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Digital Microfluidic System Integrated with Electrochemical Sensor for Multiplexed Monitoring of Immune Responses to Immunotherapy

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Immunotherapy:

Immune checkpoint inhibitor

- Down-regulation of T cell response to tumor cells
- Can be blocked by antibodies

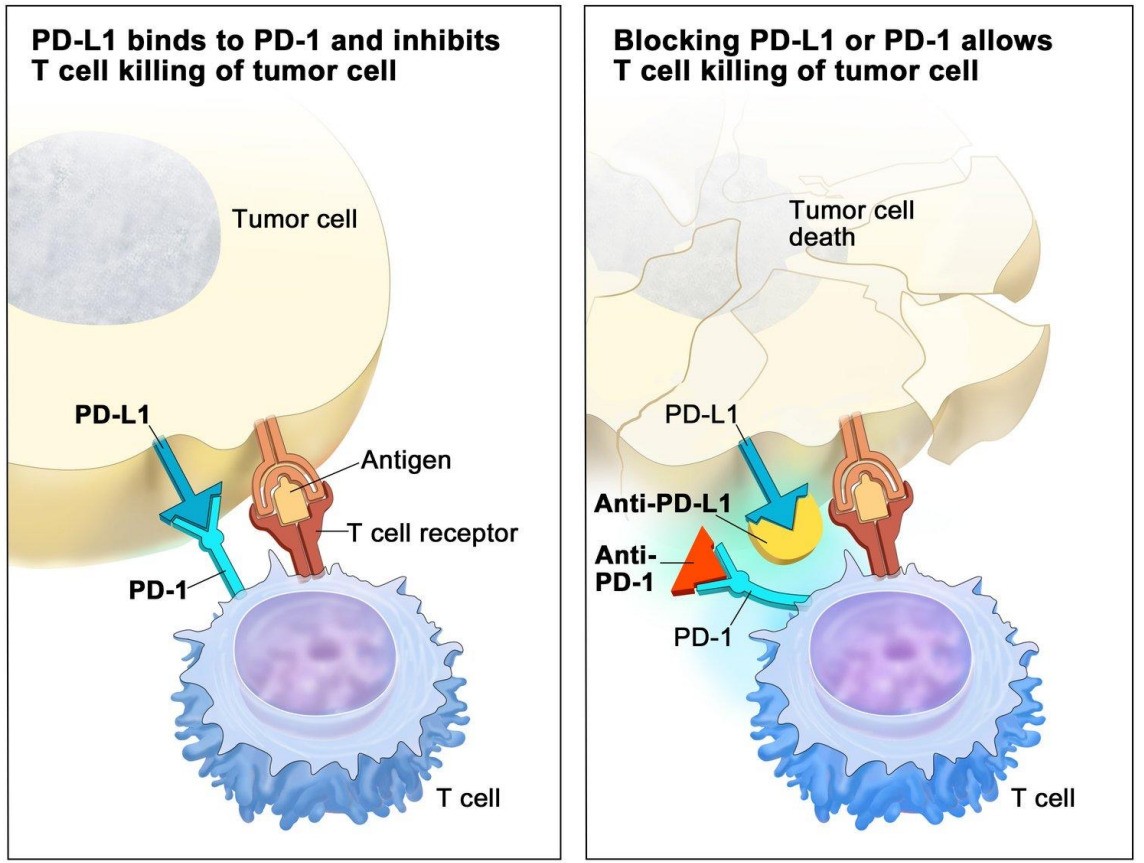
Challenges of Immunotherapy

- *Low response rate*: *15 to 20%*
- *High cost*: $200,000 per patient per year
- *Heterogeneity* in therapeutic responses

Potential solutions:

- Continuously monitor the therapeutic responses
- Detecting multiple biomarkers for a more comprehensive assessment:
  - Peripheral blood mononuclear cell (PBMC)
  - Soluble PD-L1 protein (sPD-L1)
  - Extracellular vesicles PD-L1 (evPD-L1)

Anti-PD 1/PD-L1 therapies



https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

https://www.hopkinsmedicine.org/inhealth/about-us/immunotherapy-precision-medicine-action-policy-brief

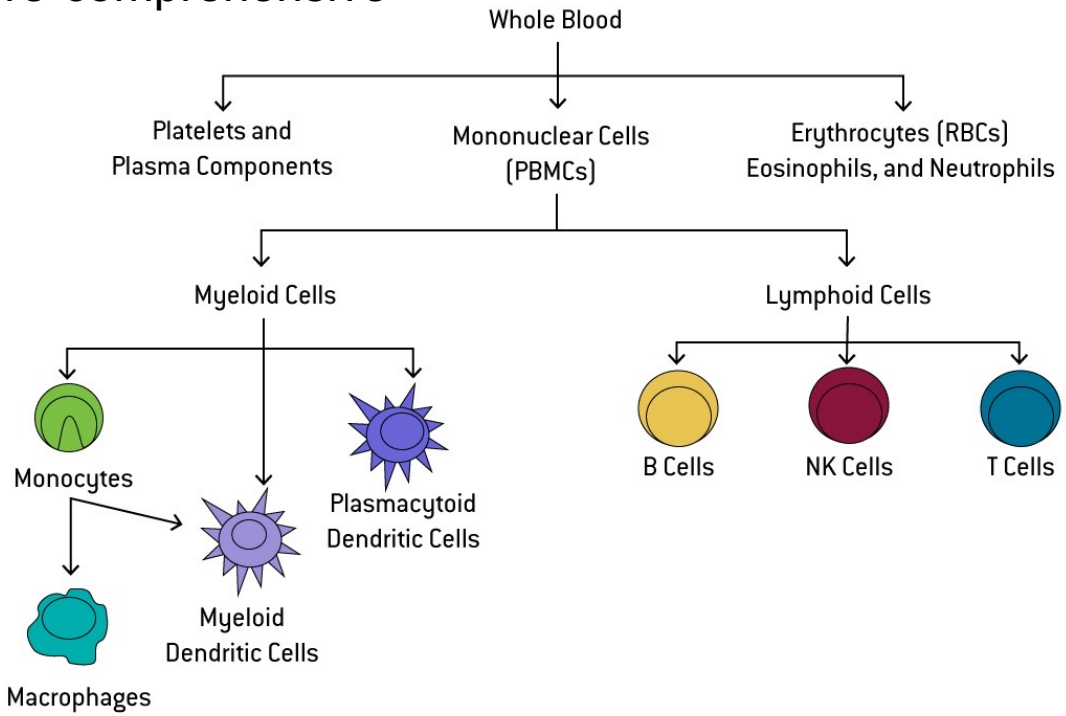
Peripheral blood mononuclear cell (PBMC)

- Key drivers of the immune responses to pathogens
- Provide a window into patient's immune response to immune checkpoint inhibitor
- Comprised of multiple immune cell subsets, providing a more comprehensive overview of the immune status

❑ **Standard detection method: Flow cytometry**

✖ Highly specialized and bulky equipment in core facilities

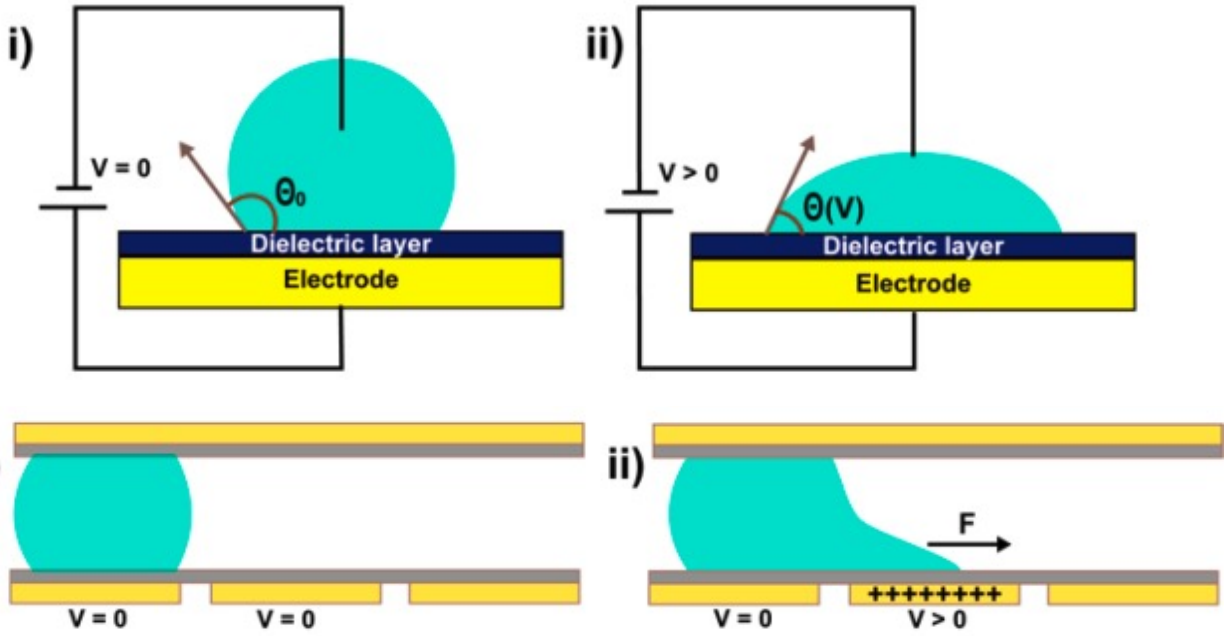
✖ Unrealistic to regularly monitor changes in immune functions

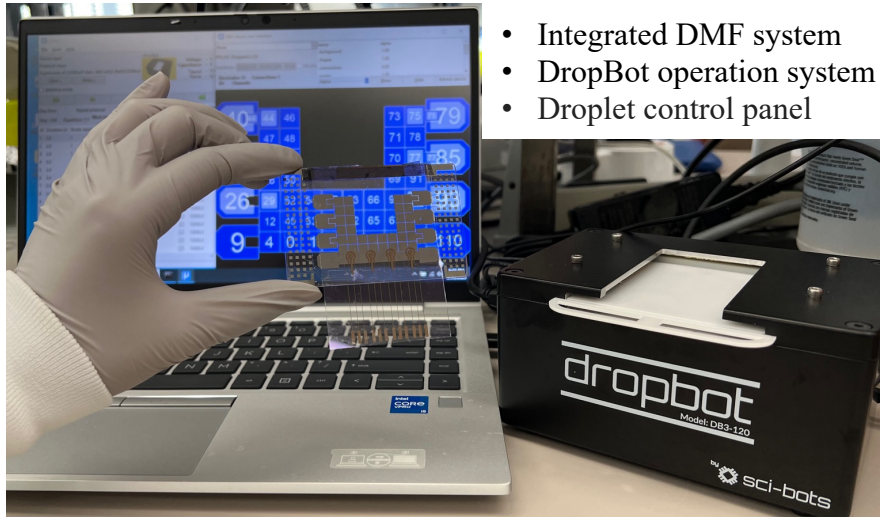


Digital Microfluidic (DMF) System integrated with electrochemical sensor

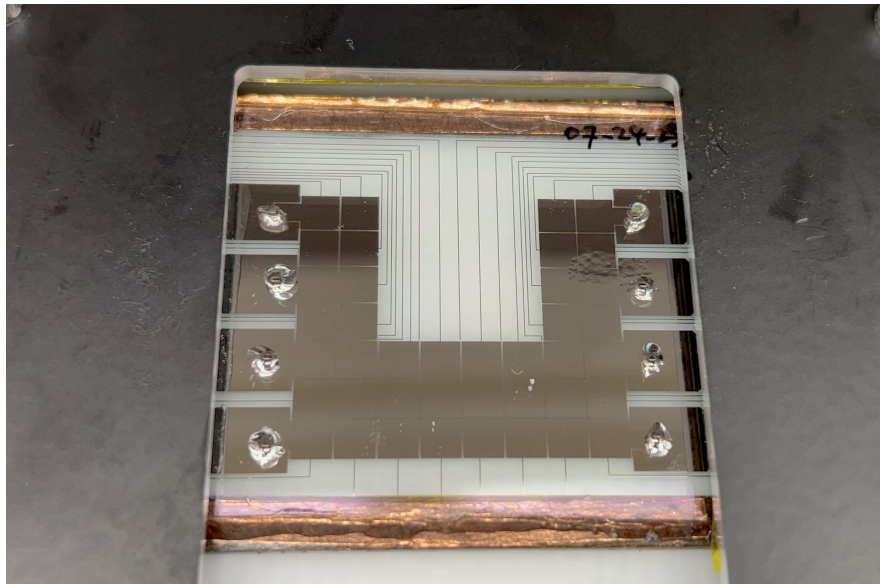
❑ Digital microfluidic lab-on-chip:

- Manipulation of discrete droplets
- Electrowetting-on-dielectric (EWOD)
- Automated and programmable, reconfigured on-demand

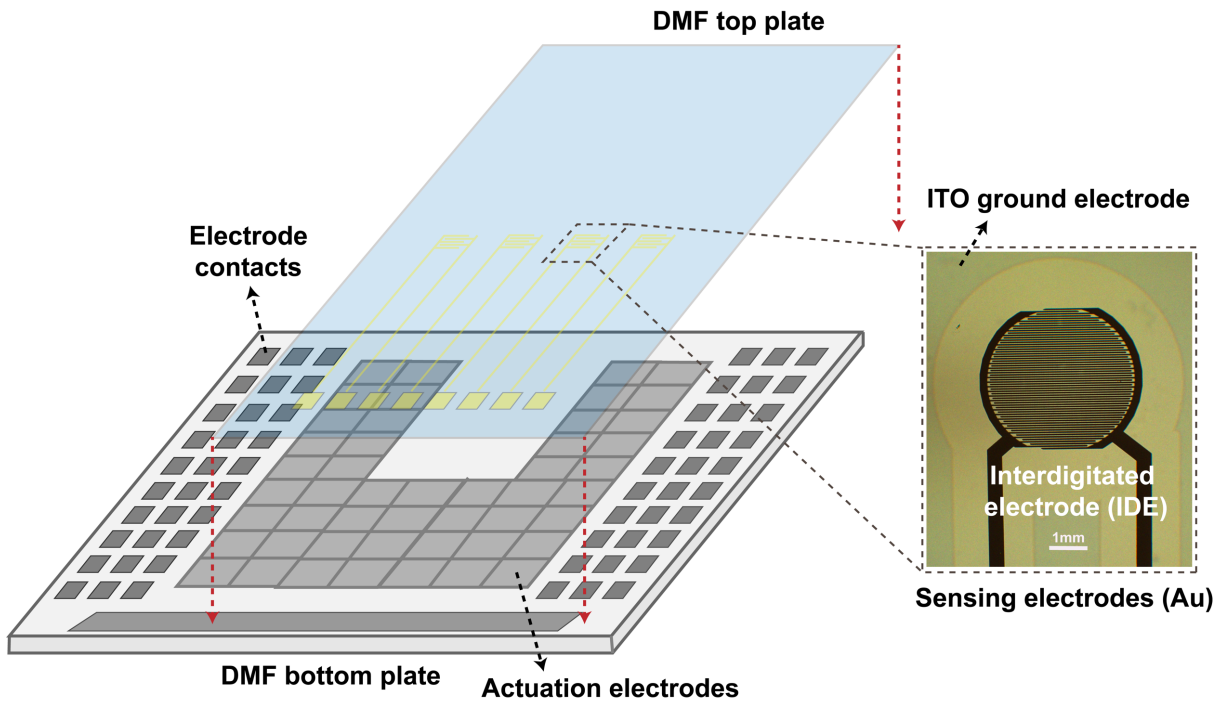


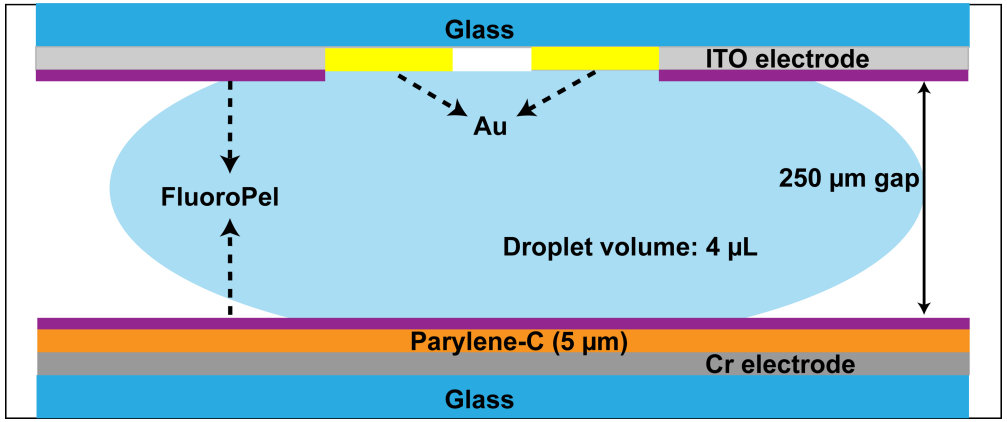


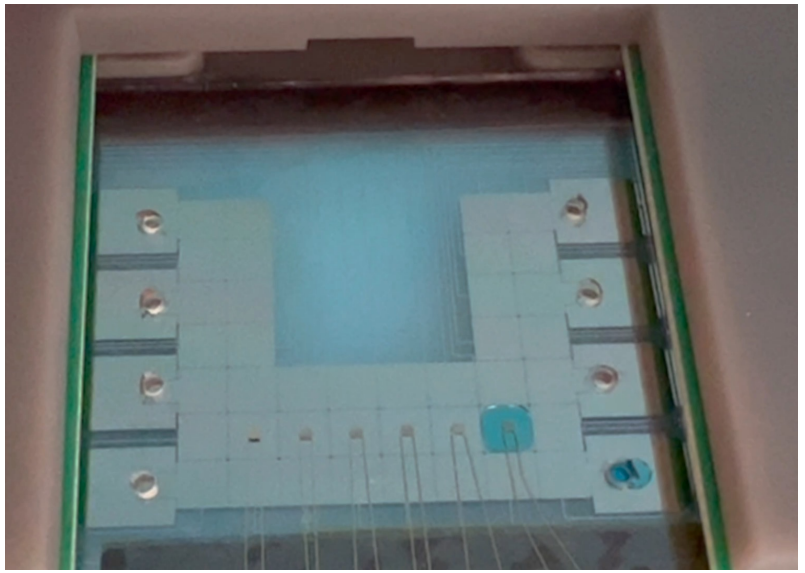
- Integrated DMF system
- DropBot operation system
- Droplet control panel



Layout of the integrated DMF device





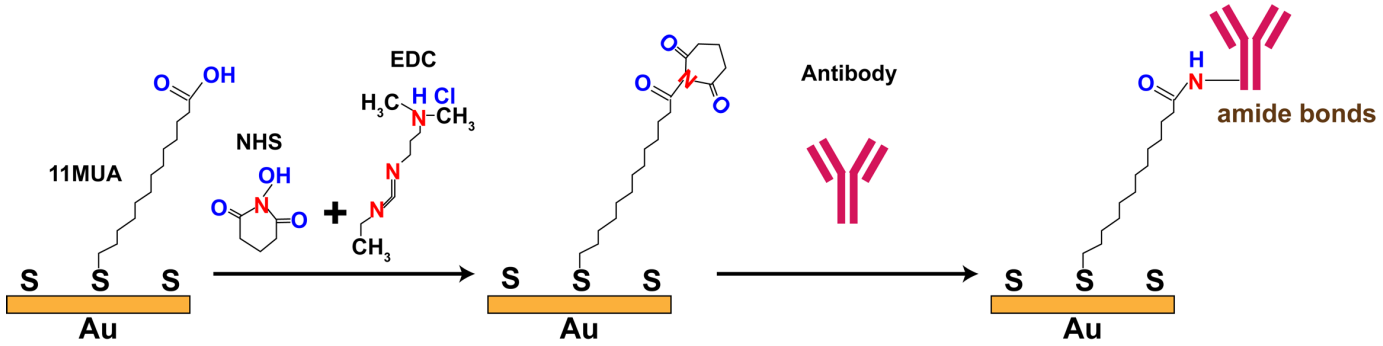


Overview of DMF device with integrated interdigitated electrodes:

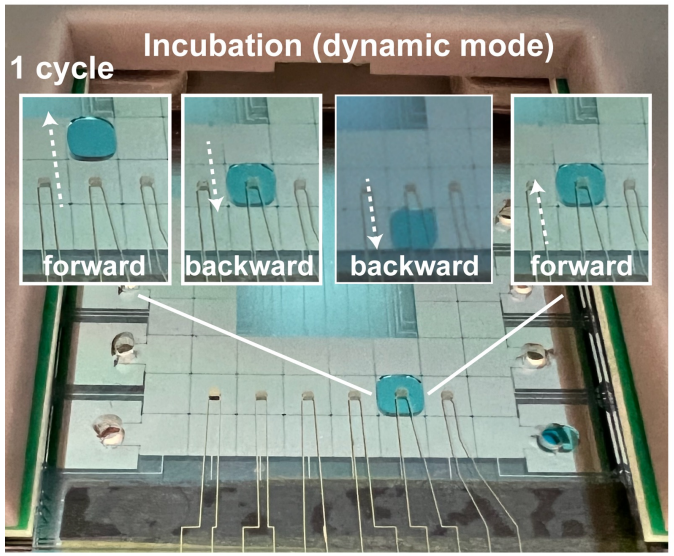
- **bottom plate**: actuation electrode array to manipulate droplet
- **top plate**: IDE sensing electrode embedded in Indium Tin Oxide (ITO) ground electrode

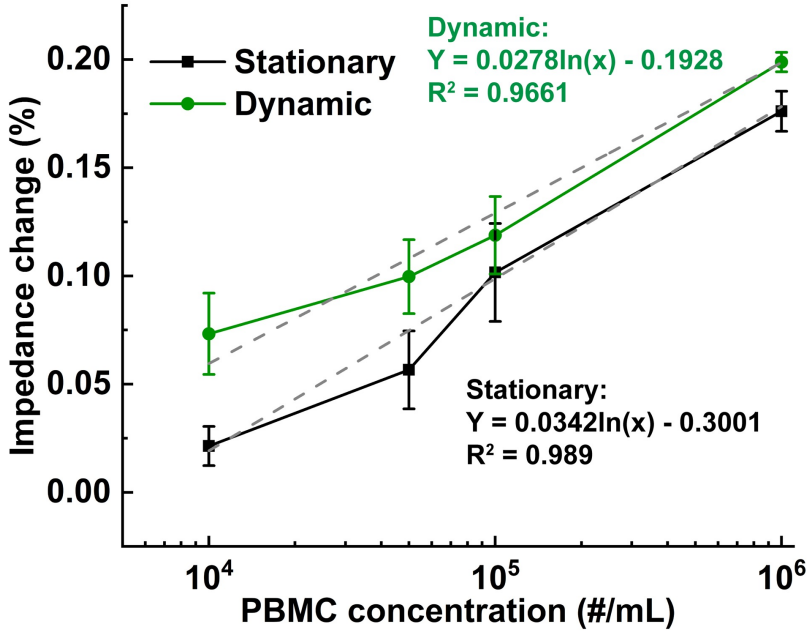
Zhang, Yuqian, and Yuguang Liu. "A digital microfluidic device integrated with electrochemical impedance spectroscopy for cell-based immunoassay." *Biosensors* 12.5 (2022): 330.

Quantification of PBMC abundance: thiol-carboxyl self-assembled monolayer (SAM) + Cell assay



Dynamic incubation mode: forward-backward-backward-forward





- Low sample volume (4 µL) for rapid detection (20 min)
- 2.4X **enhanced detection signal** in dynamic mode
- Detect as low as 10^4 PBMCs/mL, **~two orders of magnitude** less than the biologically relevant range (0.7–6.2*10^6 cells per mL of blood)

Soluble PD-L1 protein (sPD-L1)

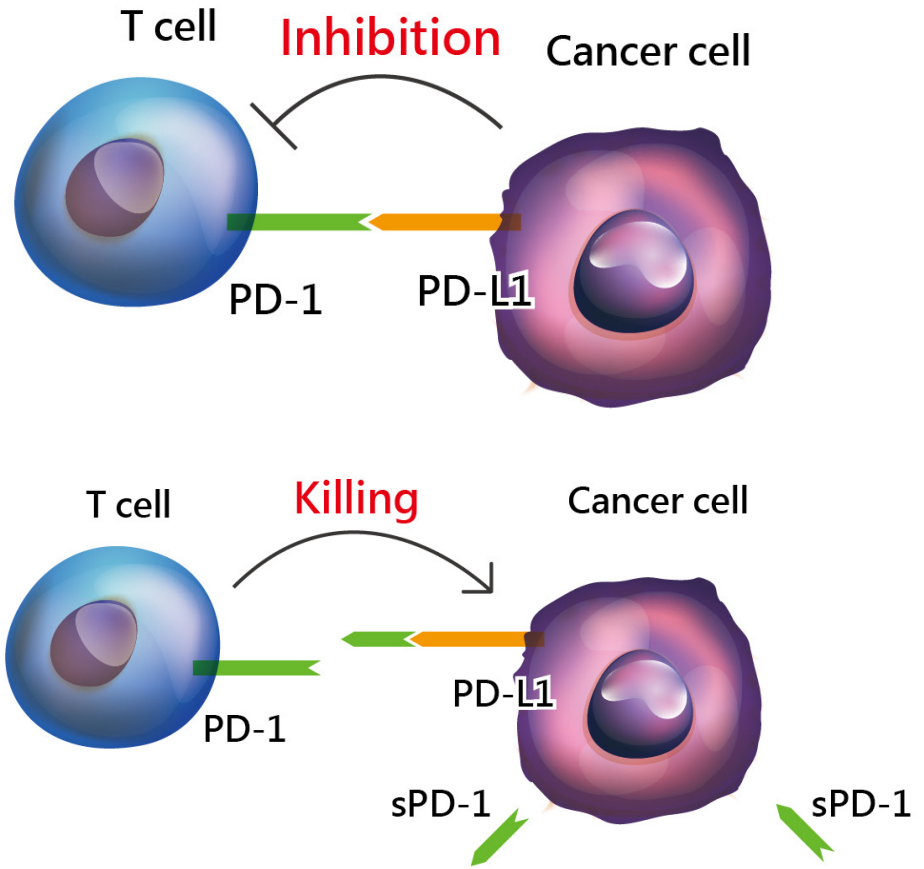
- The first functionally characterized ligand of the coinhibitory programmed death receptor 1 (PD-1)
- PD-L1 expression is generally associated with *poor prognosis*

❑ **Conventional detection method: ELISA**

✖ Labor-intensive

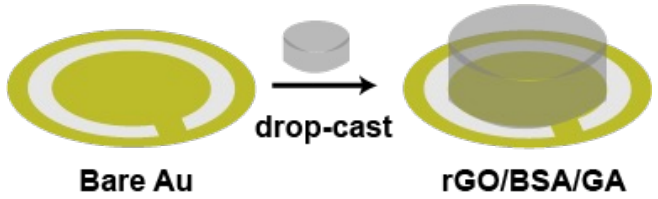
✖ Extended turnaround time: sensitivity ∝ hybridization time

- Best sensitivity performance (8 pg/ml) for commercial sPD-L1 ELISA kit



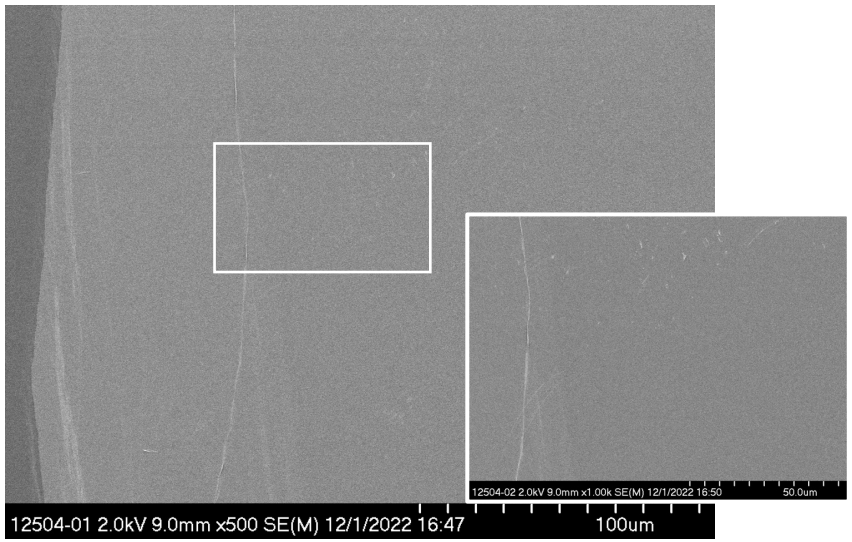
❑ A 3-dimentional microstructure for PD-L1 capture

- Reduced graphene oxide (rGO): large surface area, high conductivity
- Bovine serum albumin (BSA): reduce the non-specific binding
- Glutaraldehyde (GA): cross-link BSA molecules to create a 3D protein matrix

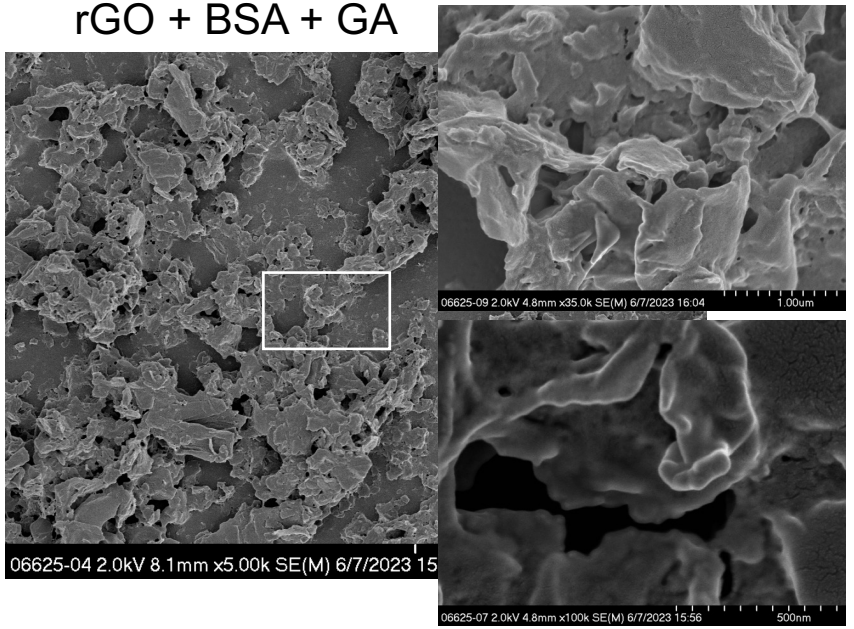


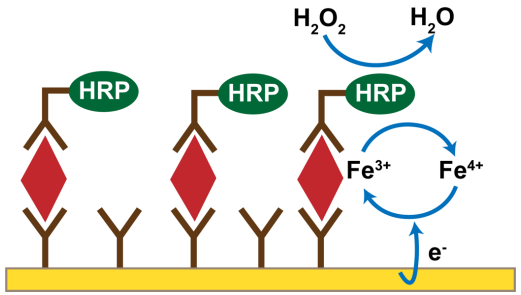
❑ SEM characterization

Bare Au electrode



rGO + BSA + GA



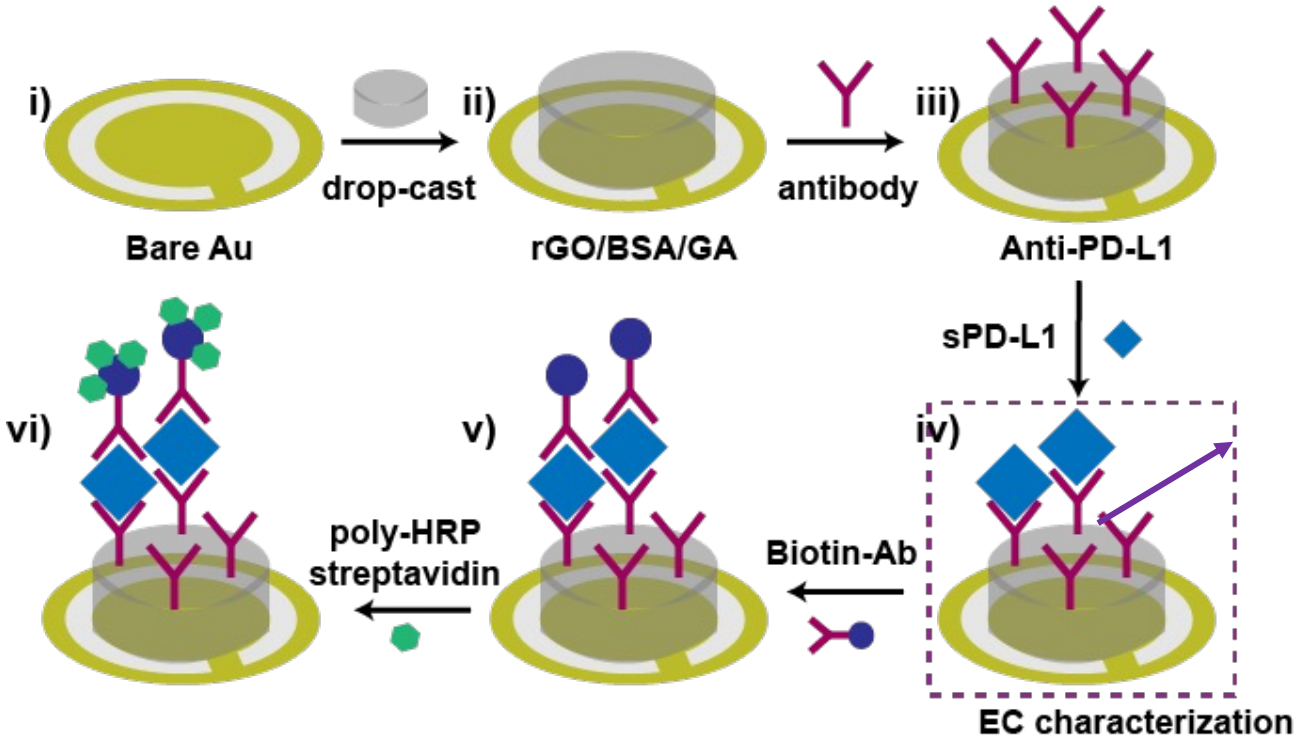


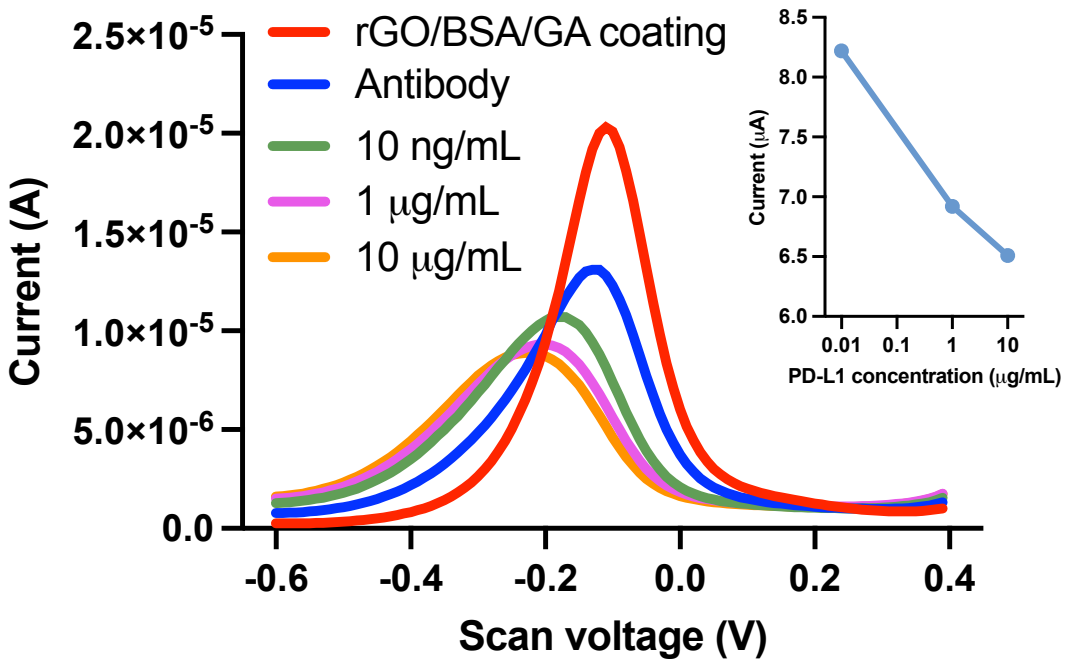
Electrochemical sensor

Zupančič, Uroš, et al. "Graphene Enabled Low-Noise Surface Chemistry for Multiplexed Sepsis Biomarker Detection in Whole Blood." *Advanced Functional Materials* 31.16 (2021): 2010638.

❑ Electrochemical validation of immunoassay

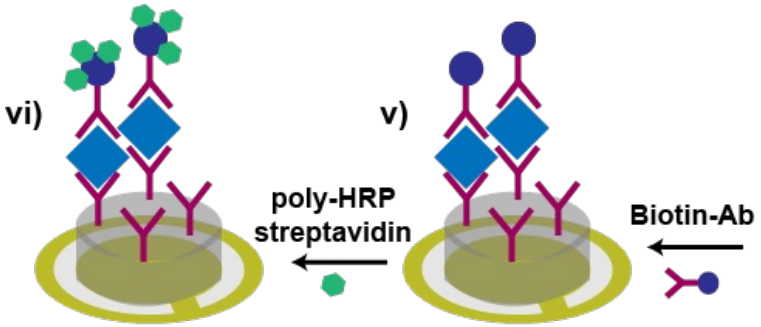
- The formation of sandwich immunoassay
- Signal shifting: the inhibition of charge transfer between the sensor surface and the electrolyte solution

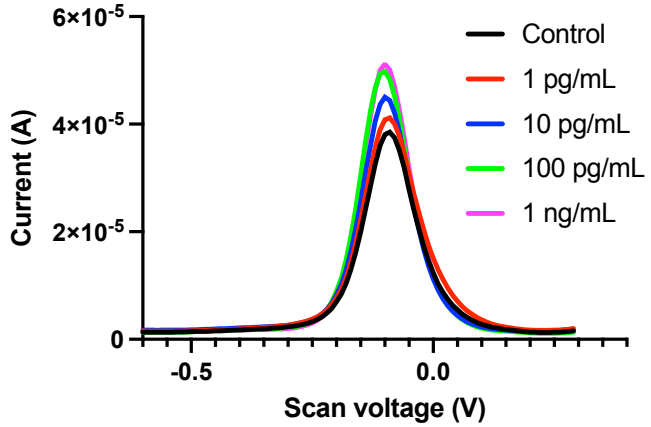


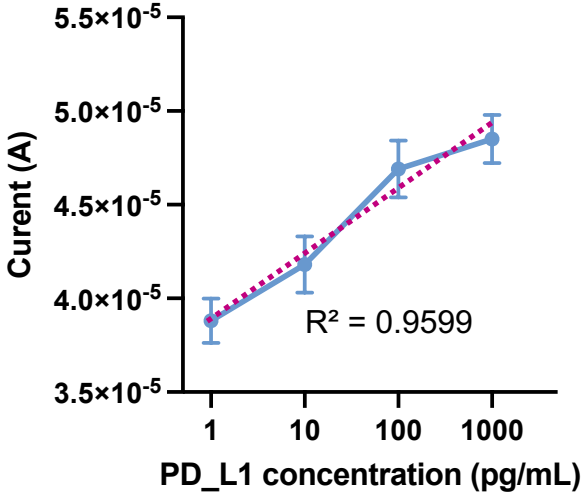


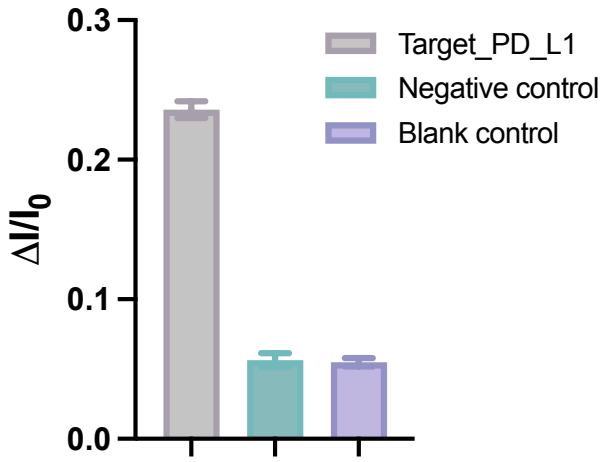
❑ Quantitative detection of sPD-L1 with electrochemical biosensor

- Able to detect sPD-L1 concentration down to 1 pg/mL
- High specificity

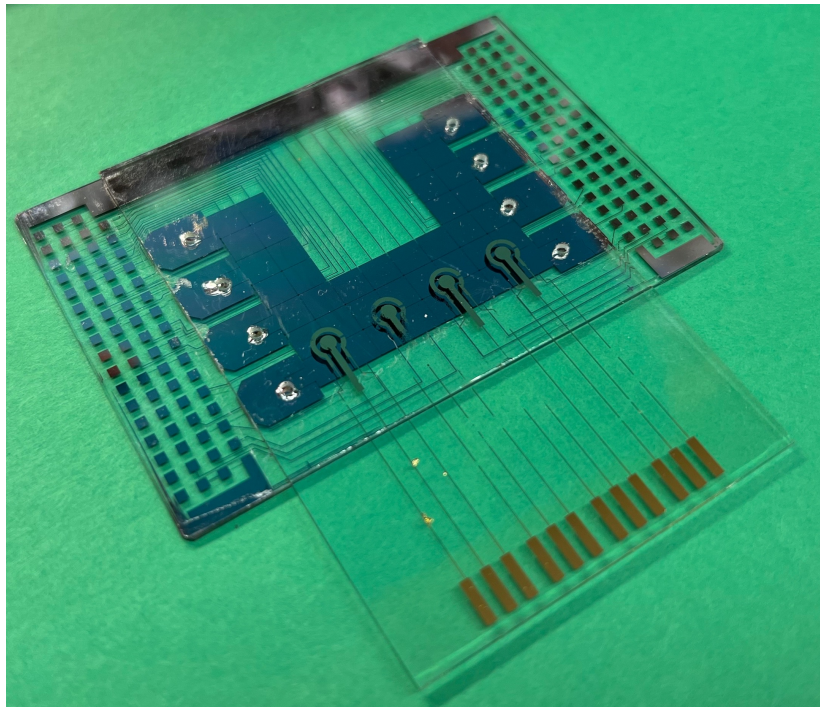






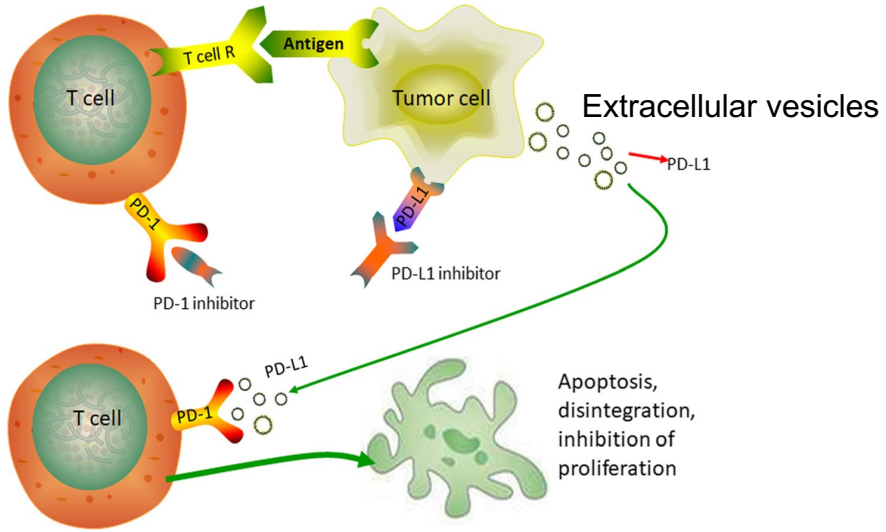


Electrochemical biosensor integrated with digital microfluidic platform



Extracellular vesicles PD-L1 (evPD-L1)

- EV derived PD-L1 has a similar function to tumor PD-L1.
- EvPD-L1 binds to PD-1 on T cells, induce T cell apoptosis, and inhibit T cell activation and proliferation



❑ Conventional separate and detection methods

- Ultracentrifugation

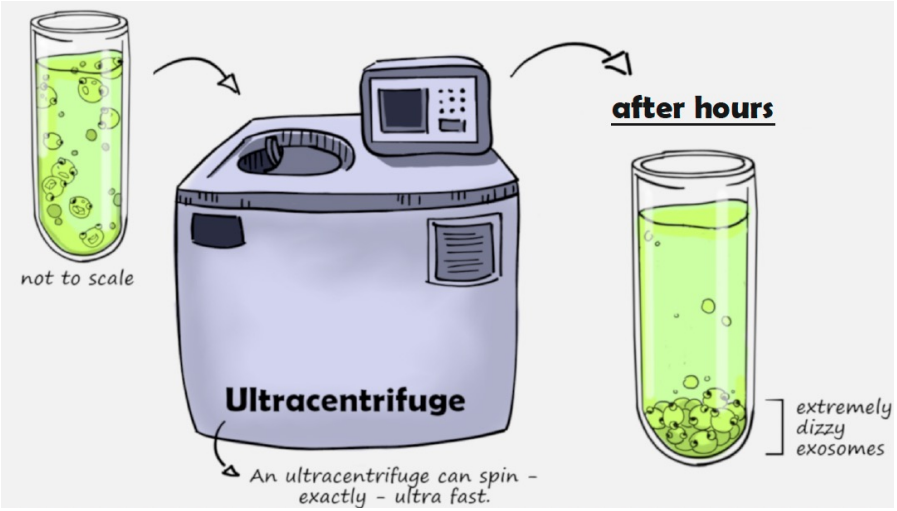
✖ Large sample volume, bulky instrumentation

✖ Time-consuming

- Western blot/nano-flow cytometry

✖ Labor-intensive

✖ Require sample pre-concentration



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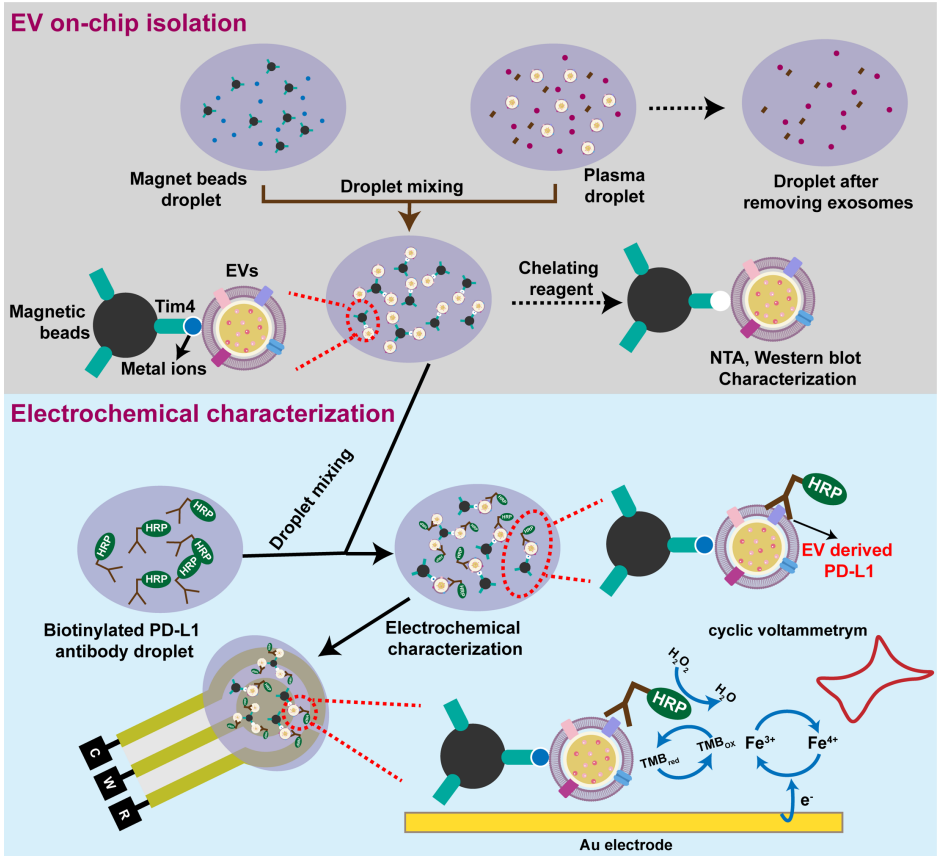
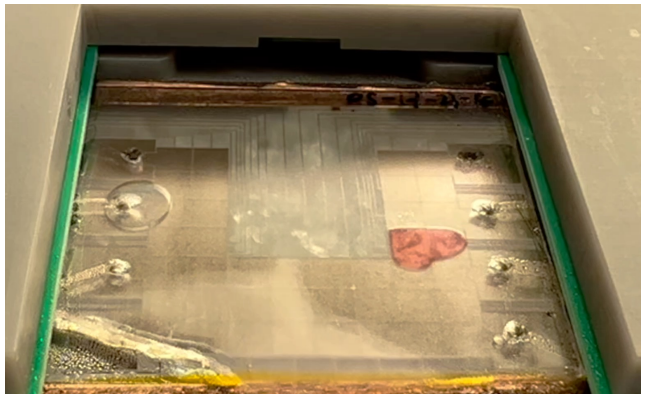
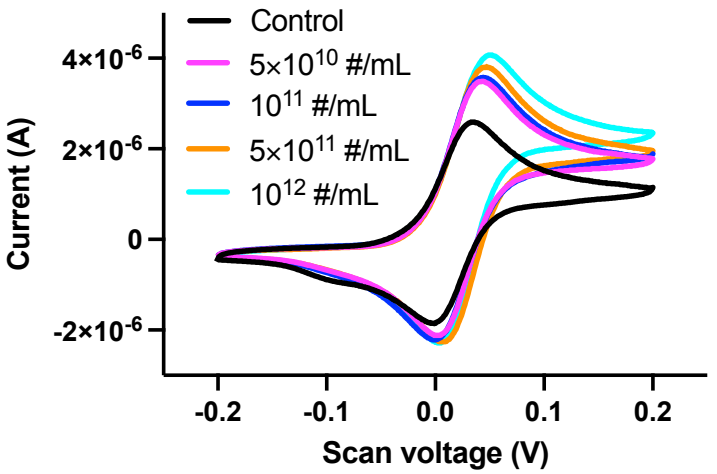
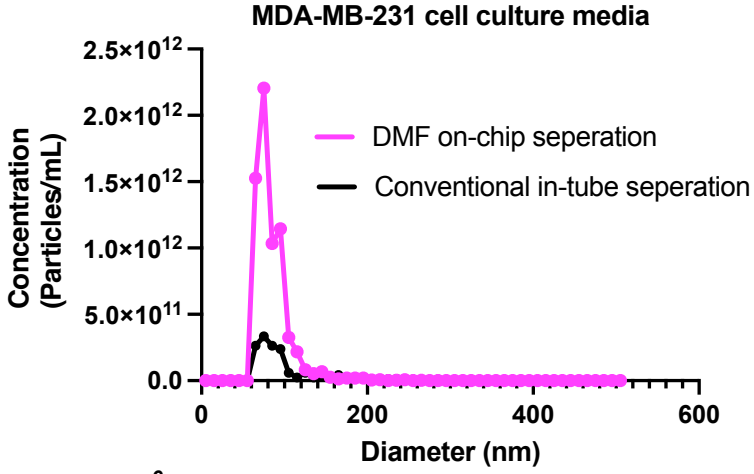
❑ On-chip separation and detection of evPD-L1

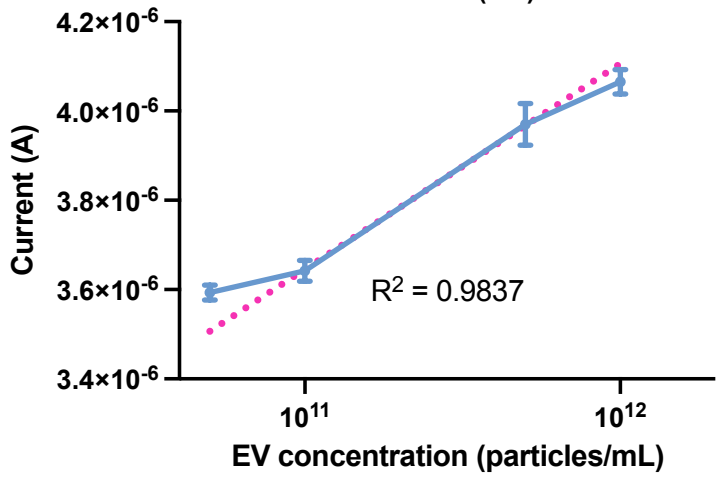
- Immunomagnetic separation on DMF chip: 2 hours, automated; ~ 6X higher capture efficiency
- Qualitative detection of EVPD-L1 with electrochemical sensor











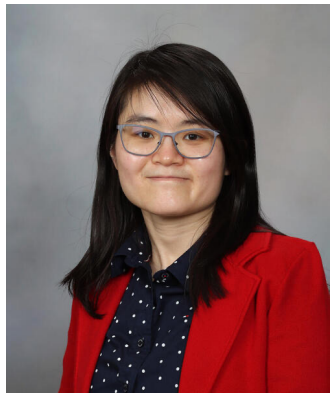
❑ Summary

- A novel DMF platform integrated with multiplexed electrochemical sensors to detect PBMCs, sPD-L1 and evPD-L1.
- Dynamic incubation enhances hybridization.
- Integrate sample prep and detection, 2 hours turnaround time.

❑ Examples of utility

- Pretreatment evaluation: PD-L1+ EVs >0.55 ng/ml: T cell over-exhaustion
- In-treatment evaluation: >2.08X changes in PD-L1+ EVs reflect successful anti-tumor immunity

❑ Acknowledgements



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- Department of Physiology & Biomedical Engineering
- Microbiome Program, Center for Individualized Medicine

NIH National Institutes of Health

Thank you for listening!
Any questions?