Brain-Derived Extracellular Vesicles as Molecular Probes and Therapeutic Vehicles in Neonatal Brain Injury

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7.13.22

UNIVERSITY of WASHINGTON

The University of Washington acknowledges the Coast Salish peoples of the land on which we teach, learn, and study – this touches the shared waters of all tribes and bands within the Suquamish, Tulalip and Muckleshoot nations

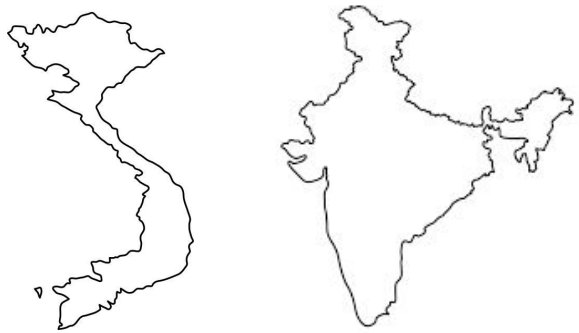
Hypoxic **I**schemic-**E**ncephalopathy (HIE) is the leading cause of neonatal mortality and morbidity.

And it has no cure.

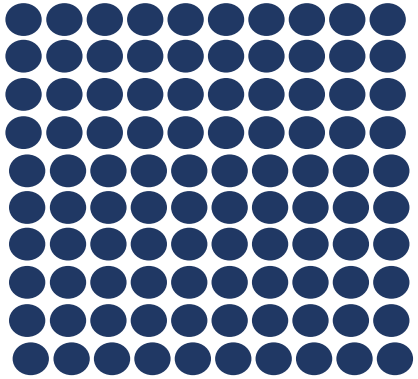
Millar et. al, 10.3389/fncel.2017.00078

Neonatal HIE is a Global Burden

Neonatal HIE most impacts the developing world…



Low-resource countries:
Incidence of 2.6%



Douglas-Escobar M, 10.1001/jamapediatrics.2014.3269

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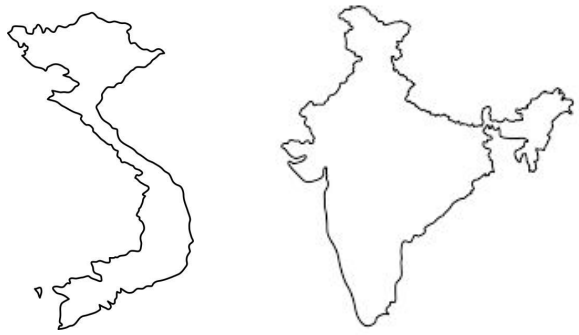

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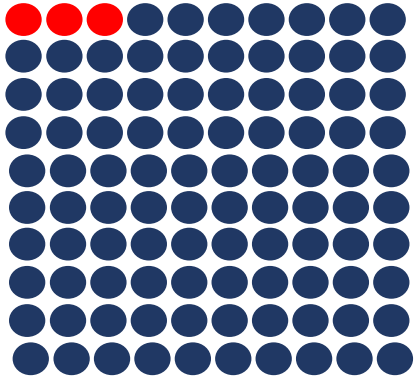

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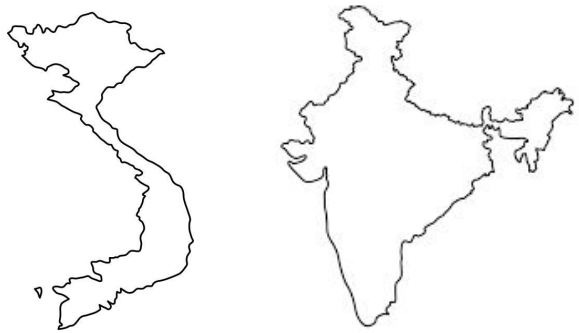

Low-resource countries:
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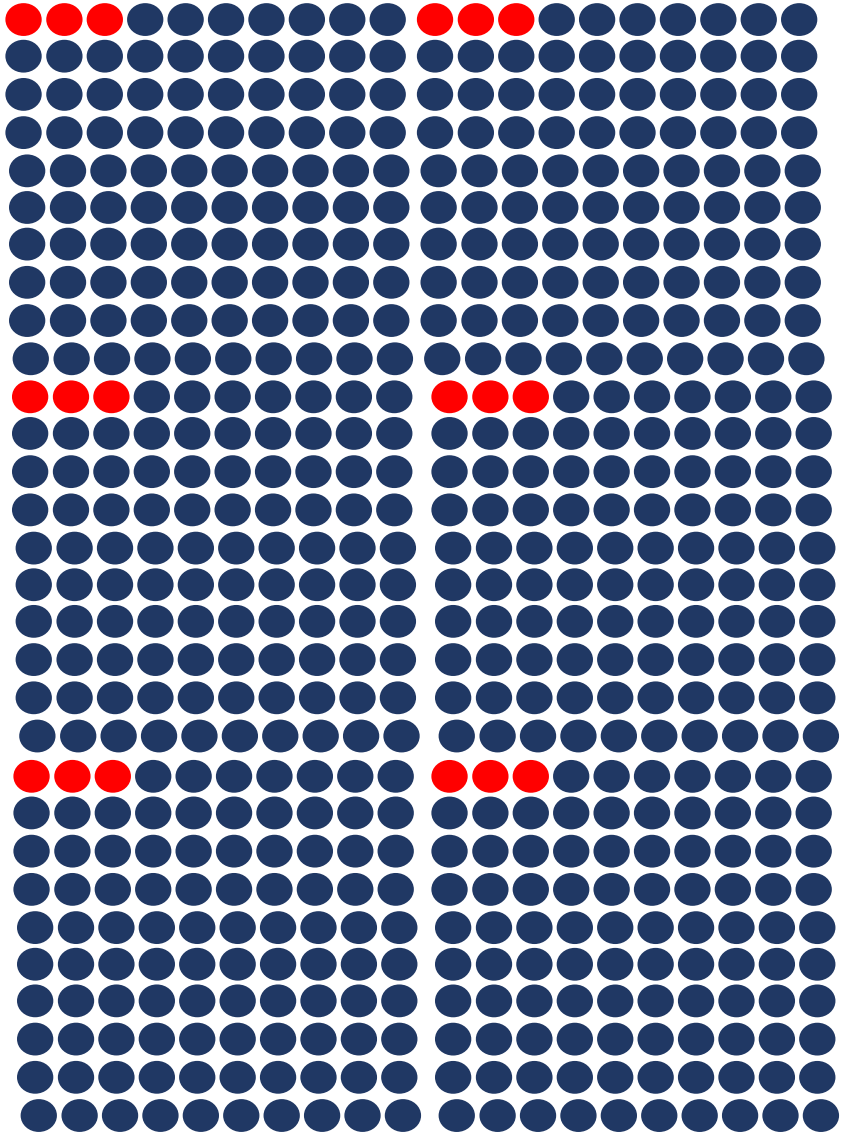

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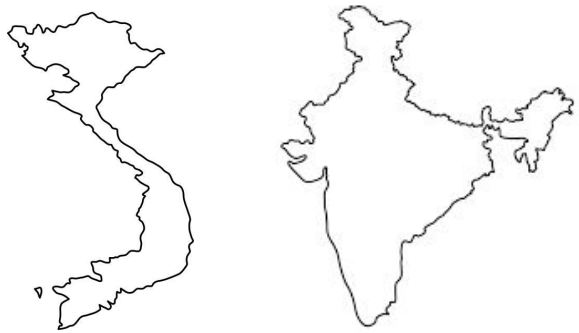
Neonatal HIE is a Global Burden

Neonatal HIE most impacts the developing world…



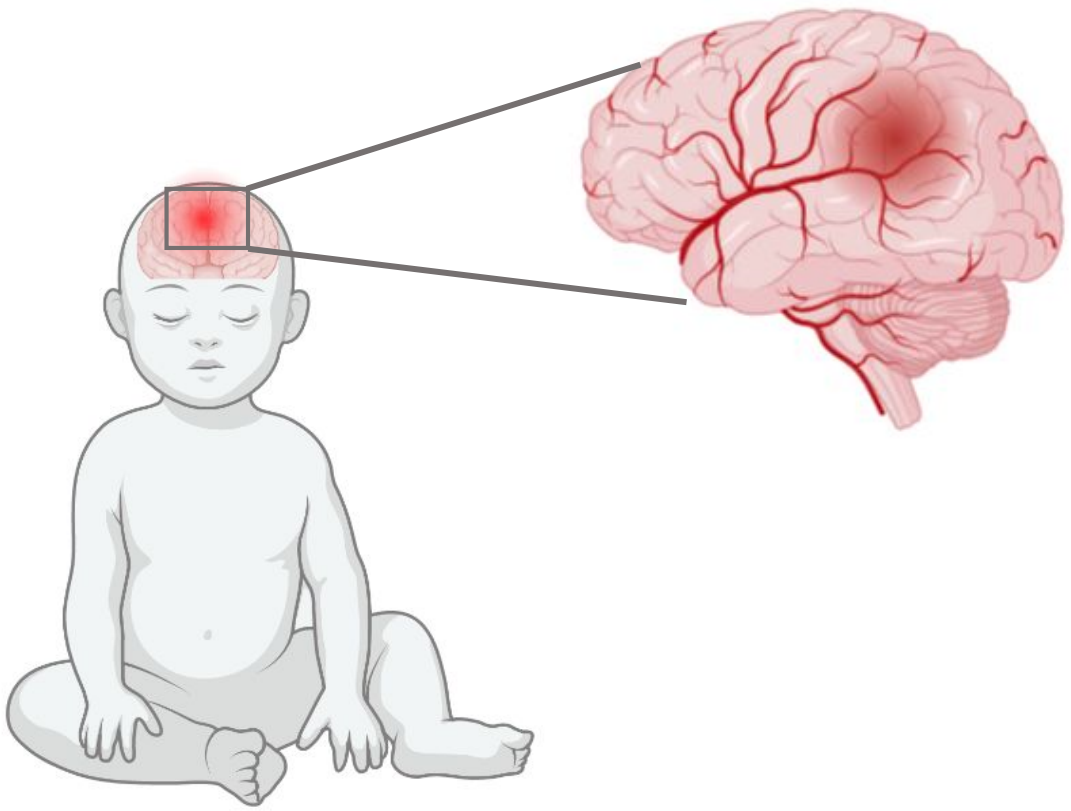
Low-resource countries:
Incidence of 2.6%

… But the developed world is affected too.

High-resource countries:
Incidence of 1%

Douglas-Escobar M, 10.1001/jamapediatrics.2014.3269

Brain Inflammation is a Hallmark of HIE



- Blood supply
- Oxygen levels
- ATP levels

- Dysregulated ionic exchange
- Production of reactive oxygen species
- Glutamate Release
- Immune cell activation

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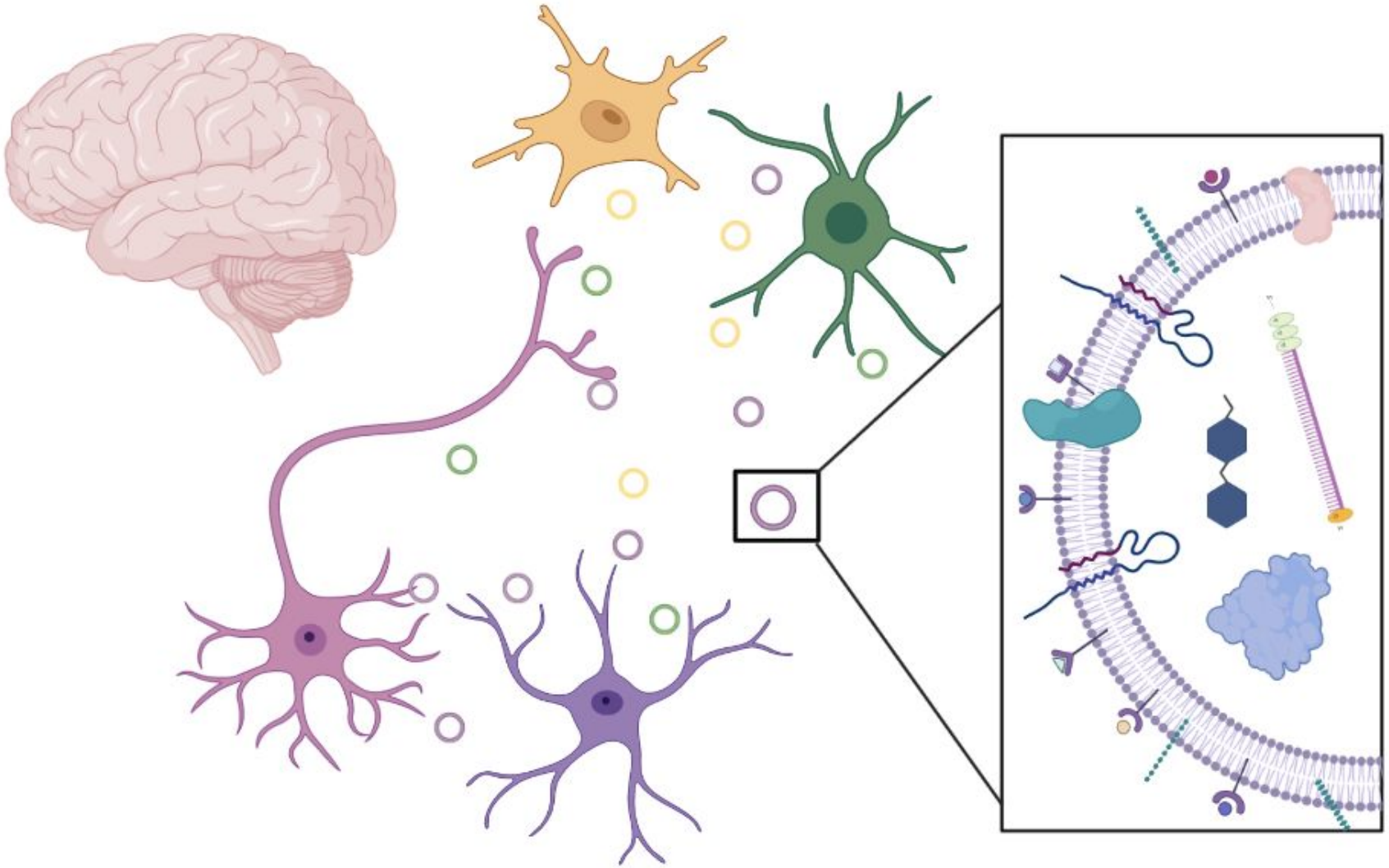
Liao et. al., *10.1177/1849543520970819*
Benakis et. al, 10.3389/fncel.2014.00461

Extracellular vesicles (EVs) are important players in HIE injury response

What Are **E**xtracellular **V**esicles (EVs)?



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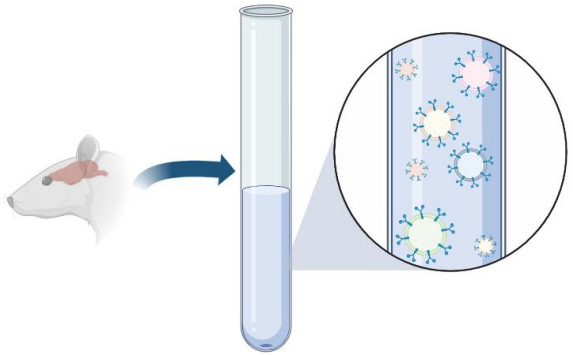
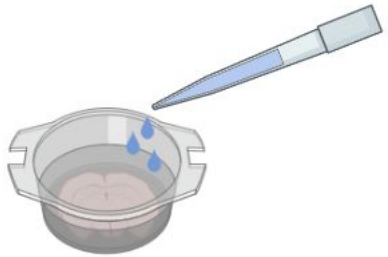
Holm et. al.,10.1016/j.tins.2018.03.006
Zheng M. et.al. 10.1021/acs.bioconjchem.9b00085.
Turturici, G., et.al., 10.1152/ajpcell.00228.2013.

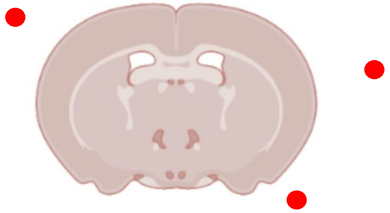
Research Objectives



Isolation & Characterization of Brain Tissue Derived EVs (BEVs)



Evaluate Therapeutic Efficacy of BEVs in an *Ex Vivo* Model of Neonatal HI



Investigate BEV Fate in the Neonatal HI Brain

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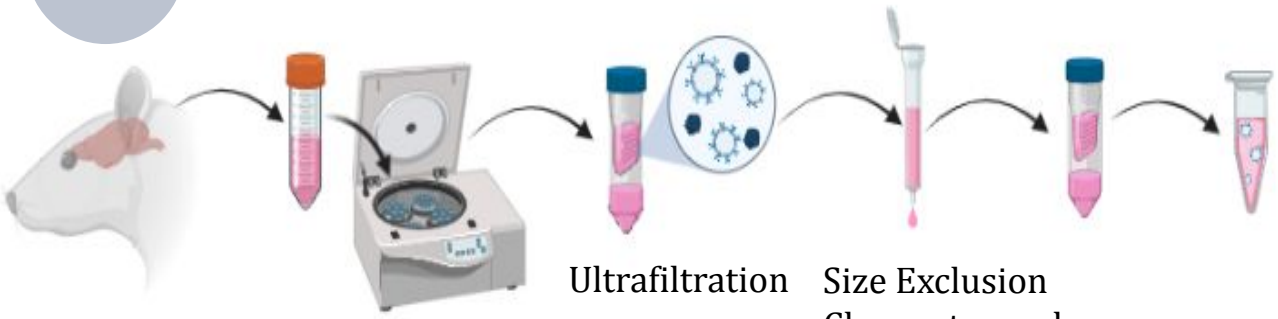
Overview of Methods

1 Isolation & Characterization of BEVs



Ultracentrifugation

Ultrafiltration

Size Exclusion Chromatography

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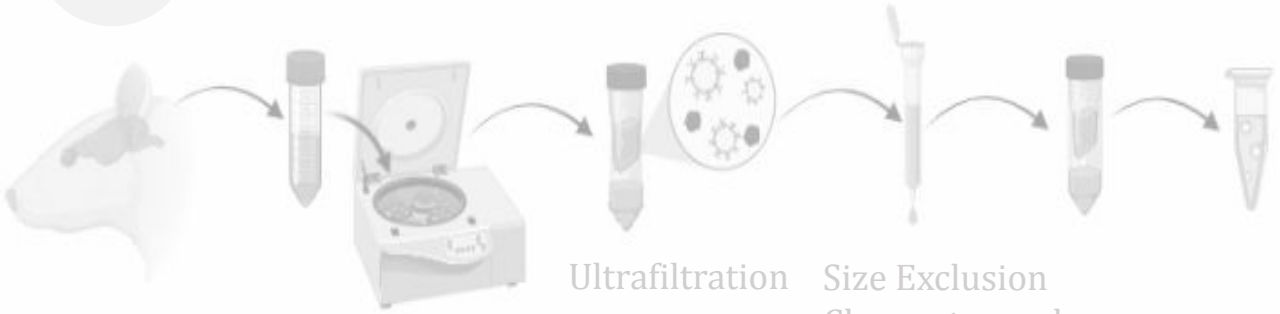
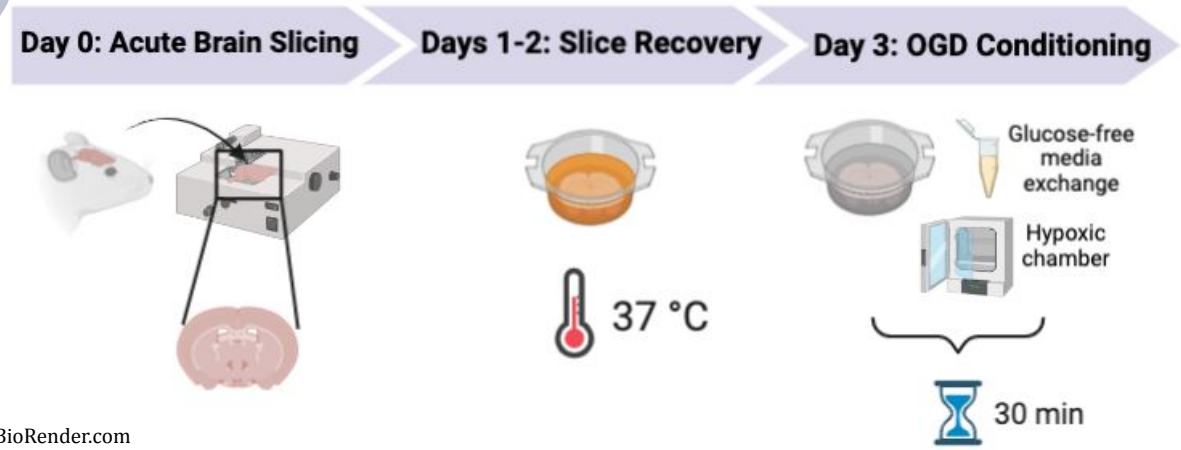
Nguyen NP. et.al., DOI: 10.3390/ijms23020620
Vella et.al, DOI: 10.1080/20013078.2017.1348885

Overview of Methods

1 Isolation & Characterization of BEVs



2 Preparing the *Ex Vivo* Neonatal HIE Model



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Nguyen NP. et.al., DOI: 10.3390/ijms23020620
Joseph et.al., DOI: 10.1002/btm2.10175

Overview of Methods

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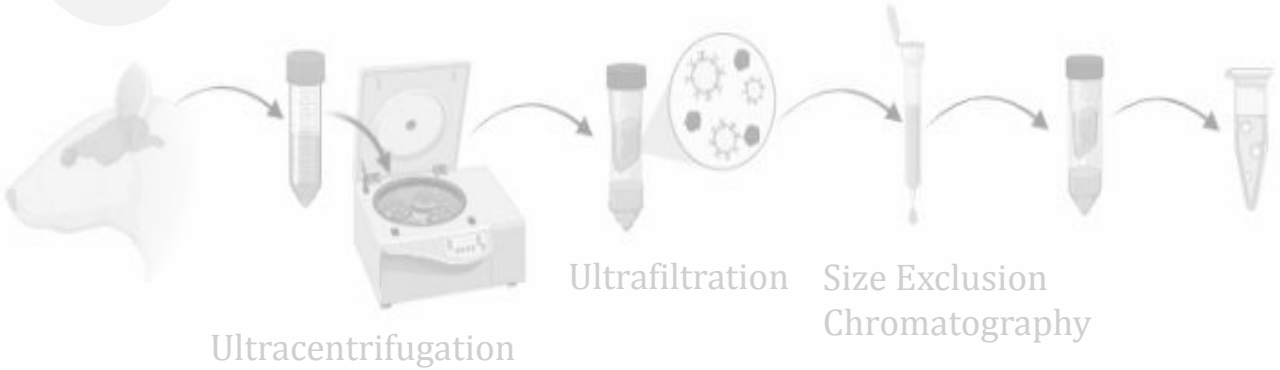

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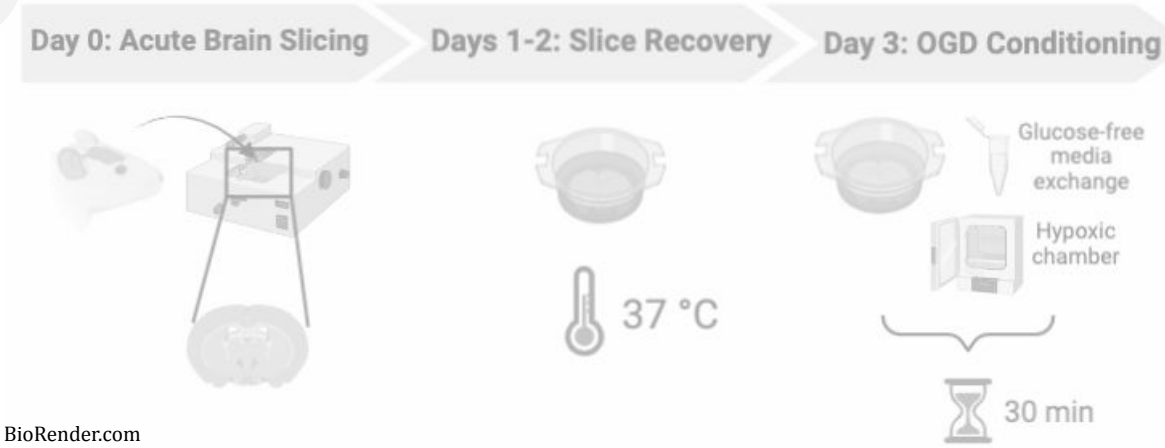

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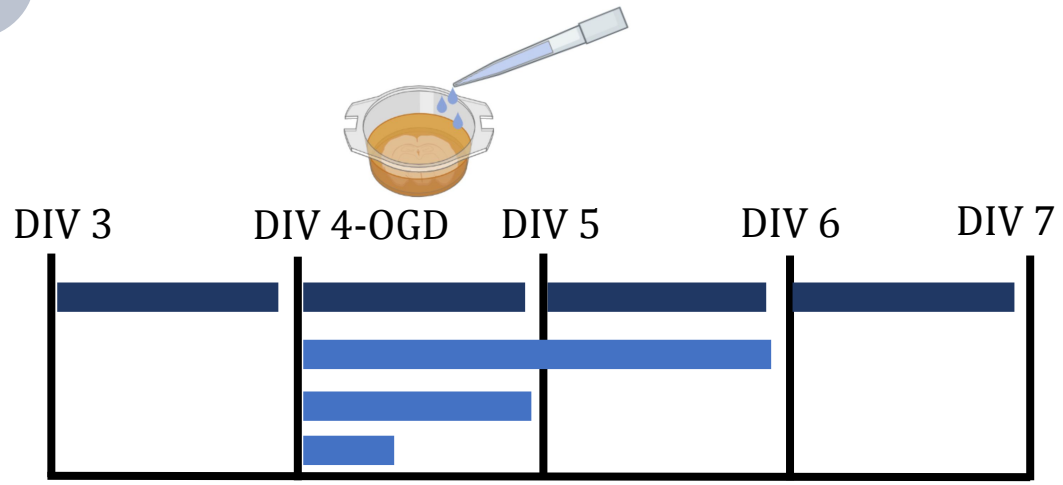
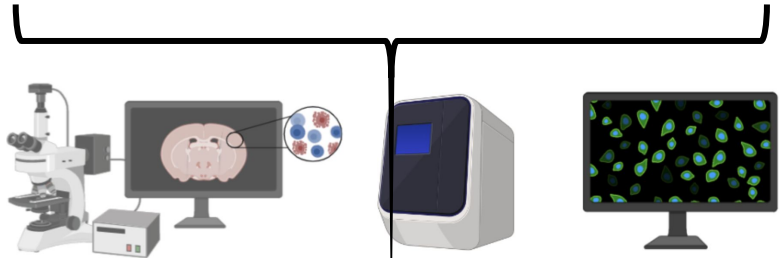
3 BEV Administration & Assessment

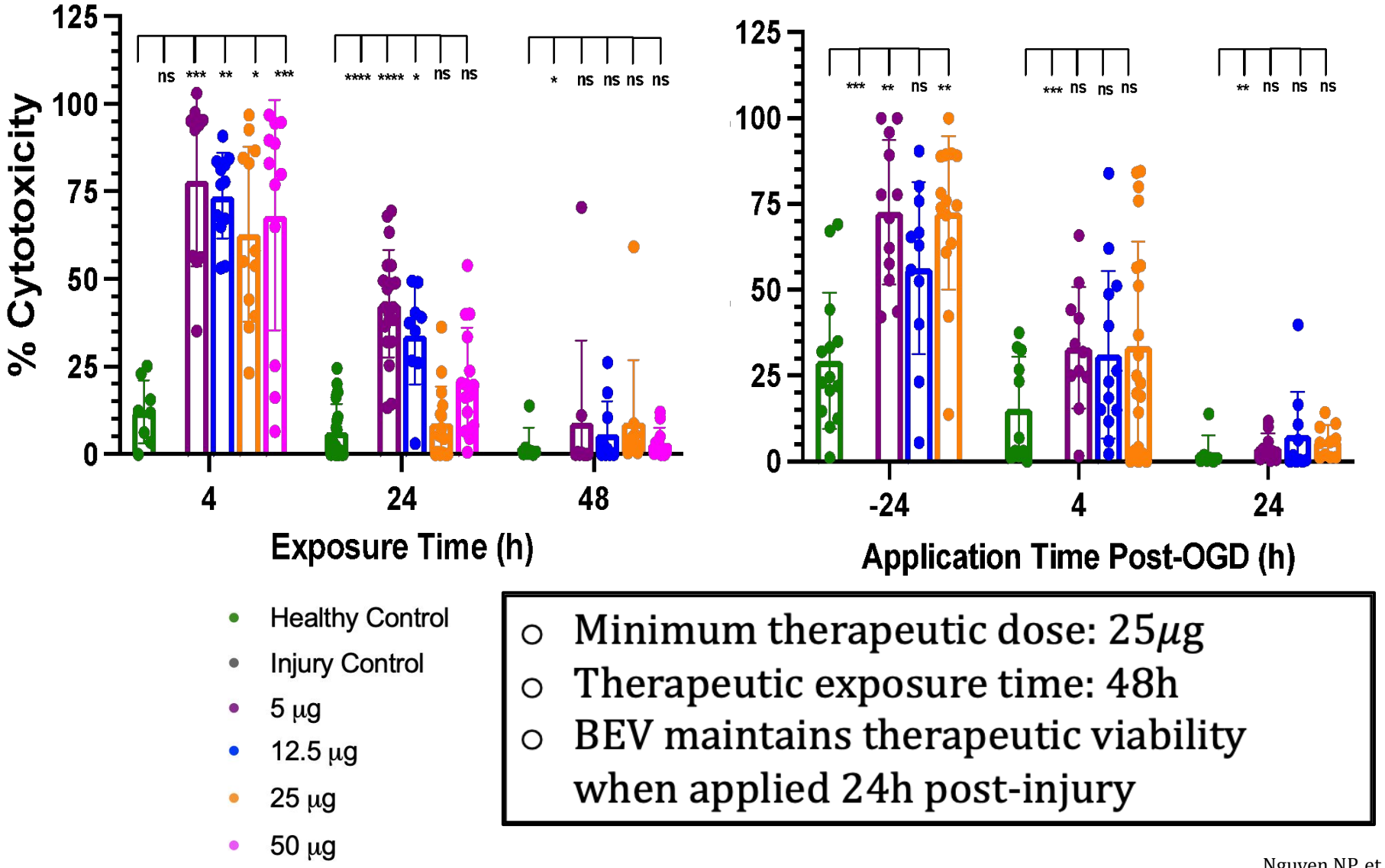


- Experiments With Changing BEV Exposure Times
- Experiments With Changing BEV Application Times



Nguyen NP. et.al., DOI: 10.3390/ijms23020620
McKenna et.al., DOI: 10.1186/s13036-022-00293-w

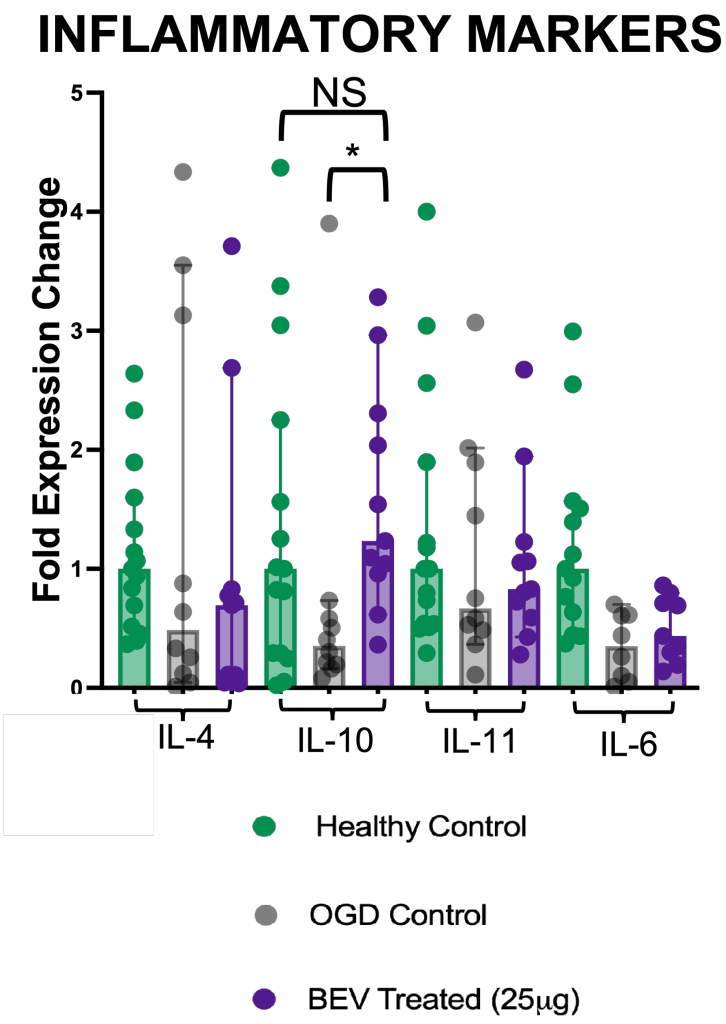
BEV Treatment Decreased Cytotoxicity in HI Slices

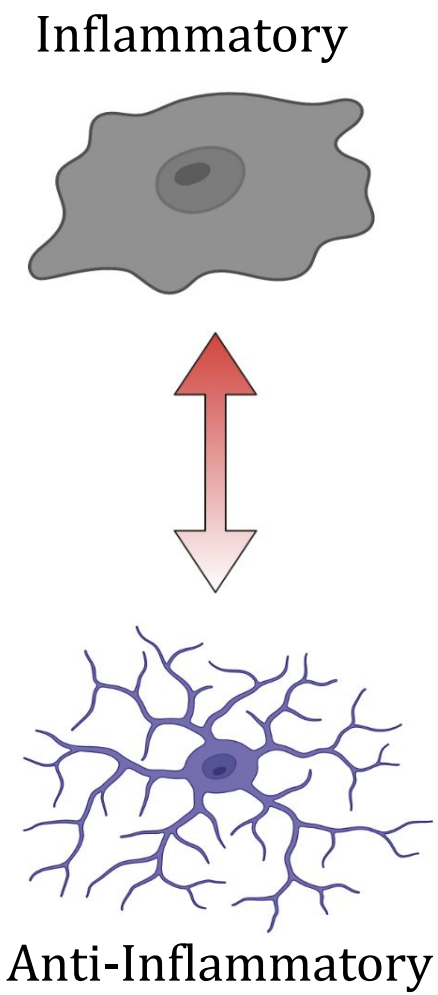


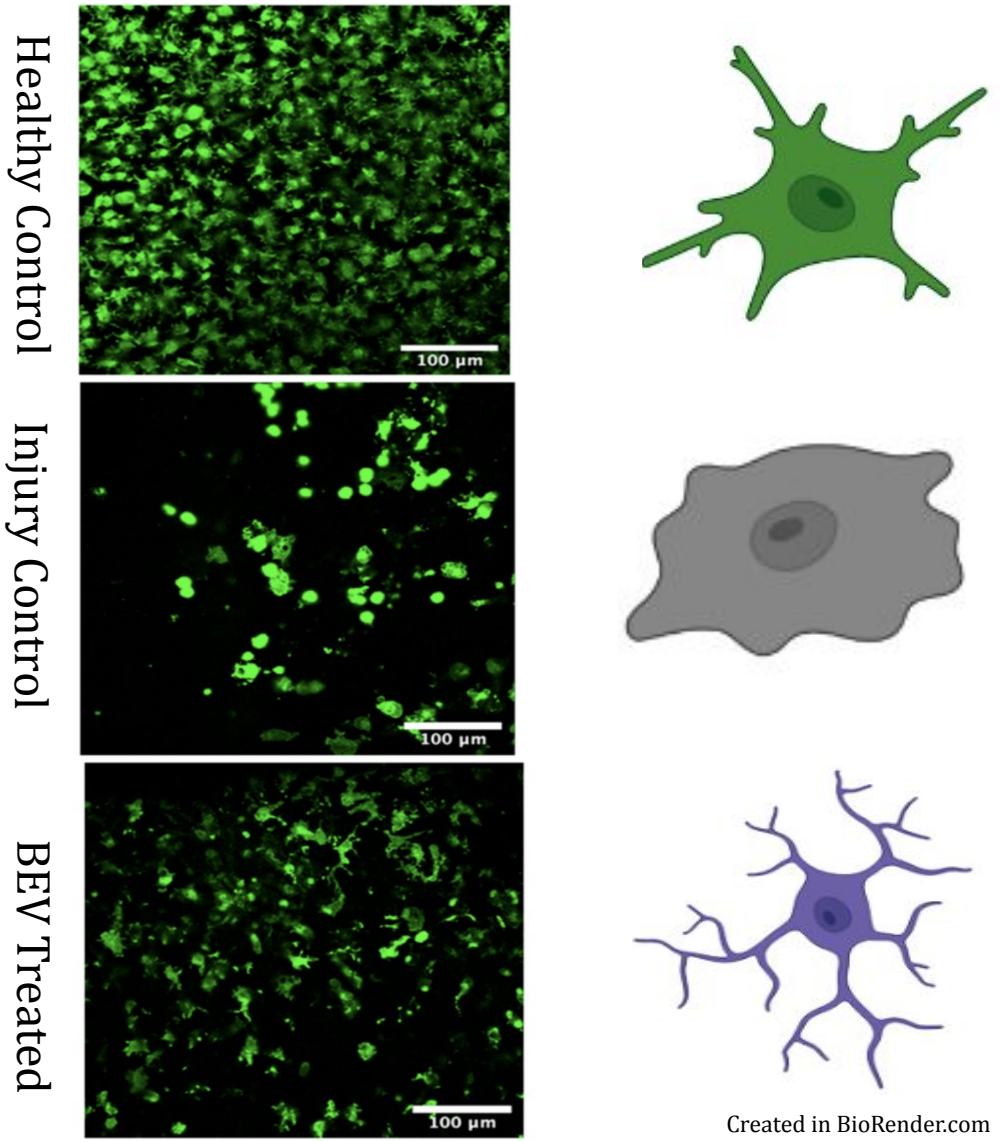
- Minimum therapeutic dose: 25μg
- Therapeutic exposure time: 48h
- BEV maintains therapeutic viability when applied 24h post-injury

Nguyen NP. et.al., DOI: 10.3390/ijms23020620

BEV Treatment Increased Anti-Inflammatory Cytokine Expression







Modified from: Nguyen NP. et.al., DOI: 10.3390/ijms23020620

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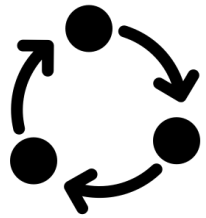
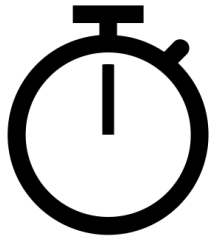
Important Conclusions



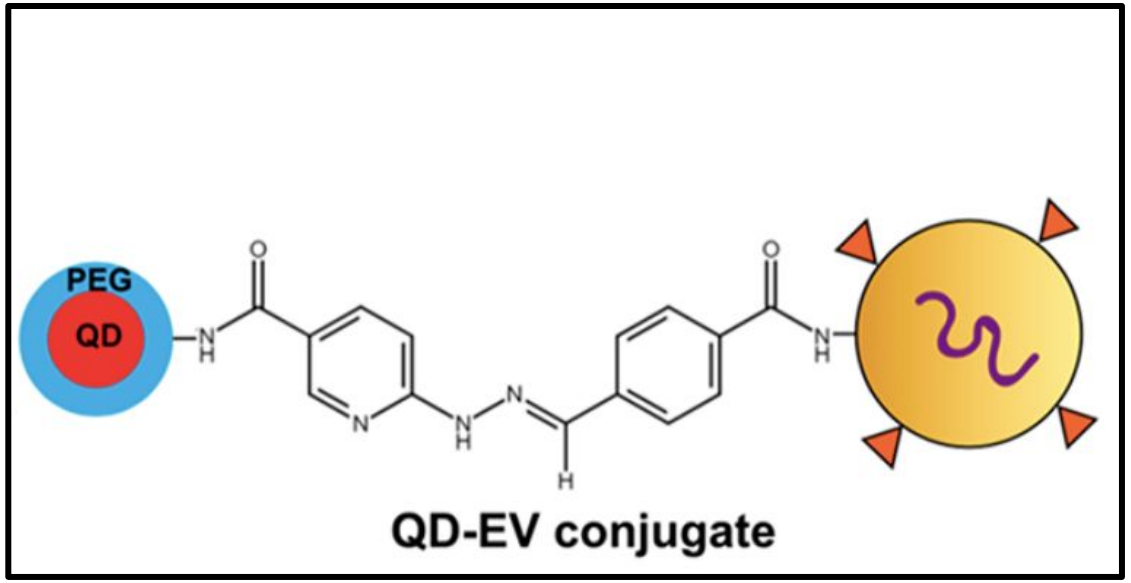
Optimized methods allow for BEV isolation & characterization



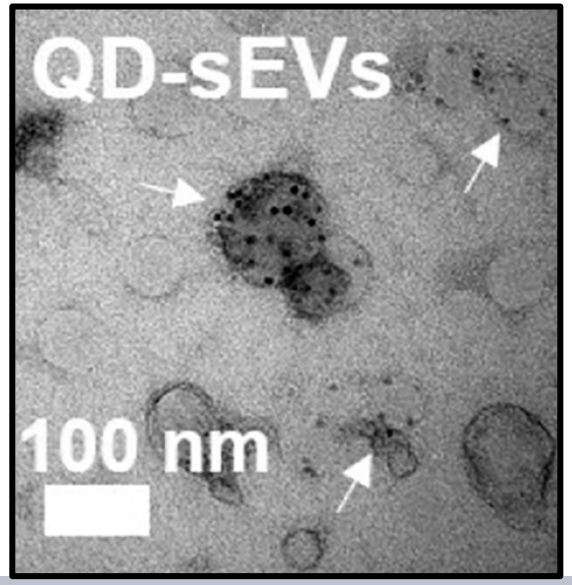
BEVs have dose- and time-dependent therapeutic effects

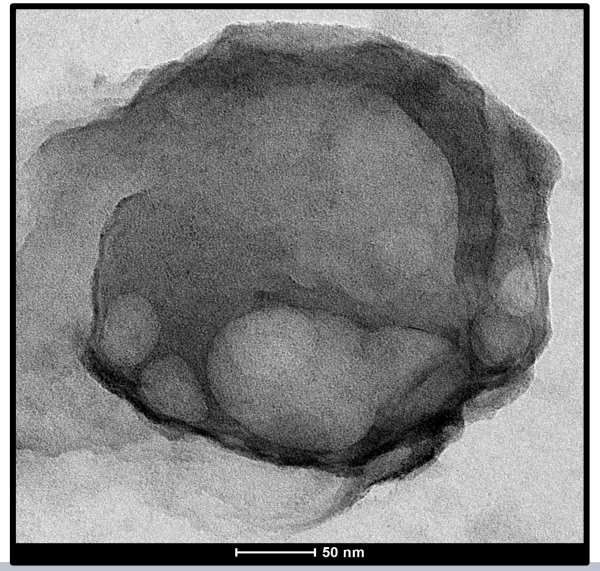
BEVs demonstrate therapeutic potential by decreasing cell cytotoxicity, increasing IL-10 expression, influencing microglia morphology

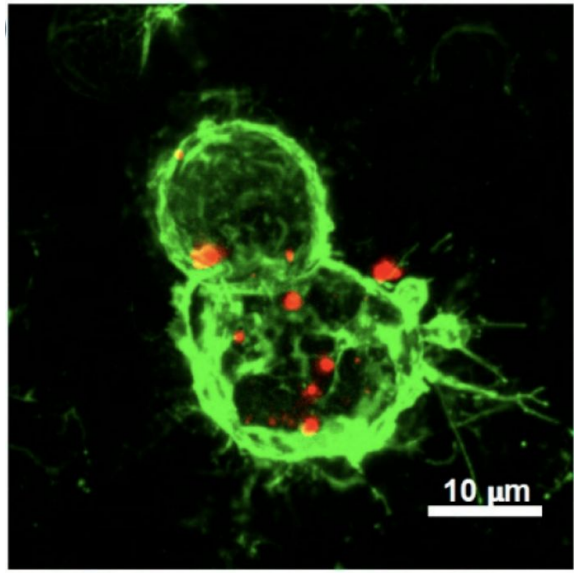
Probing BEV Fate in the Neonatal HI Brain with Quantum Dots

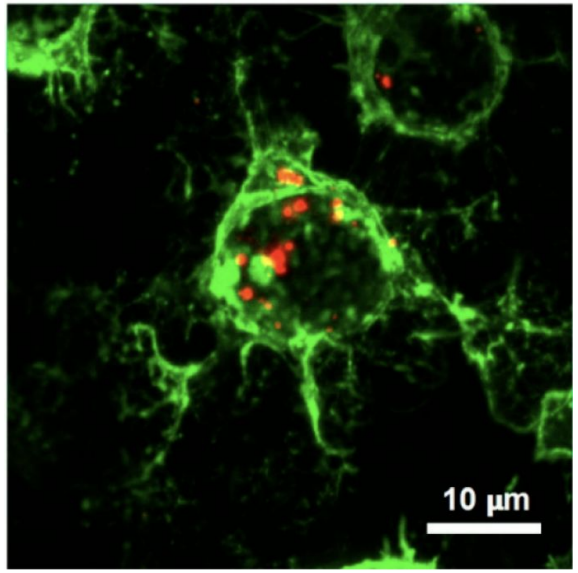


Transmission Electron Microscopy









Nguyen NP. et.al., DOI: 10.3390/ijms23020620
Zhang et.al, DOI: 10.1021/acsanm.0c01553

Our Team and Our Funding

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Dr. Cole DeForest

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CHEMICAL ENGINEERING

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[1] Douglas-Escobar M, *JAMA Pediatrics (2015)*, 10.1001/jamapediatrics.2014.3269

[2] Liao et. al., Nanobiomedicine. (2020), 10.1177/184954352097081 9

[3] Benakis et. al, *Front.. Cell. Neurosci.* (2015): 10.3389/fncel.2014.00461

[4] Holm et. al., *Trends in Neuroscience* (2018) DOI: 10.1016/j.tins.2018.03.006

[5] Zheng M. et.al., Bioconjugate Chemistry (2019) doi: 10.1021/acs.bioconjchem.9b00085.

[6] Turturici, G., et.al., American Journal of Physiology-Cell Physiology (2014),10.1152/ajpcell.00228.2013.

[7] **Nguyen NP. et.al., *IJMS (2022),* 10.3390/ijms23020620**

[8] Vella et.al, *Journal of Extracellular Vesicles* (2014), 10.1080/20013078.2017.1348885

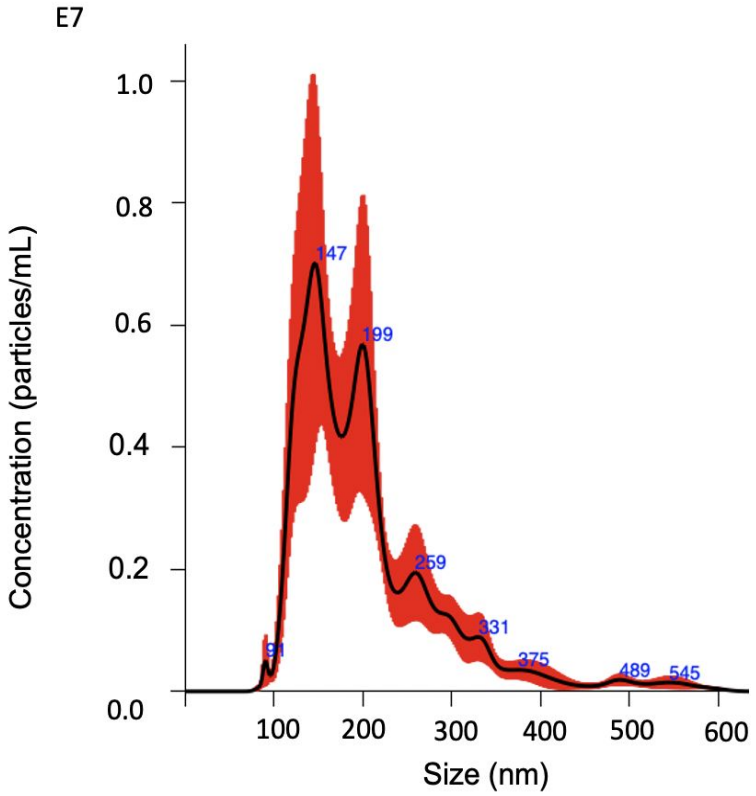
[9] Joseph, et.al., *BTM* (2020), 10.1002/btm2.10175

[10] McKenna et.al., *JBE* (2022), 10.1186/s13036-022-00293-w

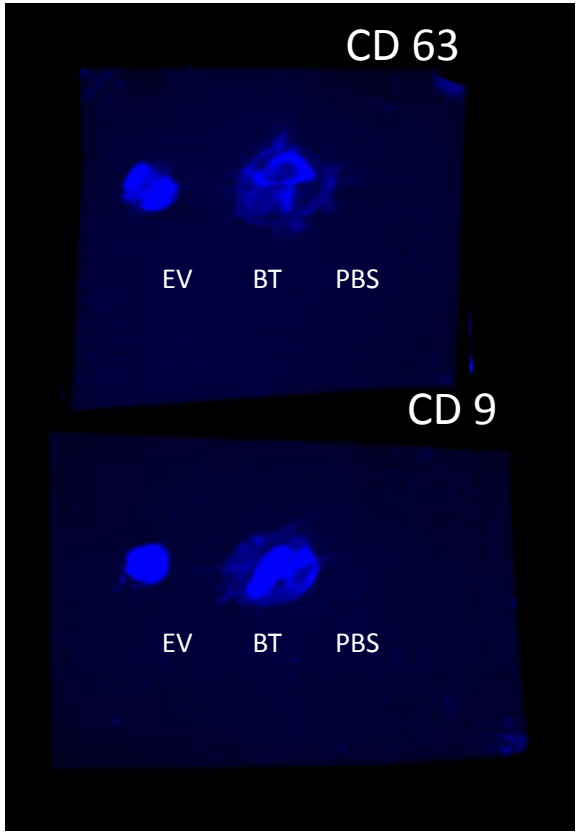
[11] Zhang et.al, *BTM* (2020): 10.1021/acsanm.0c01553

BEV Characterization

Nanoparticle Tracking Analysis



Immunoblot for EV Markers



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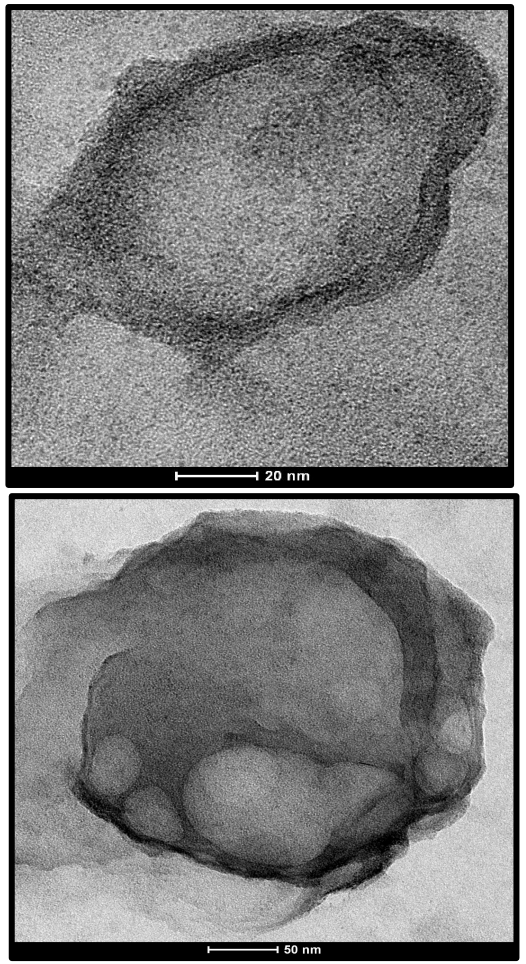
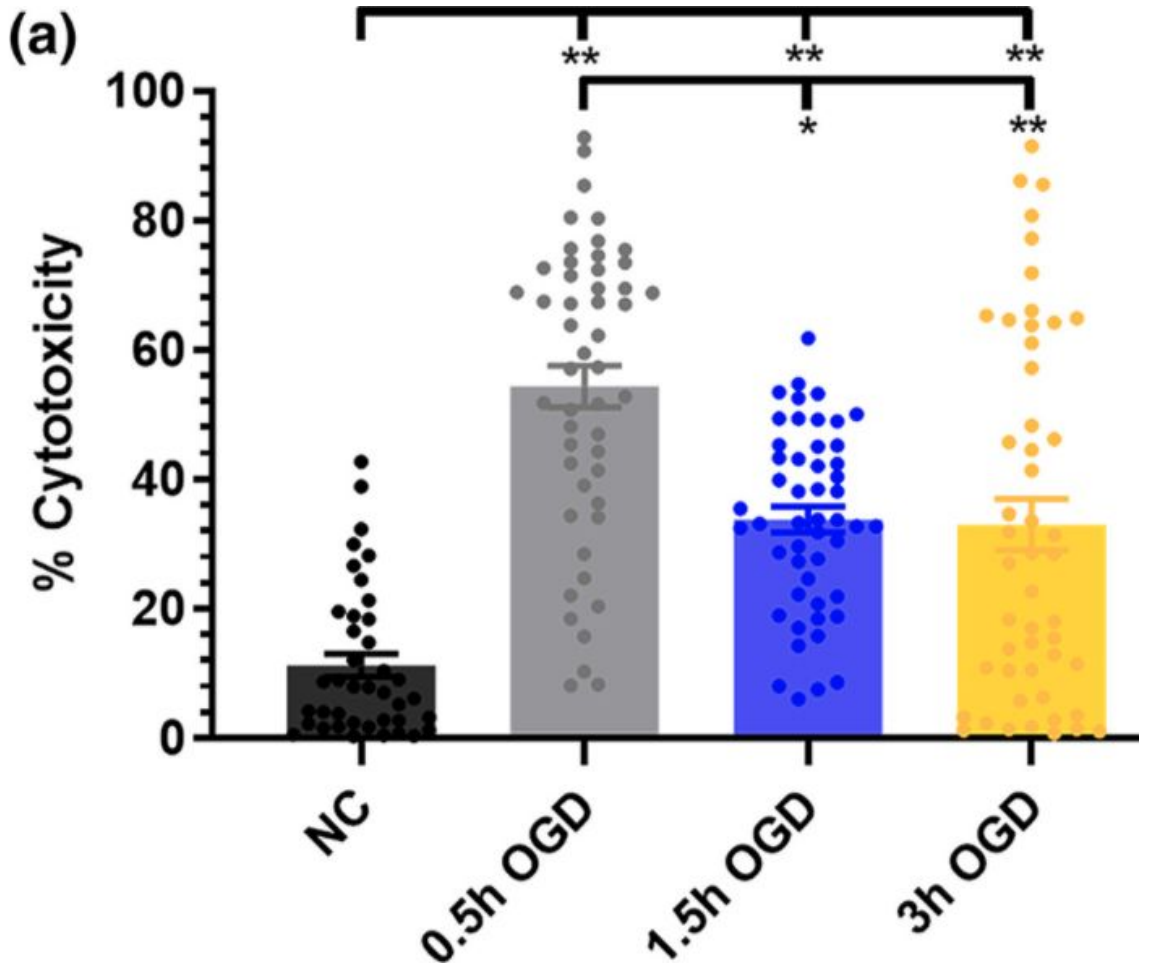


Nguyen NP. et.al., DOI: 10.3390/ijms23020620

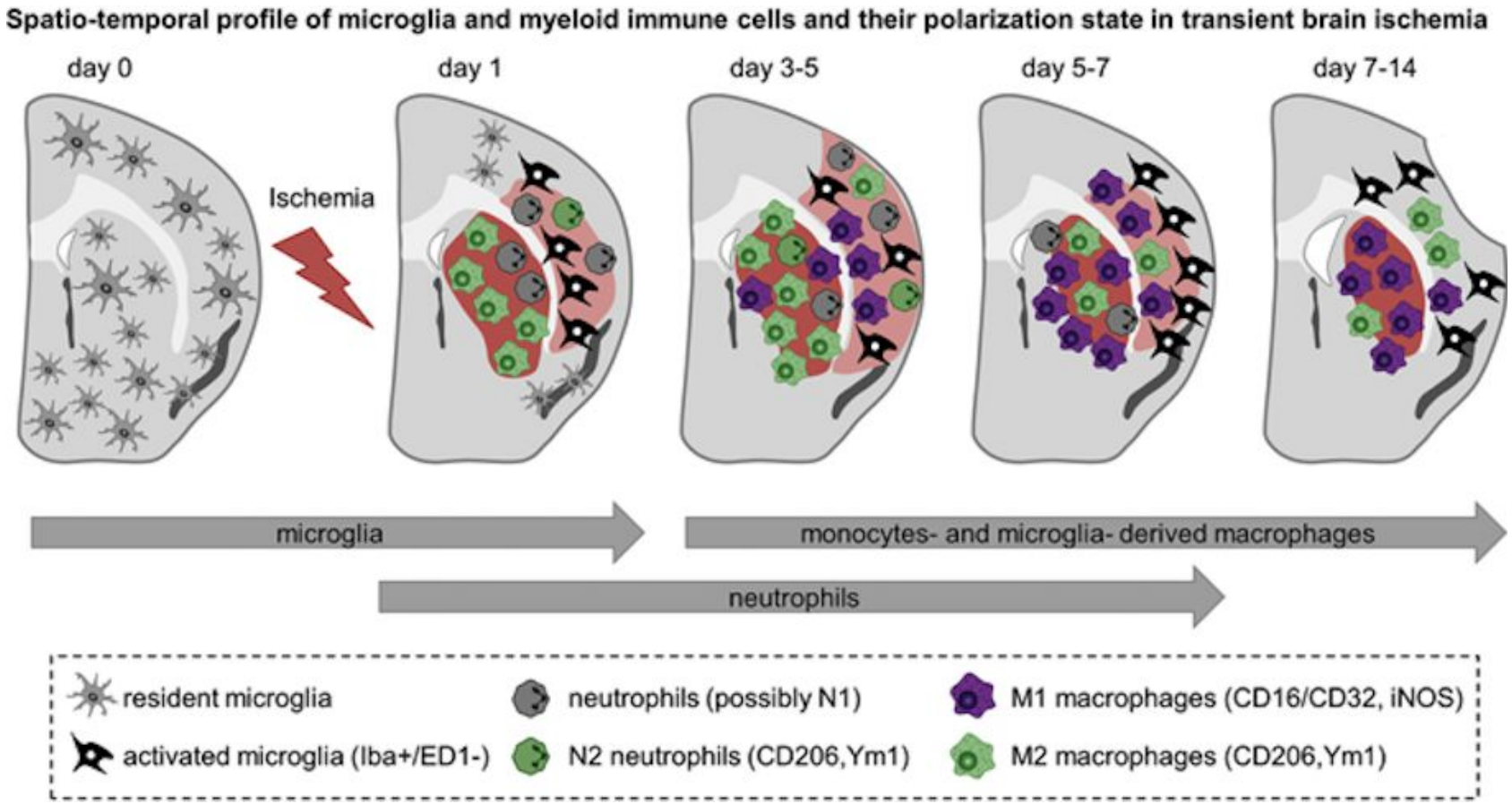
0.5 hr OGD Treatment Resulted in Highest % Cell Death



N=40-50 images per condition

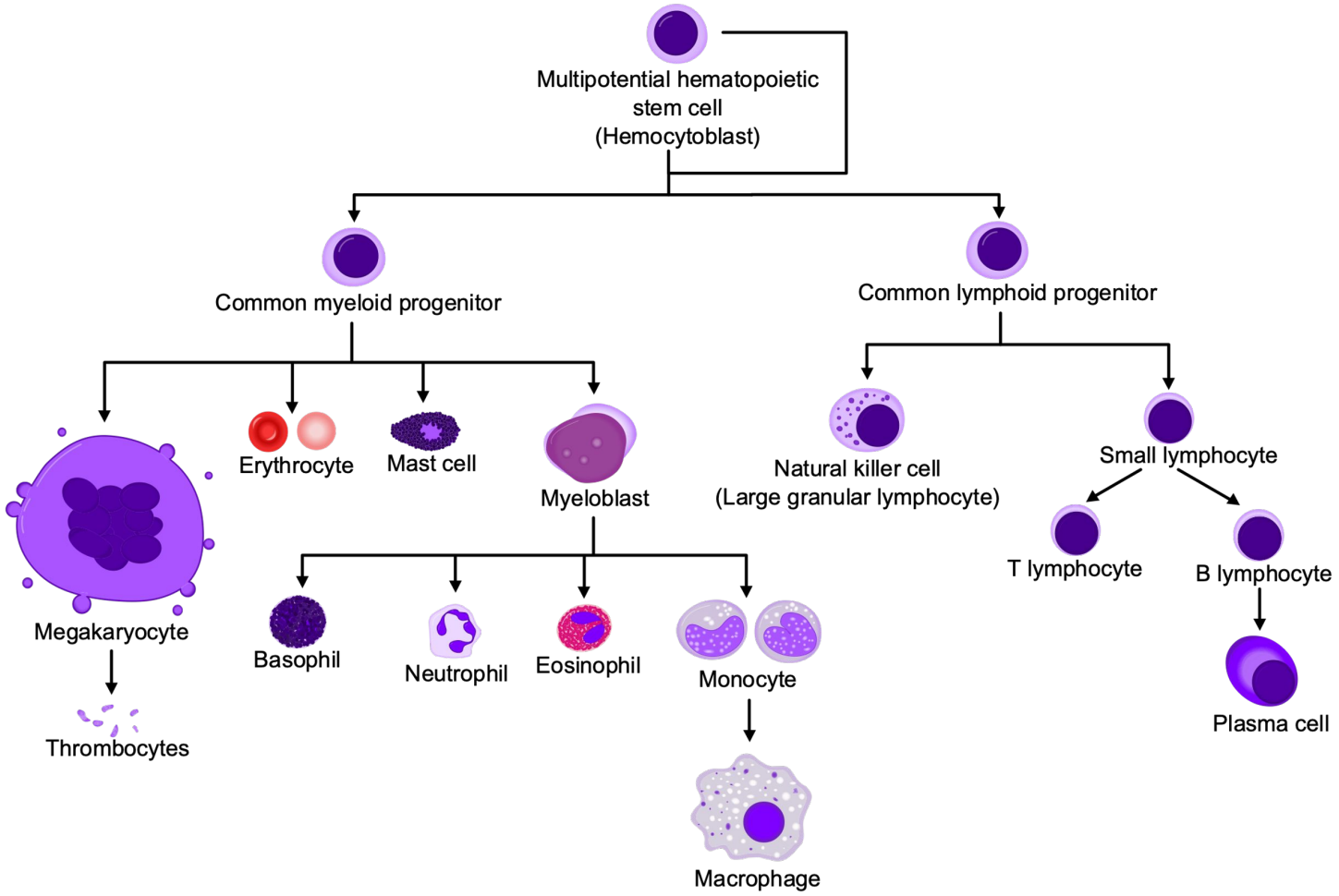
Joseph et.al, *BTM* (2020): 10.1002/btm2.10175

Microglial response post-injury



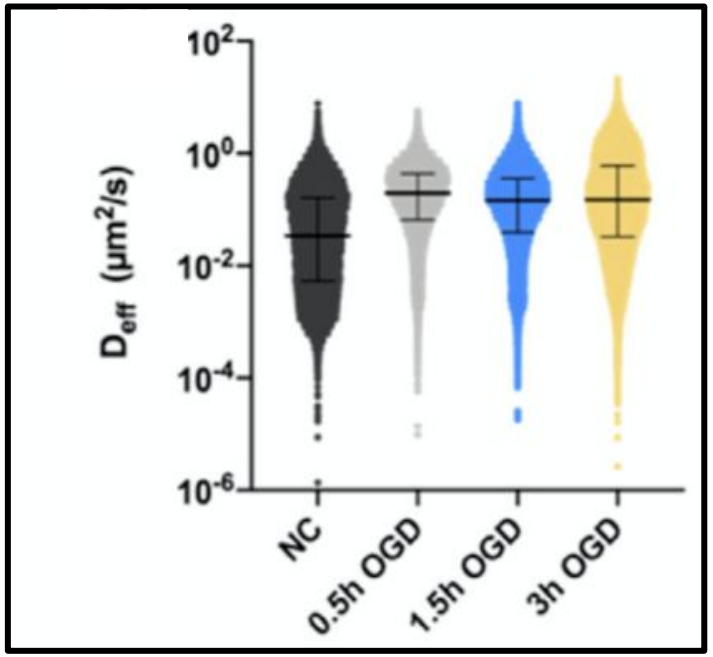
Benakis et. al, *Front.. Cell. Neurosci.* (2015): 10.3389/fncel.2014.00461

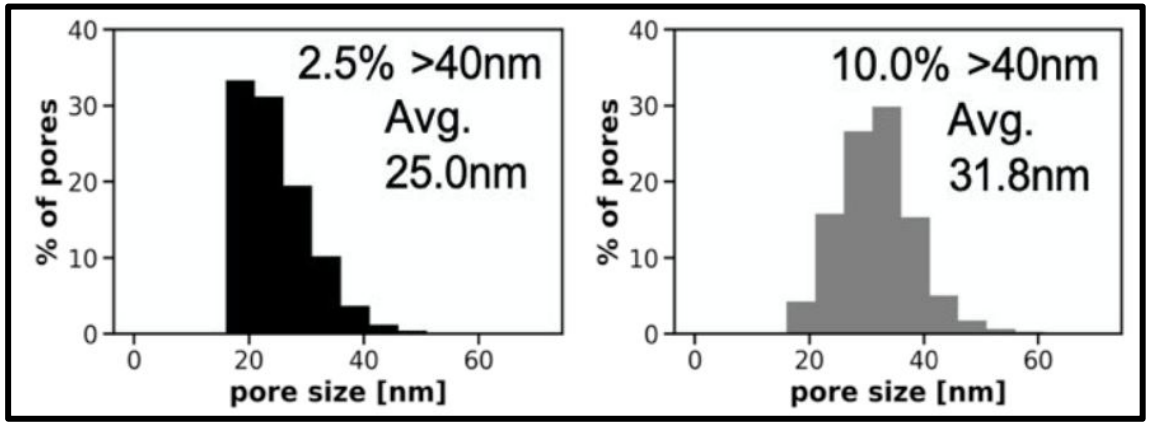
Immunology: hematopoietic stem cell



EV Transport through the brain ECM

- OGD treatment increases diffusivity of nanoparticles through ECM
- EV transport mainly governed by passive diffusion
- Cortical images taken within first 3-5 cell layers

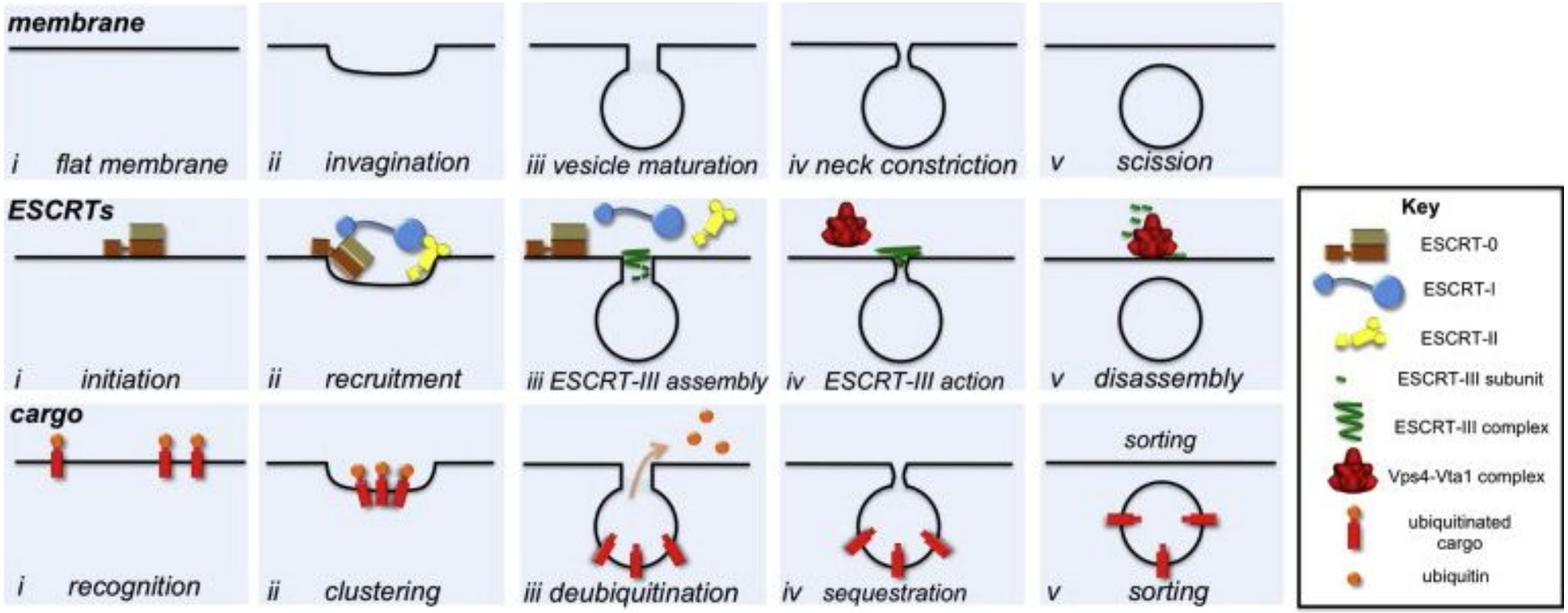




Joseph et.al, *BTM* (2020): 10.1002/btm2.10175
Lenzini et al. *Nat.Nanotech* (2020), 10.1038/s41565-020-0636-2

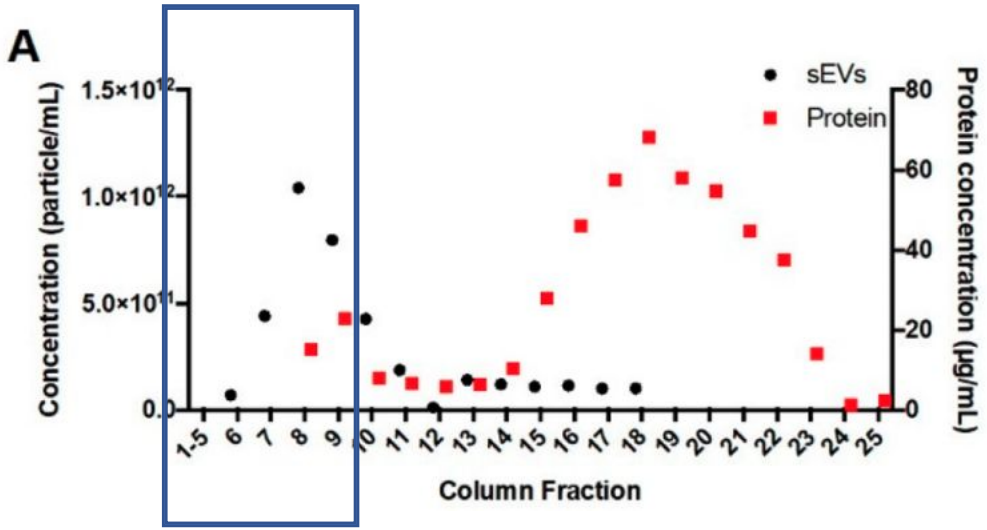
ESCRT (Endosomal Complex Required for Transport)

- ESCRT 0,I,II: recognize and sequester membrane proteins
  - ESCRT III: responsible for membrane budding



Henne et al. *Dev. Cell*(2011), 10.1016/j.devcel.2011.05.015

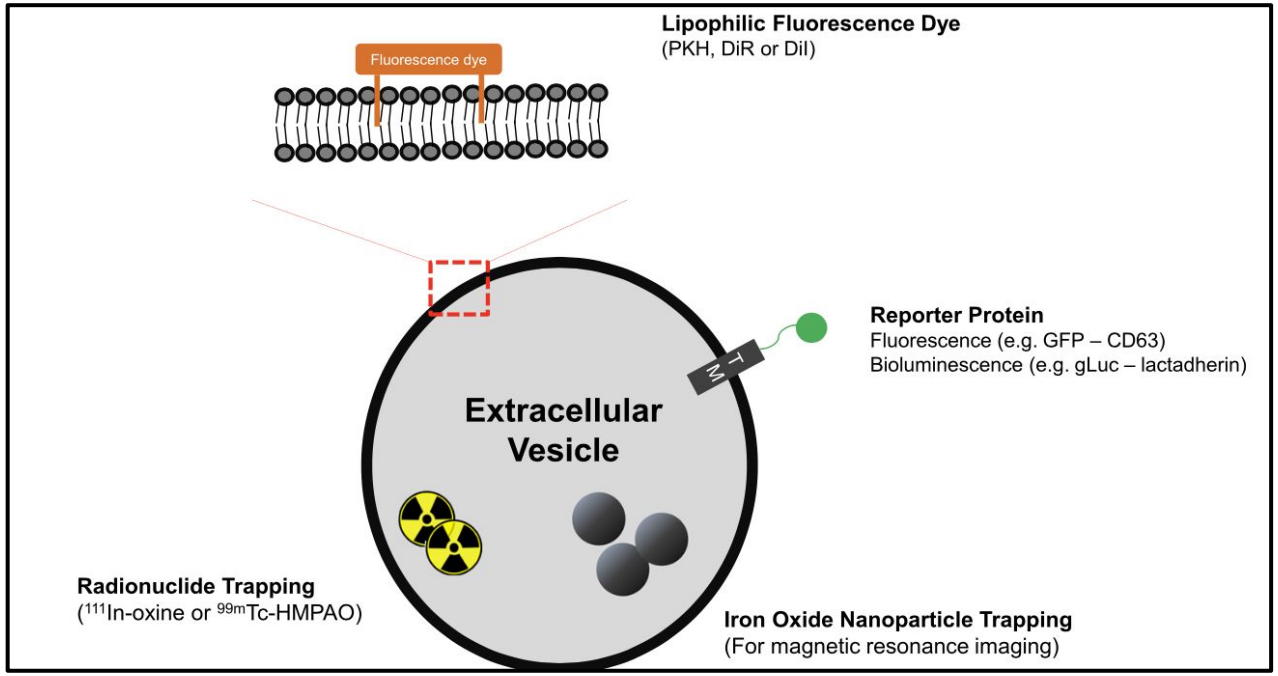
SEC fractions 7-10 yield highest sEV concentration



Zhang, Mengying, et al. *ACS* (2020), 10.1039/C9NA00334G.

Current EV labeling methods

- Reporter proteins (fluorescent markers)
  - Pros: sensitive, high signal to noise
  - Cons: Requires genetically engineered cells
- Fluorescent dyes
  - Pros: easy, accessible, well characterized
  - Cons: Quenching, photobleaching
- Quantum dots
  - Pros: tailorable, high quantum yield
  - Cons: expensive, toxic
- Radionuclide or iron oxide trapping (MRI)
  - Pros: high penetration
  - Cons: toxic, low signal to noise, cost



Choi, et al. *Stem Cell Research & Therapy* 7.1 (2016). 10.1186/s13287-016-0316-1.