

Toward an automated microfluidic platform for monitoring immune response to immunotherapy

Yuqian Zhang, PhD^{1, 2}, Yohan Kim, PhD³, Fabrice Lucien, PhD^{3, 4}, Haidong Dong, MD, PhD^{3, 4} and Yuguang Liu, PhD^{1, 2, 3, 5}

¹Department of Physiology and Biomedical Engineering, ²Microbiomics Program, Center for Individualized Medicine,

³Department of Immunology, ⁴Department of Urology and ⁵Department of Surgery

Abstract

Background: PD1/PD-L1 checkpoint inhibitors are at the forefront of cancer immunotherapies, yet, the overall response rate is only 10-30%. Even in initial responders, drug resistance evolves. Circulating biomarkers, including peripheral blood mononuclear cells (PBMCs), extracellular vesicles-derived and soluble PD-L1 are increasingly recognized as non-invasive biomarkers for evaluating the immune-mediated therapeutic responsiveness². Measuring these markers requires standard assays including flow cytometry, Western Blot and ELISA often found in core facilities. This creates practical challenges in closely monitoring patients' immune responsiveness. Yet, close monitoring is critical as it can help avoid prolonged use of a futile therapy which can lead to the loss of time, and financial burden on patients, healthcare and economy.

Objective: Our overarching objective is to create a sensitive tool that can be easily used to frequently monitor cancer patients' immune responsiveness.

Methods: We integrate electrochemical microsensors into our automated digital microfluidic (DMF) platform³ to rapidly detect PBMCs, soluble and EV-derived PD-L1 in microliters of sample.

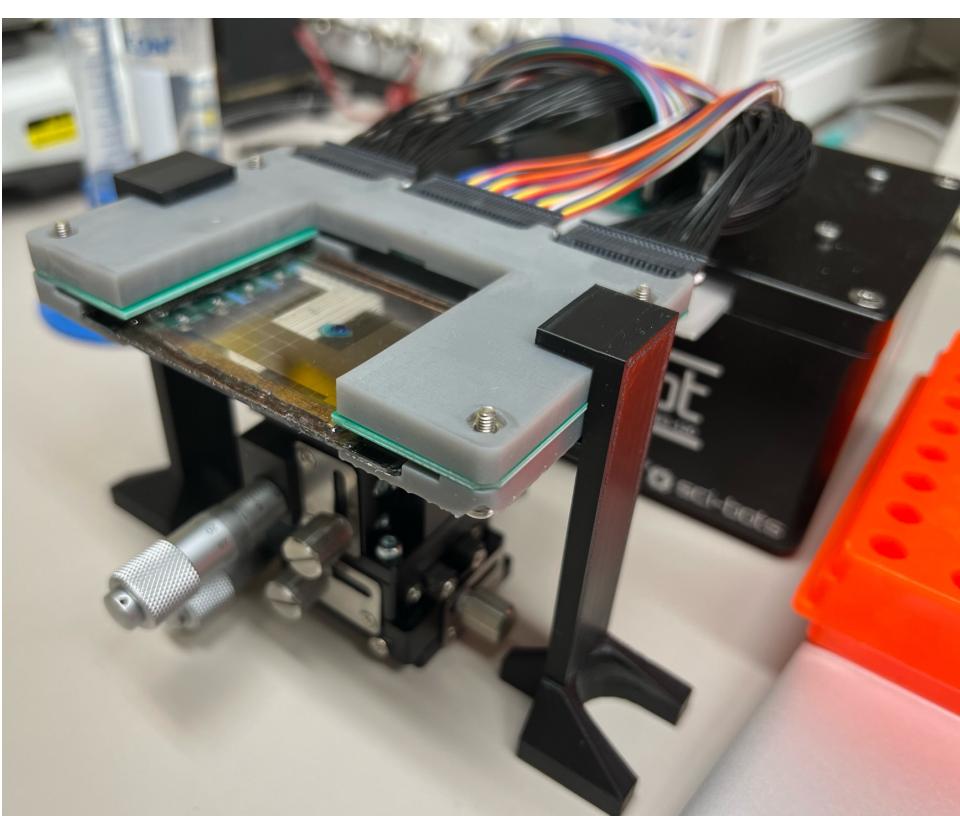
Results: We showed the feasibility of the device to: 1) Quantify as low as 5 PBMCs in 5 μ L sample.

2) Extract and detect EV-derived PD-L1 on-chip.

3) Detect soluble PD-L1 with 1 pg/mL detection limit.

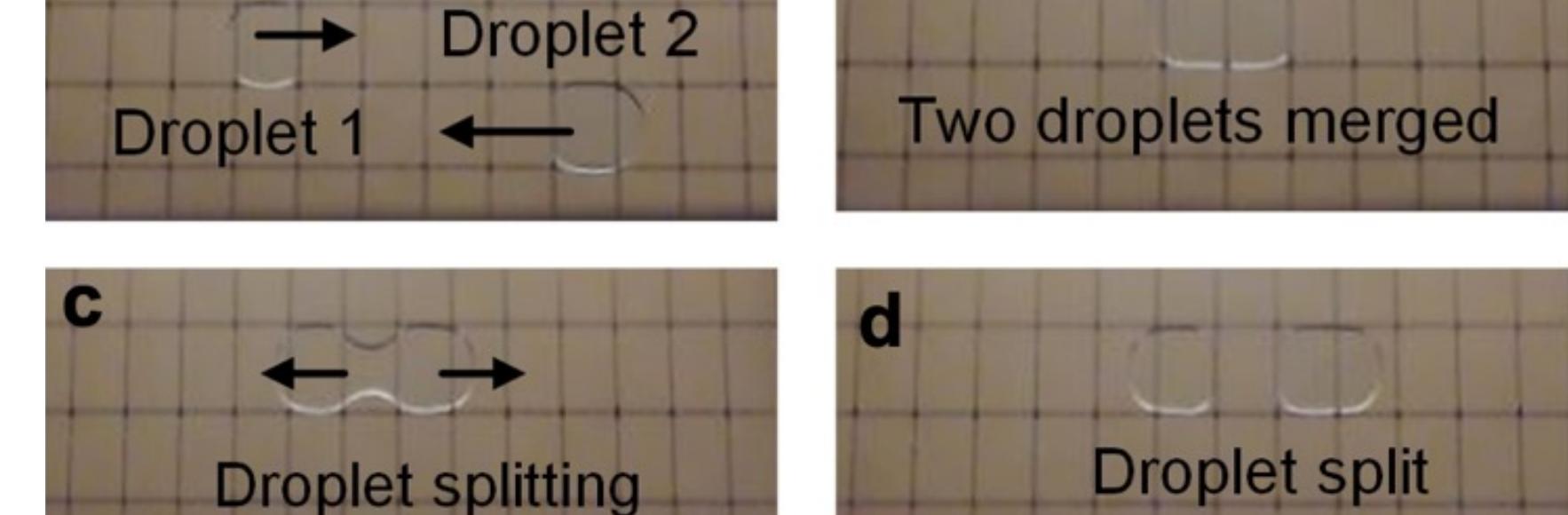
Digital microfluidic platform

□ A portable, automated microfluidic platform



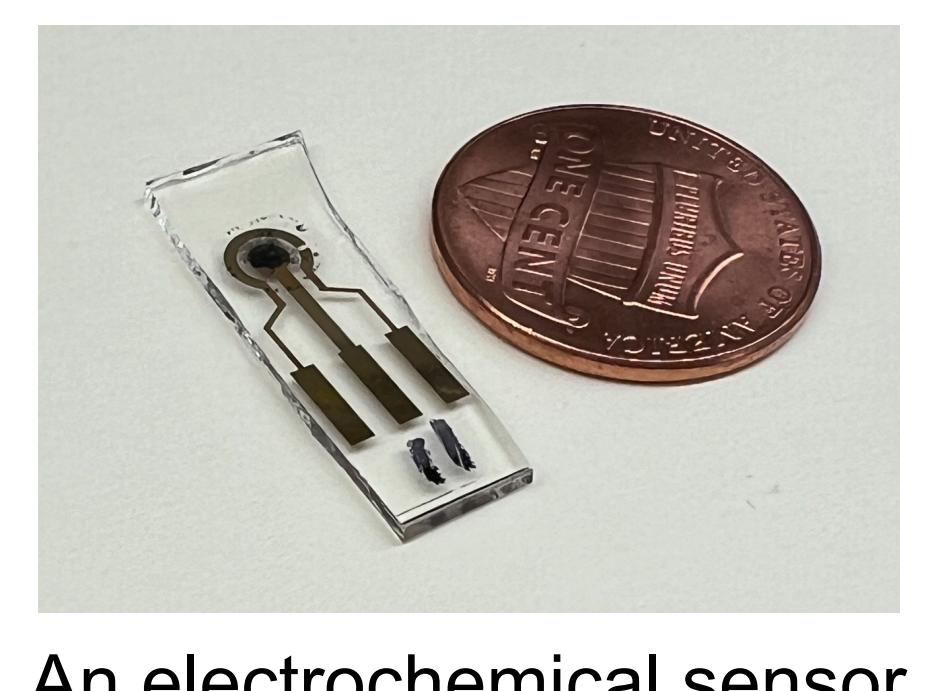
□ Programmed droplet handling on electrode arrays

□ Ideal for multiplexed biomarker detection



Electrochemical sensors integration

- Robust fluid handling
- Sensor miniaturization
- Multiplexed sensing



An electrochemical sensor

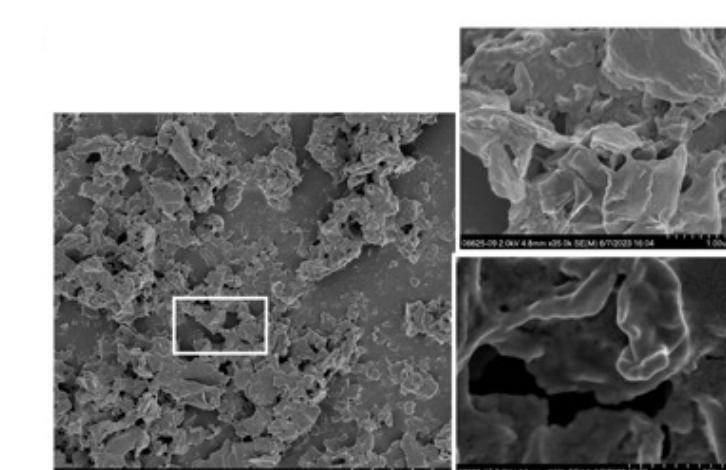
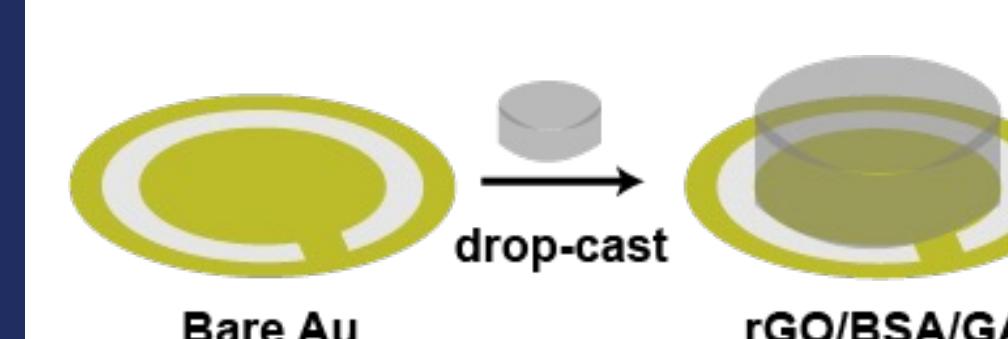
Results

Rapid PBMC detection

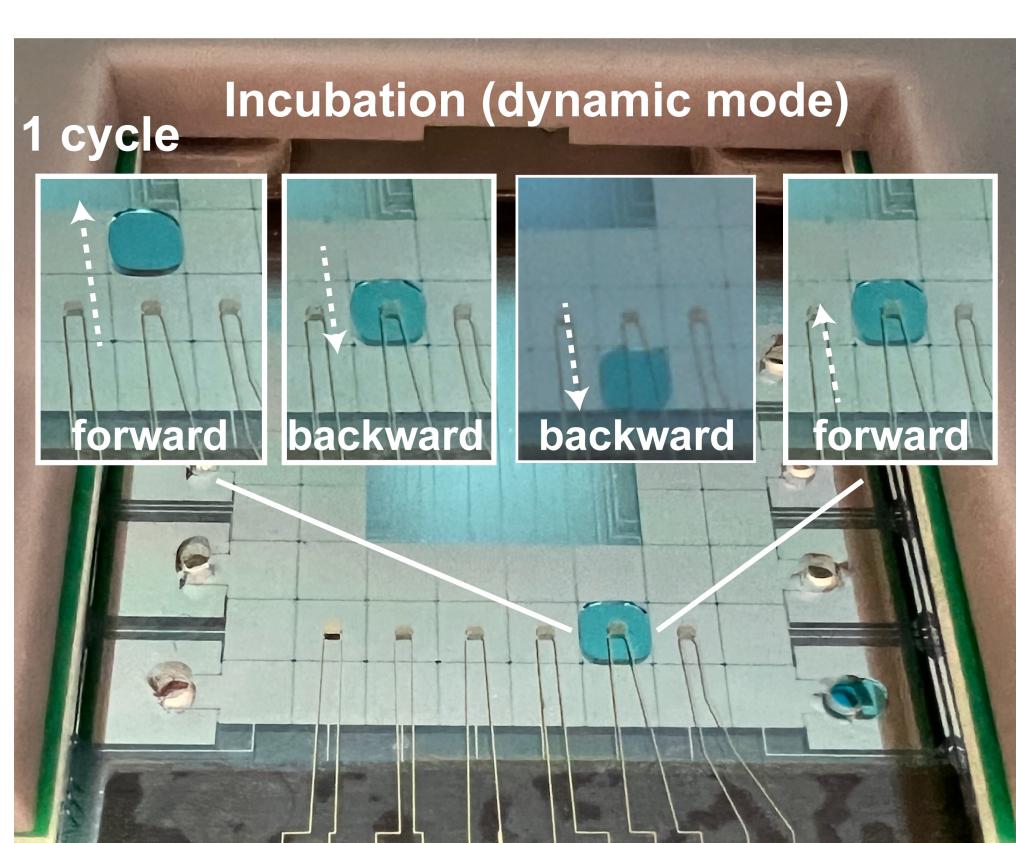
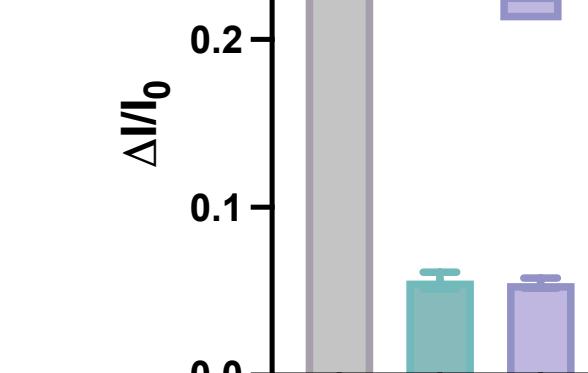
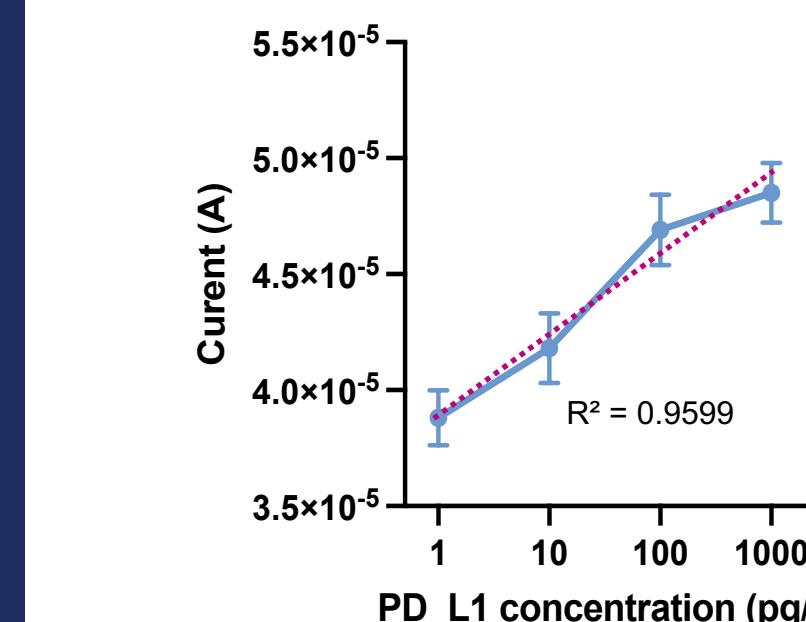
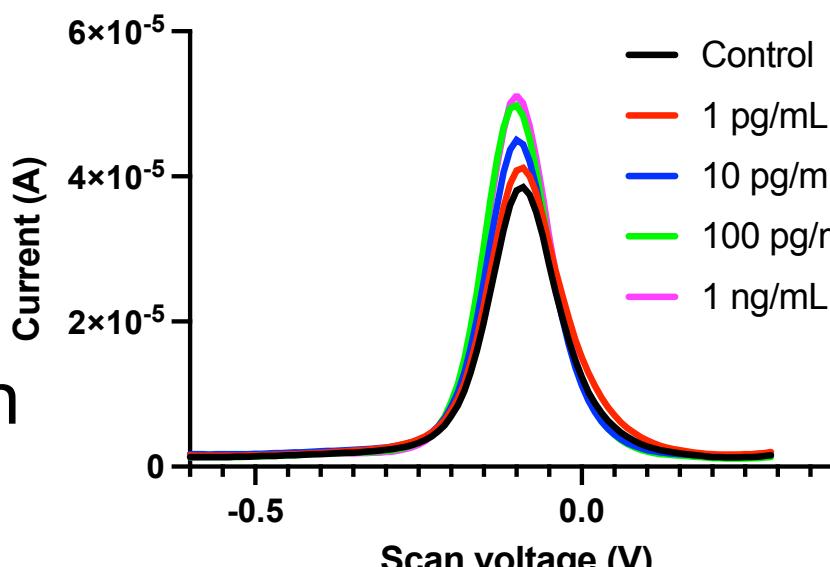
- PBMC size: 5-30 μ m
- Low sample volume: 5 μ L
- Detection time: 20 min
- Dynamic incubation mode: 2.4X signal increase⁴
- Detection limit: 10⁴ PBMCs/mL, 100X < clinically relevant range

3D hydrogel matrix for soluble PD-L1 detection

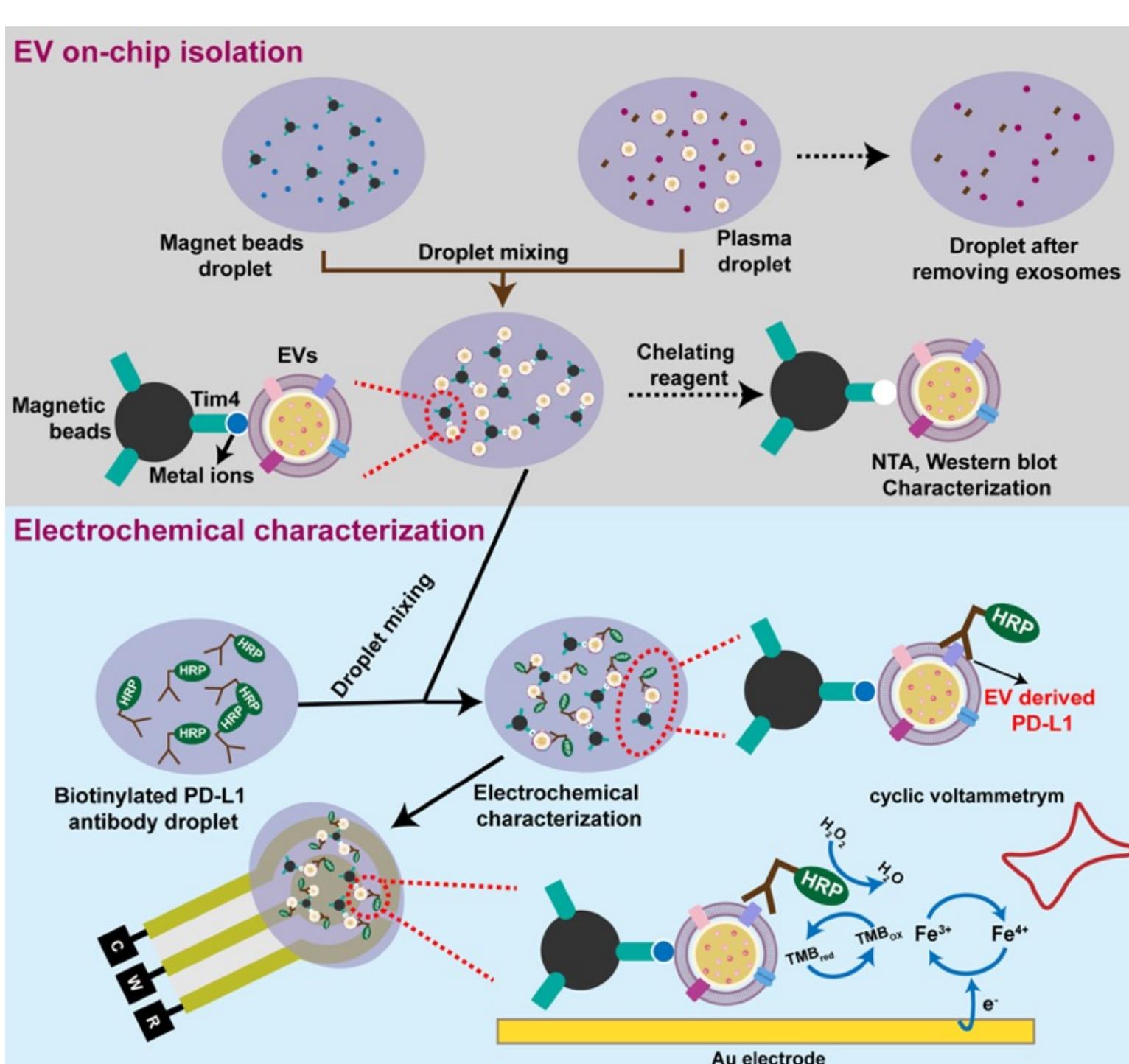
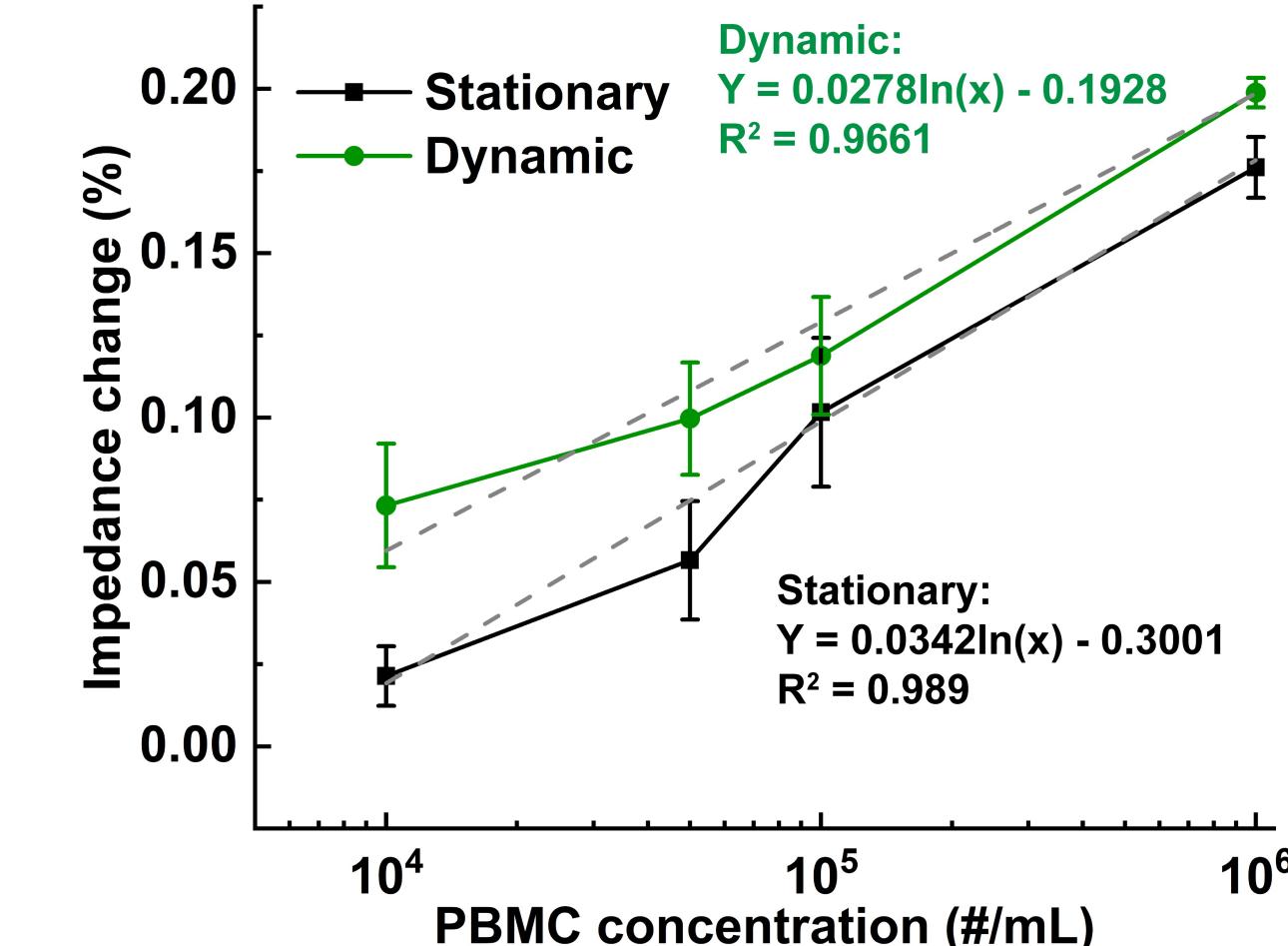
- Soluble PD-L1: <10 nm
- Hydrogel and reduced graphene oxide: increase porosity and increase electrical conductivity



- Detection limit: 1 pg/mL
- Good linearity
- Low non-specific detection

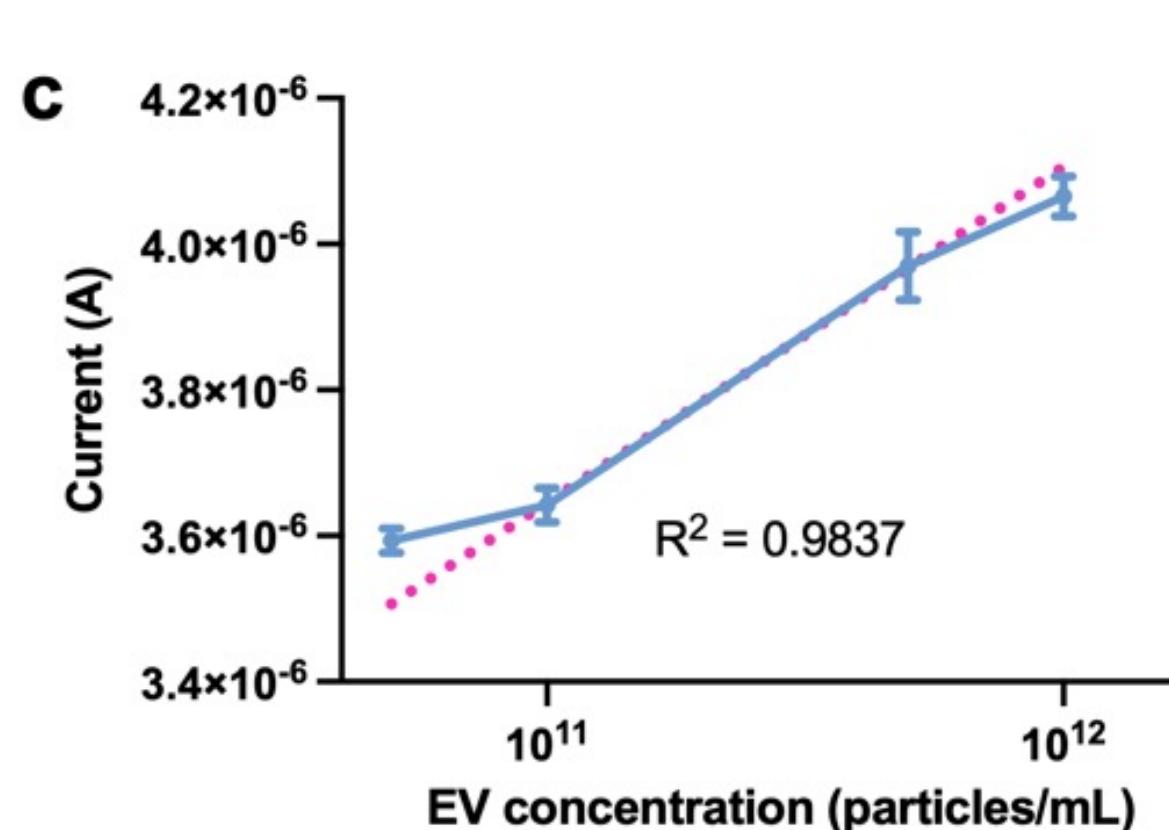
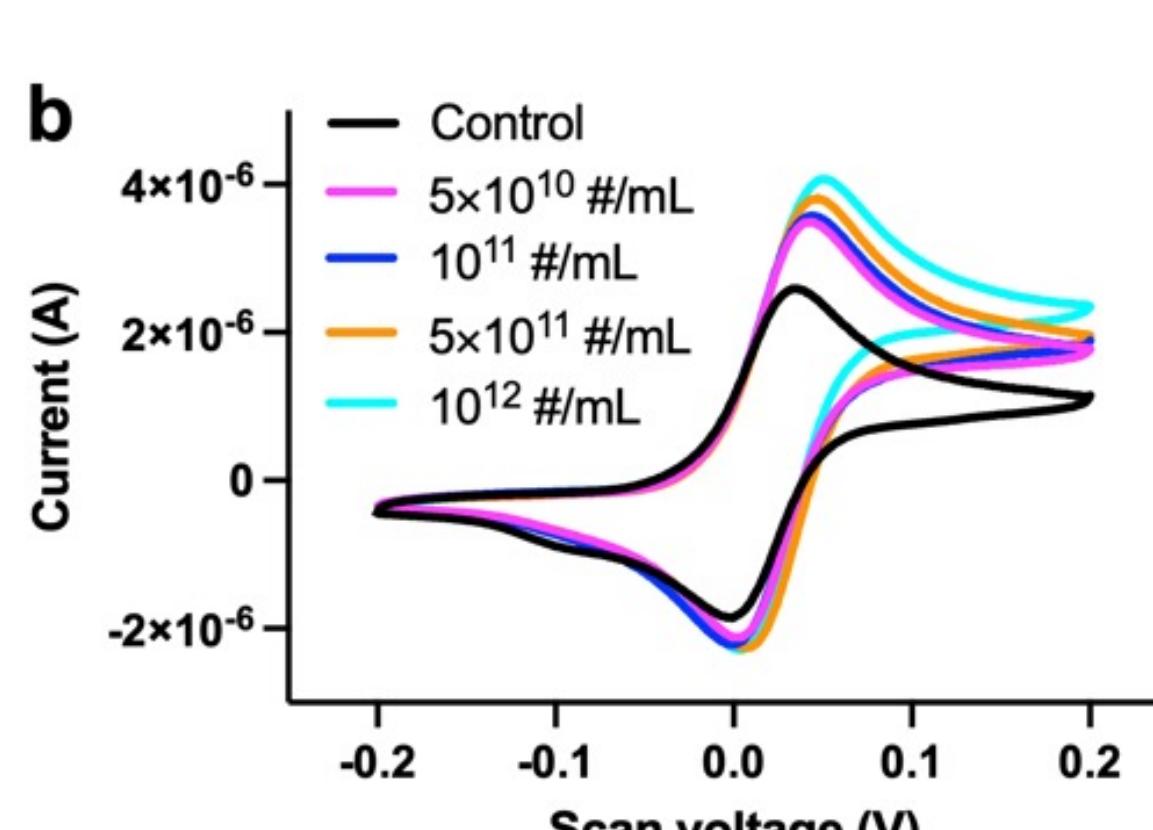
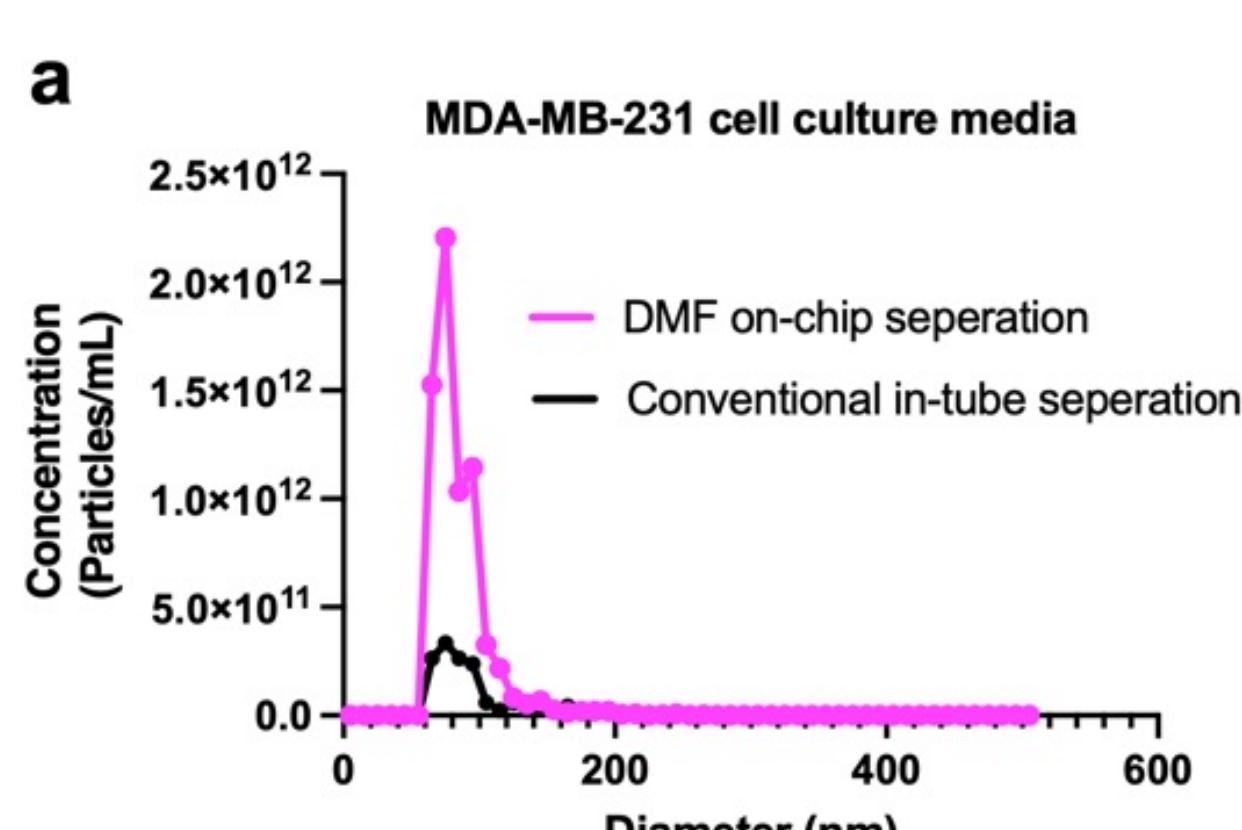


Incubation (dynamic mode)
forward backward backward forward



On-chip extracellular vesicle (EV) extraction and detection

- EV size: 150 nm - 2 μ m
- EV extraction from MDA-MB-231 breast cancer cell line
- On-chip EV extraction
 - ❖ EVs captured by immunomagnetic beads
 - ❖ 10X increase in the number of EVs extracted
- Quantitative differentiation of EV concentrations.



Future directions

- Continued platform development
 - ❖ Phenotype PBMC subsets
 - ❖ Quantify PD-L1 positive EVs on-chip
 - ❖ Whole blood processing on-chip
- Pretreatment evaluation
 - ❖ Overall immune abundance
 - ❖ T cell exhaustion (PD-L1⁺ EVs >0.55ng/mL)
- In-treatment evaluation
 - ❖ Anti-tumor immunity (PD-L1⁺ EVs changes>2X)
 - ❖ Lymphocyte-to-monocyte ratio

Acknowledgements

We gratefully acknowledge the support of the National Institute of Health (NIH) and the Ivan Bowen Family Foundation.

References

1. P. Sharma et al., *Cell*, 2017, 168, 707.
2. G. Chen et al, *Nature*, 2018, 560, 382.
3. Y. Zhang and Y. Liu., *Sensors & Diagnostics*, 2022, 1, 648.
4. Y. Zhang and Y. Liu, *Biosensors*, 2022, 12, 330.