



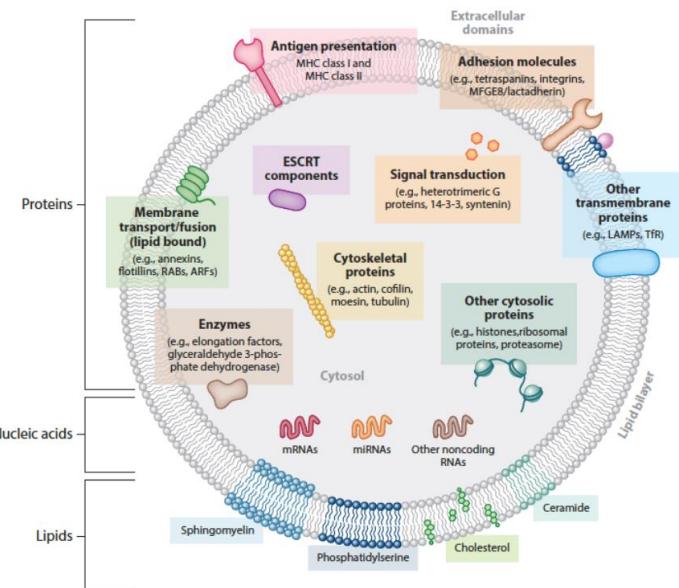
Máster en Biología Sanitaria

**Vesículas extracelulares en la fisiología y
fisiopatología vascular**

Rafael Ramirez
manuel.ramirez@uah.es

¿ Que son las vesículas extracelulares ?

Cell-Derived Vesicles

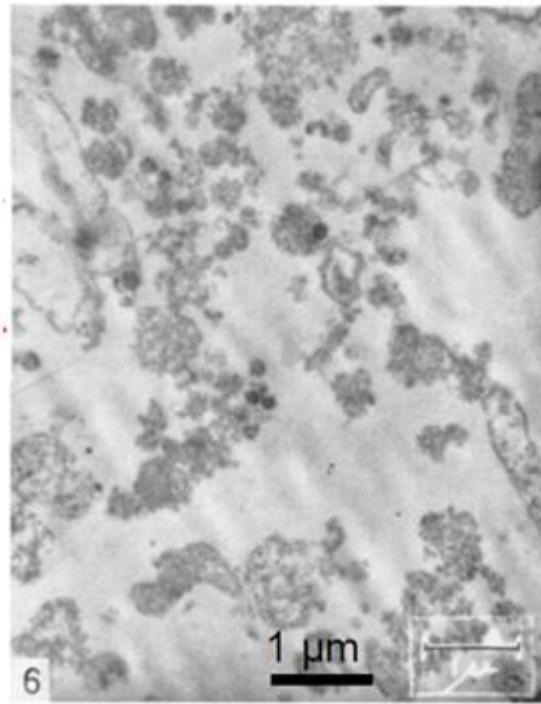


- EVs are spherical particles enclosed by a phospholipid bilayer.
- Both Eukaryotic and Prokaryotic cells release vesicles.
- Contain several types of function molecules, such as proteins, mRNAs, and miRNAs....

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

Un poco de historia....

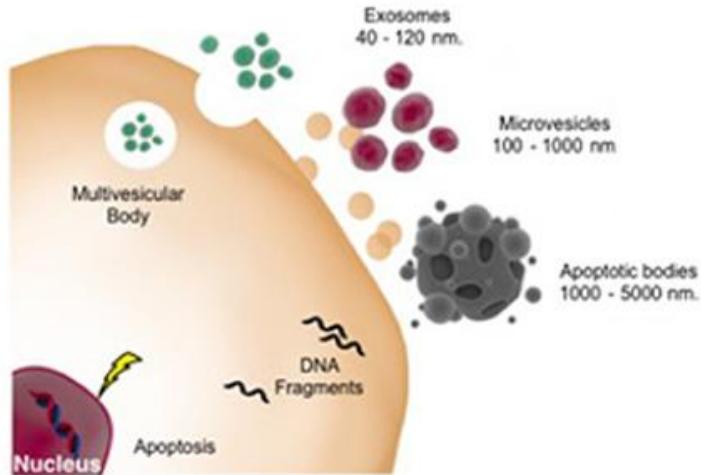
- The preliminary studies of cell-derived vesicles were addressing “cell-free plasma contains a subcellular factor that promotes clotting of blood” (Chargaff and West, 1946).
- More than 20 years later, in 1967, this subcellular fraction was identified by electron microscopy and was shown to consist 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 described 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....

The screenshot shows the homepage of the *Circulation of the Kidney International* website. The top navigation bar includes links for Home, Circulation, Articles & Issues, Focuses, For Authors & Reviewers, For Readers, and For Advertisers. The main content area features a large bar chart titled "Number of publications" on the y-axis (ranging from 0 to 30,000) and "Year" on the x-axis (ranging from 1960 to 2000). The bars show a significant increase in publications over time, particularly after 1980. Below the chart, the journal's logo is displayed, featuring the acronym ISN and the text "INTERNATIONAL SOCIETY OF NEPHROLOGY". The journal title "Circulation of the kidney INTERNATIONAL" is prominently shown, along with the subtitle "OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY". A large, bold headline reads: "Nucleic acids within urinary exosomes/microvesicles are potential biomarkers for renal disease". Below this, a sub-headline says: "THE LARGEST EXPRESSION OF NUCLEIC ACIDS IN URINARY EXOSOMES". A list of authors is provided: Kevin C. Miranda¹, Daniel T. Bond¹, Mary McKee¹, Johan Skog², Teodor G. Păunescu¹, Nicolas Da Silva¹, Dennis Brown¹, Leileata M. Russo^{1,*}. The article abstract discusses the levels of nucleic acids in urinary exosomes and their interactions with podocytes, leading to dysfunction and proteinuria. It also mentions the relationship between exosome-derived nucleic acids and renal function.

Number of publications

Home > Circulation

ISN

INTERNATIONAL SOCIETY OF NEPHROLOGY

Circulation of the kidney INTERNATIONAL

OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

Articles & Issues Focuses For Authors & Reviewers For Readers For Advertisers

Nucleic acids within urinary exosomes/microvesicles are potential biomarkers for renal disease

THE LARGEST EXPRESSION OF NUCLEIC ACIDS IN URINARY EXOSOMES

MECHANISMS OF DISEASE

Malignant effusions and exosomes

Fabrice Andre, MD • Noel EC Schatzki, MD • Philippe Morice, MD • et al. Show all authors

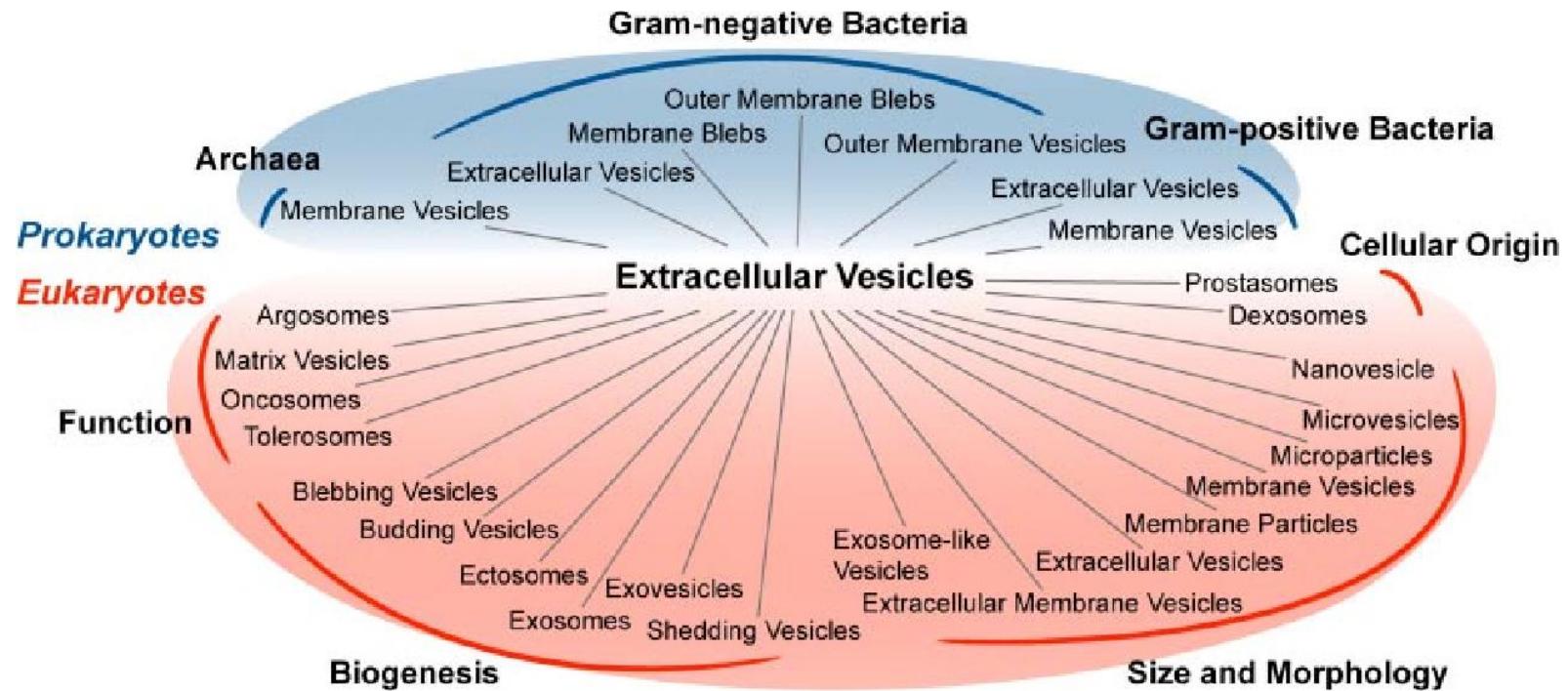
Published online July 27, 2002

Levels of nucleic acids in urinary exosomes and their interactions with podocytes in proteinuric renal diseases: a link between exosome-derived nucleic acids and renal dysfunction and proteinuria

Andres Soriano; Wenche Iy, Julio Chirinos; Martin Valdivia; Herme Ahn; Caroline Flament, BS • Patricia Pautier, MD •

Published: July 27, 2002 • DOI: [https://doi.org/10.1016/S0140-6736\(02\)09552-1](https://doi.org/10.1016/S0140-6736(02)09552-1)

¿ Como se definen: microvesículas, exosomas... ?



<https://doi.org/10.1002/mas.21420>

¿ Como se definen: microvesículas, exosomas... ?



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

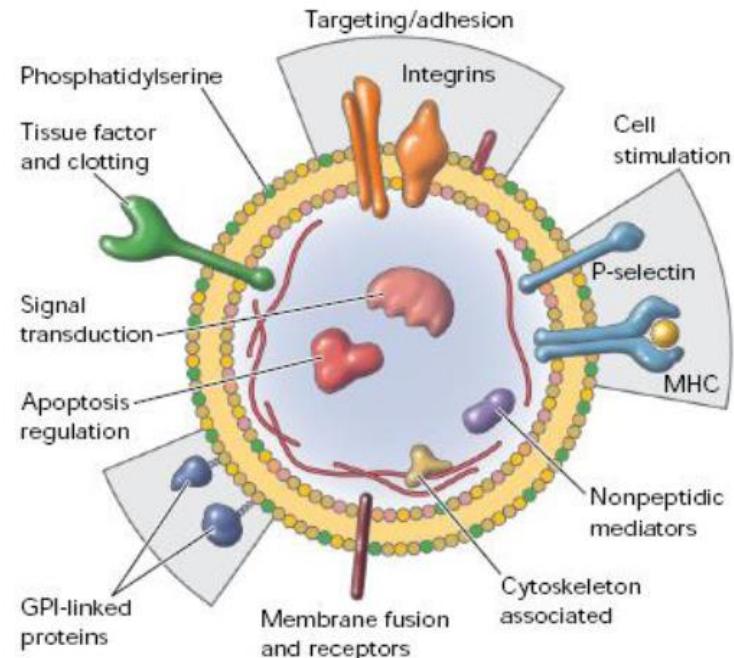
“To prevent any confusion about the nomenclature of the cell release vesicles, the ISEV community suggests to use the term “extracellular vesicle (EV)” unless the subcellular origin of the vesicle is demonstrated”

ANNUAL
MEETING
ISEV
2020
20-24 May
Philadelphia Marriott Downtown
Philadelphia, Pennsylvania



¿ Qué son las vesículas extracelulares ?

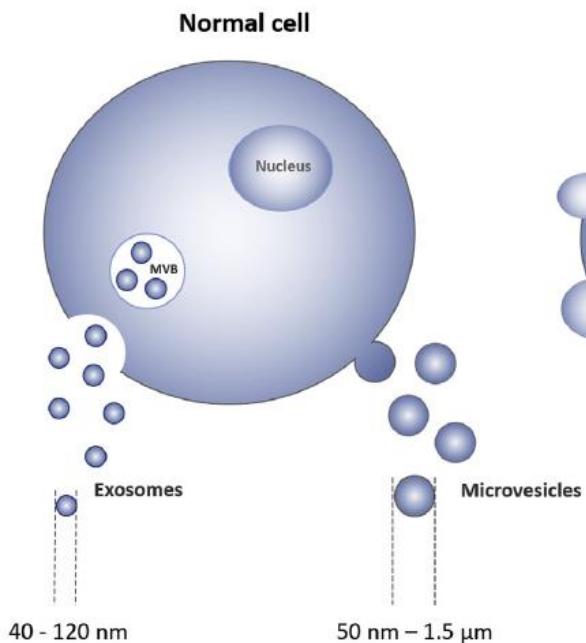
- Cells release membrane vesicles in the extracellular milieu upon activation
- Evs are found in body fluids: plasma, urine, CSF ..., and in cell culture supernatants
- Evs present surface receptors and/or contain elements (RNAs, miRNAs ..) allowing to identify their cell of origin



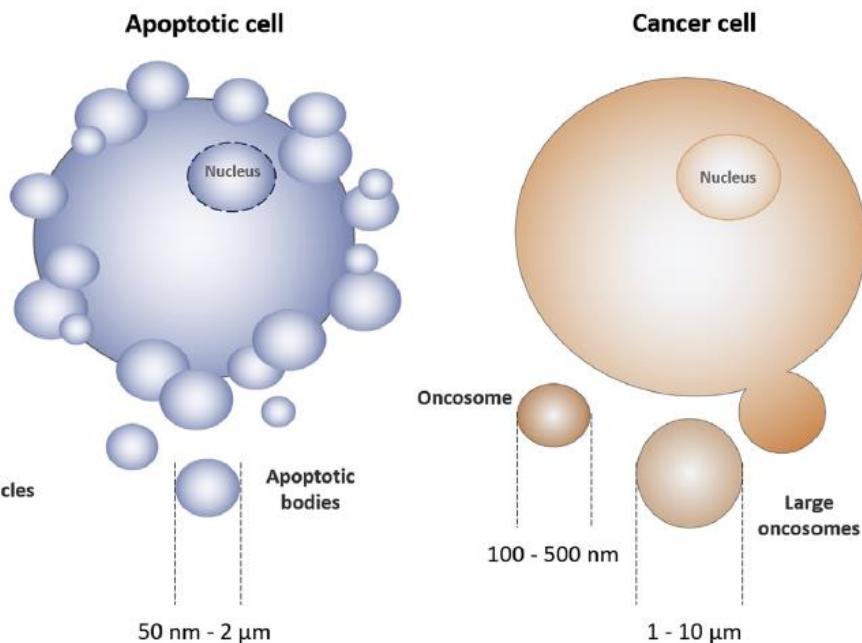
<https://doi: 10.1152/physiol.00029>.

¿ Qué son las vesículas extracelulares ?

Vesicular objects



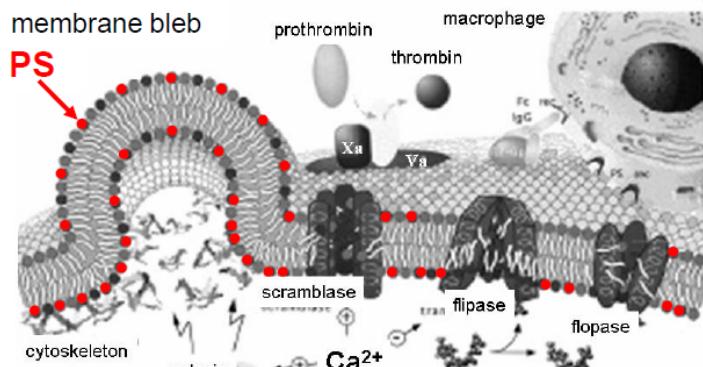
Non-vesicular objects



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

¿ Como se producen ?

Mechanisms of formation of extracellular vesicles

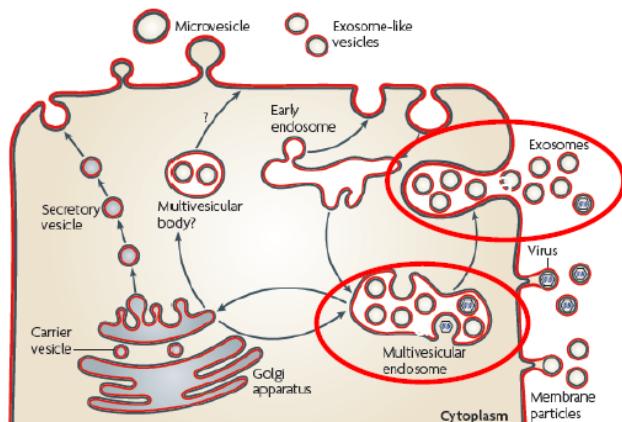


Zwaal & Schroit 1997, *Blood* 89 (4):1121-1132

Microparticles, Microvesicles

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

- entrance of Ca^{2+} & increase of $[\text{Ca}^{2+}]$
- 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

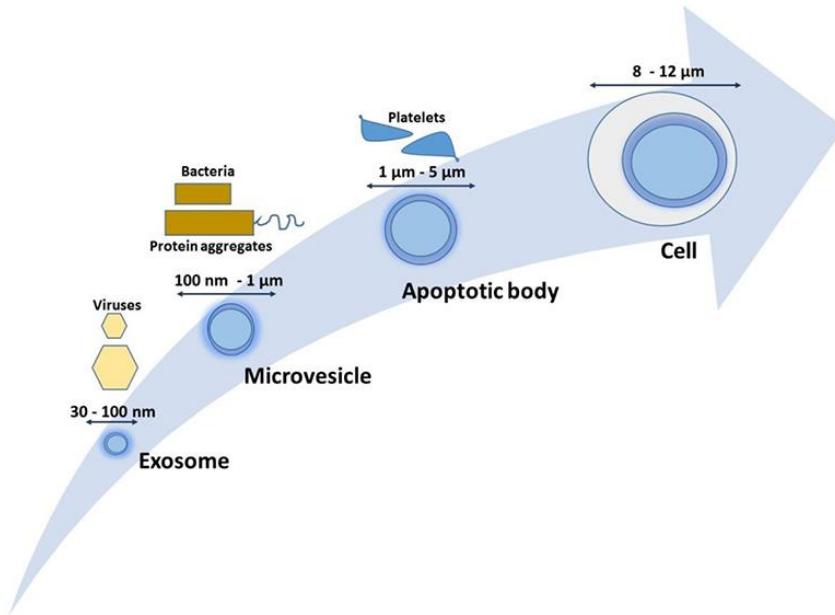
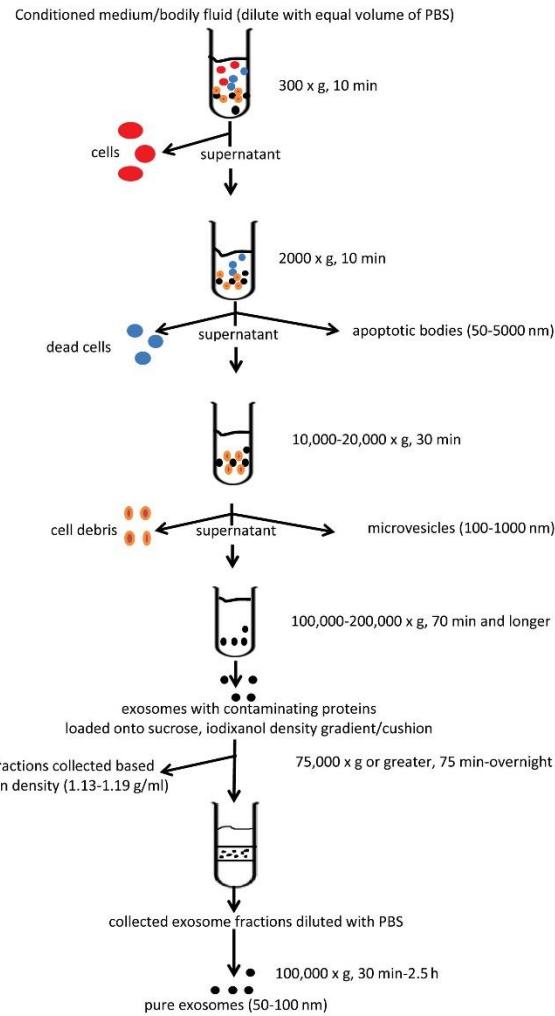


Théry et al. *Nat. Rev. Immunology* 2009, 9:581-593

Exosomes (50 - 100 nm)

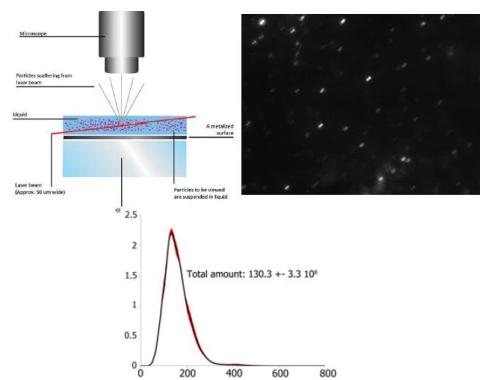
- form in multi-vesicular bodies (MVBs)
- are released in the extracellular milieu after fusion of MVBs with the cell plasma membrane
- rich in tetraspanins (CD63, CD81, CD9)

¿ 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:

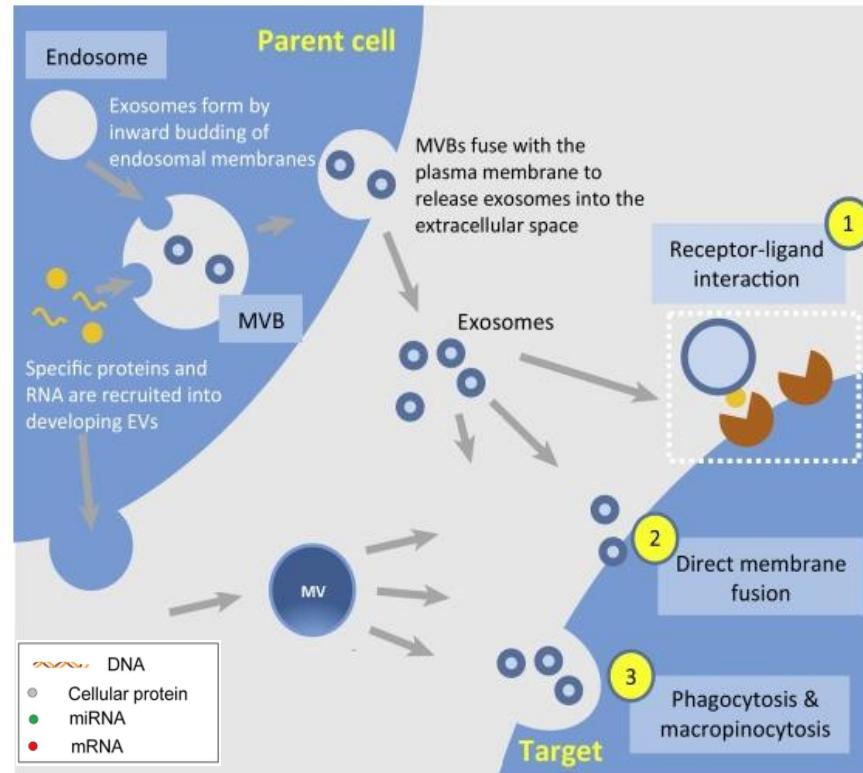
- Electron microscopy
- Flow cytometry
- Nanoparticle Tracking Analysis (NTA)
- ...

Bulk methods:

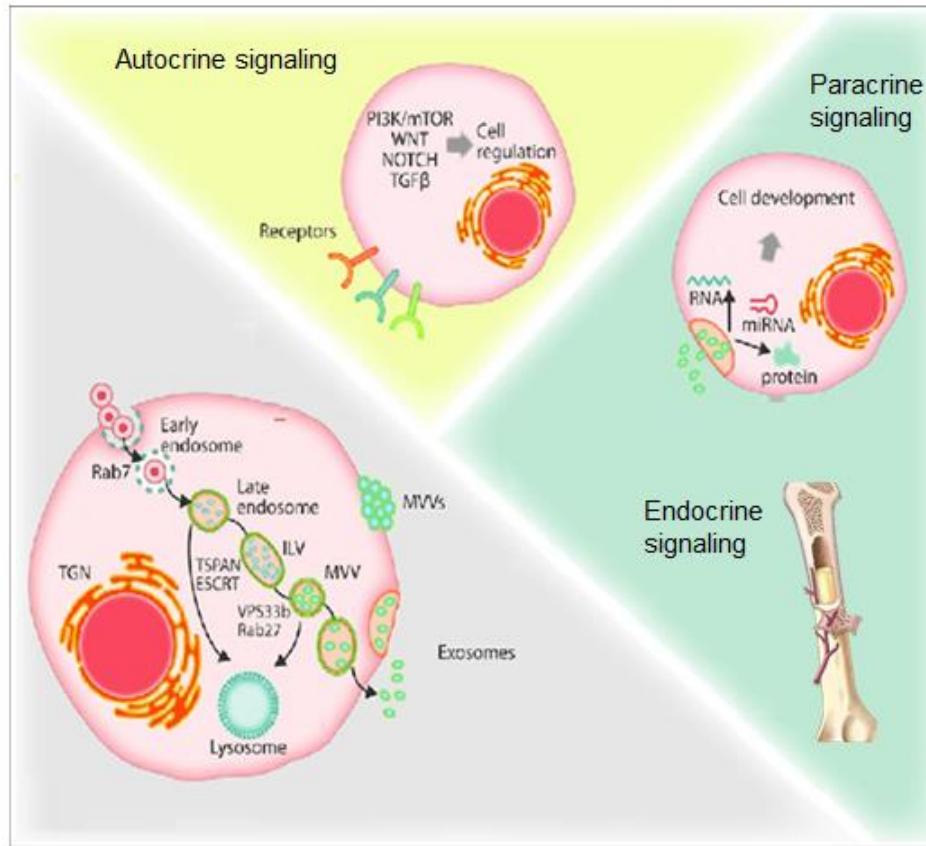
- Western blotting
- "Omic" methods: mass spectrometry, RT-qPCR.
- Functional assays: measurement of activity
- ...

¿ Que función tienen ?

Apoptotic Cell-Derived Extracellular Vesicles: More Than Just Debris



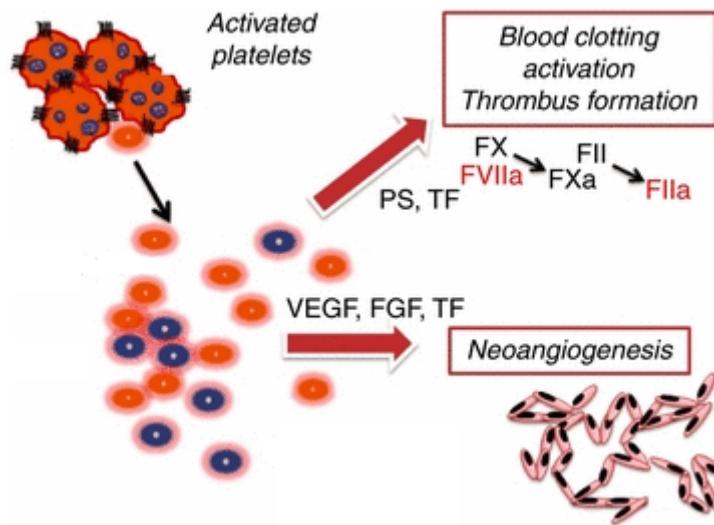
¿ Tienen un papel fisiológico ?



Modified from: doi:10.3324/haematol.2017.183335

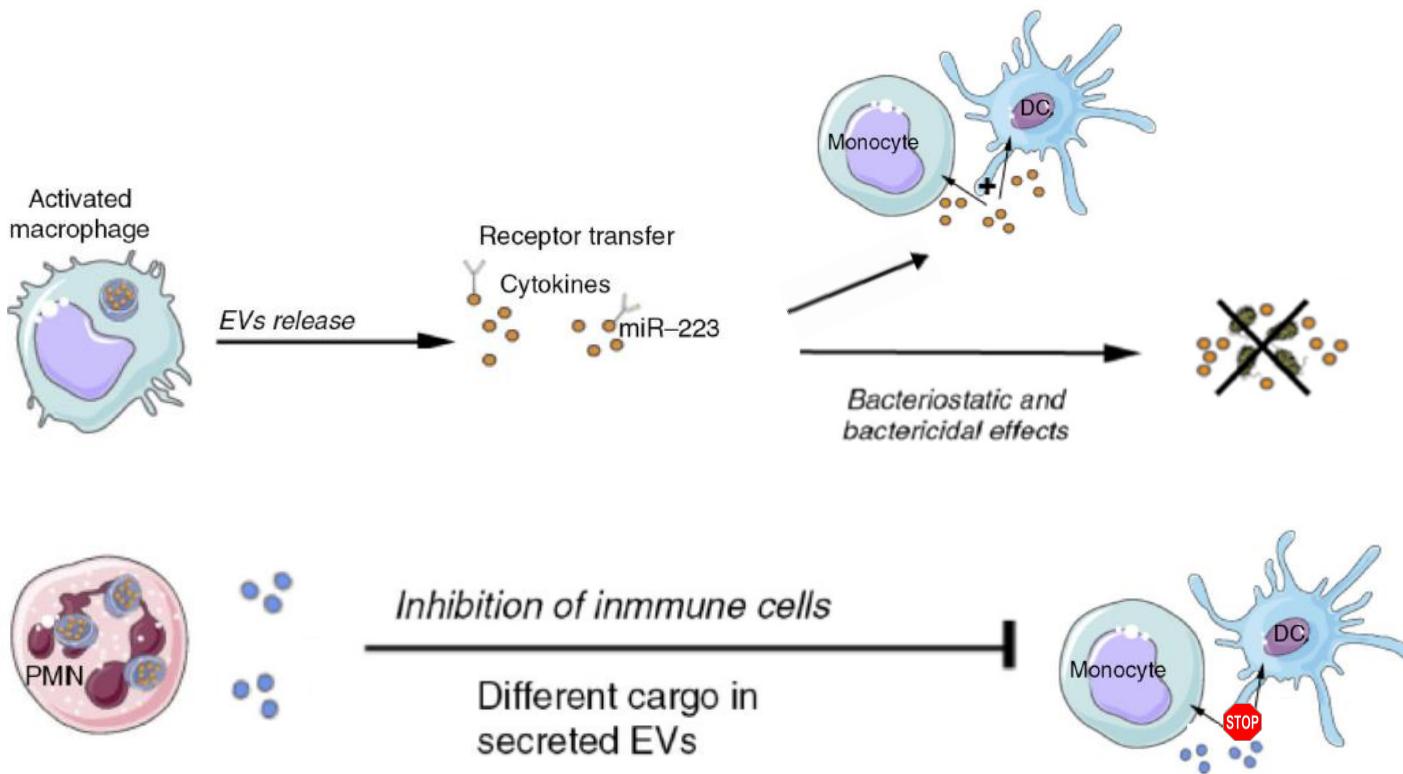
¿ Tienen un papel fisiológico ?

Cross Talk Pathways Between Coagulation and the Platelet Extracellular Vesicles



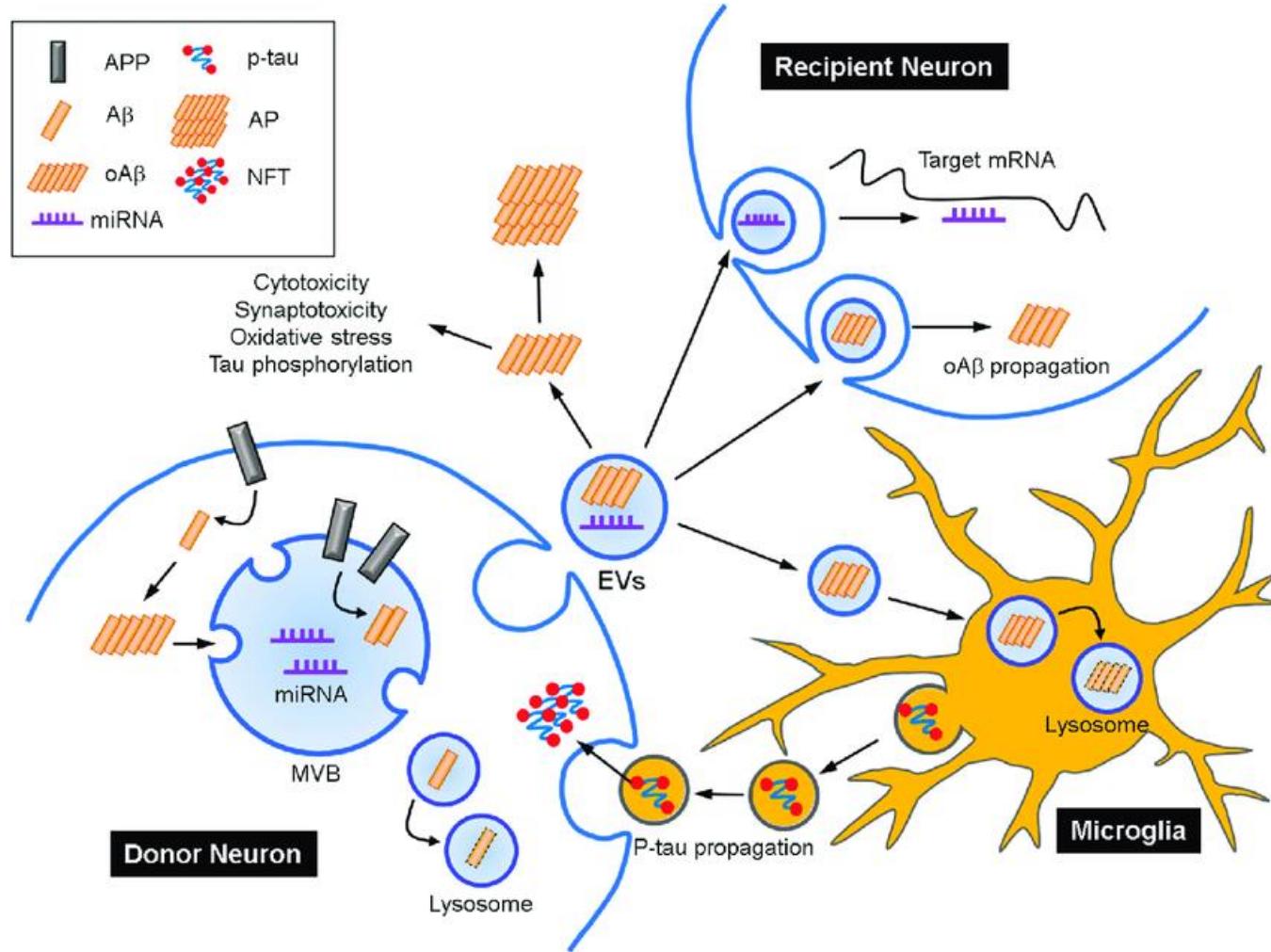
¿ Tienen un papel fisiológico ?

Extracellular Vesicle Signalling Implicated in Immunity

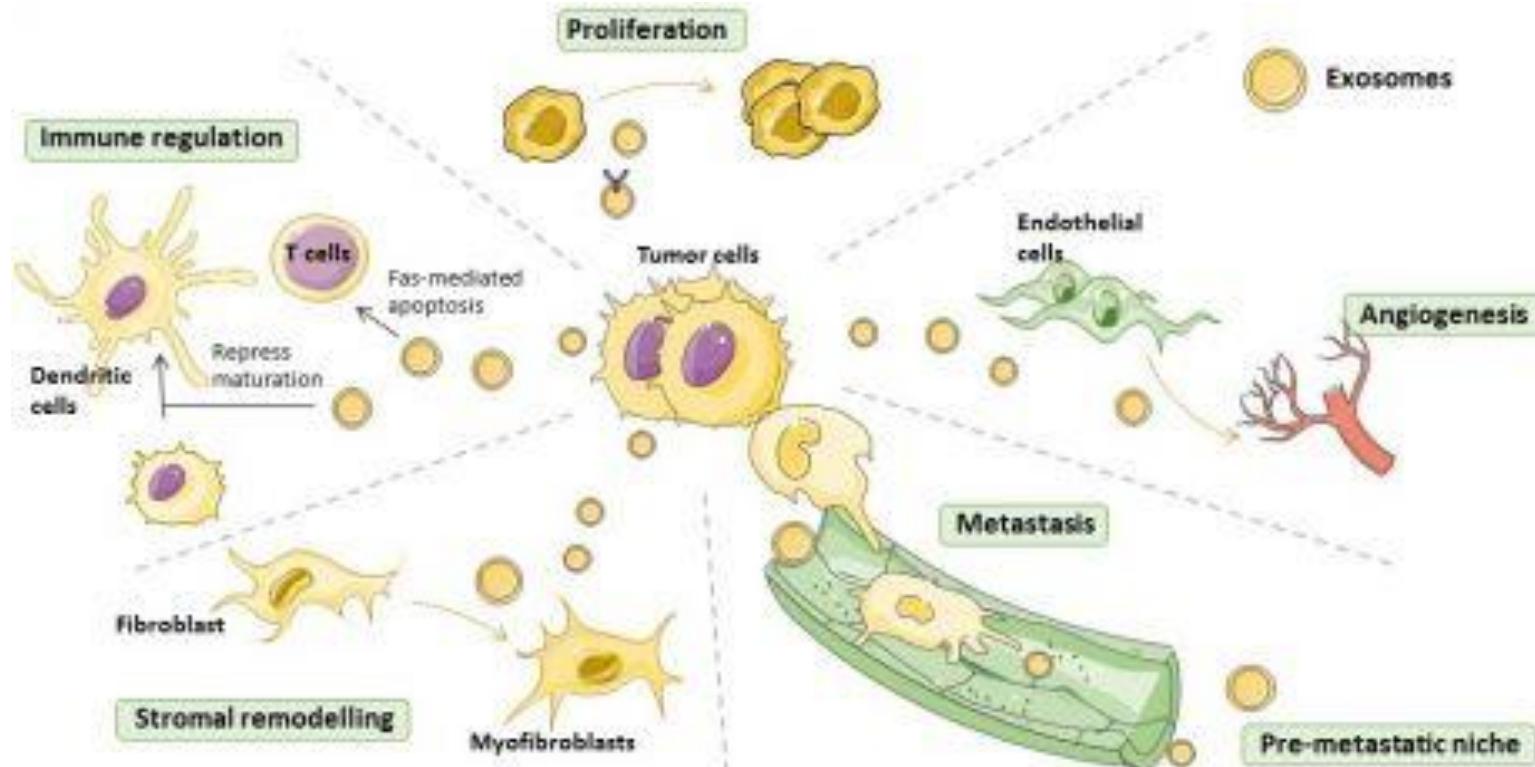


doi: 10.3402/jev.v4.27066

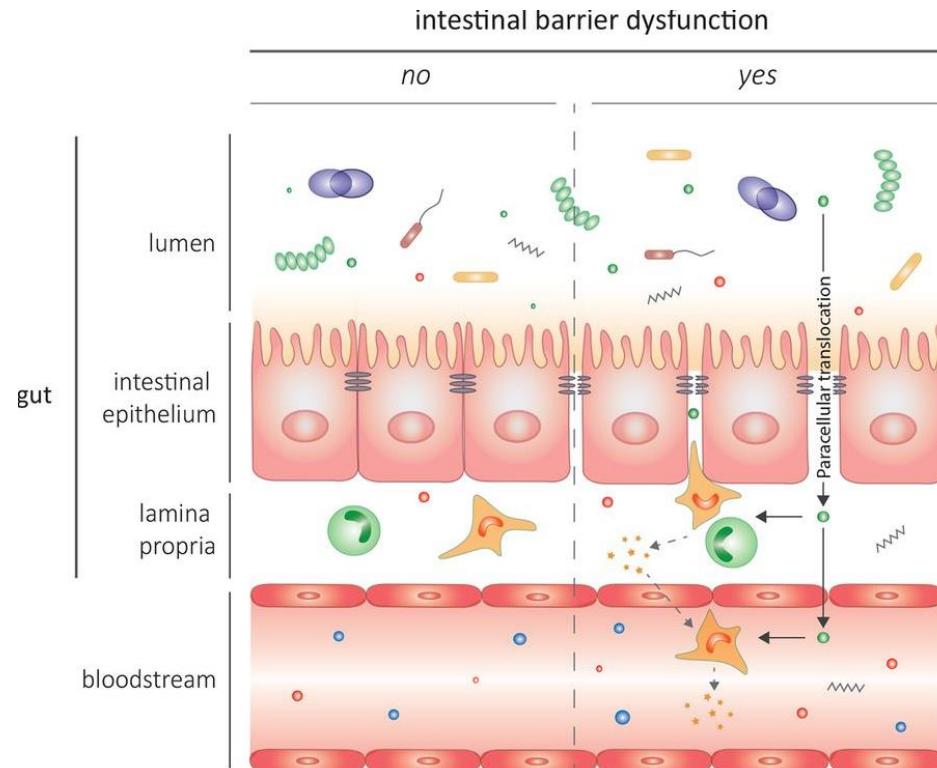
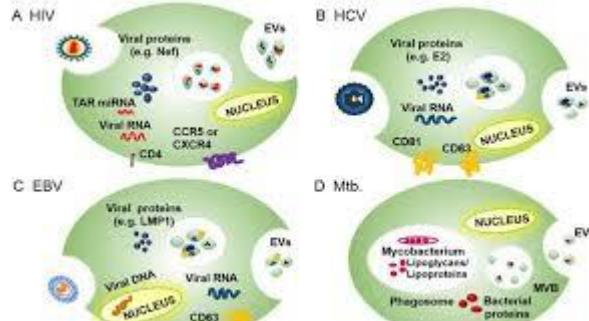
¿ Tienen un papel fisiopatológico ?



¿ Tienen un papel fisiopatológico ?

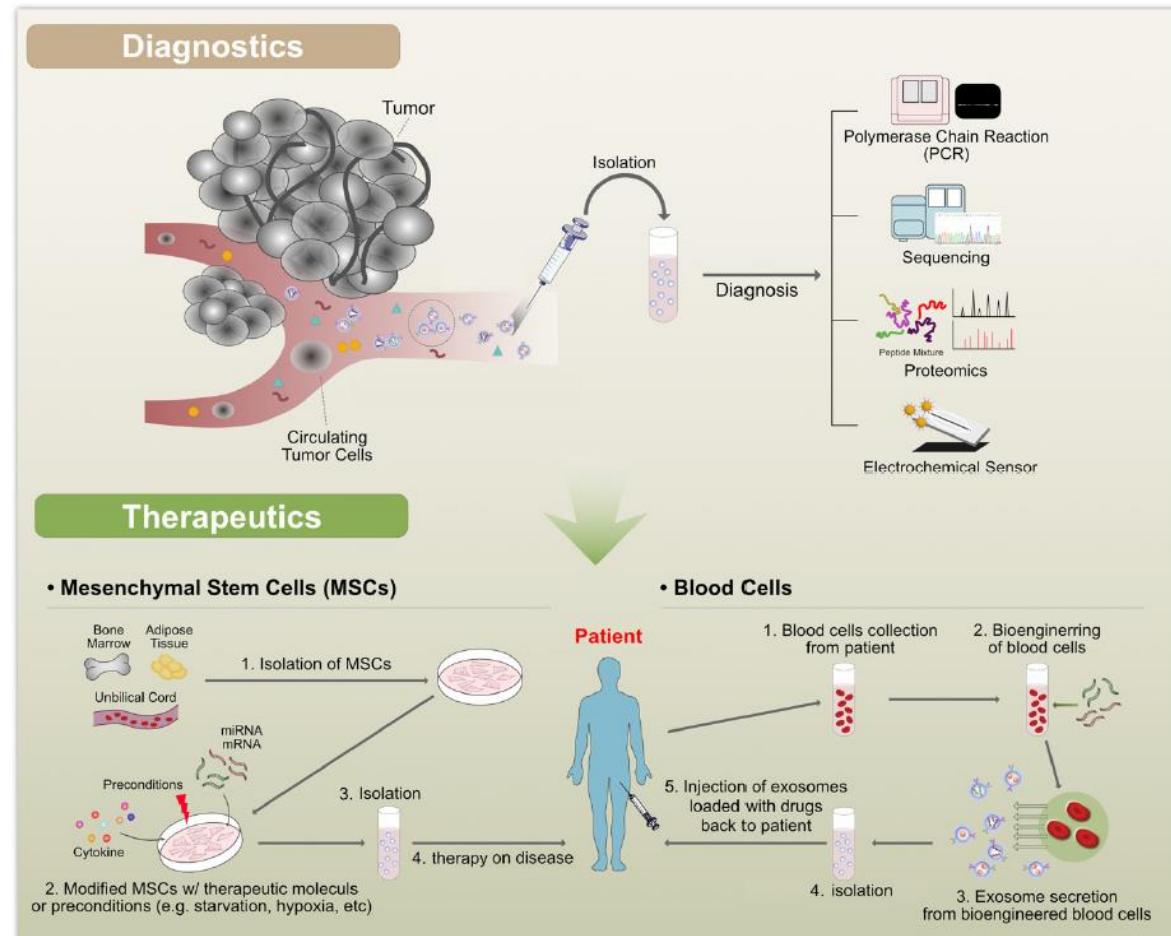


¿ Tienen un papel fisiopatológico ?



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 cardíaca en ambos sexos. España, 1975-2014. Fuente: Actualización del Informe SEA 2007

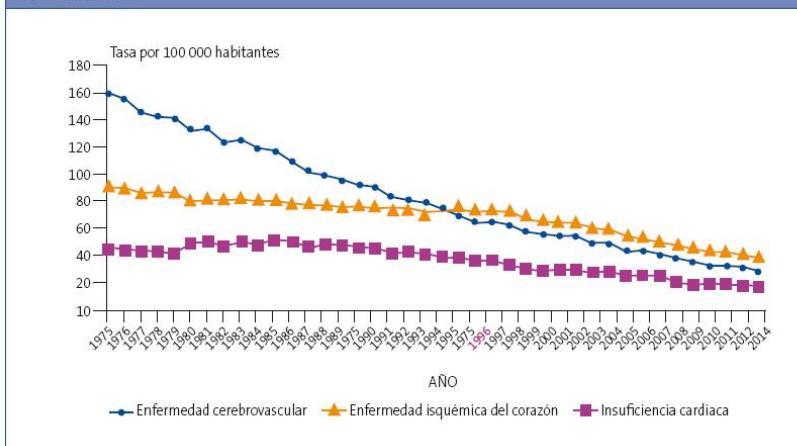
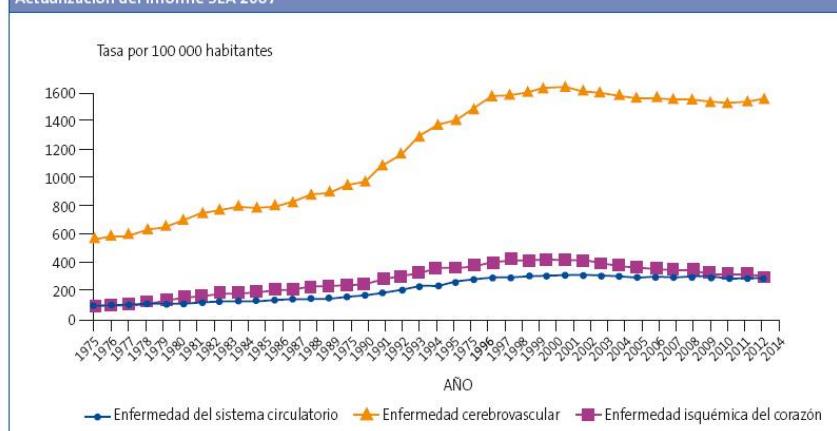


Figura 2. Tendencia de la tasa de morbilidad hospitalaria de las enfermedades del sistema circulatorio, enfermedad isquémica del corazón y enfermedad cerebrovascular en ambos sexos. España, 1975-2014. Fuente: Actualización del Informe SEA 2007



Fuente: Centro Nacional de Epidemiología.

Vesículas extracelulares en la fisiología y fisiopatología vascular

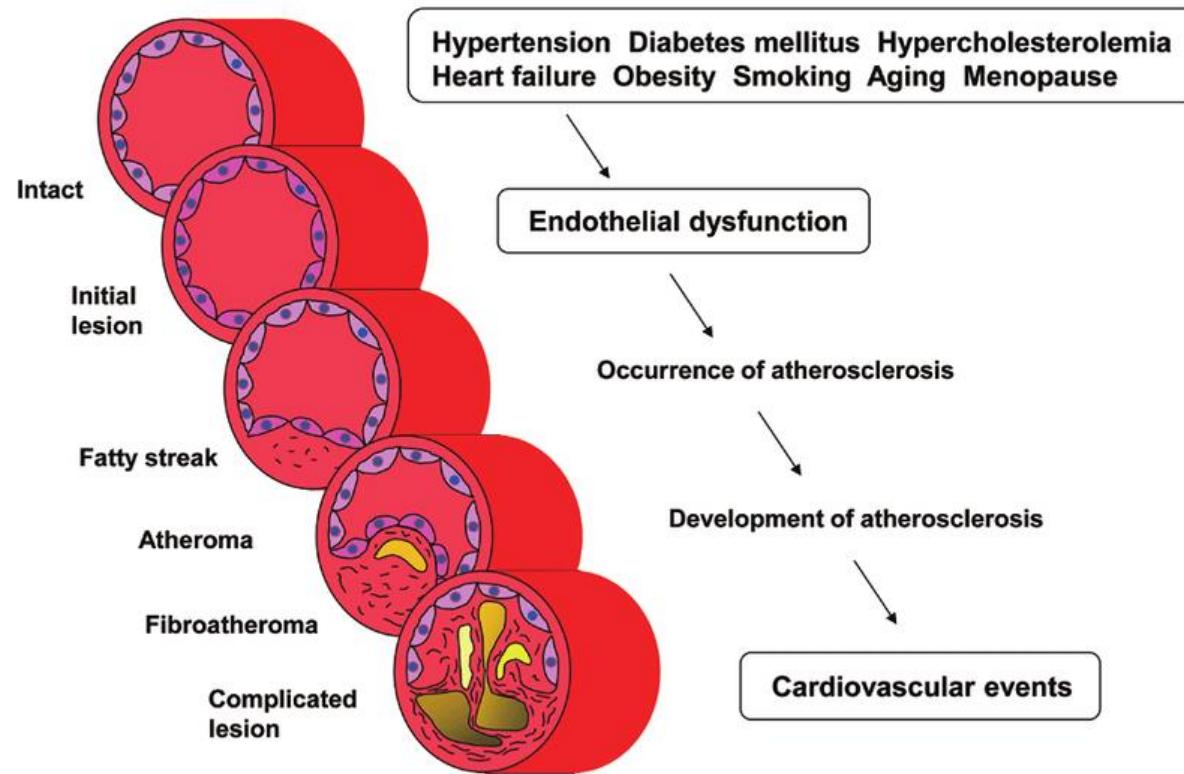
¡¡ Predicar en el desierto !!

No tengo nada ¿verdad?
¿Puedo empezar a ir
al gimnasio?
Será difícil dejar el
tabaco porque tengo
mucho estrés

Tiene la tensión algo alta
Tendría que dejar de
fumar
Debe perder peso
Puede y debe hacer
ejercicio, sin forzar



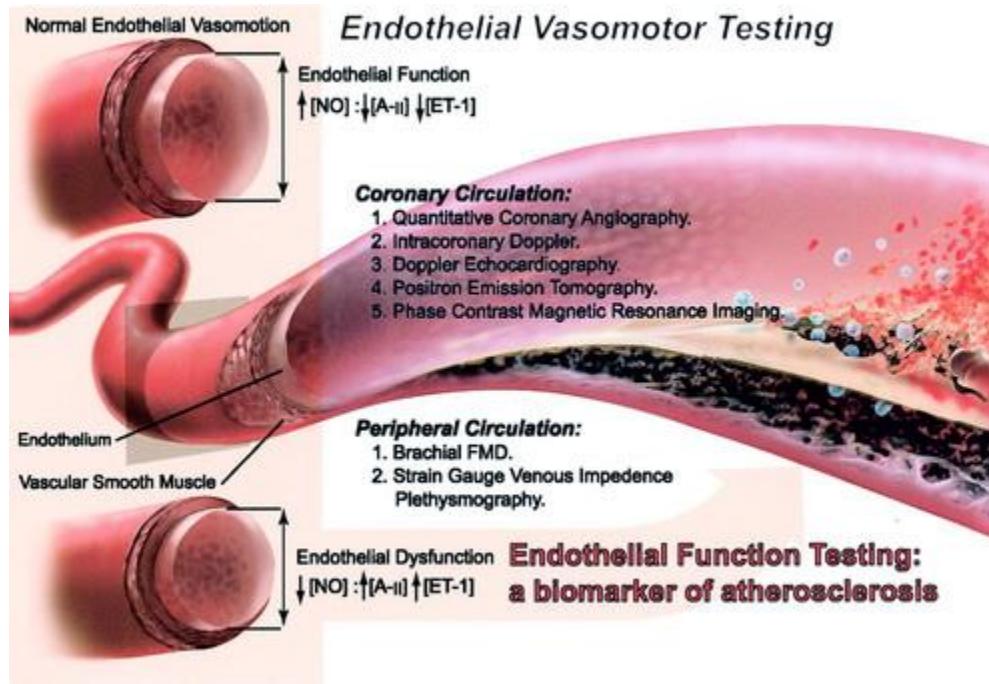
Vesículas extracelulares en la fisiología y fisiopatología vascular



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

Vesículas extracelulares en la fisiología y fisiopatología vascular

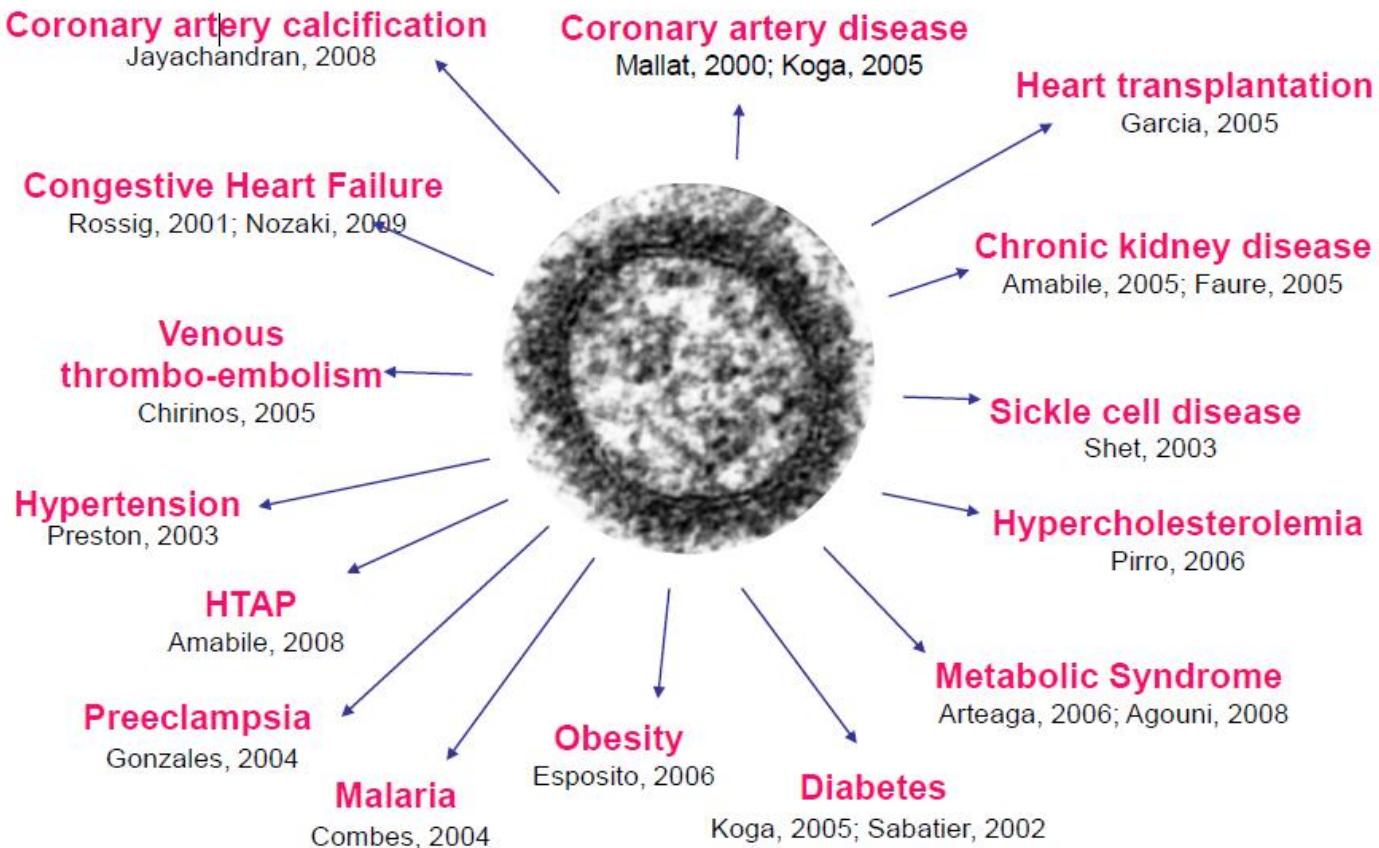
Endothelial Function Testing as a Biomarker of Vascular Disease



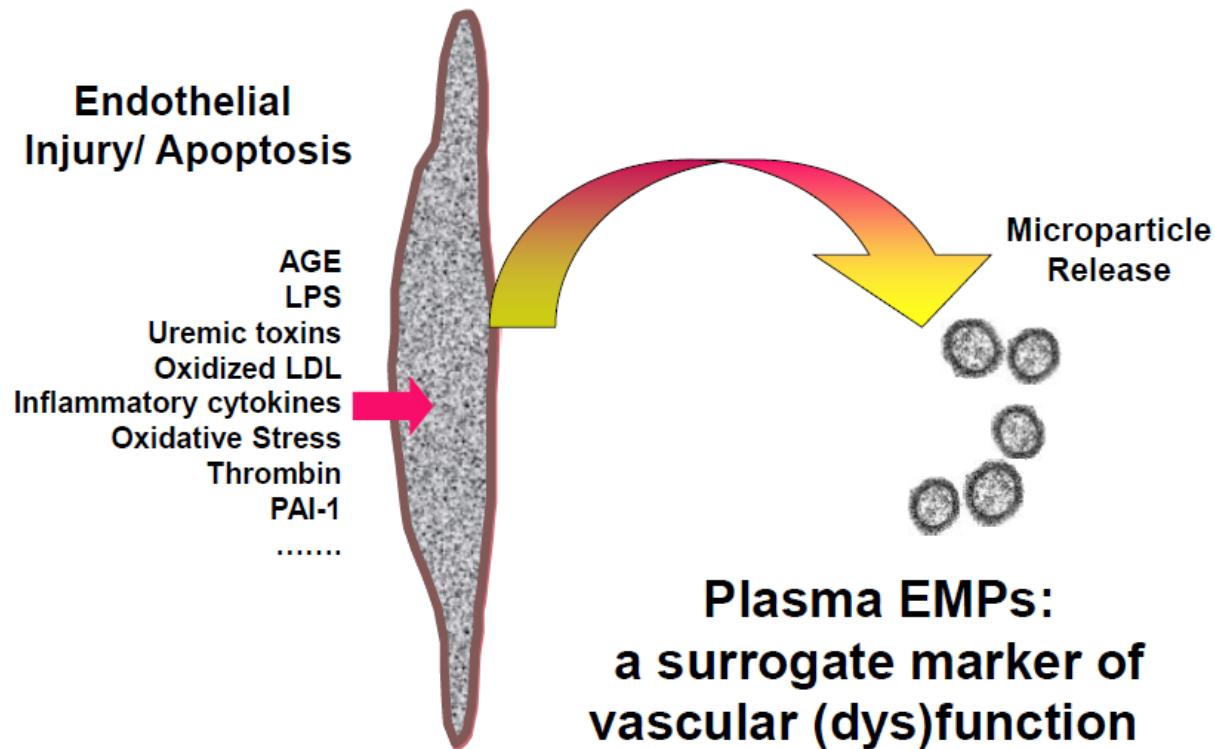
<https://doi.org/10.1161/01.CIR.0000089191.72957.ED>

Vesículas extracelulares en la fisiología y fisiopatología vascular

Increases in Circulating EMPs

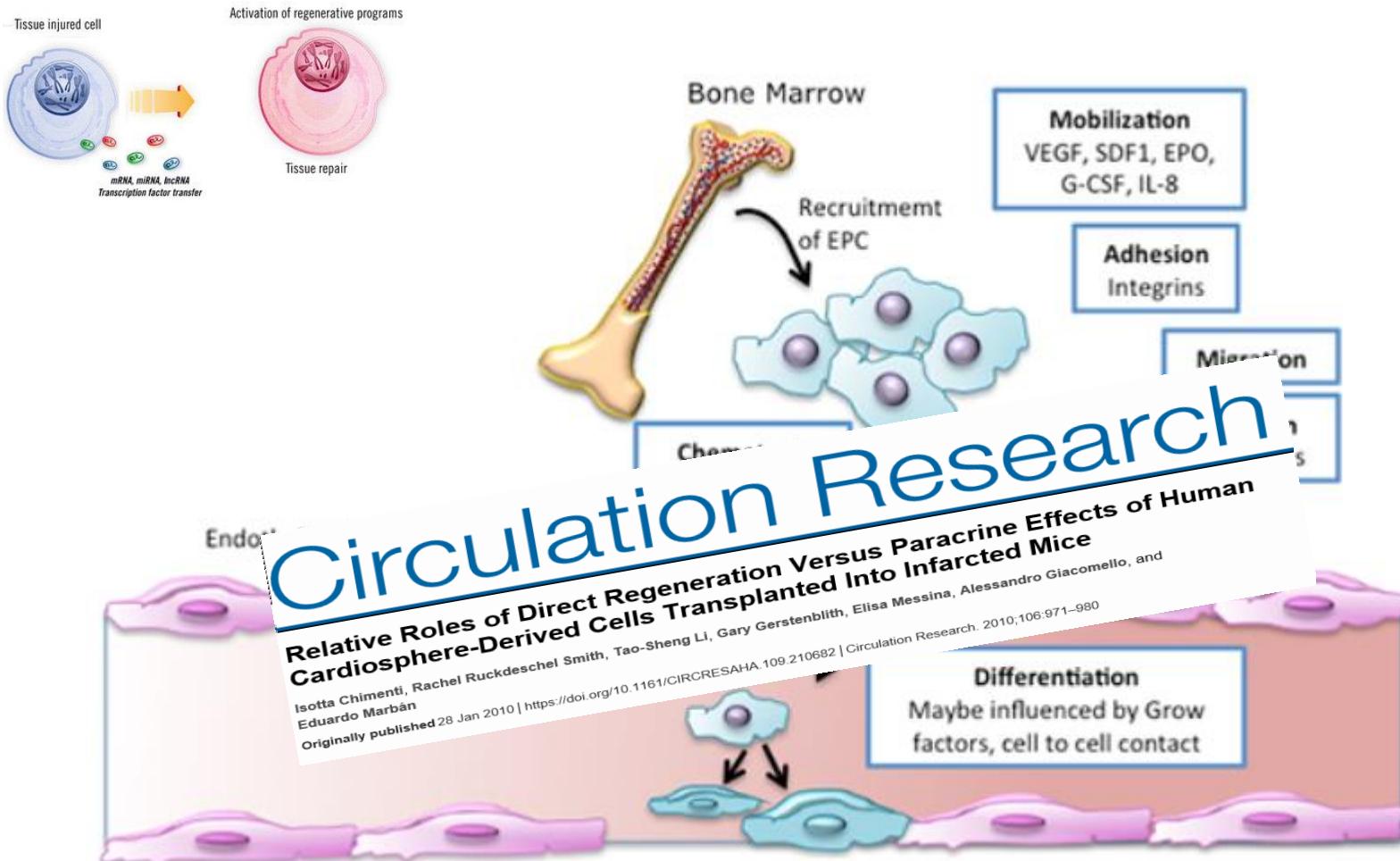


Vesículas extracelulares en la fisiología y fisiopatología vascular



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

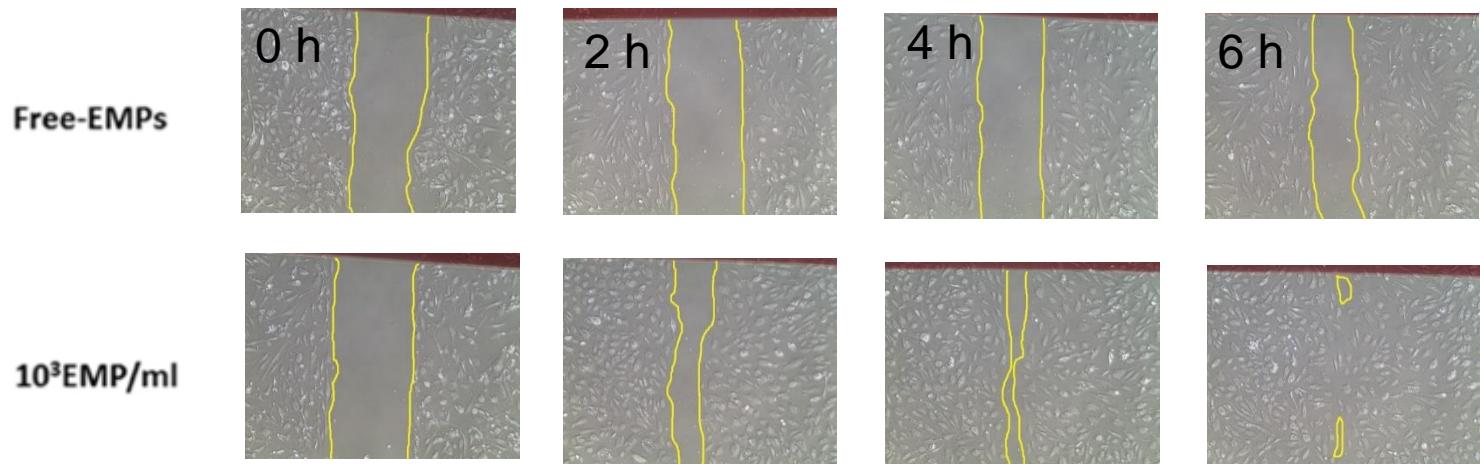
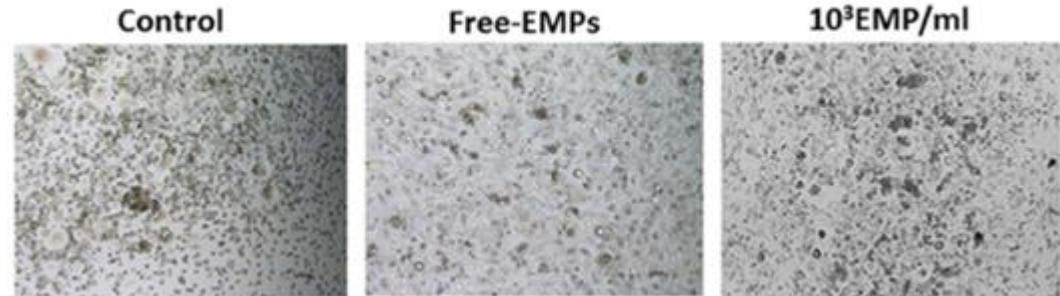
Vesículas extracelulares en la fisiología y fisiopatología vascular



<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

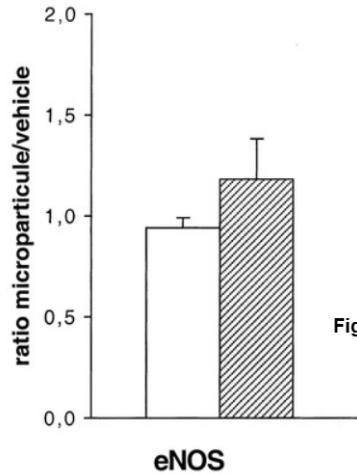
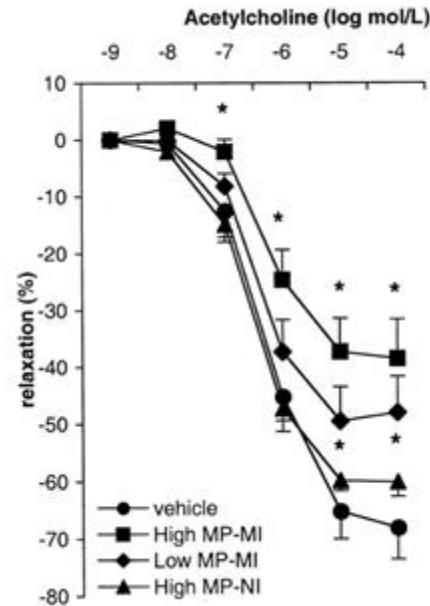


Figure 2. Semiquantitative evaluation of endothelial NO synthase (eNOS) expression in aortic rings exposed for 24 hours to high concentrations of microparticles from MI and NI patients



Vesículas extracelulares en la fisiología y fisiopatología vascular



FASEB J. 2017 Aug; 31(8): 3689–3694.

Published online 2017 May 5. doi: 10.1096/fj.201700149: 10.1096/fj.201700149

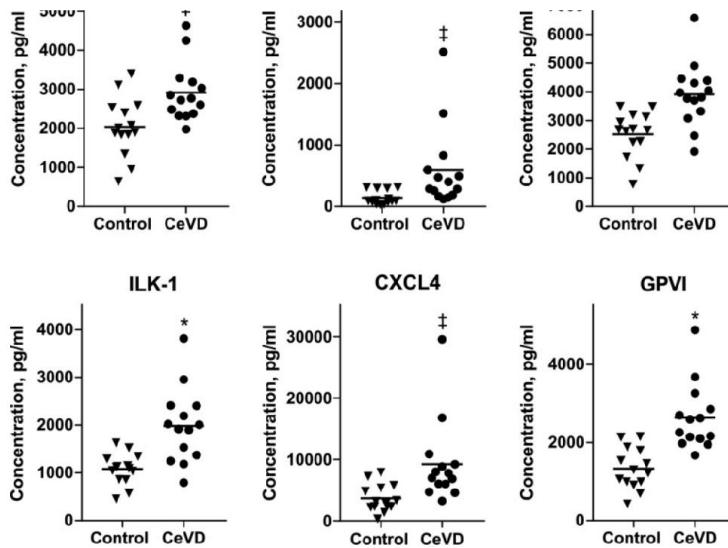
PMCID: PMC5503715

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.

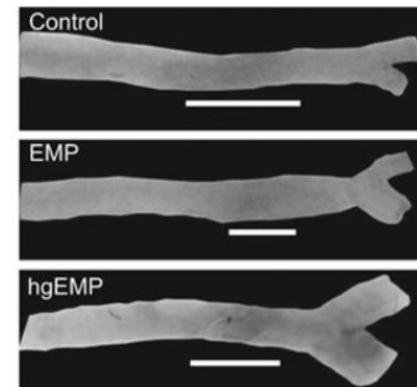
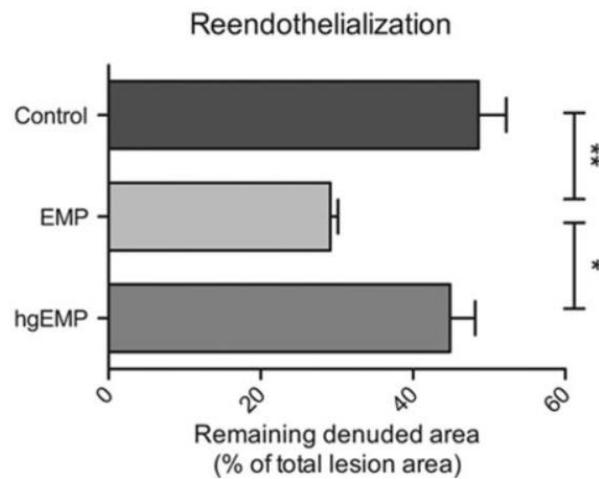
Vesículas extracelulares en la fisiología y fisiopatología vascular

Circulation

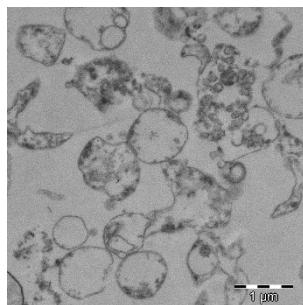
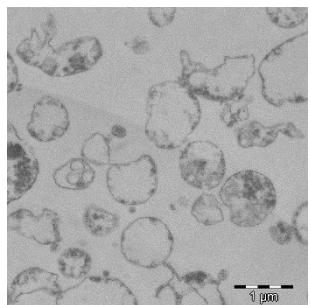
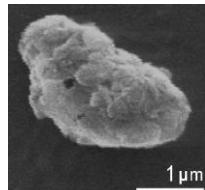
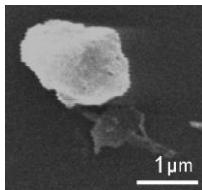
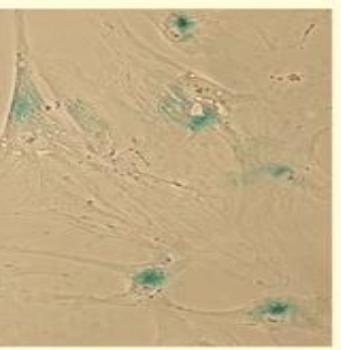
Endothelial Microparticle–Mediated Transfer of MicroRNA-126 Promotes Vascular Endothelial Cell Repair via SPRED1 and Is Abrogated in Glucose-Damaged Endothelial Microparticles

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 | 343 | 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 |

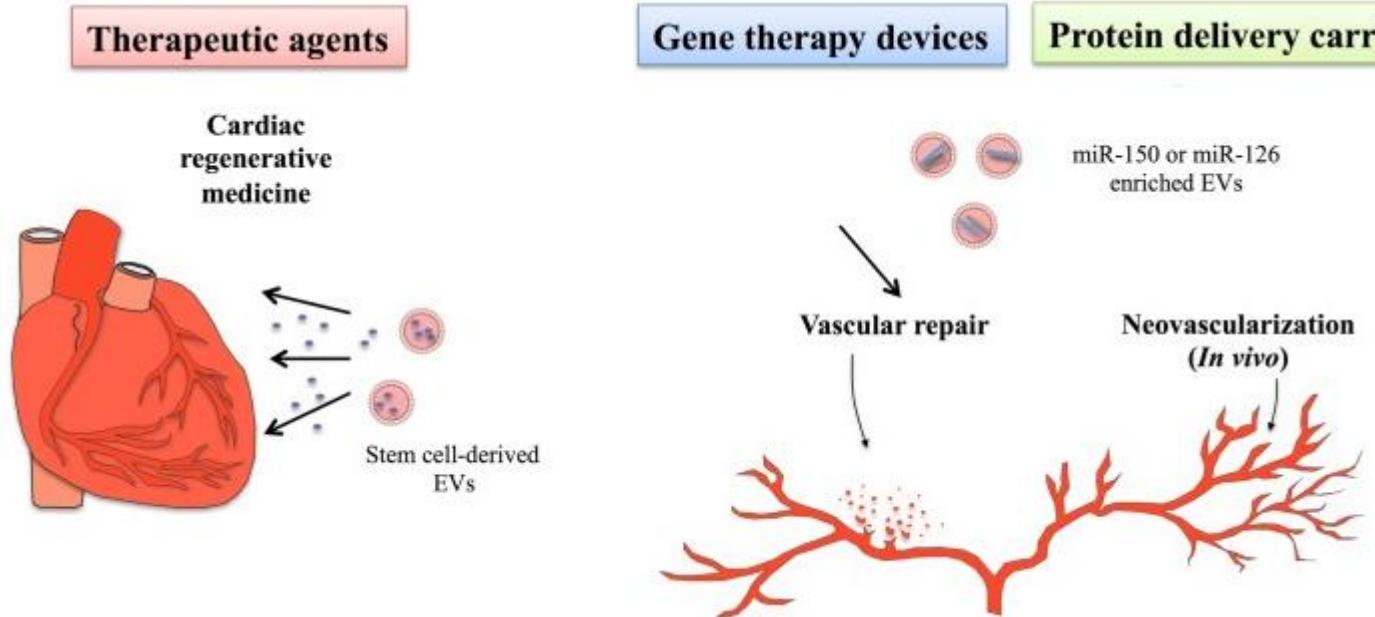
Only amplification in senescent MP.

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



Vesículas extracelulares en la fisiología y fisiopatología vascular

the end for now,

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

Thank you!

