

Multiplexed analysis of extracellular vesicles from single cells in an array with opposing microwells

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Summary:

Single-cell analysis can help to resolve the heterogeneous and complex biogenesis of extracellular vesicles (EVs), thereby improving our understanding of EVs' role in signalling and cell-cell communication. Here, we introduce a method to detect up to five membrane proteins in EVs secreted from single cells. Two microwell arrays, one for single-cell trapping and another one for capturing fluorescently barcoded beads pre-functionalized with EV-capture antibodies, are aligned during incubation. Then, membrane proteins of bead-bound EVs are analysed by immunostaining for four breast-derived cell lines.

Keywords: single cell, extracellular vesicles, microfluidics, sandwich immunoassay, epifluorescence microscopy.

Introduction

Extracellular vesicles (EVs) mediate intercellular communication between a parental cell and a recipient cell, both in healthy and pathological states. In the context of cancer, EVs can promote metastasis, tumour vascularization, suppress the local immune response, or contribute to treatment resistance via exiting pathways [1]. EV phenotypes are highly heterogeneous due to the various biogenesis and secretion pathways. To reduce complexity in the analysis of EVs, recent efforts focused on the development of single-cell EV analysis methods [2].

Here, we present a method, where we align two microwell arrays for the multiplexed analysis of EVs by immunostaining. We co-detect five distinct EV membrane proteins from the breast cancer cell line MDA-MB-231. Moreover, we compare EV secretions from four distinct breast cell lines.

Microfluidic Platform Description

The microfluidic device builds on our previous work [3]. It consists of a polydimethylsiloxane (PDMS) layer with over 2700 square wells (60x60x60 μm^3) for capturing individual cells, aligned with an upper array made of glass, which contains smaller microwells to trap paramagnetic beads functionalized with specific EV capture antibodies (Fig. 1). The intensity ratios between two far-red fluorophores serves as barcode and enables to assign a bead to the specific EV capture antibody (Fig. 2). All captured EVs are detected afterwards with another antibody targeting the tetraspanin CD9 which is labelled with the fluorescent protein phycoerythrin (PE).

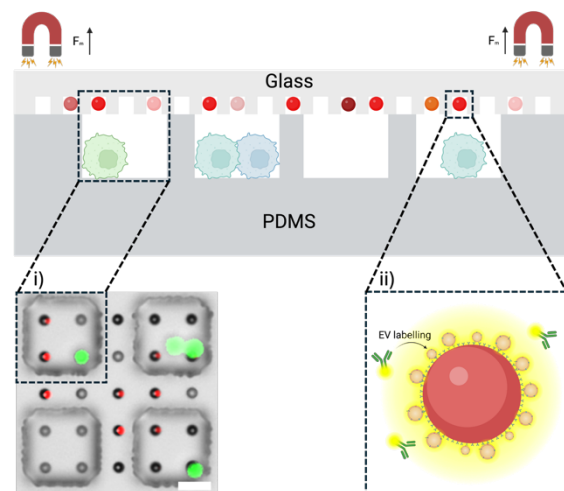


Fig. 1. Schematic of the microfluidic device during incubation. i) Microscopy image with cells (green) and beads (red). Scale bar = 60 μm . ii) Bead-bound EVs are labelled with a phycoerythrin-conjugated detection antibody via a two-step sandwich immunoassay.

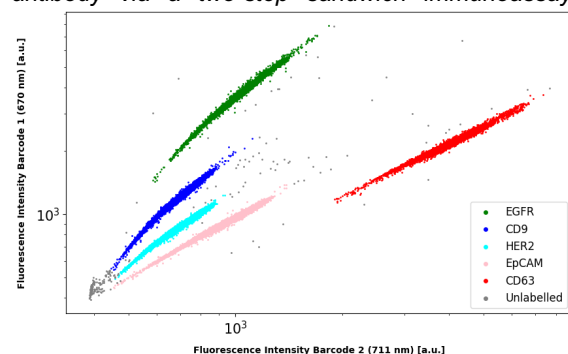


Fig. 2. Bead classification, where each bead is assigned to a specific EV capture antibody (Procarta-plexTM).

The experimental workflow consists of coating both arrays with a 1% bovine serum albumin

(BSA) solution, before loading the beads and cells. Both arrays are then aligned and clamped to seal the individual microwells. Following a 16-hour incubation, the arrays are unclamped, and bead-bound EVs are detected by addition of the PE-labelled antibody. Images of the two microwell arrays are acquired with an epifluorescence microscope, then processed and analysed with an in-house Python-based software. Thereby, the average fluorescence intensity measured on the beads in one well can be assigned to the corresponding co-incubated single cell, stained with cellTracker® Green. Wells with no cells, dead cells or multiple cells are discarded.

Results & Discussion

First, the platform was used to detect five distinct EV phenotypes from triple negative breast cancer cell line MDA-MB-231 (Fig. 3). Secreted EVs were selectively immobilised via one of five membrane proteins: tetraspanins (CD9, CD63), HER2, EGFR or EpCAM. All EVs were detected via anti-CD9 detection antibody. The overall high intercellular heterogeneity and larger signals observed for tetraspanin-positive EVs is aligned with existing literature [4]. The lower fluorescent signals for EpCAM-positive EVs confirms the mesenchymal phenotype of MDA-MB-231 cells, and the low level of HER2 is in accordance with prior observation [5].

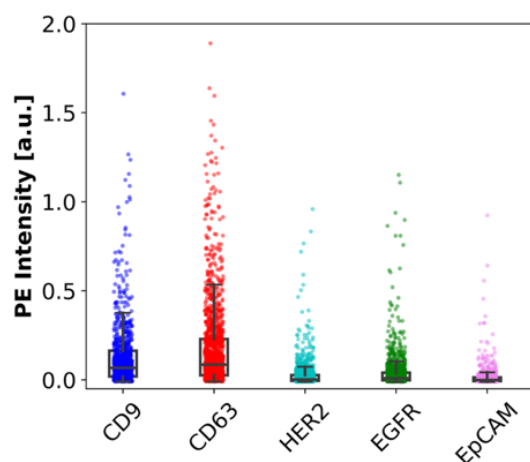


Fig. 3. EV detection from MDA-MB-231 cells. Secreted EVs were immobilized via one of five capture antibodies and labelled with CD9 detection antibody.

Last, EVs from different breast cell types were compared: one benign breast cell line MCF-10A and three breast cancer lines MCF-7, SkBr3 and MDA-MB-231. EVs were captured on the beads via CD63 and detected via anti-CD9 detection antibody (Fig. 4). Significant differences in average fluorescence intensity can be observed between the cell types. The data confirm the co-expression of the tetraspanins CD63 and CD9 in all four cell types, with high levels in at least one

of the proteins for MCF-7 and MDA-MB-231. The signal variation in all data reflect the general heterogeneity of EVs with regard to protein levels.

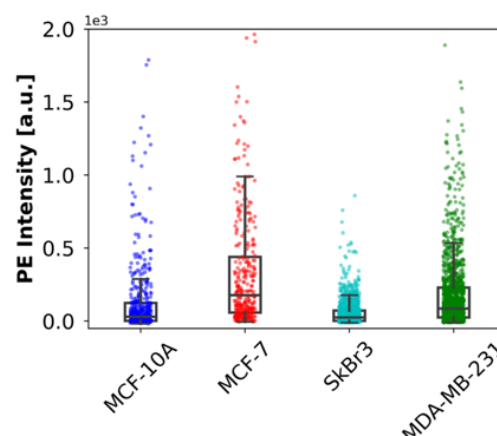


Fig. 4. EV detection from four breast cell lines. Secreted EVs were immobilized via CD63 capture antibody and labelled with CD9 detection antibody.

Conclusions

We present a microwell array for the analysis of extracellular vesicles derived from single cells. The use of barcoded functionalised beads enables to measure distinct EV phenotypes on a single device. In future work, we intend to investigate the changes in EV secretions upon drug treatment to evaluate the potential of EVs as a biomarker for therapy success.

References

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