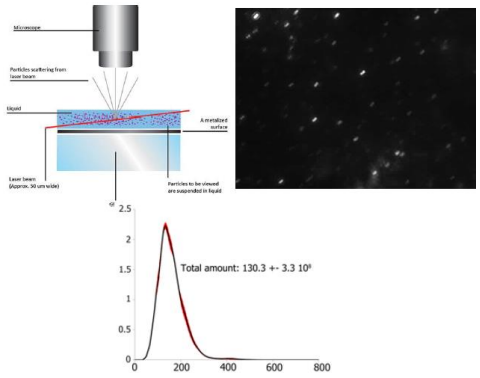
¿ Como se caracterizan ?





¿ Como se determinan ?

Nanoparticle Tracking Analysis



NTA detects particles down to ~20 nm
but detects also non EV material,
e.g. lipoproteins

Single EV detection methods:

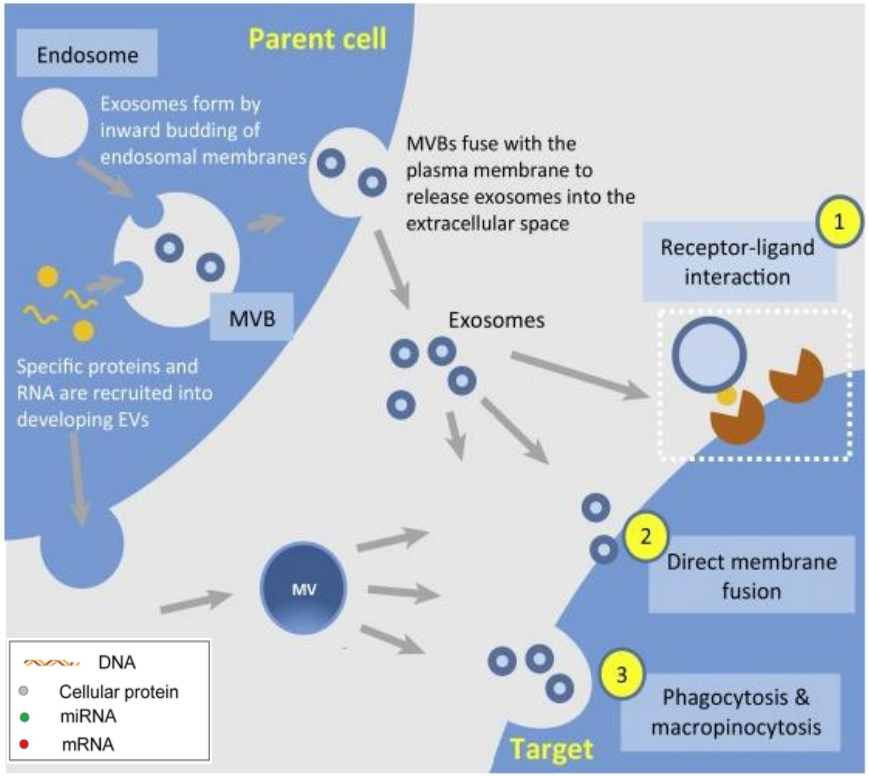
- Electron microscopy
- Flow cytometry
- NanoparticleTracking Analysis (NTA)
- …

Bulk methods:

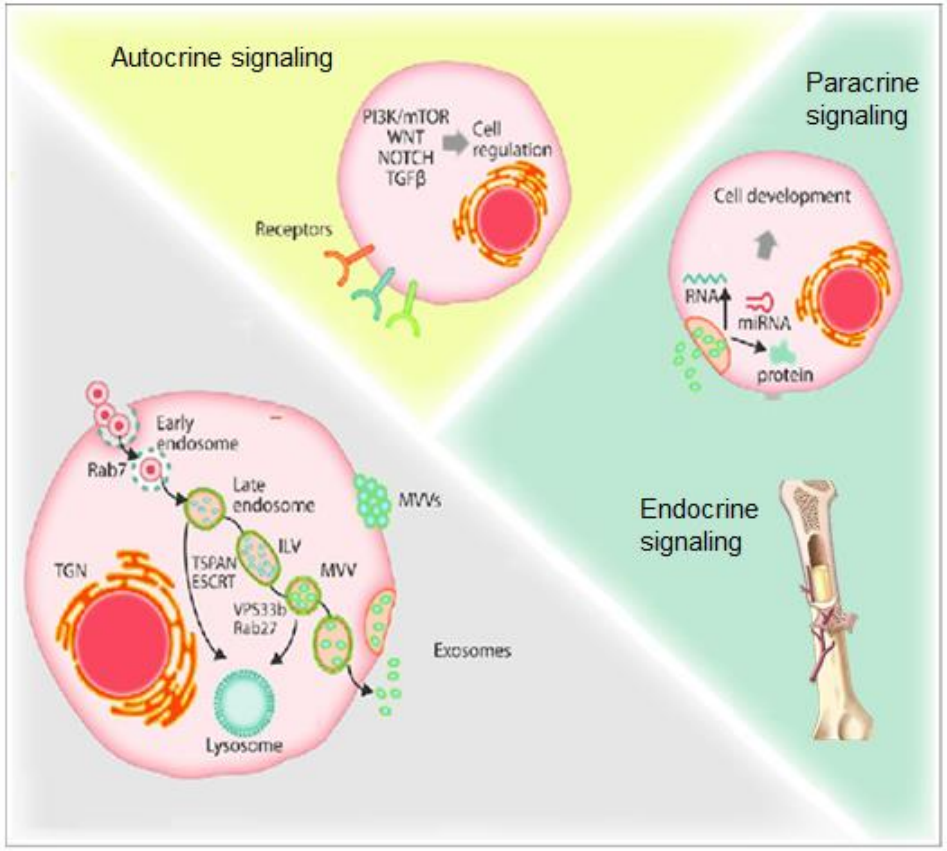
- Western blotting
- "Omic" methods: mass spectrometry, RT-qPCR.
- Functional assays: measurement of activity
- …

¿ Que función tienen ?

Apoptotic Cell-Derived Extracellular Vesicles: More Than Just Debris



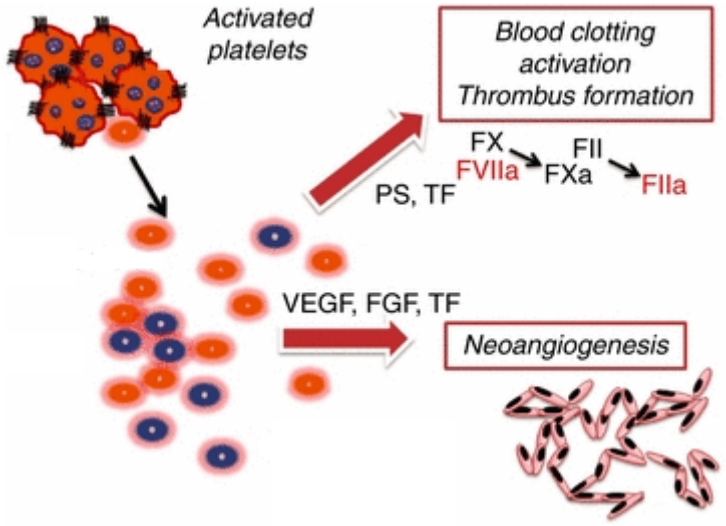
¿ Tienen un papel fisiológico ?



Modified from: doi:10.3324/haematol.2017.183335

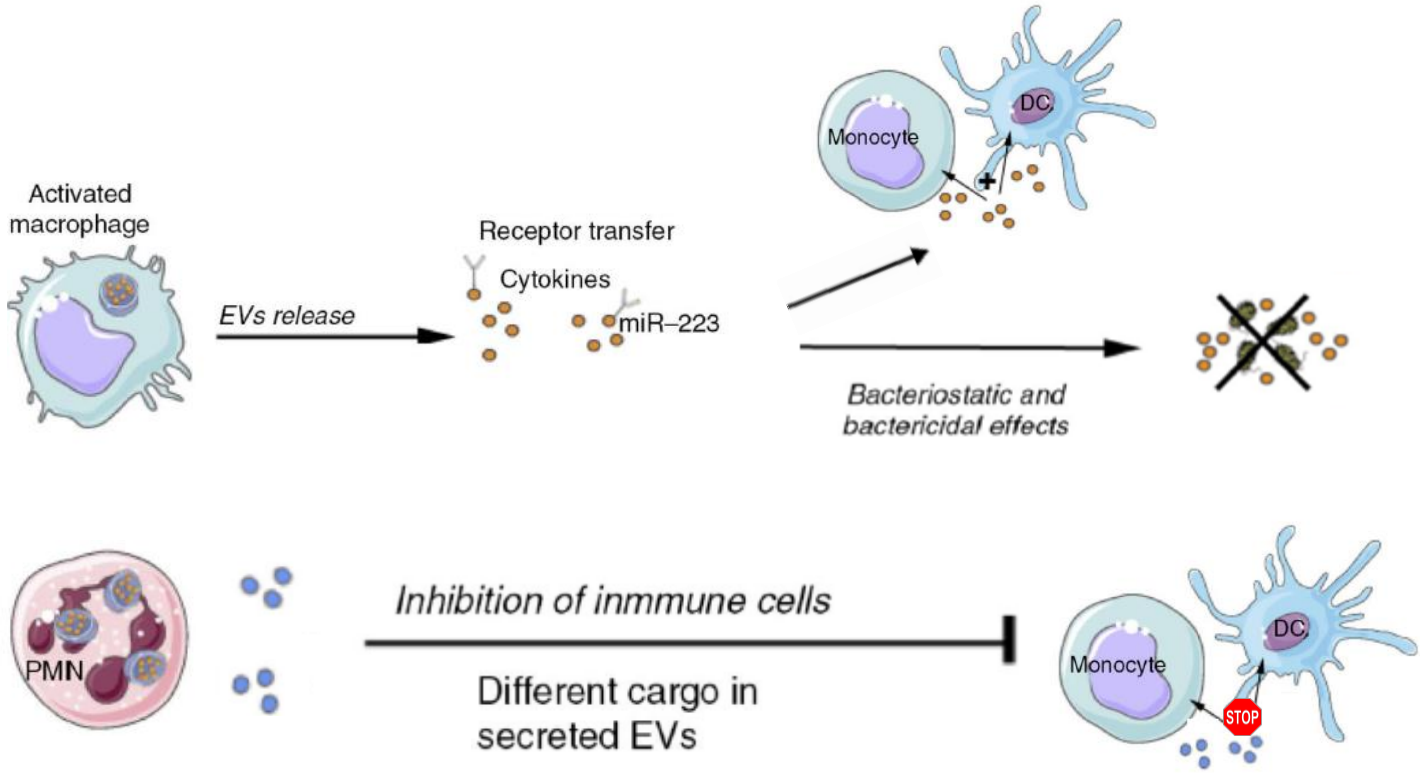
¿ Tienen un papel fisiológico ?

Cross Talk Pathways Between Coagulation and the Platelet Extracelular Vesicles



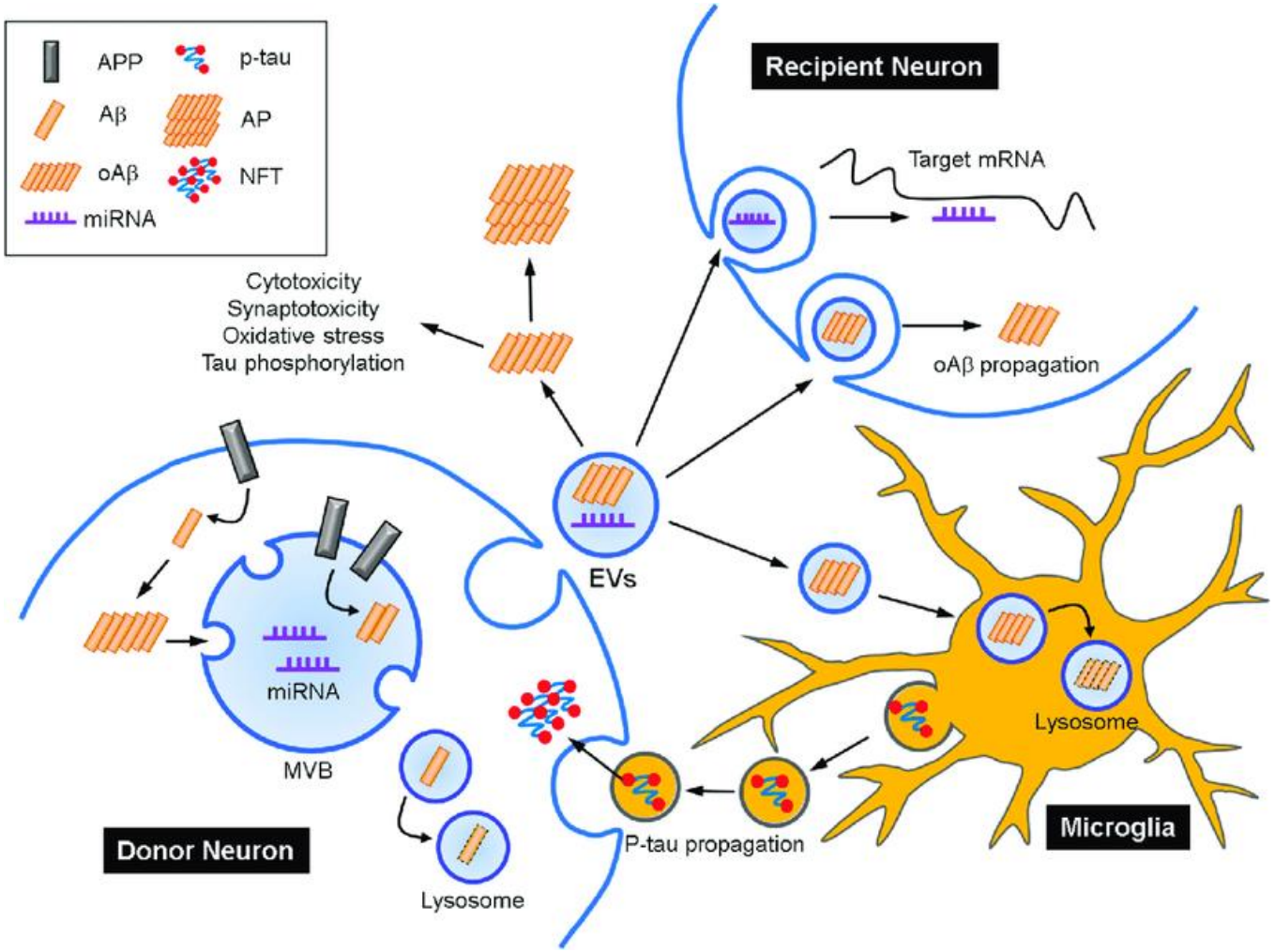
¿ Tienen un papel fisiológico ?

Extracellular Vesicle Signalling Implicated in Immunity

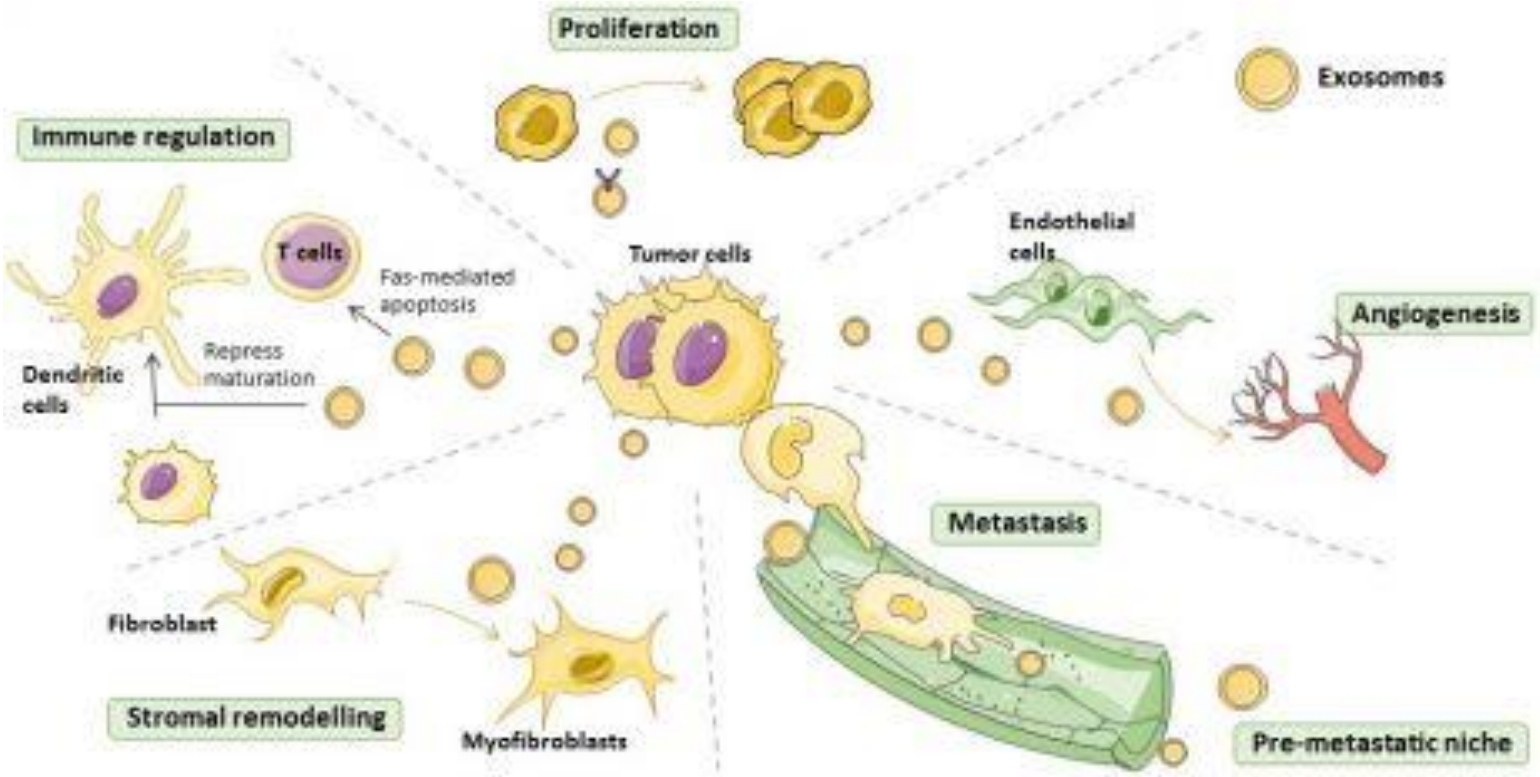


doi: 10.3402/jev.v4.27066

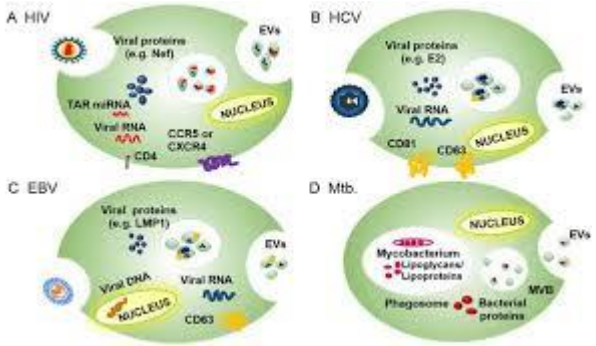
¿ Tienen un papel fisiopatológico ?

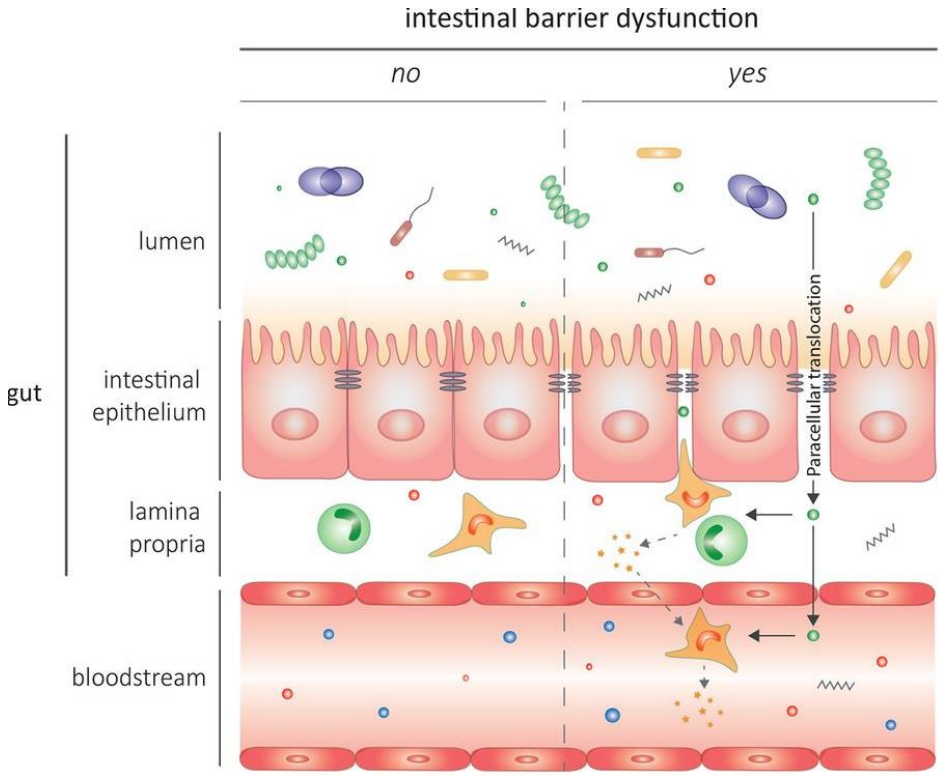


¿ Tienen un papel fisiopatológico ?



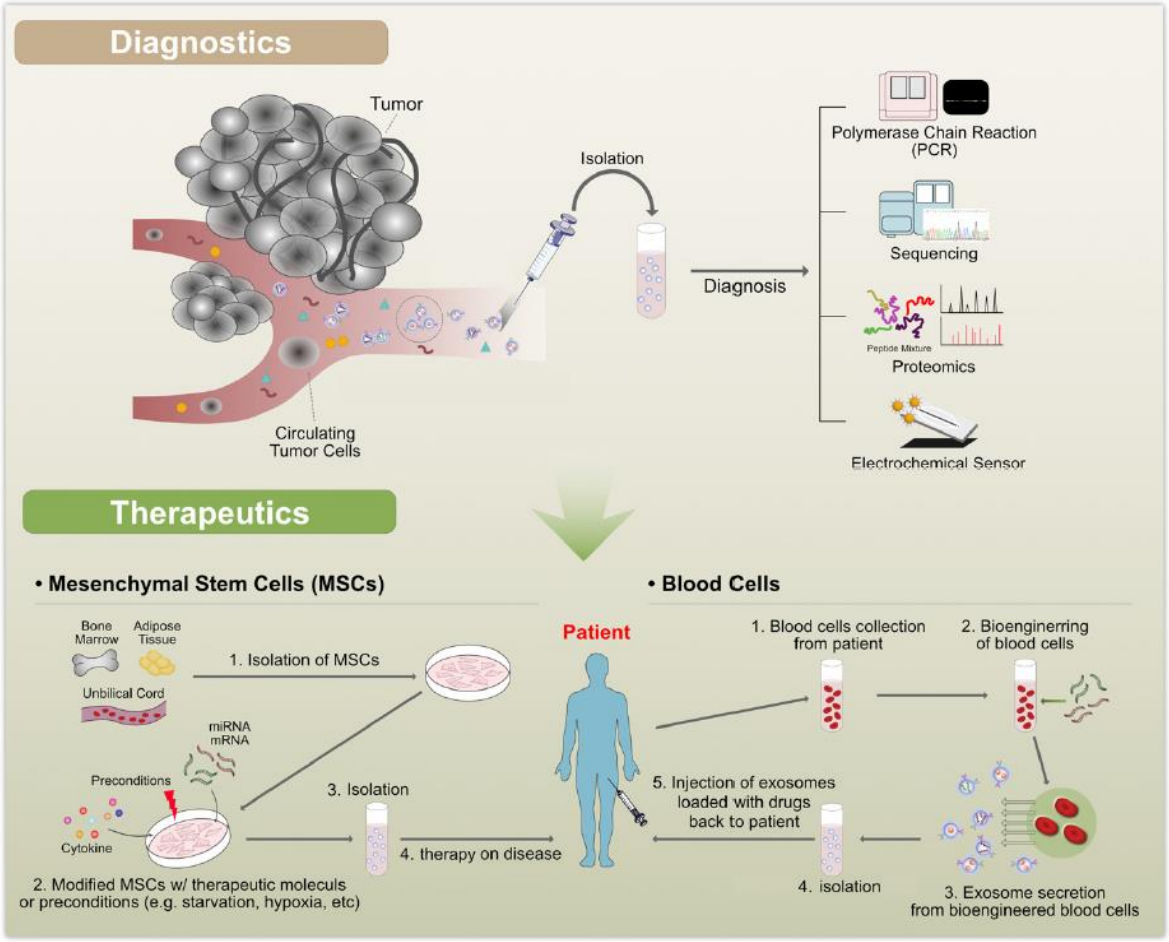
¿ Tienen un papel fisiopatológico ?





Nuevas perspectivas, que son una realidad

Nowadays Vesicles can be used for therapy, prognosis, and biomarkers for health and disease.



Vesículas extracelulares en la fisiología y fisiopatología vascular

- **Las ECV siguen siendo la epidemia principal en el siglo XXI**

Figura 1. Tendencia de la tasa de mortalidad ajustada por edad de la enfermedad cerebrovascular, enfermedad isquémica del corazón e insuficiencia cardiaca en ambos sexos. España, 1975-2014. Fuente: Actualización del Informe SEA 2007

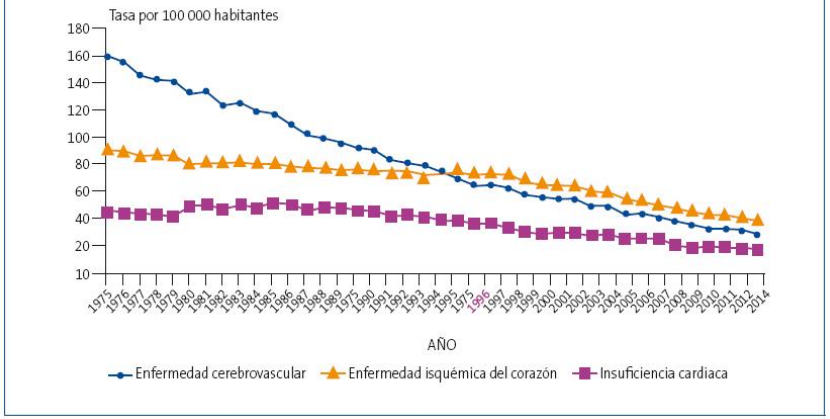
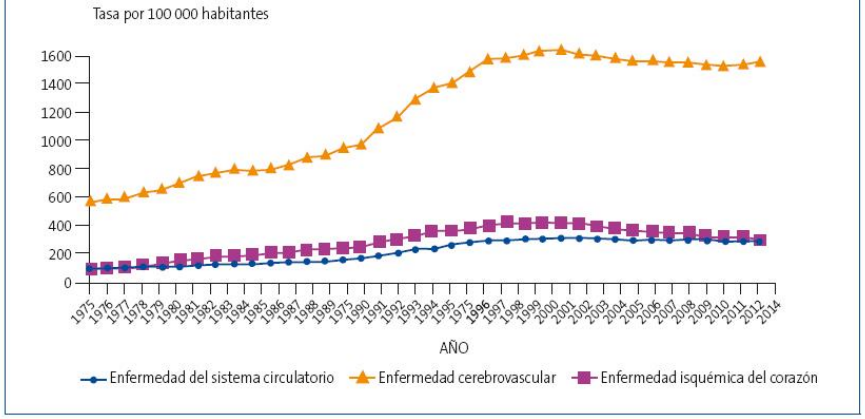


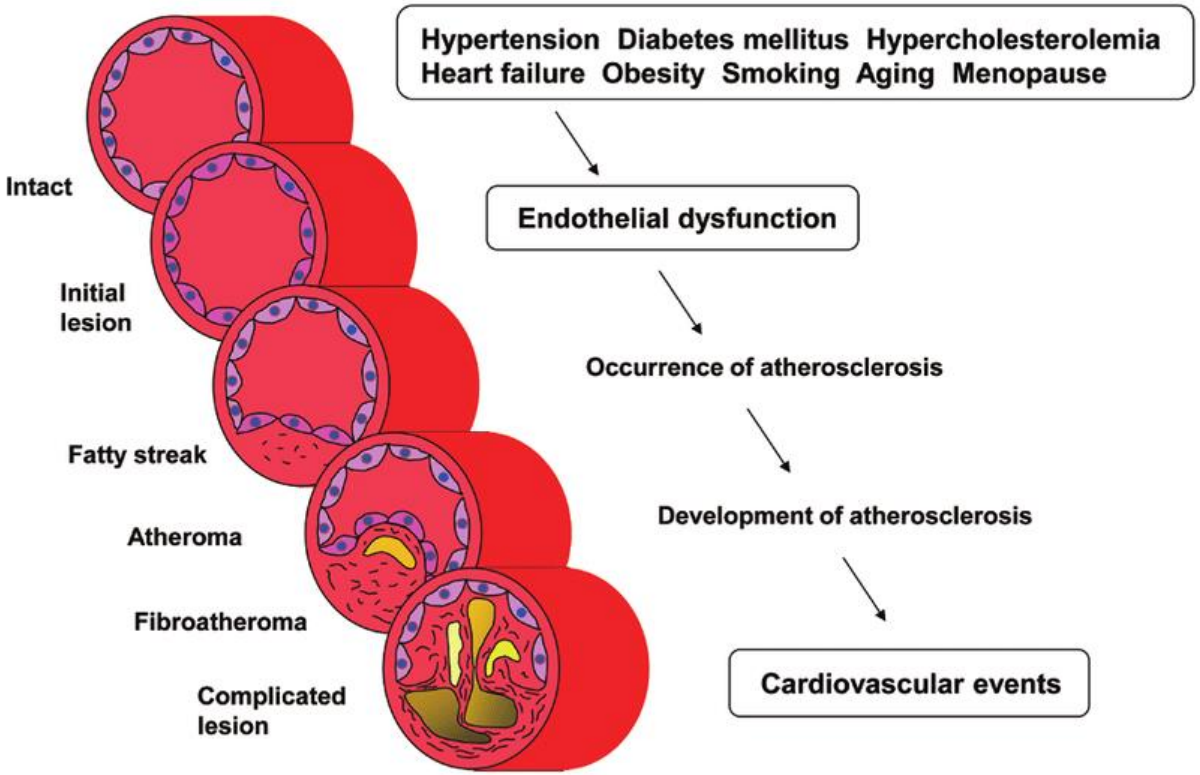
Figura 2. Tendencia de la tasa de morbilidad hospitalaria de las enfermedades del sistema circulatorio, enfermedad isquémica del corazón y enfermedad cerebrovascular en ambos sexos. España, 1977-2014. Fuente: Actualización del Informe SEA 2007



Fuente: Centro Nacional de Epidemiología.

¡¡ Predicar en el desierto !!

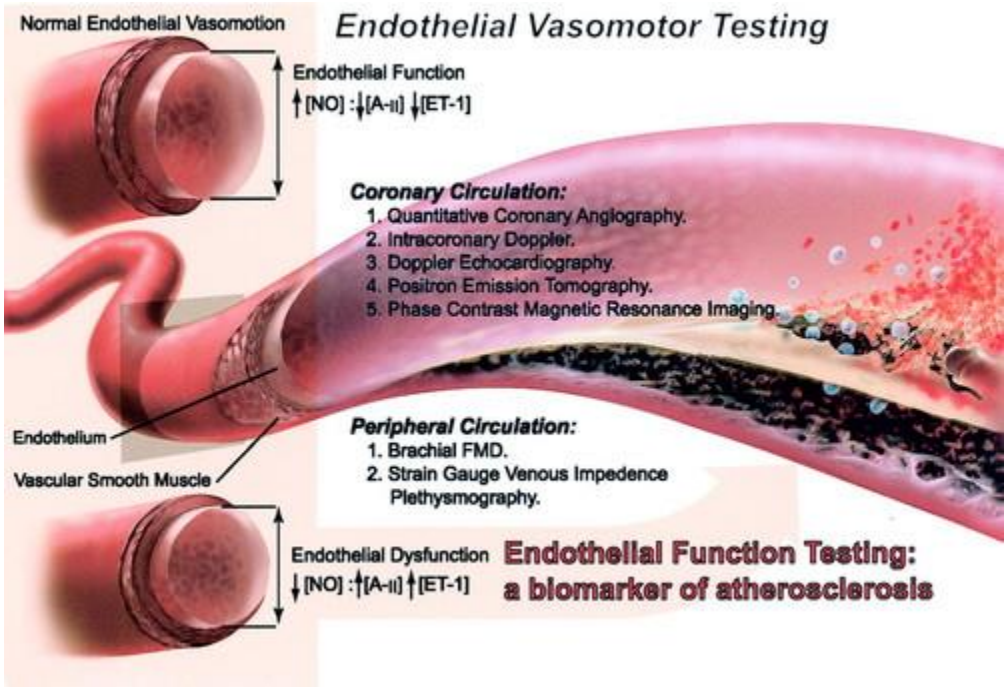
Vesículas extracelulares en la fisiología y fisiopatología vascular



Modified from: DOI: 10.1253/circj.cj-08-1102

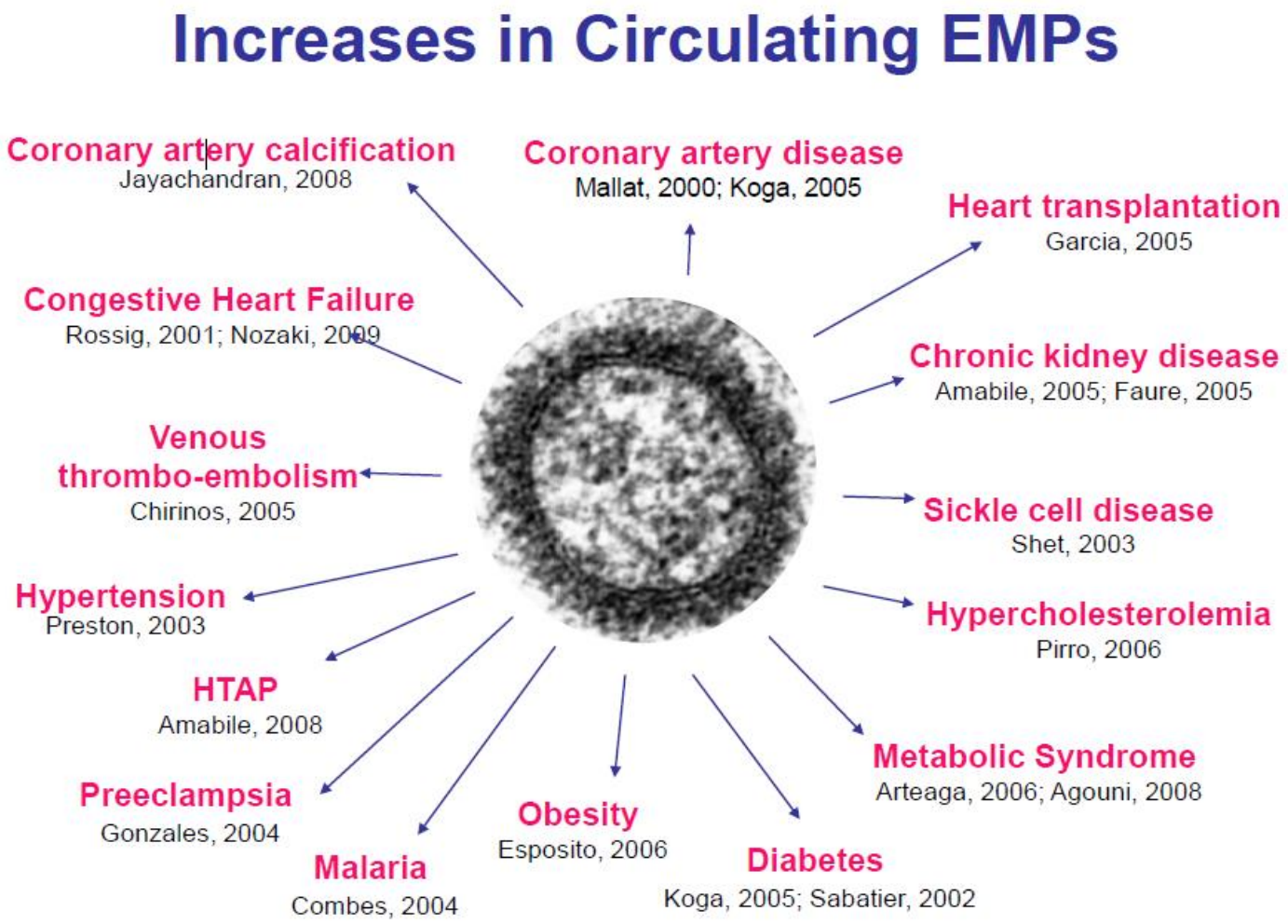
Vesículas extracelulares en la fisiología y fisiopatología vascular

Endothelial Function Testing as a Biomarker of Vascular Disease

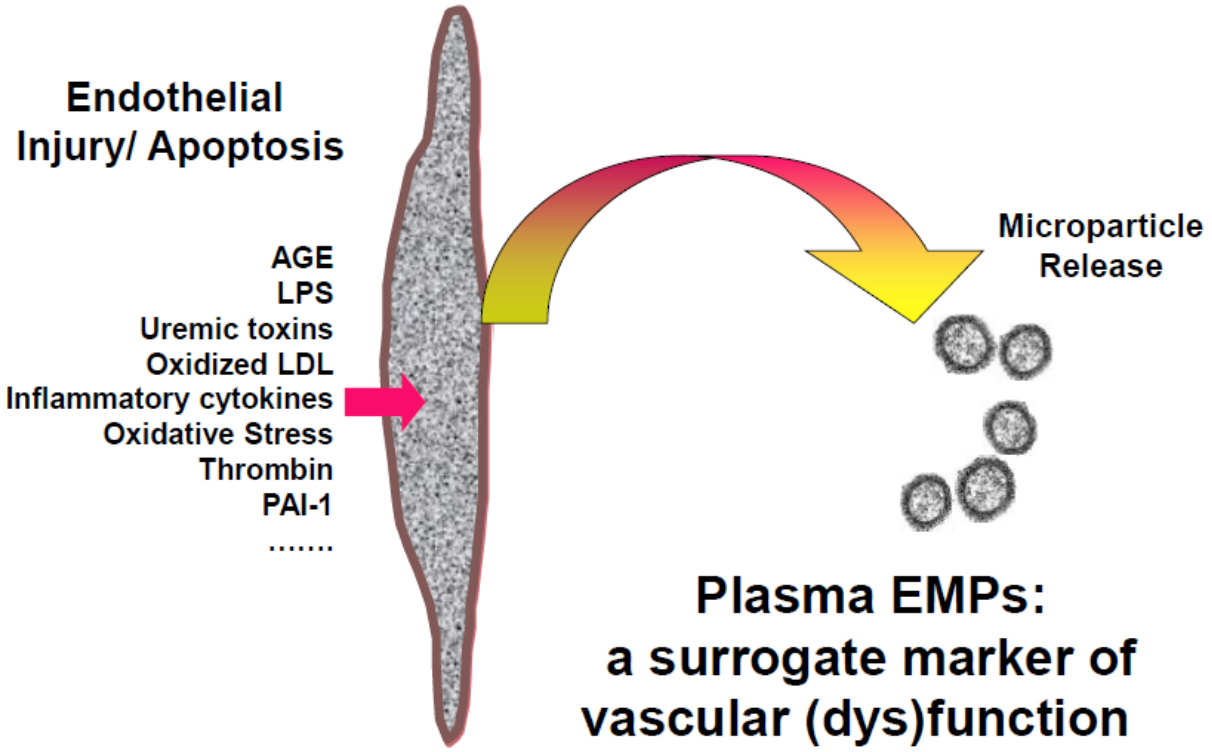


https://doi.org/10.1161/01.CIR.0000089191.72957.ED

Vesículas extracelulares en la fisiología y fisiopatología vascular

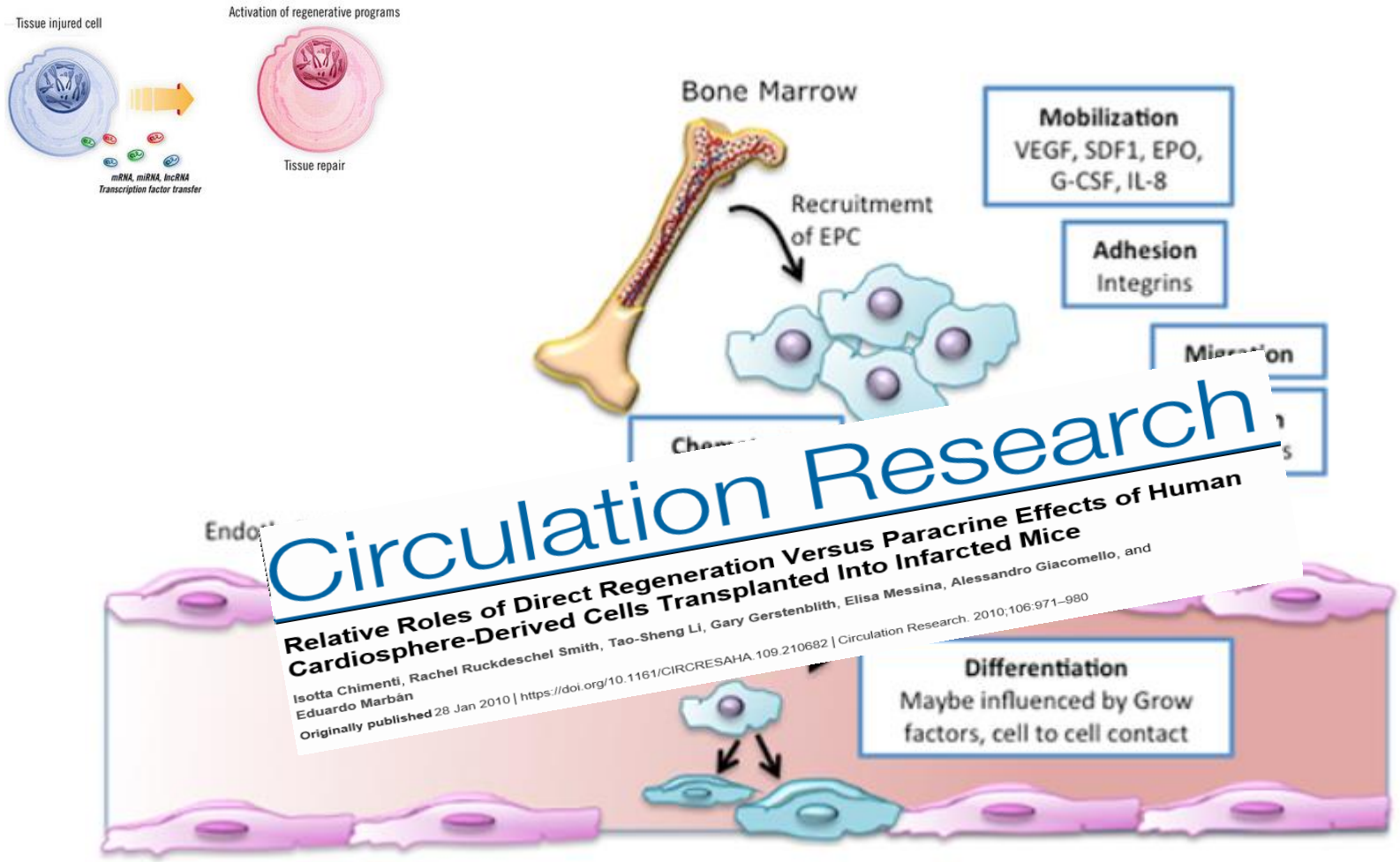


Vesículas extracelulares en la fisiología y fisiopatología vascular



Modified from: DOI: (10.1161/ATVBAHA.110.218123)

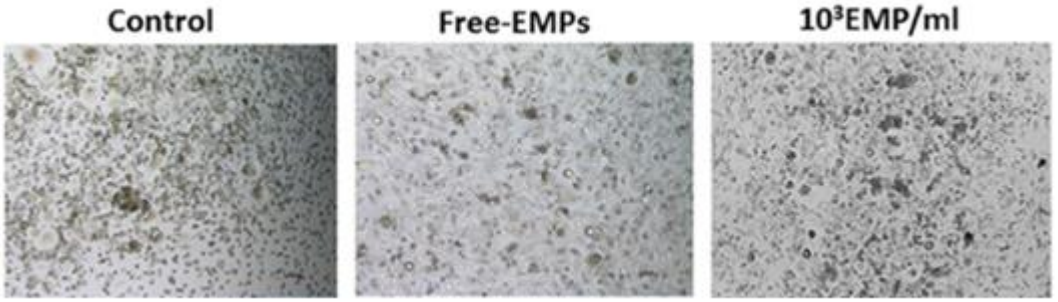
Vesículas extracelulares en la fisiología y fisiopatología vascular

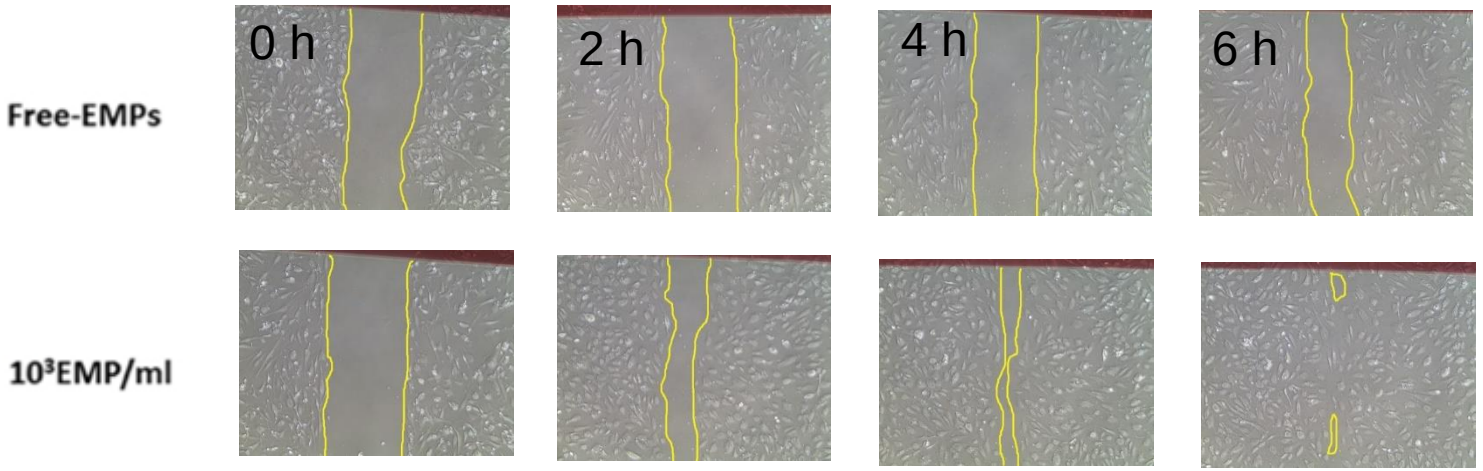


https://DOI: 10.5772/64529

Vesículas extracelulares en la fisiología y fisiopatología vascular

Wound healing assay in HUVECs cultured with EPCs in the presence of VEE





https://doi.org/10.3389/fphys.2015.00395

Vesículas extracelulares en la fisiología y fisiopatología vascular

Circulation

Circulating Microparticles From Patients With Myocardial Infarction Cause Endothelial Dysfunction

Chantal M. Boulanger, Alexandra Scoazec, Talin Ebrahimian, Patrick Henry, Eric Mathieu, Alain Tedgui, and Ziad Mallat

Originally published 27 Nov 2001 | https://doi.org/10.1161/hc4701.100516 | Circulation. 2001;104:2649–2652

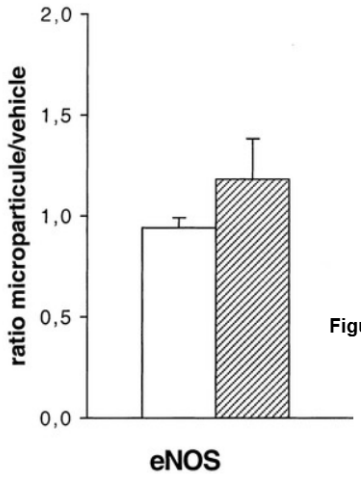
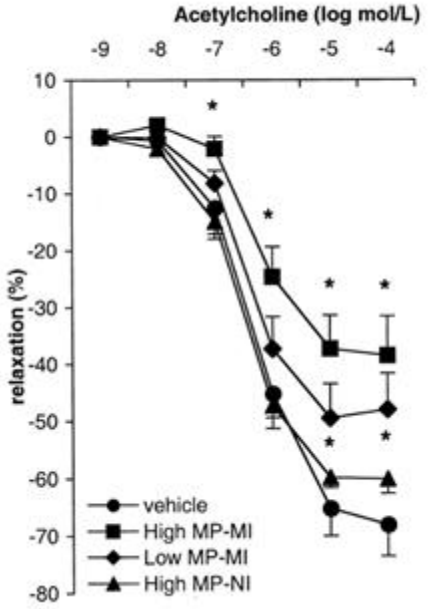


Figure 2. Semiquantitative evaluation of endothelial NO synthase (eNOS) expression in aortic rings exposed for 24 hours to high concentrations of microparticles from MI and NI patients



Vesículas extracelulares en la fisiología y fisiopatología vascular

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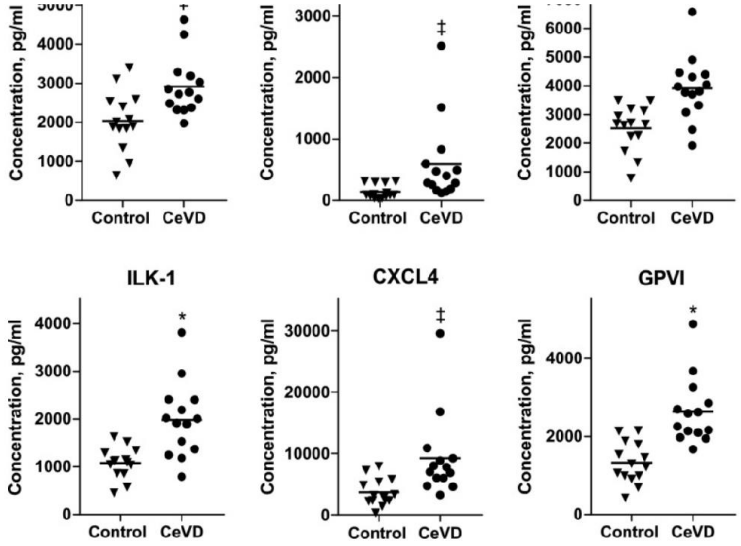
FASEB J. 2017 Aug; 31(8): 3689–3694.
Published online 2017 May 5. doi: 10.1096/fj.201700149: 10.1096/fj.201700149

PMCID: PMC5503715
PMID: 28476896

Altered cargo proteins of human plasma endothelial cell–derived exosomes in atherosclerotic cerebrovascular disease

Edward J. Goetzl,[*†,1] Janice B. Schwartz,[†‡] Maja Mustapic,[§] Iryna V. Lobach,[¶] Richard Daneman,[‖#] Erin L. Abner,[**] and Gregory A. Jicha[**]

Figure 2.



Elevated levels of platelet biomarkers and proteins implicated in atherosclerosis in plasma PDEs of patients with CeVD relative to those of matched control subjects.

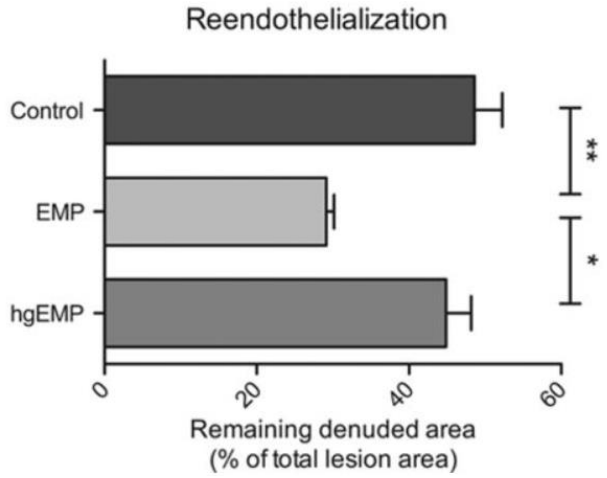
Vesículas extracelulares en la fisiología y fisiopatología vascular

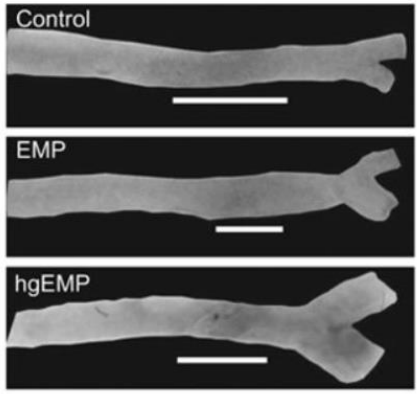
Circulation

Endothelial Microparticle–Mediated Transfer of MicroRNA-126 Promotes Vascular Endothelial Cell Repair via SPRED1 and Is Abrogated in Glucose-Damaged Endothelial Microparticles

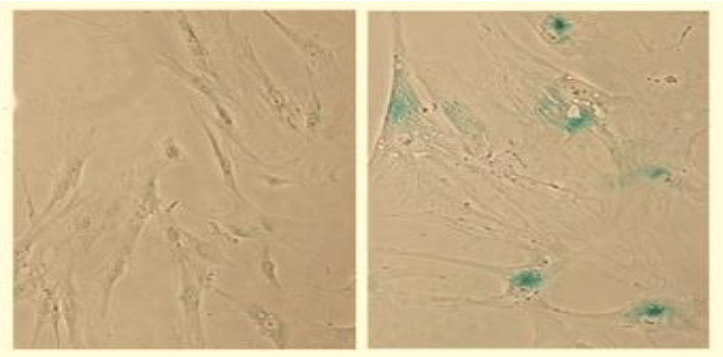
Felix Jansen, Xiaoyan Yang, Marion Hoelscher, Arianna Cattelan, Theresa Schmitz, Sebastian Proebsting, Daniela Wenzel, Sarah Vosen, Bernardo S. Franklin, Bernd K. Fleischmann, Georg Nickenig, and Nikos Werner

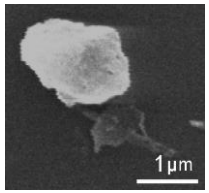
Originally published 6 Sep 2013 | https://doi.org/10.1161/CIRCULATIONAHA.113.001720 | Circulation. 2013;128:2026–2038

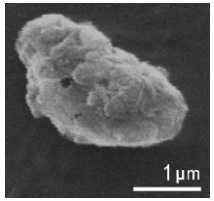


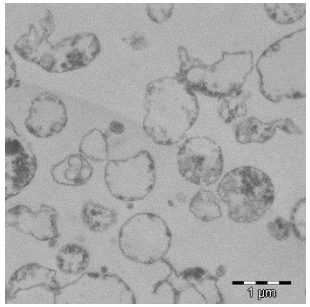


Vesículas extracelulares en la fisiología y fisiopatología vascular

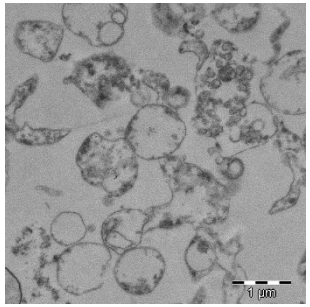








Microparticles Young HUVEC



Microparticles Senescent HUVEC

B) miRNA expression panel in senescent microparticles vs non senescent microparticles

	A	B	C	D	E	F	G	H	I	J	K	L
1	let7a	let7b	let7c	let7d	let7e	let7f	let7g	let7i	101	106b	125a-5p	125b
2	128	130a	130b	1324	144	145	15a	15b	16	17	181a	181b
3	181c	181d	186	195	19a	19b	202	20a	20b	21	211	23a
4	23b	29a	29b	29c	300	301a	301b	302a	302b	302c	30a	30b
5	30c	30d	30e	340	34a	34c-5p	372	373	374a	381	410	424
6	449a	449b	454	487	511	513b	519c-3b	519d	520d-3p	520e	524-5p	543
7	545	548c-3p	548b-3p	548e	590-5p	607	655	656	875-3p	9	93	98

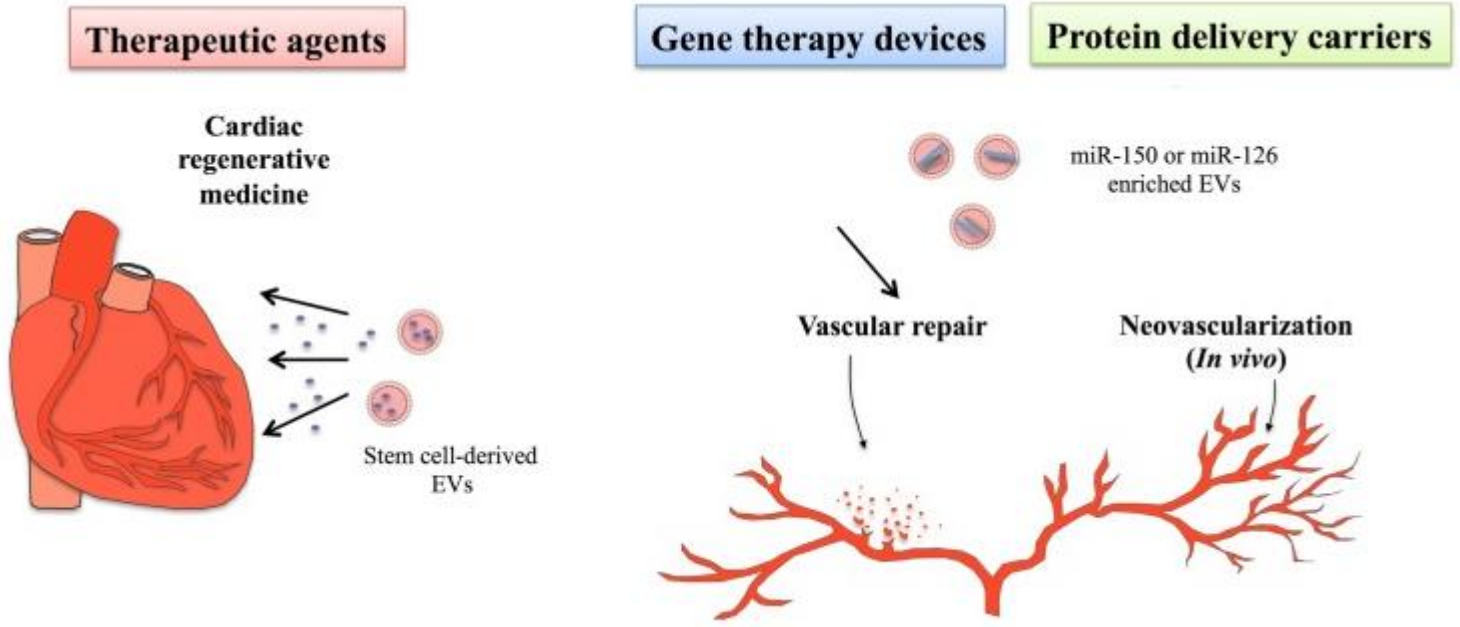
Only amplification in senescent MP.

Only amplification in non senescent MP.

> Log 10 Upregulated in senescence Mp

> Lin 10 Upregulated in senescence Mp

Vesículas extracelulares en la fisiología y fisiopatología vascular



the end for now,

...BUT THERE IS MUCH MORE TO LEARN!!!!

Thank you!