Dielectrophoretic Analysis of Peripheral Blood Mononuclear Cells in Stages III and IV Breast Cancer Models

NATIONAL ACADEMIES Sciences Engineering Medicine — National Academy of Sciences · National Academy of Engineering · National Academy of Medicine

National Cancer Policy Forum

Poster Session - Cancer Engineering: The Convergence of Engineering and Health to Advance Cancer Research and Care

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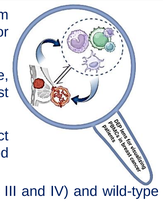
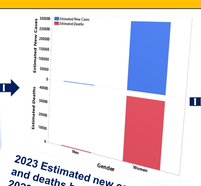
ABSTRACT

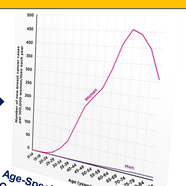
- Peripheral blood mononuclear cells (PBMCs), produced from hematopoietic stem cells (HSCs), are crucial in surveilling for signs of infection, including cancer.
- Early detection of breast cancer remains a clinical challenge, especially in younger patients and those with dense breast tissue.
- Dielectrophoresis (DEP) provides a label-free approach to detect tumor-induced bioelectric changes in circulating peripheral blood mononuclear cells (PBMCs).
- PBMCs were isolated from FVB/N MMTV-PyMT+ mice (stages III and IV) and wild-type controls to evaluate their DEP profiles.
- Key DEP parameters measured included membrane conductance, cytoplasm conductivity, membrane permittivity, membrane capacitance, and crossover frequencies.
- Significant differences in DEP properties were observed between cancer and control groups, with membrane conductance higher in cancerous PBMCs ($p < 0.05$).
- These findings suggest DEP-based biomarkers may distinguish tumor-bearing mice from healthy controls, supporting DEP's potential as a non-invasive diagnostic tool for breast cancer detection.



MOTIVATION



2023 Estimated new cancer cases and deaths by sex, United States, 2023



Age-Specific Rate of Breast Cancer in the United States

$580.6 million

Breast Cancer Projects

NATIONAL CANCER INSTITUTE 2022

- Breast cancer is the leading cancer among women worldwide; early detection is critical for improving survival outcomes.
- Breast cancer deaths are typically due to metastasis of cancer cells from the breast to other parts of the body.
- The spread is a result of **late detection**, **poor diagnosis**, and lack of effective treatment.

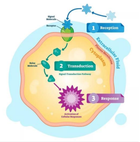
OBJECTIVES

- **Long-term goal**: To non-invasively diagnose breast cancer early
  - This technology will minimize false positives and negatives often seen in standard screening methods like mammography.
  - This is adaptable as a point-of-care screening tool.
- To realize the long-term goal, we are probing the dielectric properties of the peripheral blood mononuclear cells (PBMCs) from peripheral blood sources of MMTV-PyMT mice at stage III and stage IV.

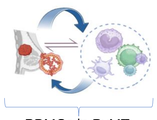
DEP-based Breast Cancer Diagnostic Tool

HYPOTHESIS

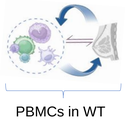
Changes triggered in the subcellular components of PBMCs at the onset of carcinoma regulate dielectric properties, affecting the bioelectric signals aiding in the detection of breast cancer.



Bio Signaling
- Reception
- Transduction
- Response



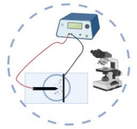
PBMCs in PyMT Mice Model



PBMCs in WT Mice Model

DEP properties of PBMCs in PyMT and WT mice models will be different

Changes detected via DEP



DIELECTROPHORESIS THEORY

Single Shell Model

$$F_{DEP} = 2\pi R^3 \varepsilon_0 \varepsilon_m \mathrm{Re}[CM]\nabla E^2$$

$$R * f_{x01} = \frac{\sqrt{2}}{2\pi C_{mem}}\sigma_{med} - \frac{\sqrt{2}}{8\pi C_{mem}} R\, G_{sp-mem}$$

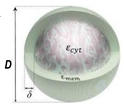
$$m = \frac{\sqrt{2}}{2\pi C_{mem}}, \quad \text{and} \quad c = -\frac{\sqrt{2}}{8\pi R C_{mem}} R\, G_{mem}$$

$$CM = \frac{\sigma_p^* - \sigma_m^*}{\sigma_p^* + 2\sigma_m^*} \quad or \quad \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$

$\varepsilon_p > \varepsilon_m$ - Positive DEP (pDEP)

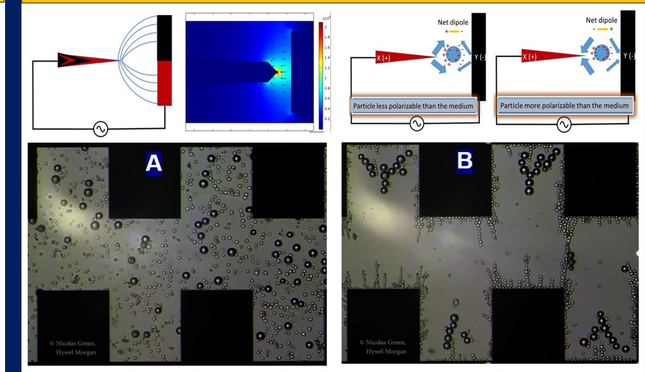
$\varepsilon_p < \varepsilon_m$ - Negative DEP (nDEP)

C, r, sp-mem, mem, p, G, and m represent capacitance, radius, specific membrane, membrane, particle (cell), conductance, and medium, respectively.



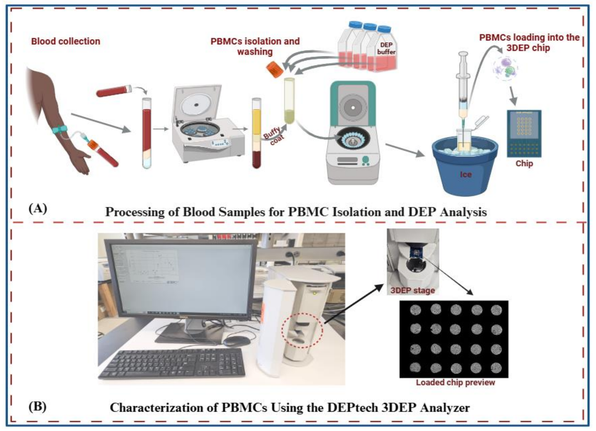
p = particle
R = radius of the particle
C_{mem} = membrane capacitance
G_{mem} = membrane conductance
ε_m = permittivity of the medium
ε_p = permittivity of the particle

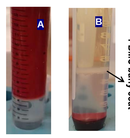
THEORY (Cont…)



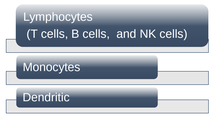
(A) Absence of non-uniform electric field (B) Presence of a non-uniform electric field, Nicholas Green and Hywel Morgan [1]

MATERIALS & METHODS





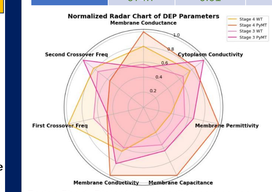
(A) Whole blood suspended on ficoll media (B) Separated PBMC buffy coat



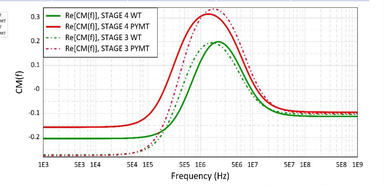
RESULTS & DISCUSSIONS

Table 1: Average, SEM, and STDEV.P of the estimated DEP-based biomarkers for distinguishing paired mouse model groups

Stage Group	Membrane conductance G_{mem} (S/m²)	Cytoplasm conductivity, σ_{cp}(S/m)	Membrane permittivity, ε_{mem}	Membrane capacitance, C_{mem} (mF/m²)	Membrane conductivity, σ_{mem}(S/m) (*E-5)	First Crossover Frequency, f_{x01} (kHz)	Second Crossover Frequency, f_{x02} (MHz)
	Mean SEM STDEV.P	Mean SEM STDEV.P	Mean SEM STDEV.P	Mean SEM STDEV.P	Mean SEM STDEV.P	Mean SEM STDEV.P	Mean SEM STDEV.P
STAGE IV WT	2481.2 1137.5 1608.7	0.04 0.02 0.03	1.5 0.3 0.5	1.4 0.3 0.5	2.2 1.02 1.5	700.1 92.8 92.8	11.2 6.8 6.8
STAGE IV PyMT	3068.2 308.5 534.3	0.04 0.01 0.03	4.0 0.8 1.1	3.9 0.8 1.1	3.5 0.7 1.5	340.7 102.5 145.0	7.6 3.1 4.4
STAGE III WT	1750.7 336.8 583.3	0.03 0.01 0.01	2.2 0.2 0.4	2.1 0.2 0.4	2.1 0.5 1.1	465.0 47.7 82.7	10.3 1.6 2.8
STAGE III PyMT	1611.5 562.7 974.7	0.05 0.01 0.01	2.7 0.3 0.5	2.5 0.3 0.6	2.9 0.3 0.5	332.4 62.5 88.4	13.5 2.4 3.4

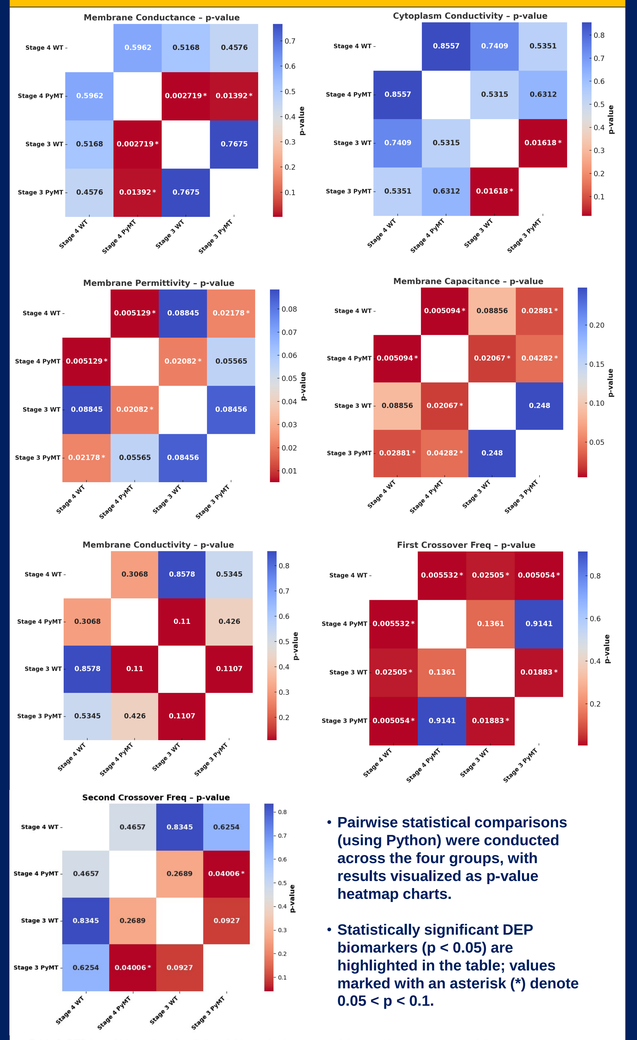


Radar Chart: Dielectric properties comparison of stages III & IV of PyMT and WT mice models



Re and Im plot of the Clausius–Mossotti factor, (CM) for MMTV-PyMT and WT PBMCs as a function of frequency at 0.03 S/m medium conductivity.

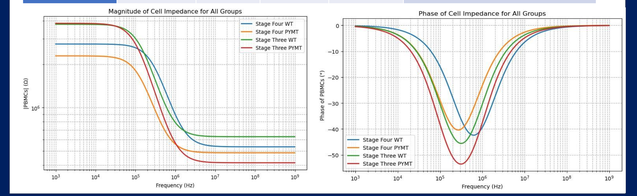
RESULTS & DISCUSSIONS (CONT….)



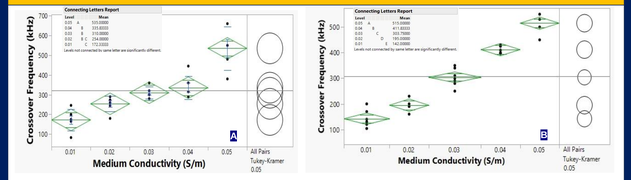
- **Pairwise statistical comparisons (using Python) were conducted across the four groups, with results visualized as p-value heatmap charts.**
- **Statistically significant DEP biomarkers ($p < 0.05$) are highlighted in the table; values marked with an asterisk (*) denote $0.05 < p < 0.1$.**

Table 2: DEP-based biomarkers for distinguishing paired mouse model groups between cancer and their controls

Group	STAGE IV WT	STAGE IV PyMT	STAGE III WT	STAGE III PyMT	DIELECTRIC PARAMETER (BIOMARKERS)
STAGE IV WT		ε_{mem} C_{mem} f_{x01}	$\boldsymbol{\varepsilon_{mem}}^*$ $\boldsymbol{C_{mem}}^*$ f_{x01}	ε_{mem} C_{mem} f_{x01}	Membrane conductance G_{mem}
STAGE IV PyMT	C_{mem} ε_{mem} f_{x01}		C_{mem} ε_{mem} G_{mem}	C_{mem} $\boldsymbol{\varepsilon_{mem}}^*$ G_{mem} f_{x02}	Cytoplasm conductivity, σ_{cp} Membrane permittivity, ε_{mem} Membrane capacitance, C_{mem}
STAGE III WT	$\boldsymbol{C_{mem}}^*$ $\boldsymbol{\varepsilon_{mem}}^*$ f_{x01}	C_{mem} ε_{mem} G_{mem}		σ_{cp} $\boldsymbol{\varepsilon_{mem}}^*$ f_{x01} $\boldsymbol{f_{x02}}^*$	Membrane conductivity, σ_{mem} First Crossover Frequency, f_{x01}
STAGE III PyMT	ε_{mem} C_{mem} f_{x01}	C_{mem} $\boldsymbol{\varepsilon_{mem}}^*$ G_{mem} f_{x02}	$\boldsymbol{\varepsilon_{mem}}^*$ σ_{cp} f_{x01} $\boldsymbol{f_{x02}}^*$		Second Crossover Frequency, f_{x02} (*) indicates $0.05 < p < 0.1$

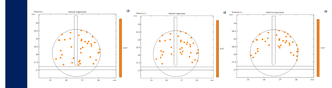


RESULTS & DISCUSSIONS (CONT….)

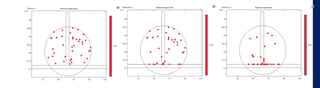


One-way analysis of variance of the crossover frequency for (A) MMTV-PyMT PBMCs and (B) MMTV-WT PBMCs

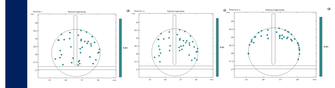
COMSOL Multiphysics computation analysis using the of Stage IV PPM experimental results for DEP response validation



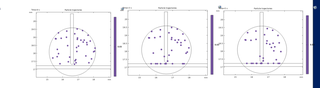
Negative dielectrophoresis (nDEP) response of PyMT PBMCs at 0.03 S/m, 200 kHz, and 8 V_{pp}



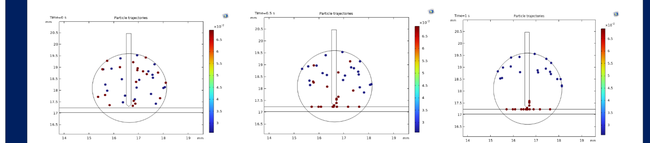
Positive dielectrophoresis (pDEP) response of PyMT PBMCs at 0.03 S/m, 300 kHz, and 8 V_{pp}



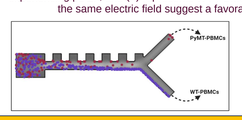
Negative dielectrophoresis (nDEP) response of WT PBMCs at 0.02 S/m, 200 kHz, and 8 V_{pp}



Positive dielectrophoresis (pDEP) response of WT PBMCs at 0.02 S/m, 300 kHz, and 8 V_{pp}



DEP response of PyMT and WT-PBMCs at 0.02 S/m, 250 kHz, and 8 V_{pp}. (●) represents PyMT-PBMCs, experiencing pDEP, and (●) represents WT-PBMCs, experiencing nDEP. The distinct responses of the cells under the same electric field suggest a favorable separation frequency region for the two cell types.



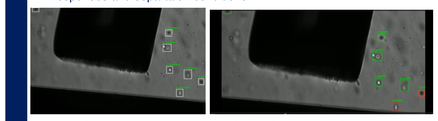
**** COMSOL computational modeling for separating PyMT and WT PBMCs at 175 µm/s, 250 kHz, and 8 Vpp***

**** Stage IV PPM estimated dielectric properties of MMTV-PyMT and MMTV-WT PBMCs.***

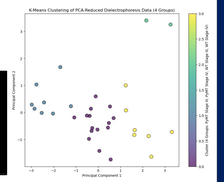
CONCLUSIONS & FUTURE OUTLOOK

- Successfully identified dielectric differences between PyMT PBMCs (cancer) and WT (control) mice in stages III and IV.
- Stage III and IV PyMT PBMCs showed higher membrane capacitance (C_{mem}) and membrane conductance (G_{mem}) compared to WT controls.
- Crossover frequencies (f_{x01}, f_{x02}) were significantly altered in PyMT PBMCs, supporting their use as DEP-based biomarkers.
- Stage IV PyMT PBMCs exhibited the most "leaky" membrane, reflected by the lowest impedance (Z).
- Pairwise statistical analysis confirmed significant differences in key DEP properties between cancer and control groups ($p < 0.05$).
- Distinct dielectrophoretic responses at 0.02 S/m, 250 kHz, and 8 Vpp indicate optimal conditions for separating PyMT from WT PBMCs.
- COMSOL simulations validated experimental dielectric responses and separation conditions.

- **Future work 1**: Future work will apply DEP profiling to earlier cancer stages (I and II) and human PBMC samples..
- **Future work 2**: Integrate computer vision and machine learning to automate DEP response tracking and classification.
- **Future work 3**: Develop a microfluidic device for DEP-based biomarker profiling as a rapid, point-of-care diagnostic tool for breast cancer detection



Visualizing the dielectric behavior of immune cells from a breast cancer model using computer vision



K-means clustering on PCA-reduced dielectrophoresis data

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