
Bioengineering
Devika S Manickam, Ph.D.
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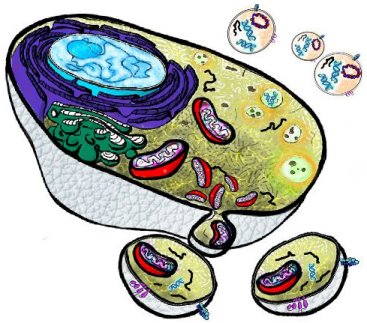
CRS 2023 ANNUAL MEETING & EXPOSITION
JULY 24-28, 2023 Paris Hotel » Las Vegas, NV, USA
THE FUTURE OF DELIVERY SCIENCE



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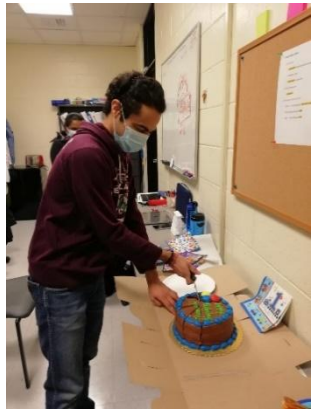
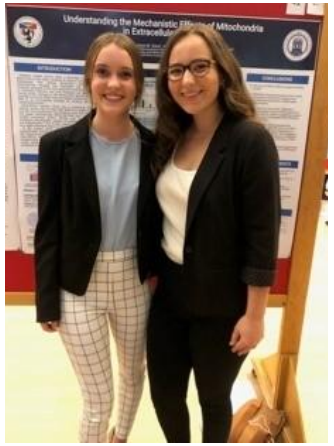
Engineered microvesicles co-deliver their innate mitochondria and an exogenous heat shock protein to protect the BBB post-stroke

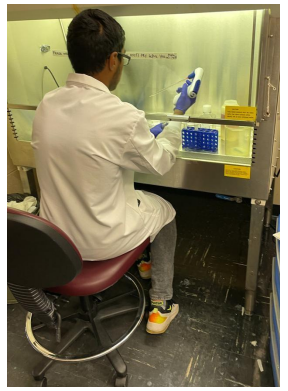
Devika S Manickam, Ph.D.
Associate Professor, Pharmaceutical Sciences
Assistant Editor, *JCR*

July 26, 2023
2023 CRS AM
Young Investigator Award, Bioengineering

soundaramanickd@duq.edu
@manickam_lab

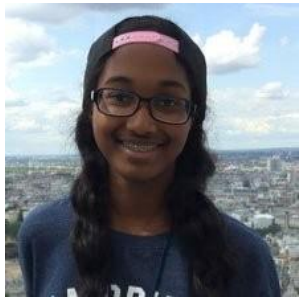






The team!

@Manickam lab


collaborators' team






collaborators

Outline

- The need for increasing mitochondrial (bioenergetic) function
- Mitochondrial crisis in stroke
- Extracellular vesicles (**EVs**)
- Biological effects of mitochondria-containing EVs
- Conclusions

Mitochondrial dysfunction

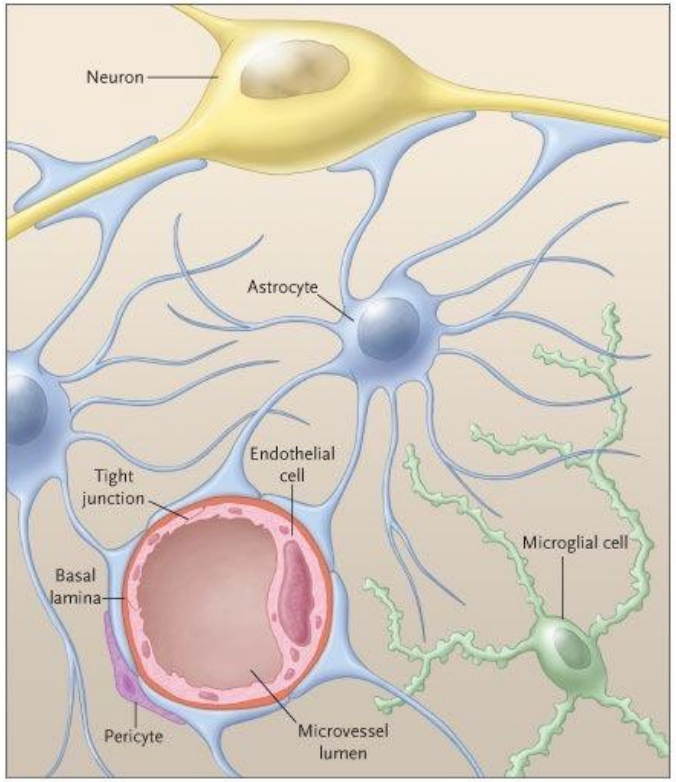
Table 1 A select list of acute and chronic diseases that exhibit mitochondrial dysfunction

Disease	Mitochondrial effect
Acute diseases	
Ischemia/reperfusion injuries: Myocardial infarction (11) Stroke (12) Acute kidney injury (10) Spinal cord injury (13)	Ischemia: ↓ATP and ATP-dependent cell functions, ↑ion imbalance (Na^+, K^+, Ca^{2+}), ↑reducing equivalents, and ↓tissue pH Reperfusion: ↑ROS, ↑oxidative stress, and ↑increased apoptosis/necrosis
Drug/toxin-induced injuries: Nephrotoxicity (14) Acute liver injury (15)	↑Apoptosis/necrosis, ↑mtDNA release, ↑oxidative stress, and ↑systemic inflammatory responses
Chronic diseases	
Type II diabetes (16)	↓Metabolic capacity, ↓PGC-1α-dependent gene expression, and ↑ lipids, possible contribution of mitochondrial dysfunction to insulin resistance
Neurodegenerative diseases: AD (17) PD (18) HD (19) ALS (20)	AD: ↓mitochondrial proteostasis, ↓cellular ATP, and ↑oxidative stress resulting in neuron loss and microvascular dysfunction PD: defective mitophagy and fission/fusion dynamics, ↑oxidative stress, ↓energetics, and ↑neuron loss HD: ↓ETC complex numbers and activity, mitochondrial trafficking disrupted by mutant Huntingtin, ↑mitochondria fragmentation, disrupted Ca^{2+} handling, and ↑MPTP opening and apoptosis ALS: ↓ATP from ETC, ↑ROS and oxidative stress, and ↑Ca^{2+} imbalance
Cardiovascular diseases: PAH (21) HF (22, 23)	PAH: ↑Mitochondrial fragmentation, ↓ETC flux, ↓ROS, ↑HIF-1α expression, ↑vasoconstriction HF: ↓MB, ↓mtDNA copy number, ↓overall mitochondrial content, and ↑ROS and oxidative stress

J Clin Invest. 2018;128(9):3716-3726

Ann Rev Pharmacol Toxicol 2016 Vol. 56 Pages 229-49

Stroke is a neurovascular disease



Neurovascular unit (NVU)

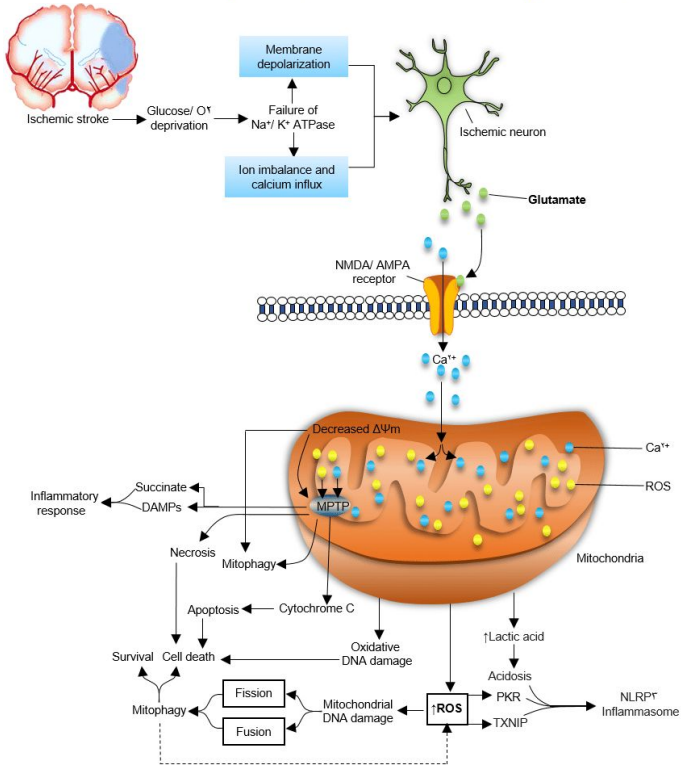
- The damage borne by the brain endothelial cells (**BECs**) disrupts the structure and function of the BBB, the NVU and contributes to poor patient outcomes post-ischemia/reperfusion injury.
- BECs also secrete trophic factors that can be directly neuroprotective.

Global hypothesis: Targeting drug delivery to the blood-brain barrier (BBB) may be a viable approach to increase BEC survival and rescue its protective functions

N Engl J Med, 2006. 354(6): p. 553-5
Proceedings of the National Academy of Sciences, 2008. 105(21): p. 7582-7587

Mitochondrial Crisis in Cerebrovascular Endothelial Cells Opens the Blood–Brain Barrier

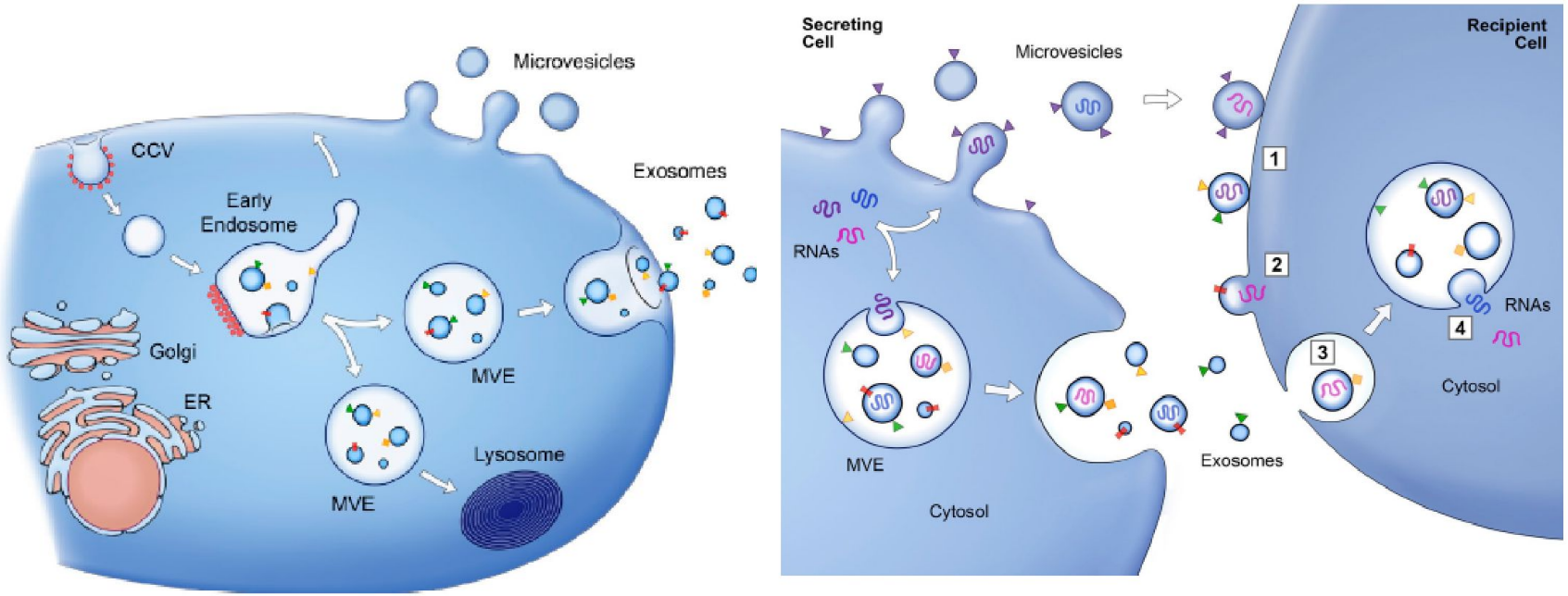
Danielle N. Doll, BSc; Heng Hu, MD; Jiahong Sun, BSc; Sara E. Lewis, MS;
James W. Simpkins, PhD; Xuefang Ren, Dr Med



BECs contain mitochondria at a ~5-fold higher concentration compared to peripheral ECs

J Cerebral Blood Flow and Metabolism, 2013. 33(1): p. 22-32

Extracellular vesicles (EVs)



EVs are tiny messengers that play key roles in cell-to-cell communication

Extracellular vesicles: Exosomes, microvesicles, and friends

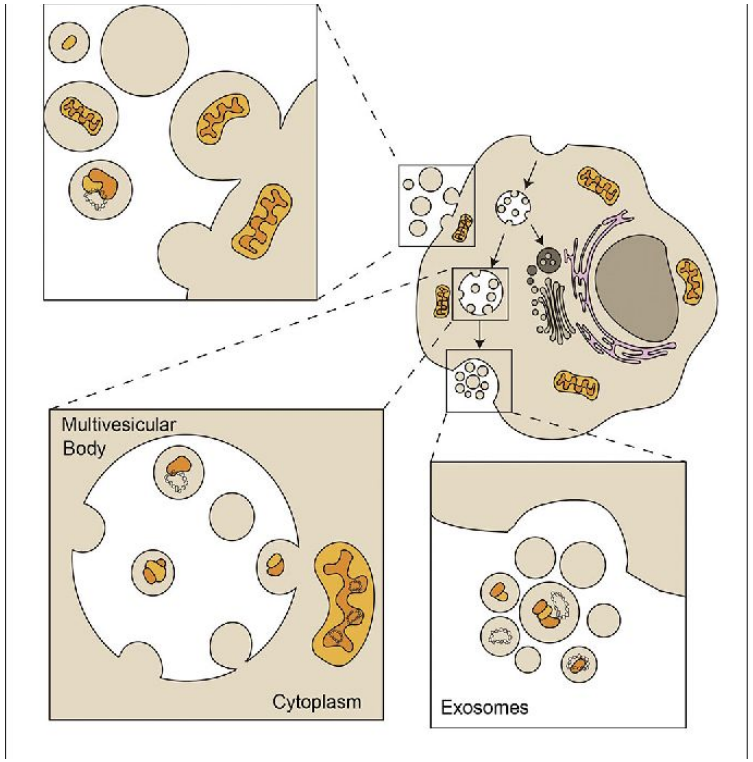
Graça Raposo[1,2] and Willem Stoorvogel[3]

J. Cell Biol. Vol. 200 No. 4 373–383

frontiers in Cell and Developmental Biology

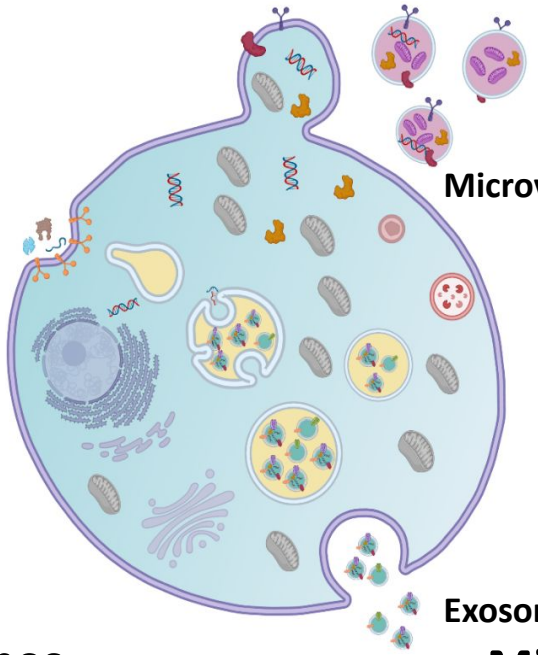
REVIEW
published: 28 September 2016
doi: 10.3389/fcell.2016.00107

Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer



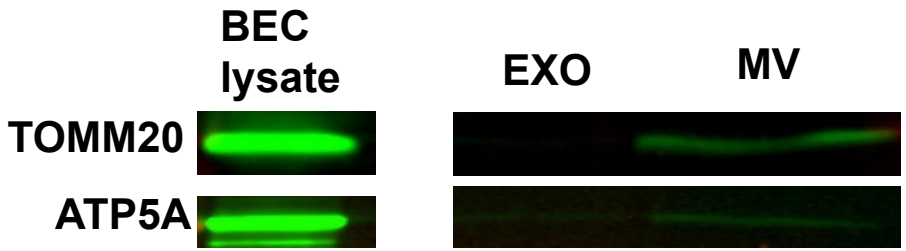
The smaller EVs (**exosomes: EXOs**) contain mitochondrial components such as mtDNA and proteins whereas the larger EVs (**microvesicles: MVs**) contain mitochondria

EV particle characteristics and marker proteins



Sample	Z-avg. (nm)	PdI	Z-potential mV	EV number x10^8/mL
MVs	394.6±19.1	0.41±0.06	-11.9±0.06	5.1±0.6
EXOs	129.3±11.2	0.27±0.04	-10.3±1.1	4.6±0.4

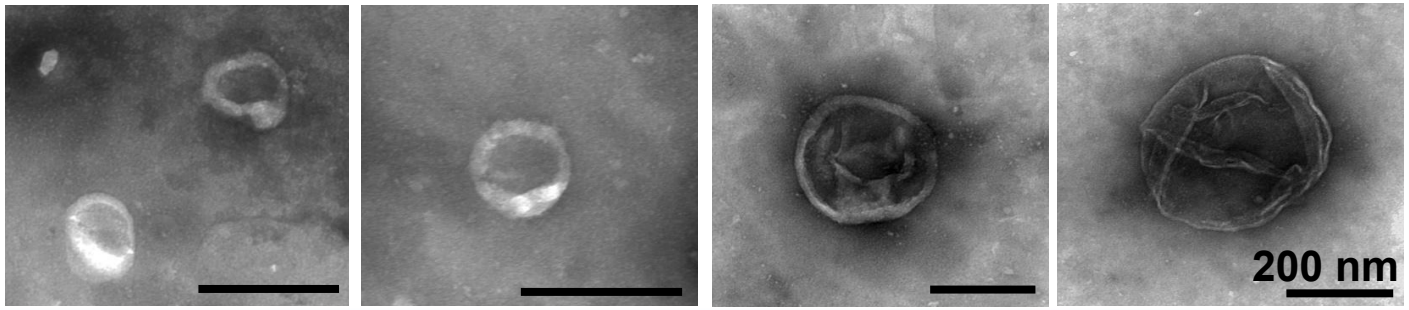
DLS: [EV] = 0.1 mg protein/mL in 10 mM HEPES, pH 7.4



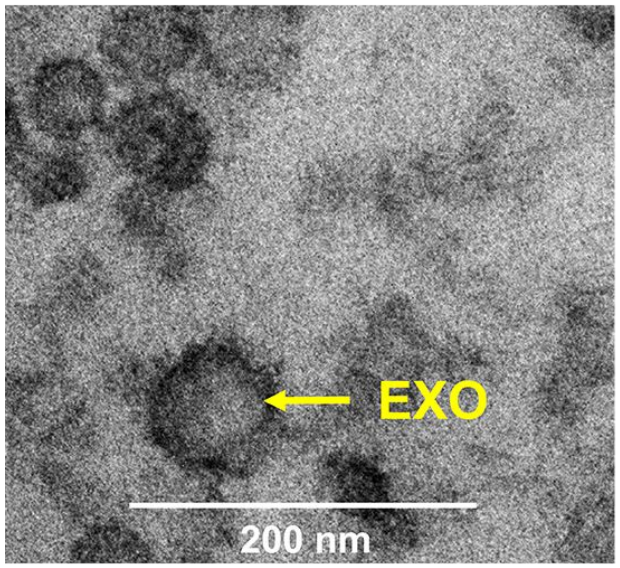
Both EXOs and MVs contain mitochondrial matrix protein ATP5A but only MVs contain mitochondrial membrane marker TOMM20

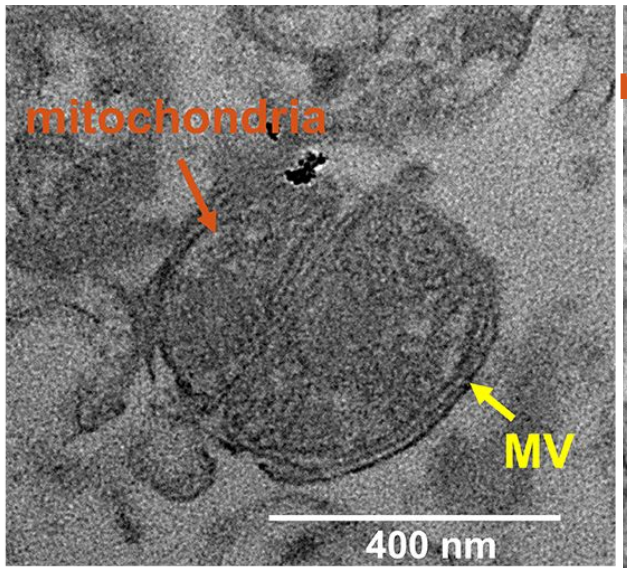
Exosomes

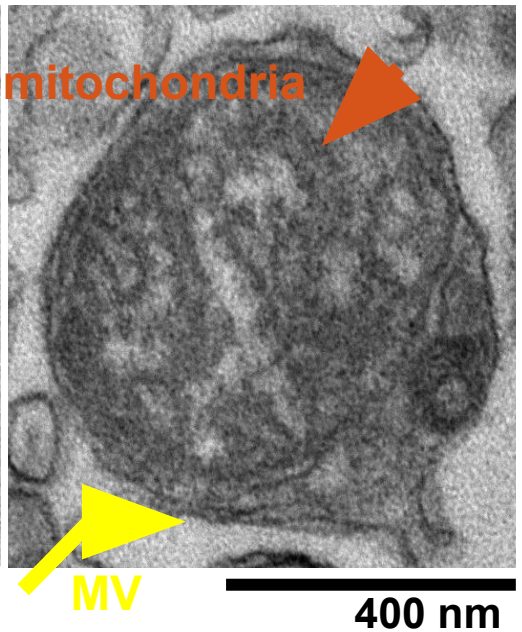
Microvesicles



MVs but not EXOs contain mitochondria







Journal of Controlled Release 354 (2023) 368–393

Contents lists available at ScienceDirect

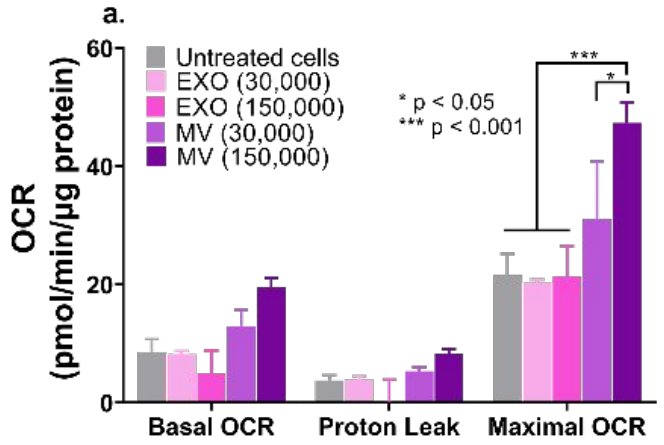
Journal of Controlled Release

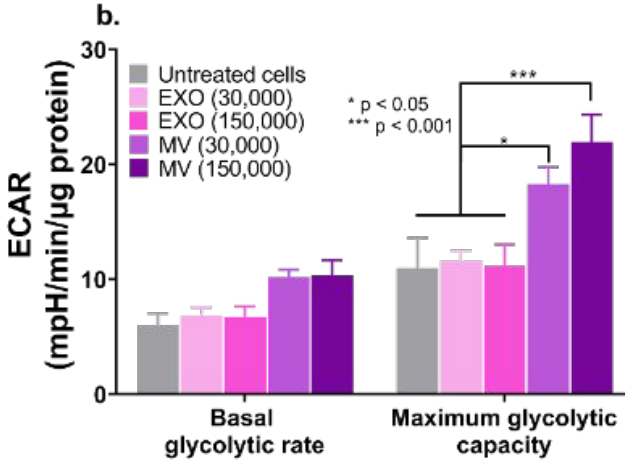
journal homepage: www.elsevier.com/locate/jconrel

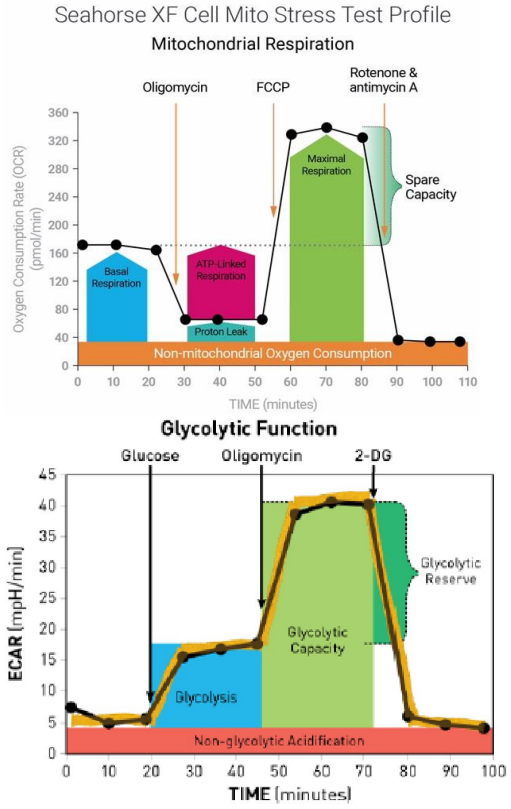
Mitochondria-containing extracellular vesicles (EV) reduce mouse brain infarct sizes and EV/HSP27 protect ischemic brain endothelial cultures

Kandarp M. Dave[a], Donna B. Stolz[b], Venugopal R. Venna[c], Victoria A. Quaicoe[c], Michael E. Maniskas[c], Michael John Reynolds[d], Riyan Babidhan[a], Duncan X. Dobbins[a], Maura N. Farinelli[a,c], Abigail Sullivan[a,f], Tarun N. Bhatia[a], Hannah Yankello[g], Rohan Reddy[a], Younsoo Bae[h], Rehana K. Leak[a], Sruti S. Shiva[d,i], Louise D. McCullough[c], Devika S. Manickam[a,*]

MV, but not EXOs, increase mitochondrial function in the recipient ischemic BECs







OCR: Oxygen Consumption Rate
ECAR: Extracellular Acidification Rate

Journal of Controlled Release 338 (2021) 505–526

Contents lists available at ScienceDirect

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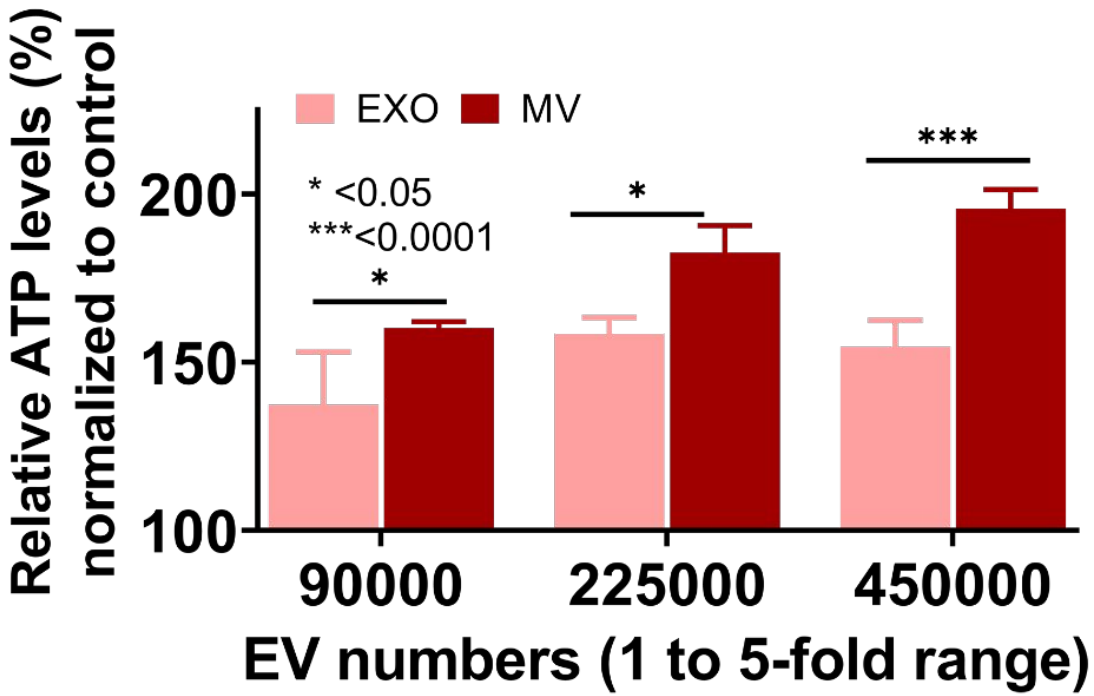
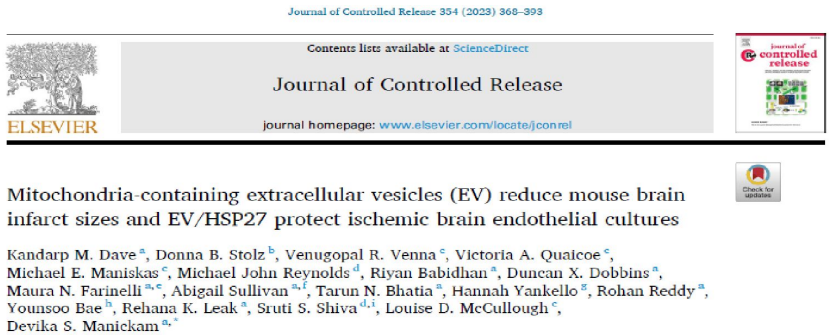
Microvesicles transfer mitochondria and increase mitochondrial function in brain endothelial cells

Anisha D'Souza[a,3], Amelia Burch[b], Kandarp M. Dave[a,1], Aravind Sreeram[c,1], Michael J. Reynolds[d], Duncan X. Dobbins[a], Yashika S. Kamte[a], Wanzhu Zhao[a], Courtney Sabatelle[a], Gina M. Joy[a], Vishal Soman[f], Uma R. Chandran[f], Sruti S. Shiva[d,e], Nidia Quillinan[b], Paco S. Herson[b,2], Devika S Manickam[a,*]

MVs increase ATP levels to a greater extent compared to EXOs in ischemic HBMECs



Primary human brain endothelial cells (HBMECs)



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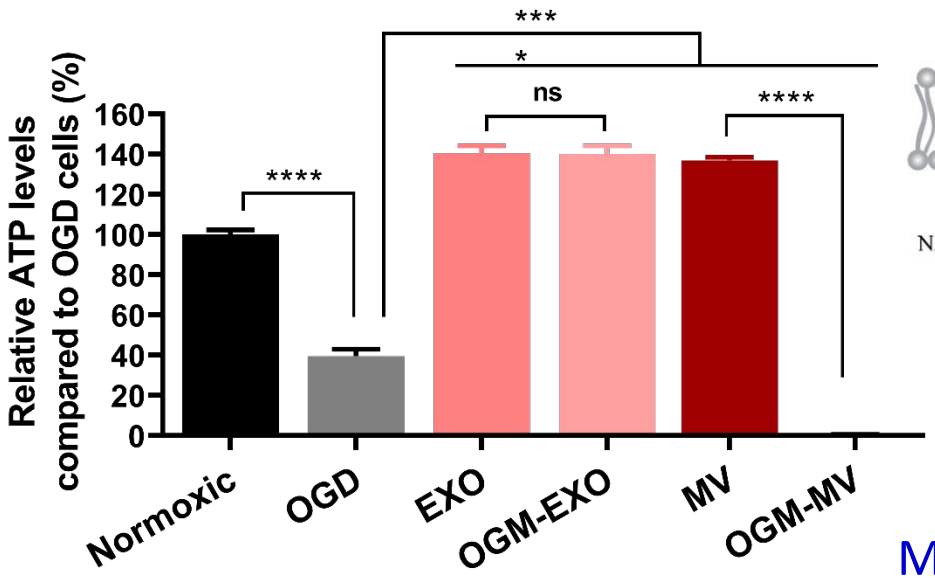
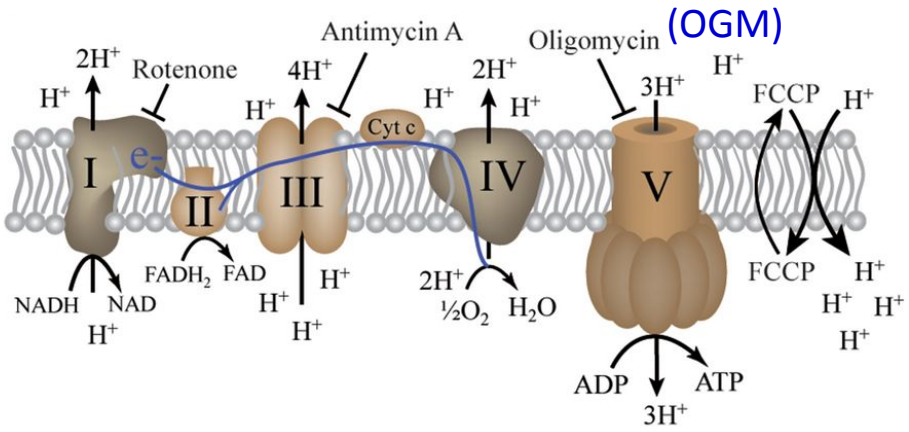
Are the MV-mediated ATP increases related to their mitochondrial load?





Scientific Reports 2020 Vol. 10 Issue 1 Pages 13179

[MV-mediated increase in recipient BEC ATP levels is a function of the innate MV mitochondria]

Cellular and Molecular Bioengineering (© 2022)
https://doi.org/10.1007/s12195-022-00738-8

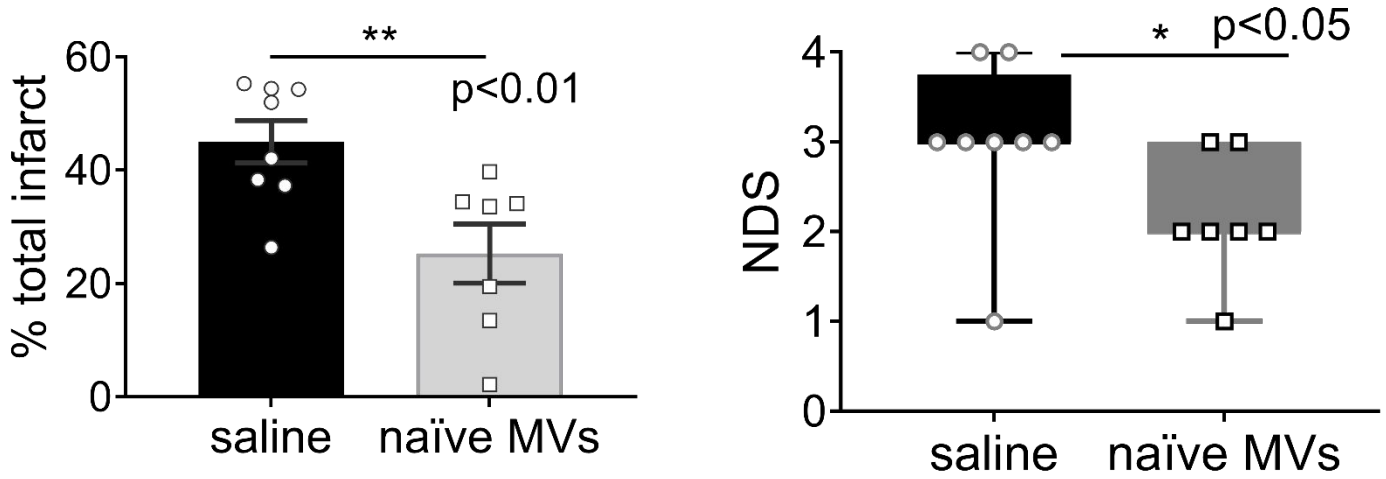
BMES BIOMEDICAL ENGINEERING SOCIETY

S.I. : 2022 CMBE Young Innovators

Engineering Extracellular Vesicles to Modulate Their Innate Mitochondrial Load

Kandarp M. Dave,[1] Duncan X. Dobbins,[1] Maura N. Farinelli,[1,2] Abigail Sullivan,[1,3] Jadranka Milosevic,[4,5] Donna B. Stolz,[6] Jeongyun Kim,[4] Siyang Zheng,[4] and Devika S. Manickam[1]

Microvesicles mediate cerebroprotection resulting in post-stroke neurological recovery



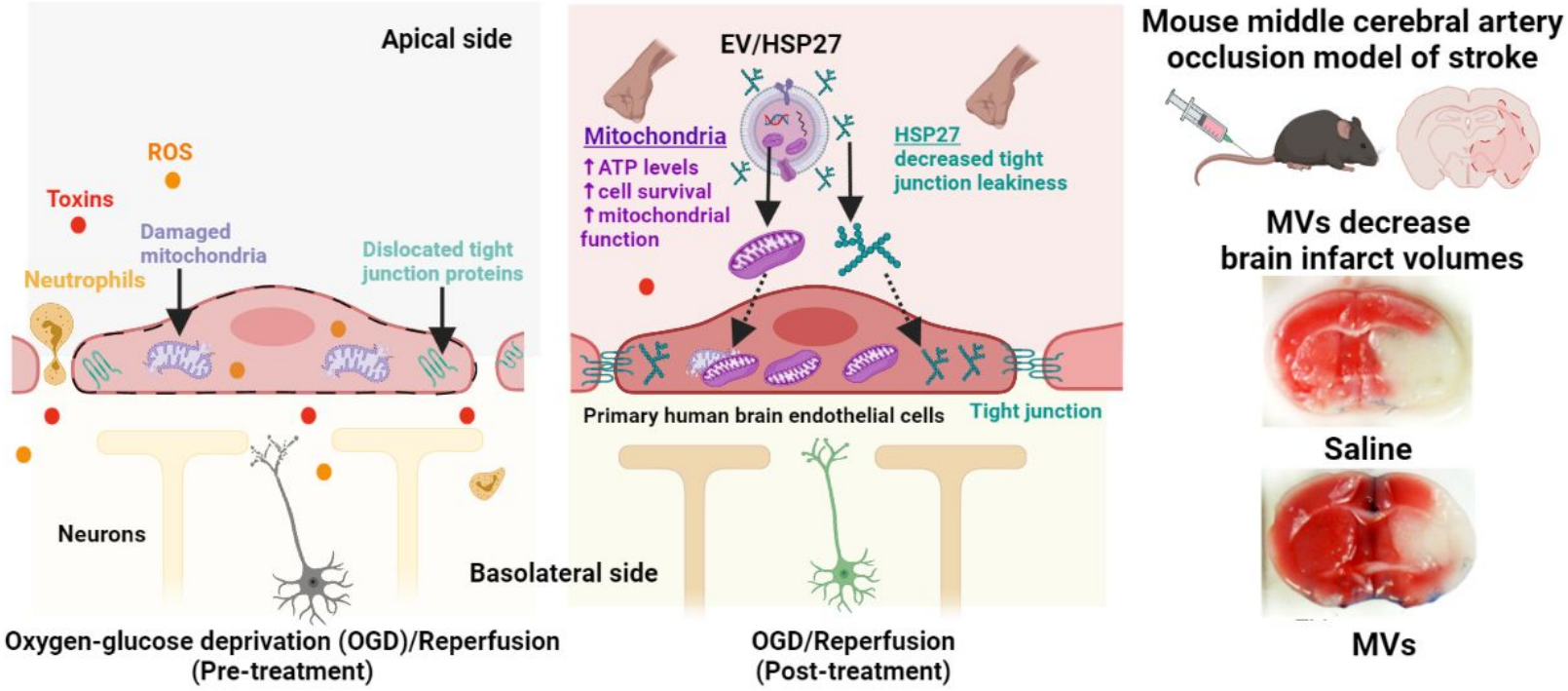
Neurological deficit score (**NDS**): 0 = no deficit; 1 = forelimb weakness and torso turning to the ipsilateral side when held by tail; 2 = circling to affected side; 3 = unable to bear weight on affected side; and 4 = no spontaneous locomotor activity or barrel rolling

Mouse BEC-derived MVs show significant cerebroprotection and behavioral recovery in a mouse model of stroke

Dave KM…Manickam DS, in preparation

Mitochondria-containing EVs (microvesicles/MVs) increase BEC metabolic function and EV/HSP27 mixtures protect BEC integrity

MVs show neuroprotection in a mouse model of ischemic stroke



- MVs transfer mitochondria to recipient BECs and mouse brain slice neurons
- MVs increase mitochondrial function in the recipient BECs
- Naïve MVs mediate cerebroprotection and neurological recovery post-stroke
- **A combination of innate MV mitochondrial cargo in addition to the exogenous drug cargo (like HSP27) can improve therapeutic outcomes**

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Microvesicles transfer mitochondria and increase mitochondrial function in brain endothelial cells

Journal of Controlled Release 343 (2022) 400–407

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Delivery of mitochondria via extracellular vesicles – A new horizon in drug delivery

Cellular and Molecular Bioengineering (© 2022)
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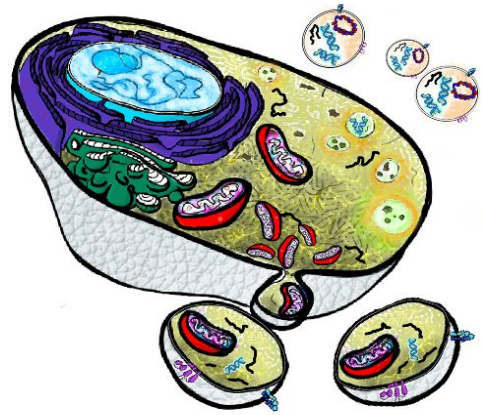
Engineering Extracellular Vesicles to Modulate Their Innate Mitochondrial Load

Manickam Lab of Biotherapeutics Delivery



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