Characterization of exosomes based on their unique dielectric properties by a novel electrical impedance measurement system

Leyla Esfandiari, Ph.D.
Assistant professor of Biomedical Engineering
Assistant professor of Electrical Engineering and Computer Science
College of Engineering & Applied Science
University of Cincinnati, USA

Characterization of Exosomes

Conventional characterization methods:

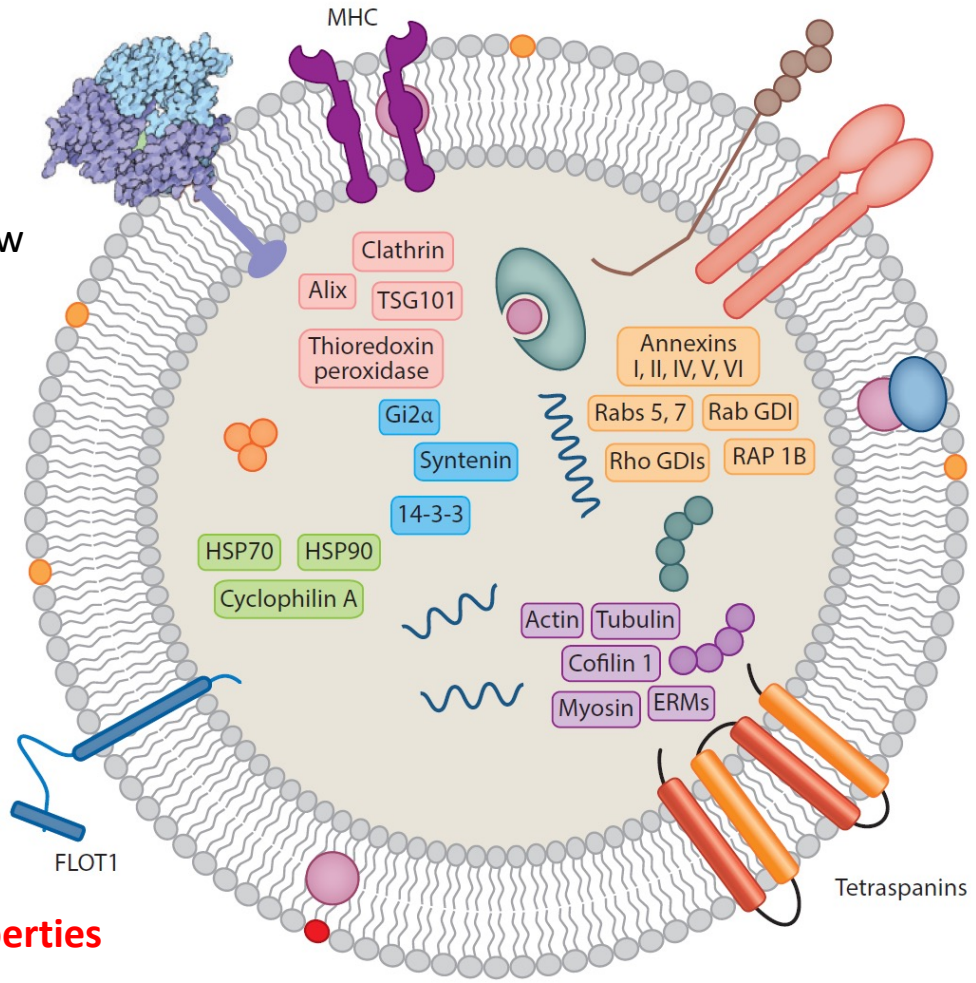
- Proteomics: enzyme-linked immunosorbent assay (ELISA), western blot, flow cytometry, and chromatography
- Genomics: qRT-PCR, microarrays, and next-generation sequencing (NGS)

Shortcomings: *break the structure of the exosomes* in labelling and lysing steps

Characterization of biophysical properties of exosomes:

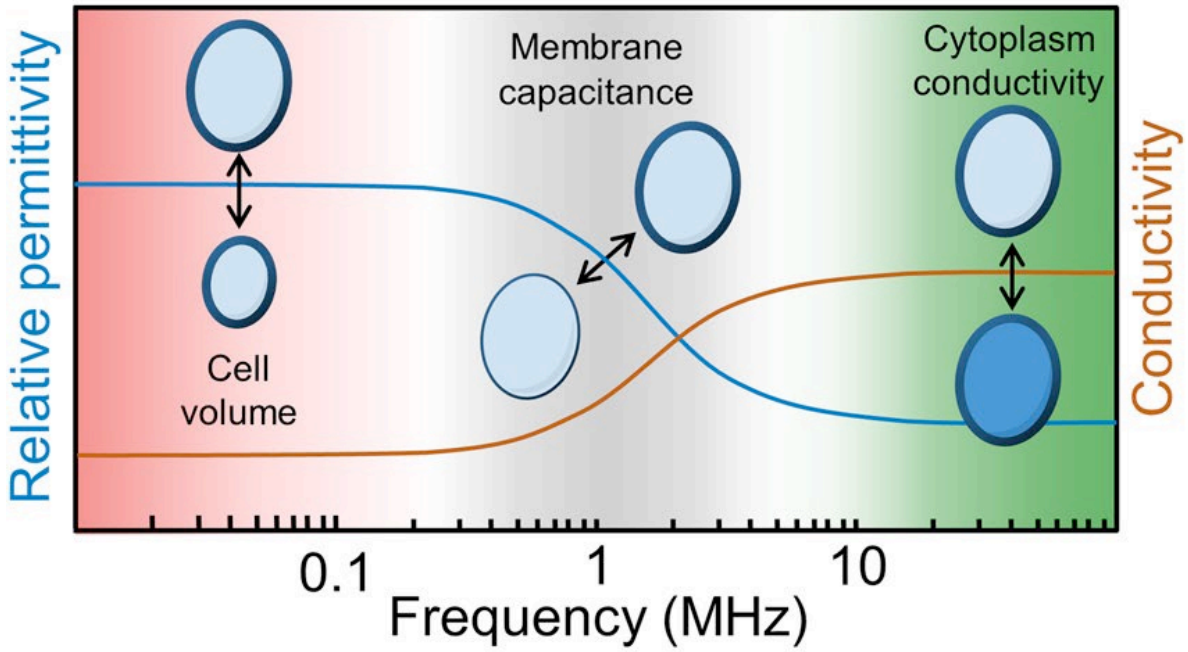
- Size
- Density
- Shape

Non-invasive characterization of exosomes based on their unique dielectric properties

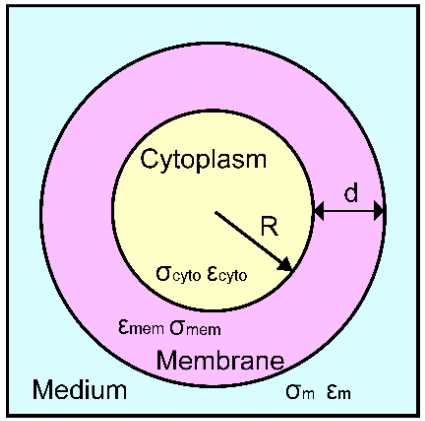


Kourembanas, Stella. "Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy." Annual review of physiology 77 (2015): 13-27.

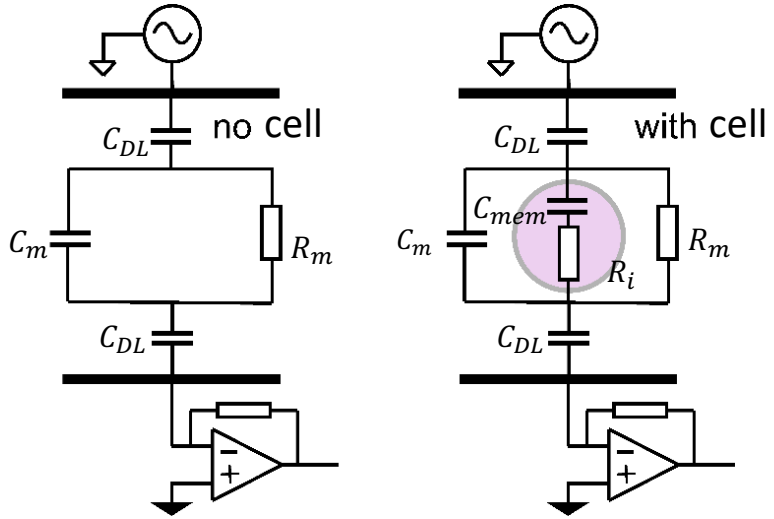
Impedance cytometry



Single-shell model



Foster and Schwan's circuit model



Maxwell's Mixing Theory is applied to analyze the dielectric properties of cells in suspension under an AC field over a wide range of frequencies.

$$Z_{mix} = \frac{1}{j\omega\tilde{\varepsilon}_{mix}G}$$

Could we characterize exosomes based on their unique dielectric properties and classify them based on their biogenesis?

McGrath, J. S., et al. "Analysis of parasitic Protozoa at the single-cell level using microfluidic impedance cytometry." *Scientific reports* 7.1 (2017): 2601.

Sun, Tao, and Hywel Morgan. "Single-cell microfluidic impedance cytometry: a review." *Microfluidics and Nanofluidics* 8.4 (2010): 423-443.

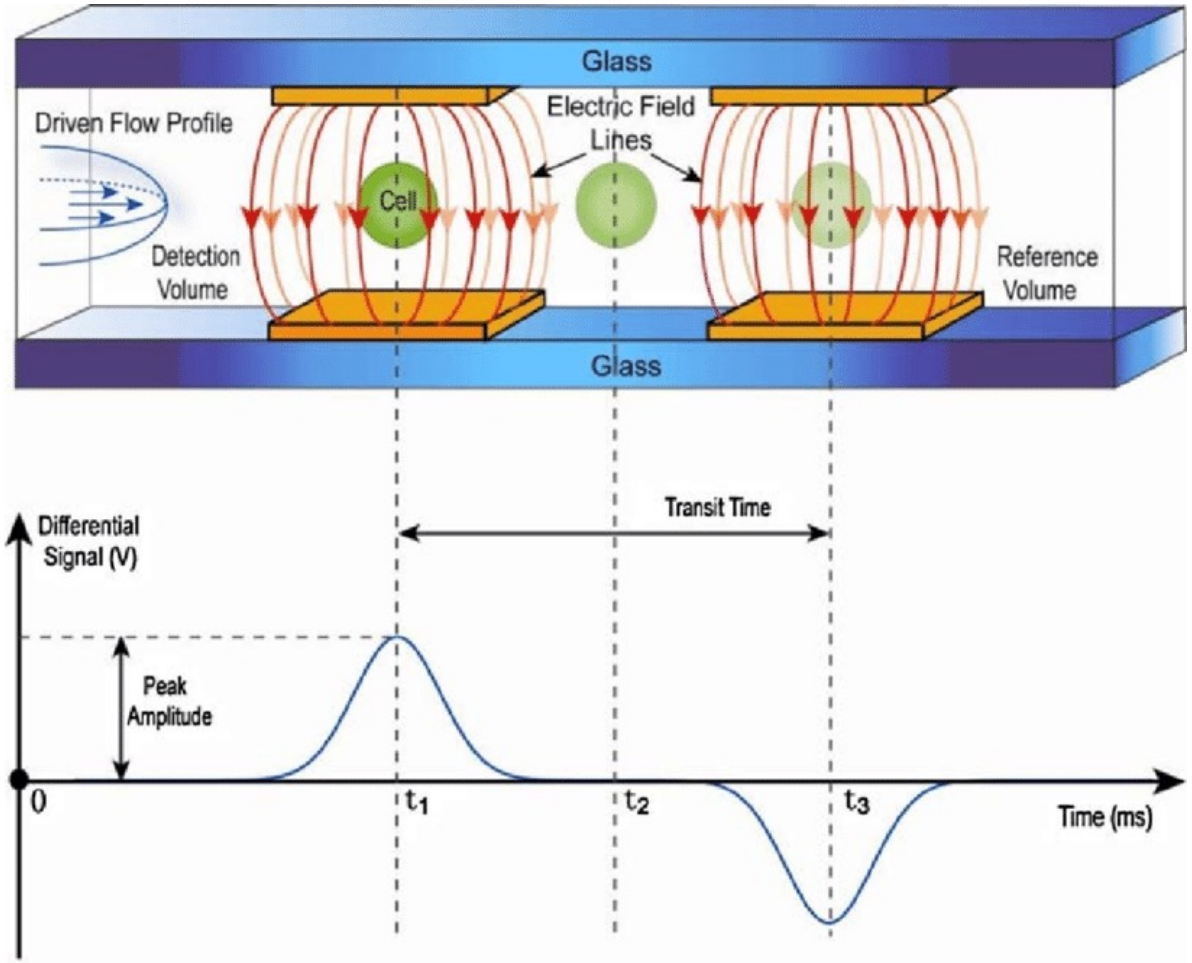
University of CINCINNATI

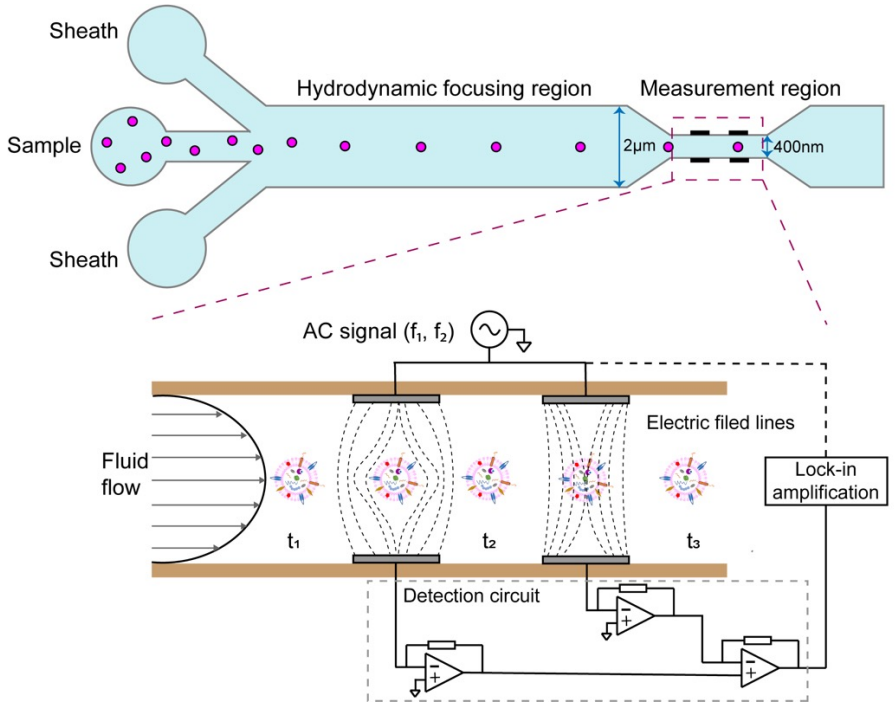
Characterization of cells' dielectric properties at different frequencies

Cell Type	Dielectric properties	Frequency Range	Ref
Human leukocytes	Membrane capacitance	1.7MHz for membrane	Holmes et al.
Stem cells	Membrane capacitance	1MHz for membrane	Song et al.
Parasite infected RBCs	Membrane permittivity	8.7MHz for membrane	Küttel et al.
Tumor cells (MCF7)	Membrane capacitance	2MHz for membrane	Spencer et al.
Erythrocytes	Membrane capacitance and plasm conductivity	Multifrequency measurement from 500kHz to 20MHz; >10MHz for cytoplasm conductivity	Cheung et al.
Schwann cell	Membrane potential and intracellular free calcium	4MHz for membrane 6MHz for intracellular free calcium	Pierzchalski et al.
Yeast cell	Intercellular fluid	>50MHz for cytoplasm	Pierzchalski et al.
Leukocytes	Cell membrane	2MHz for cell membrane	Haandbæk et al.
Parasitic Protozoa	Interior fluid conductivity	25MHz	Spencer et al.

Single Cell Impedance cytometry

Impedance micro-cytometer used for single cell analysis



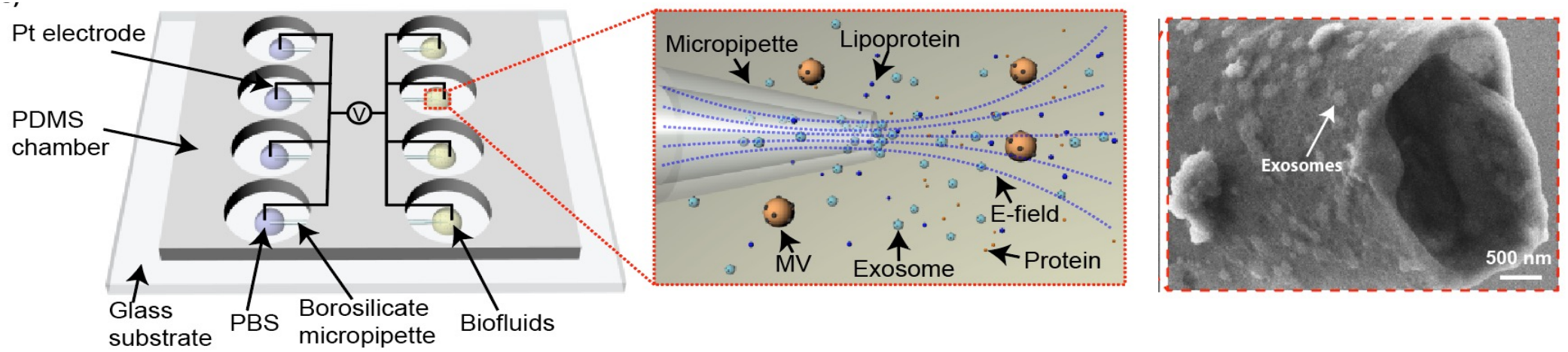


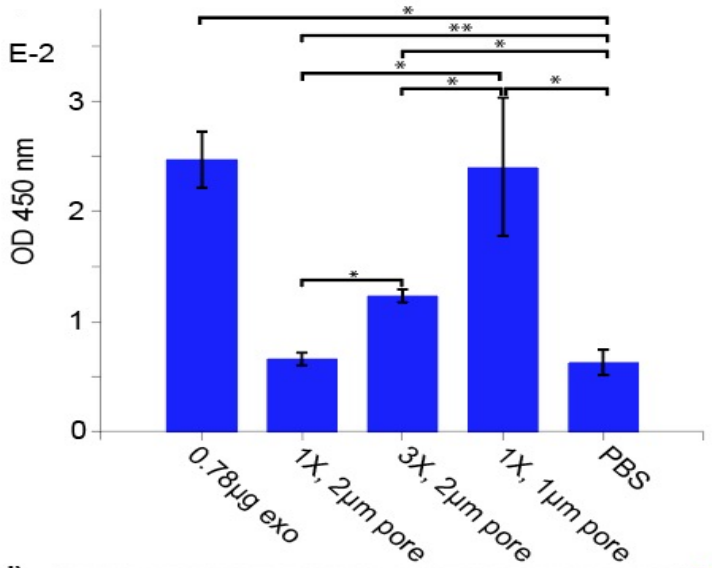
Transfer the cell impedance cytometry for exosomes:

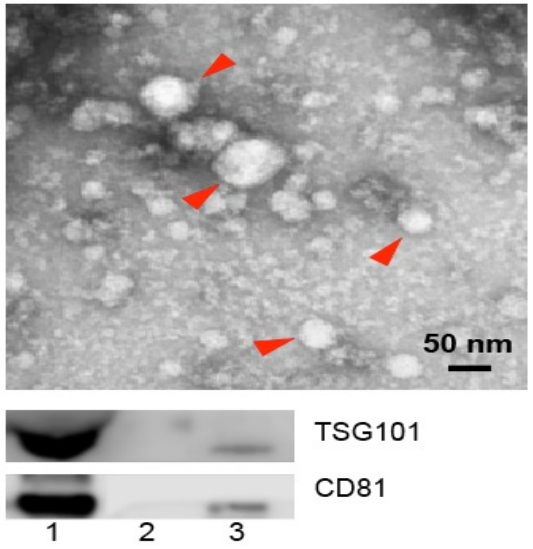
- Challenges in nano-sized channel fabrication
- Needs high pressure pump
- Low signal-to-noise ratio
- Requires high sampling rate and thus, high resolution data acquisition

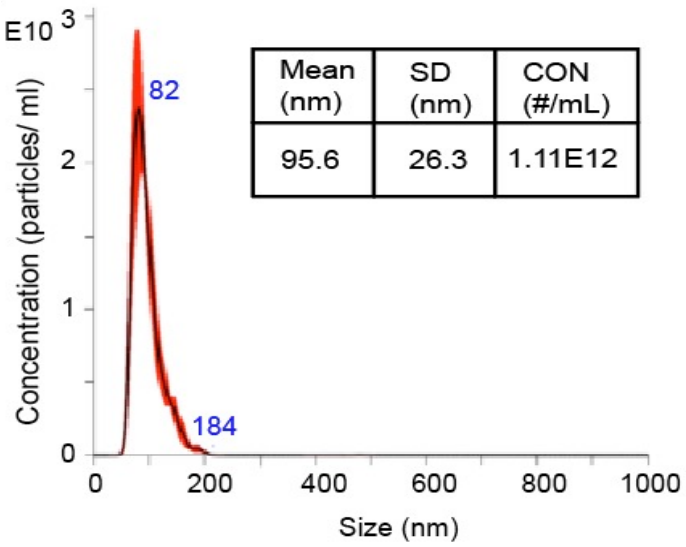
McGrath, J. S., et al. "Analysis of parasitic Protozoa at the single-cell level using microfluidic impedance cytometry." *Scientific reports* 7.1 (2017): 2601.

New nanopipette Dielectrophoretic Isolation Device







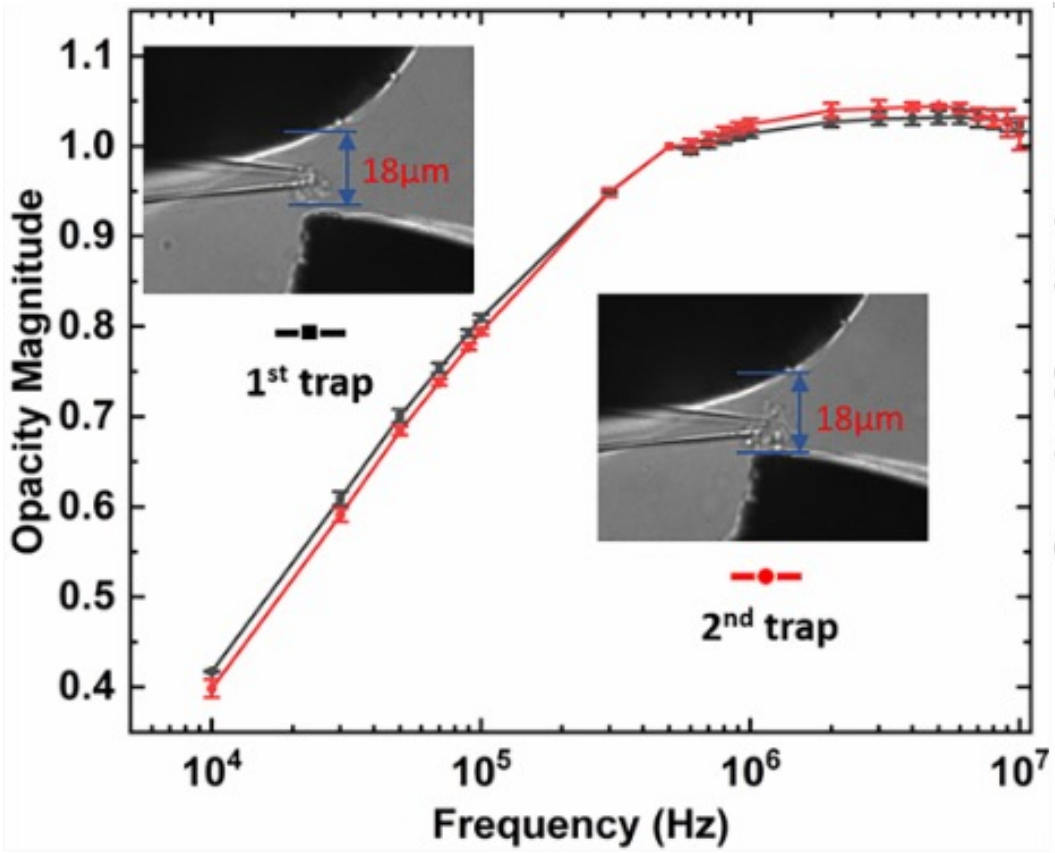


Mean (nm)	SD (nm)	CON (#/mL)
95.6	26.3	1.11E12

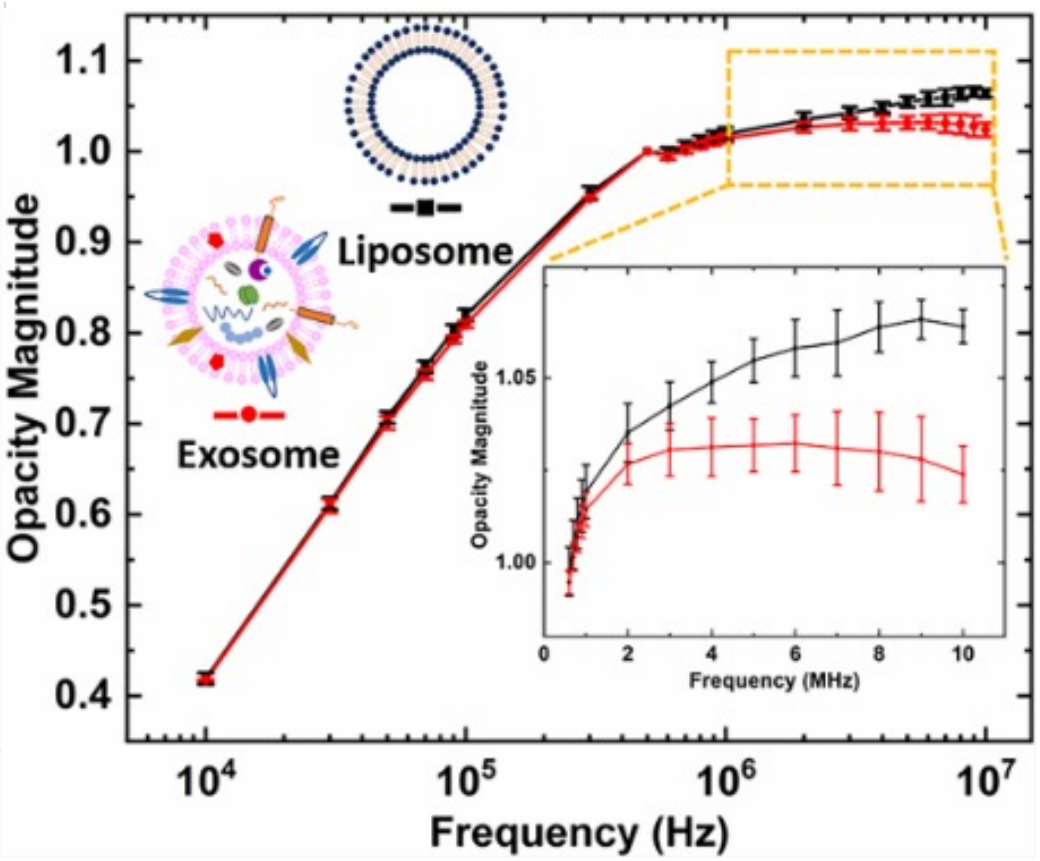
Shi, Leilei, Ankit Rana, and Leyla Esfandiari. "A low voltage nanopipette dielectrophoretic device for rapid entrapment of nanoparticles and exosomes extracted from plasma of healthy donors." Scientific reports 8 (2018).

Integrated Impedance Spectroscopy for Exosomes characterization

Ruling out the effect of number of particles



Difference of 100 nm liposomes and exosomes

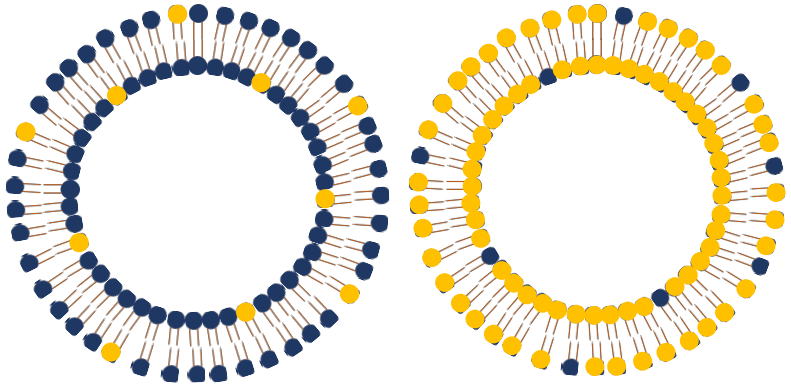


The effect of membrane capacitance

Cholesterol (CH)

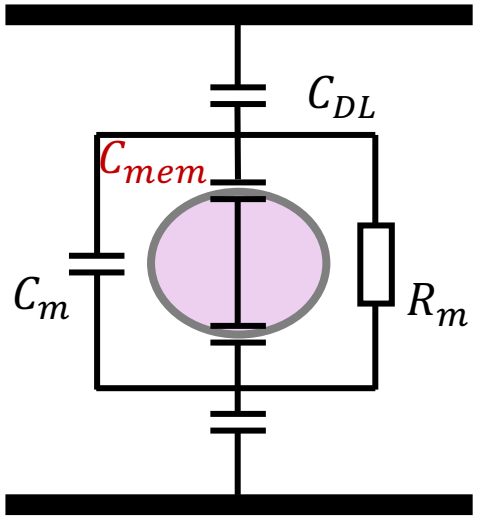
Lecithin (PC)

100 nm liposomes



1:10 cholesterol/lecithin

10:1 cholesterol/lecithin



C_{DL}: double layer capacitance

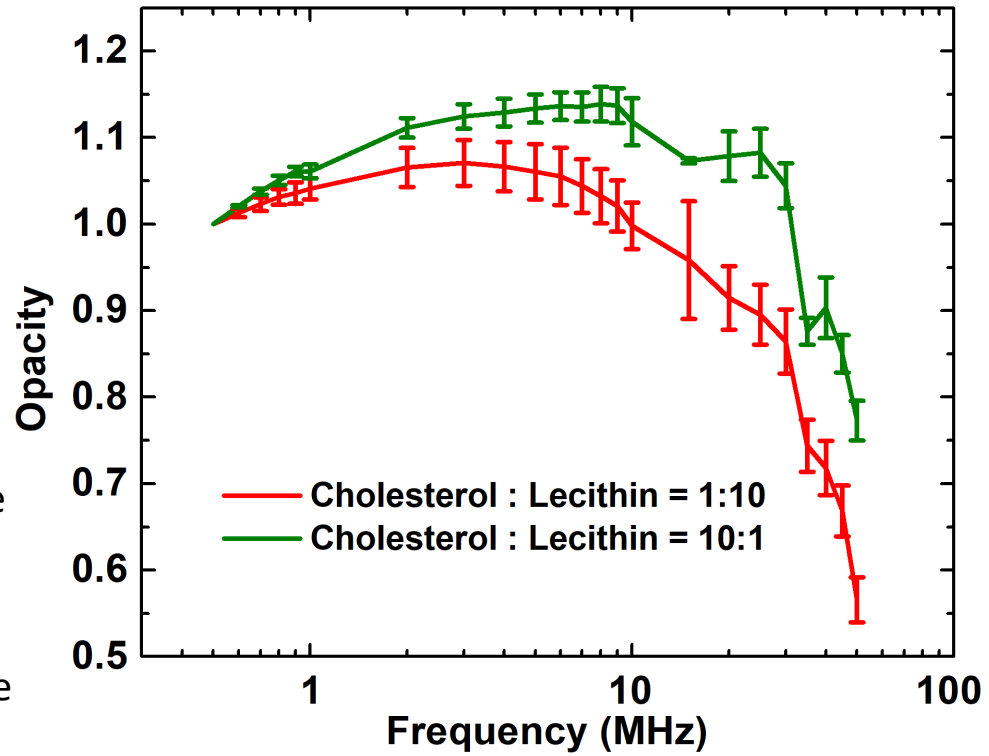
C_m: medium capacitance

R_m: medium resistance

C_{mem}: membrane capacitance

$$C_{mem/1:10} < C_{mem/10:1}$$

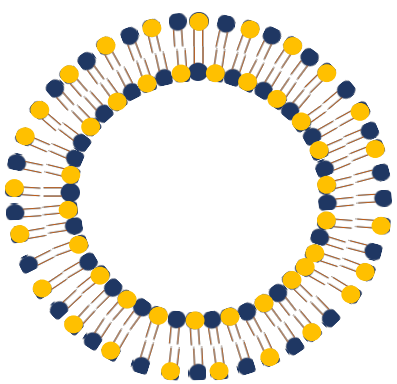
$$Opacity_{1:10} < Opacity_{10:1}$$



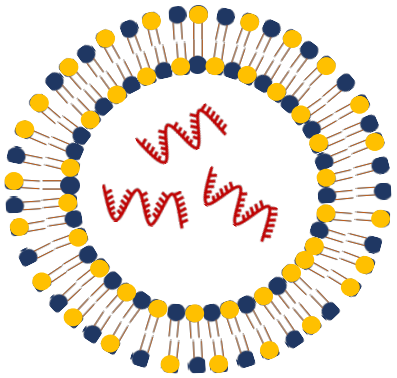
The effect of cytosolic conductance

transfer RNA (tRNA)

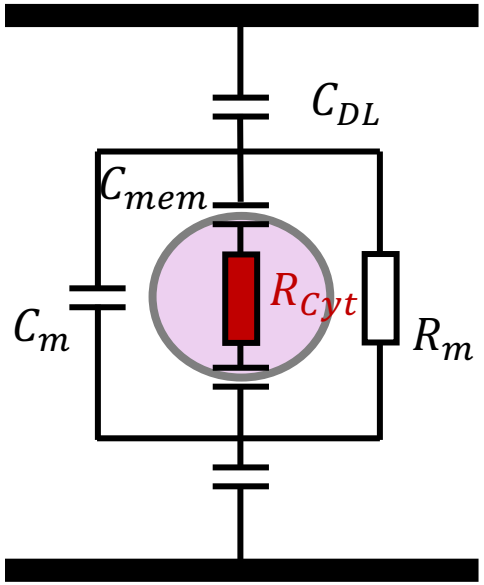
100 nm liposomes



DSPC/Chol/PEG/DODPA



DSPC/Chol/PEG/DODPA + tRNA



C_{DL}: double layer capacitance

C_m: medium capacitance

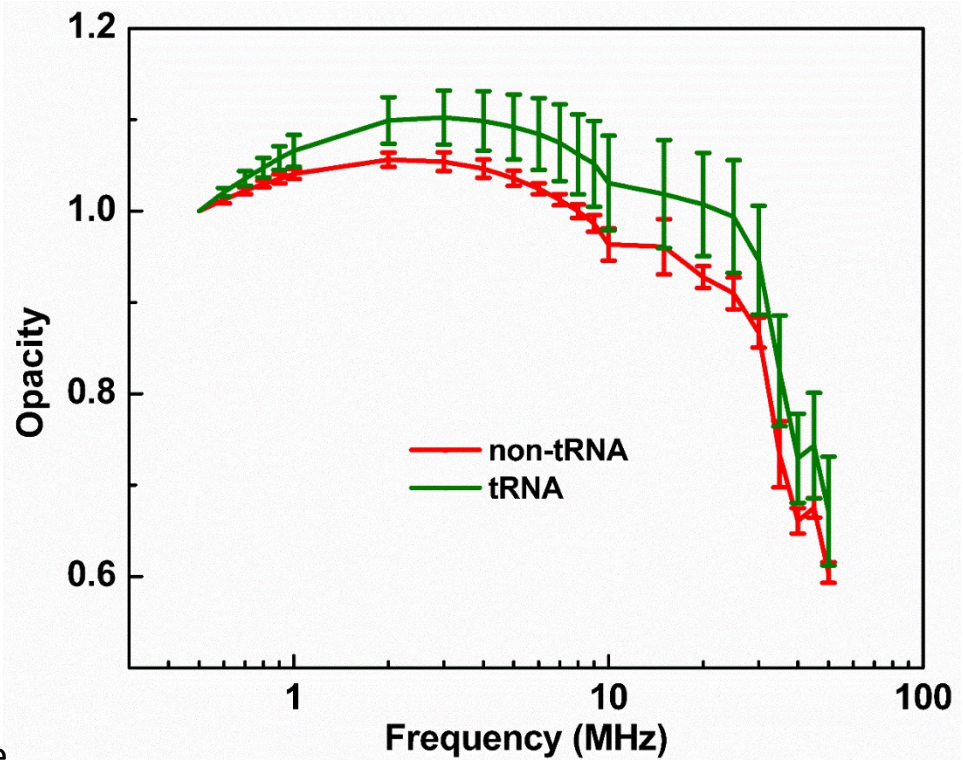
R_m: medium resistance

C_{mem}: membrane capacitance

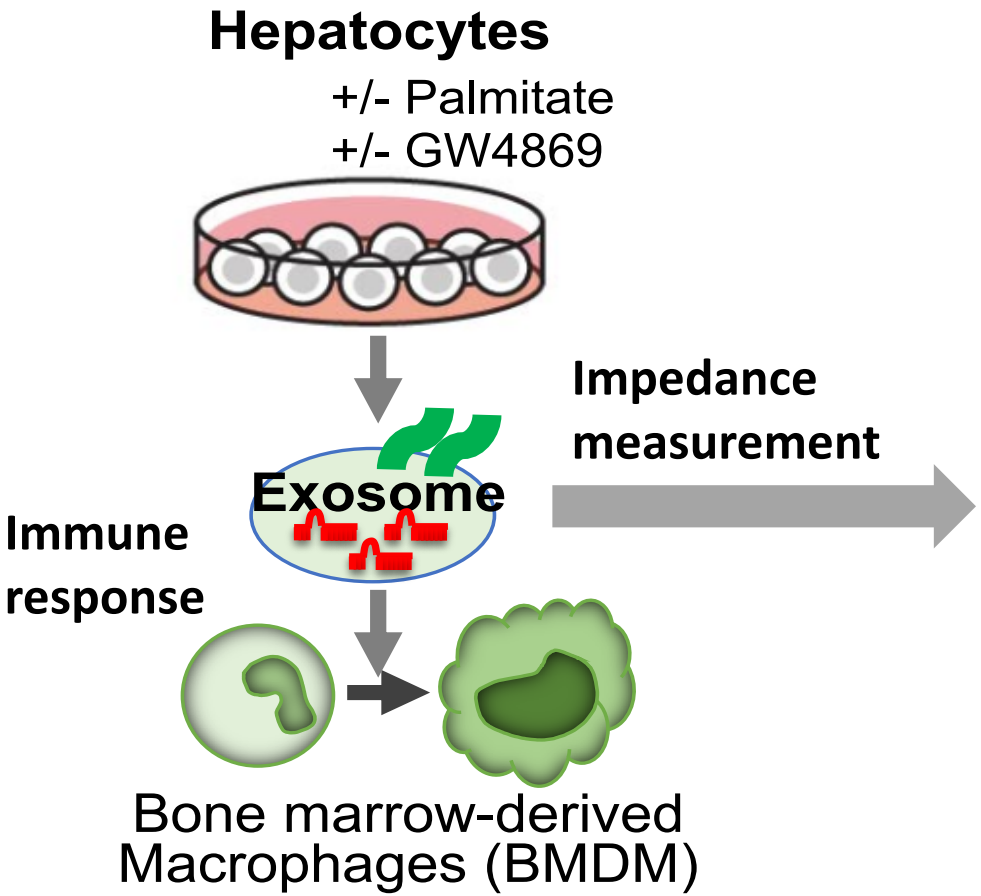
R_{Cyt}: Cytosolic Resistance

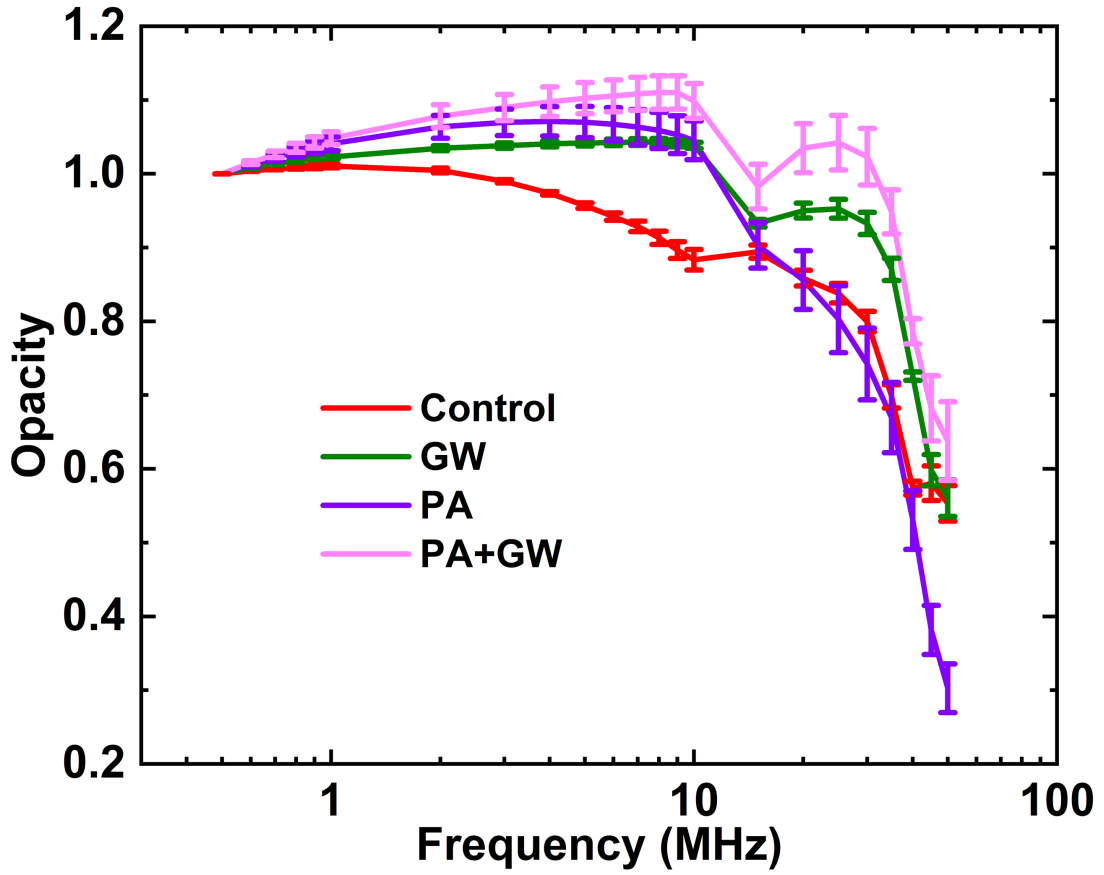
$$R_{Cyt+tRNA} < R_{Cyt}$$

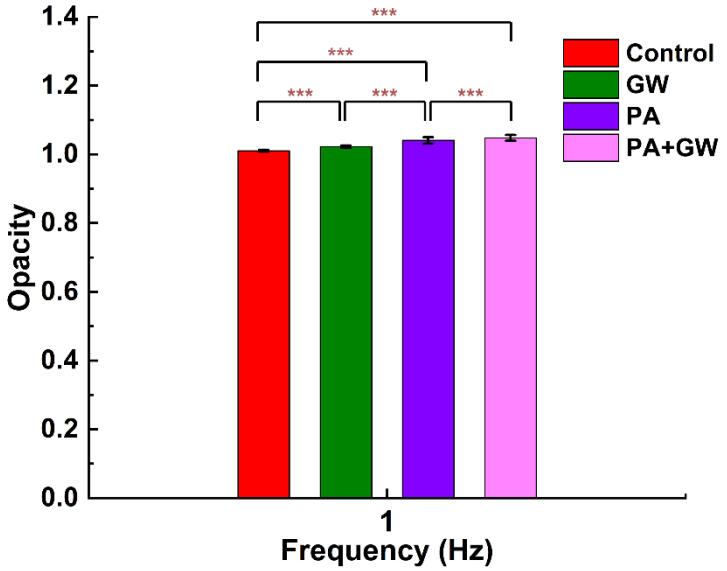
$$Opacity_{cyt+tRNA} > Opacity_{cyt}$$

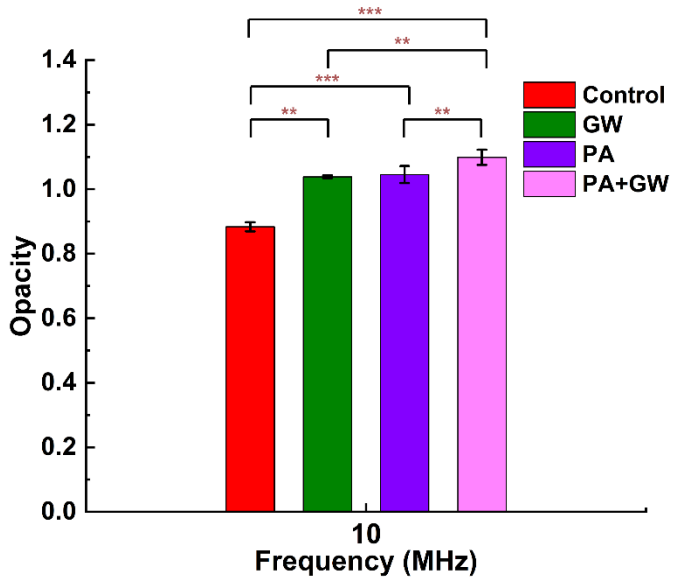


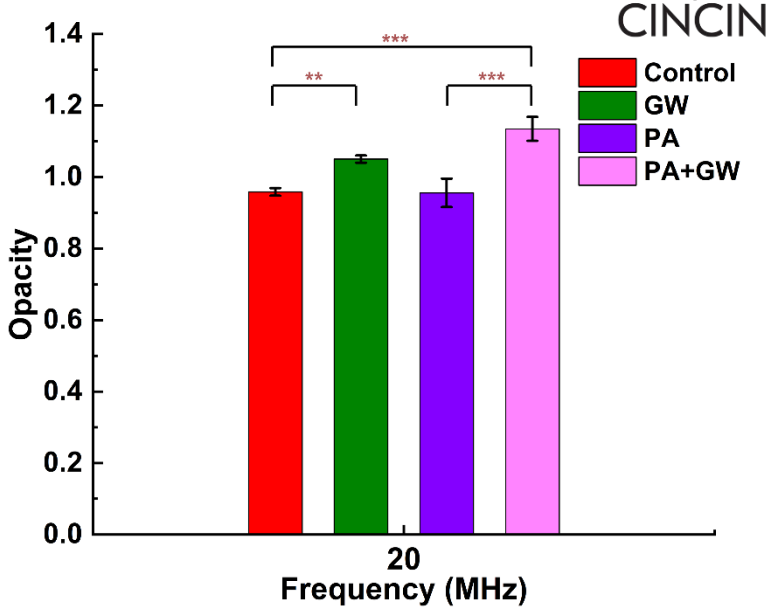
Impedance measurement of exosomes with different biogenesis

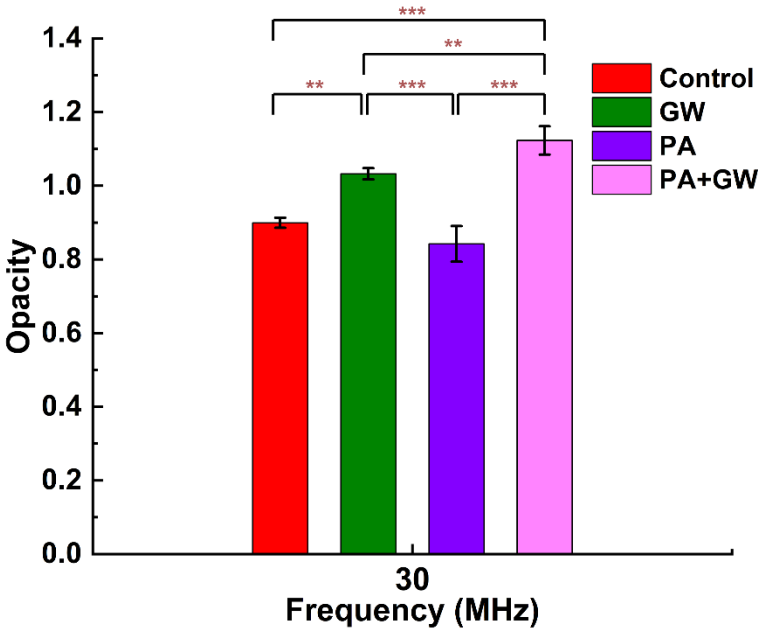


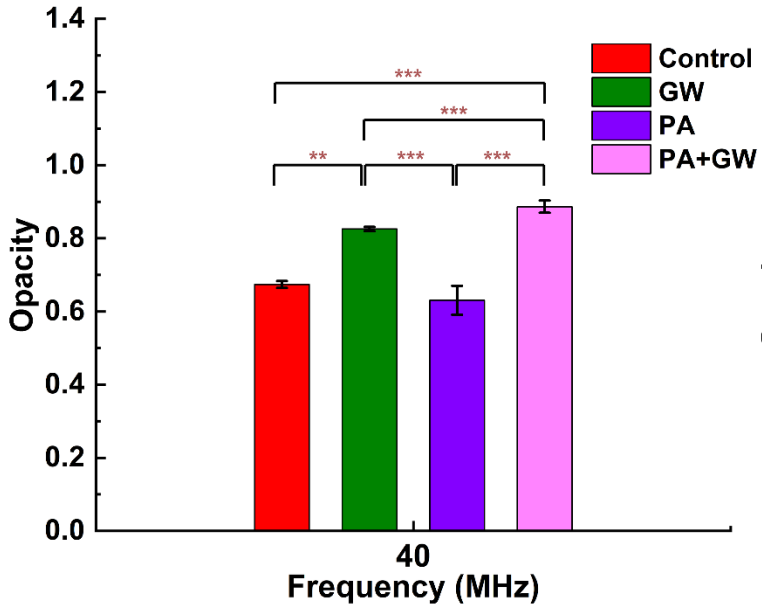


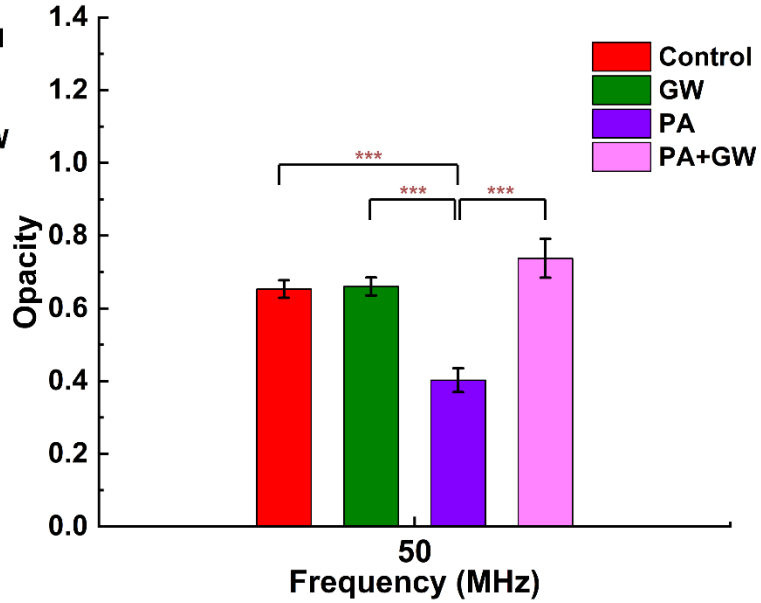

Opacity
Frequency (Hz)
1
Control
GW
PA
PA+GW


Opacity
Frequency (MHz)
10
Control
GW
PA
PA+GW

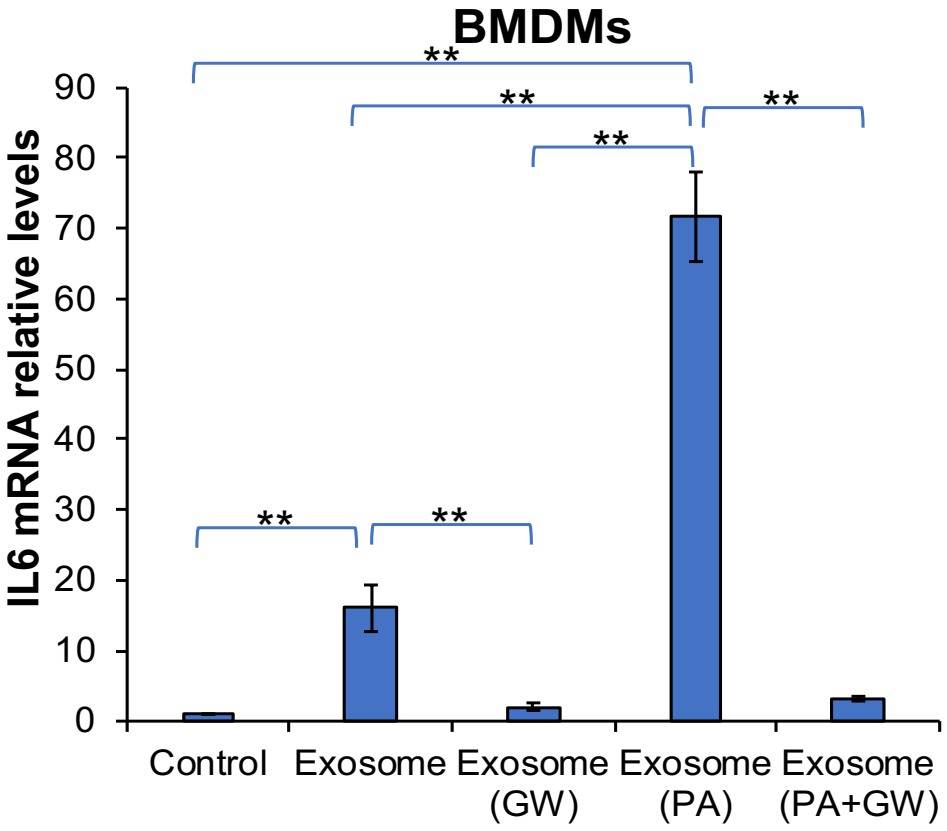

Opacity
Frequency (MHz)
20
Control
GW
PA
PA+GW


Opacity
Frequency (MHz)
30
Control
GW
PA
PA+GW

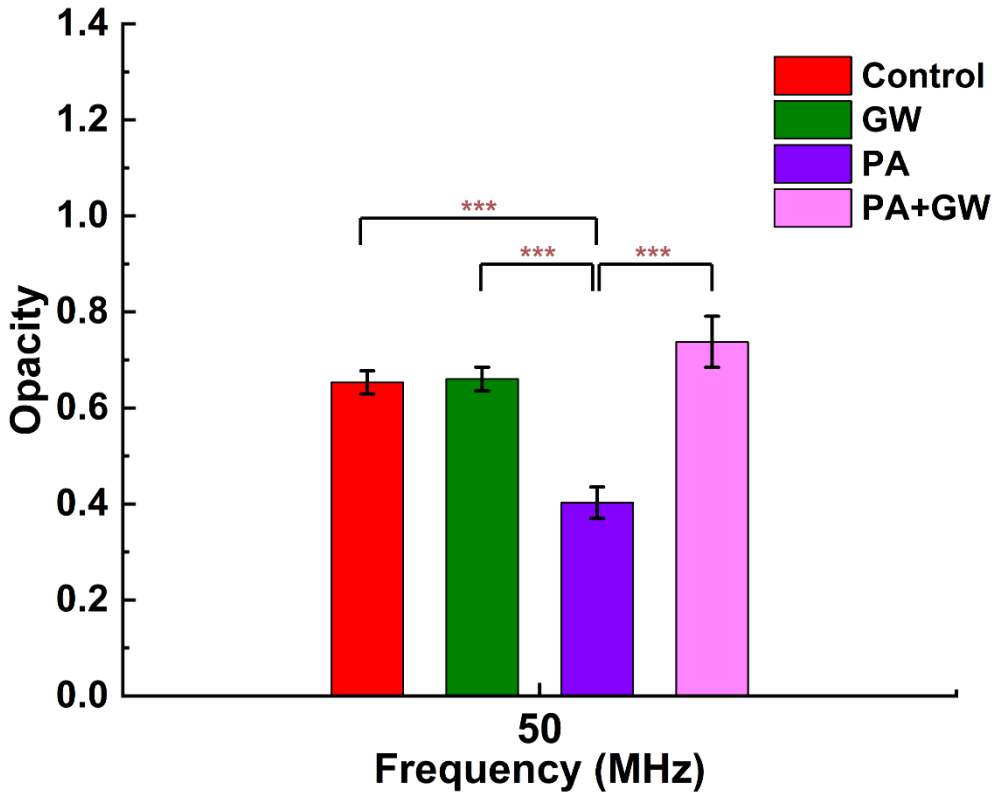

Opacity
Frequency (MHz)
40
Control
GW
PA
PA+GW


Opacity
Frequency (MHz)
50
Control
GW
PA
PA+GW

Comparison of immune response with electrical impedance measurements

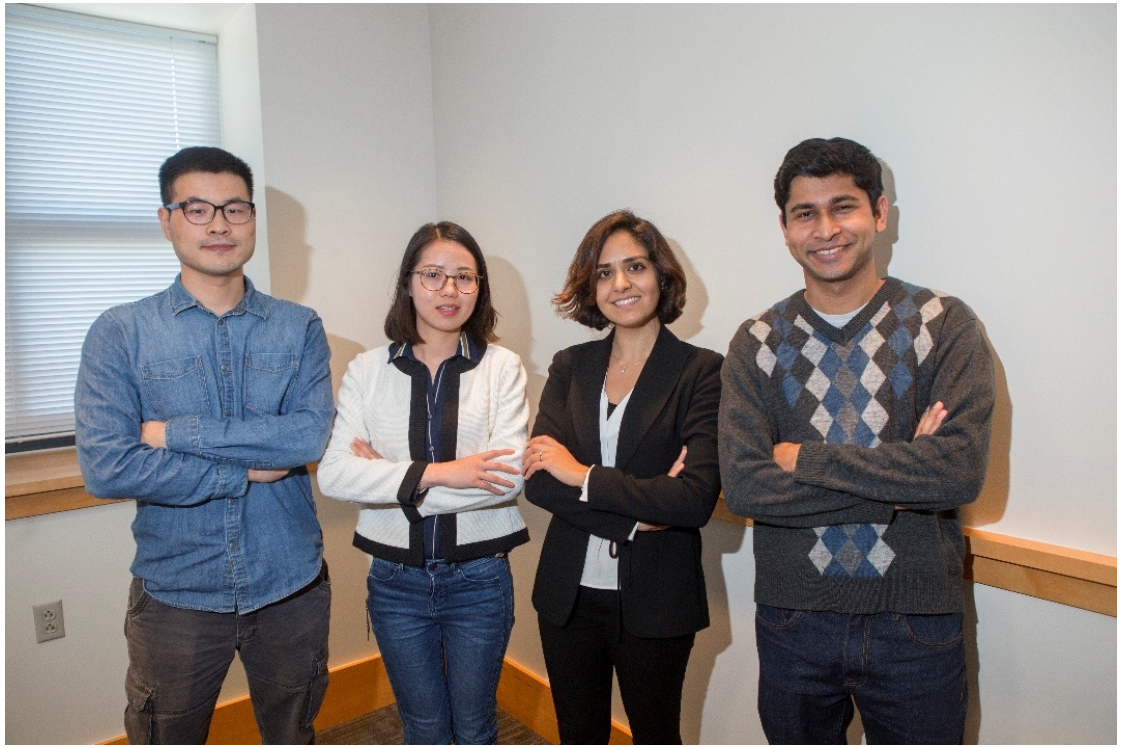


?



Conclusion and future direction

- ✓ Electrical impedance sensor for characterization of exosomes based on their unique dielectric properties
- ✓ Both membrane capacitance and cytosolic conductance of exosomes influence the impedance signal at frequency > 1MHz
- ➢ Classification of exosomes based on their cell of origin
- ➢ A diagnostic tool for detection of pathogenic exosomes



Members of IBL:

Yuqian Zhang
EECS Ph.D. student

Leilei Shi
EECS Ph.D. Student

Ankit Rana
EECS MSc. Student

Collaborators:

Takahasi Nakamura, Ph.D.
Assistant Professor, Department of Pediatrics
Cincinnati Children's hospital

Scott M. Langevin, MHA, Ph.D.
Assistant Professor of Environmental Health
University of Cincinnati College of Medicine