Sorting cell subtypes from murine melanoma on Highway1:
A comparison with conventional droplet sorting

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Introduction

Isolation of various cell types from the tumour microenvironment is essential for a range of downstream processes including outgrowth for disease models, sequencing, or metabolic profiling. For all these techniques, cells must be in a viable, unperturbed state to ensure analysis is reflective of physiological conditions.

Here, we present a simple workflow (Figure 2) for the selection of various cell types from a murine melanoma using the Highway1, the fastest microfluidic cell sorter, using the patented VACS (Vortex Actuation Cell Sorting) technology to gently sort target cells.

We also compare the outgrowth of cells from the tumour microenvironment sorted on the Highway1 with the incumbent selection method (droplet sorting) and show the Highway1 can preserve the viability, growth and function of tumour cells more effectively.

With a strong focus on gentleness, sterility and ease of use, we show the Highway1 is the ideal sorter for isolating cells from the tumour microenvironment.

Figure 1: *The Highway 1 cell sorter and sterile cartridges.*

In collaboration with the VIB in Leuven, we present a workflow to select and sort cell subtypes from a subcutaneous murine melanoma. Using microscopy to assess vaiblity and outgrowth, we show cells sorted with the Highway1 (Figure 1) survive and grow better than cells sorted with the incumbent droplet sorter.

Workflow

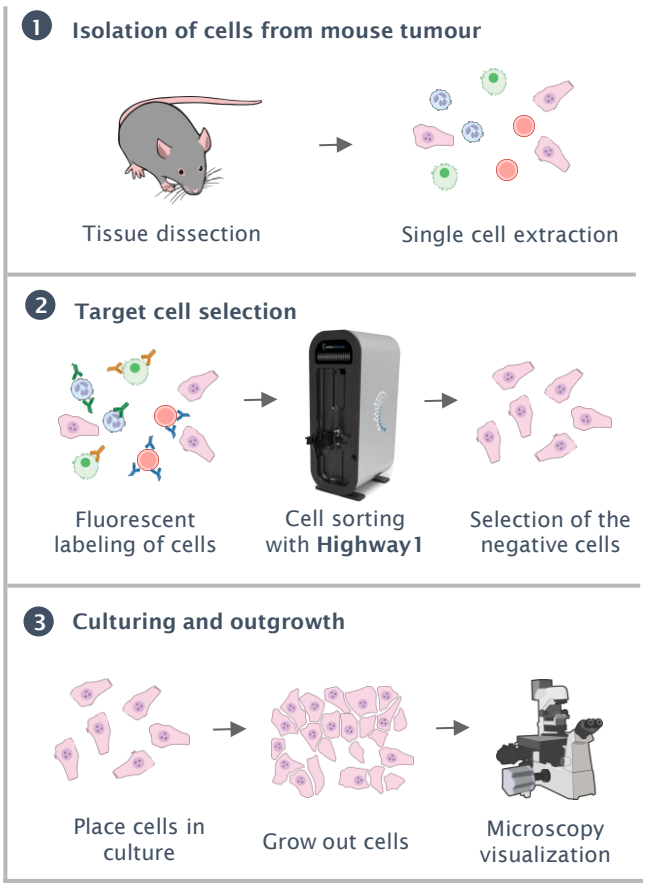

Figure 2: *Experimental workflow overview.*

Illustrations from NIAID NIH BIOART Source.

Sample preparation

Subcutaneous murine melanoma tumours were collected, minced and digested at 37°C for 45 minutes in digestion mix (1 mg/mL Collagenase P, 5 mg/mL Dispase II and 0.4 mg/mL DNase in 0.1% BSA RPMI) followed by an additional digestion step in TrypLE Express for 2 minutes at 37°C. Digested material was incubated in Red Blood Lysis Buffer for 5 minutes on ice before proceeding to immunostaining.

Cells were stained with fluorescently labelled antibodies targeting either CD45, Notch3, SMA or CD31 to identify the immune cells, pericytes, fibroblasts, and endothelial cells respectively (Table 1).

Identification and selection of tumour microenvironment cell subtypes

Single stained samples for immune cells, pericytes, fibroblasts, and endothelial cells were sorted on the Highway1. To identify cells, each sample was first gated on FSC-A vs SSC-A, then voltages for the fluorescent channels were set and compensation was applied. Gates for each of the fluorescent markers were set using the unstained control (Figure 3).

To sort, cells were diluted in DPBS (Calcium and Magnesium free) + 0.1% P188 at a concentration of 1.5million/mL, then sorted on Highway1 at 6,000 events per second in Purity mode at 4 °C. Post sort, all cells were viable and the pericytes, fibroblasts and endothelial cells adhered when placed in culture.

Sorting CD31 CD45 double negative cells on Highway1 vs the incumbent droplet sorter

Cells were stained with CD45 FITC and CD31 APC, then resuspended in DPBS (Calcium and Magnesium free) + 0.1% P188 and kept at 4 °C. The gating strategy for Highway1 and the incumbent droplet sorter is shown in Figure 4. On the Highway1, cells were run at 6,000 events per second in Purity mode. On the incumbent droplet sorter, cells were sorted at 2,500 events per second using the 130µm nozzle in four-way purity mode.

Target	Fluorochrome	Cell Types
CD45+	FITC	Immune cells
Notch3+	PE	Pericytes
SMA+	eFluor660	Fibroblasts
CD31+	APC	Endothelial cells

Table 1: *Antibody panel used to identify cell subtypes within the tumour microenvironment.*

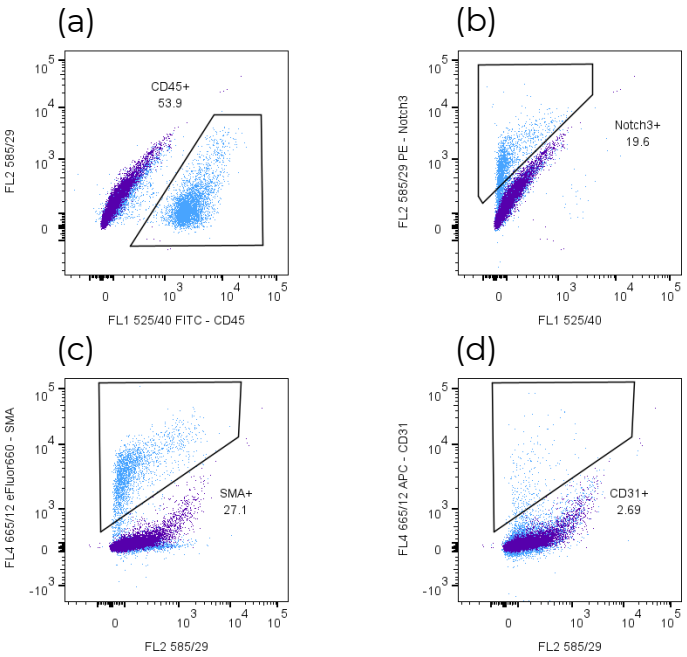

Figure 3: *Gating strategy for CD45+ cells (a) Notch3+ cells (b) SMA+ cells (c) and CD31+ cells (d). Unstained control used to set the gate is shown in purple.*

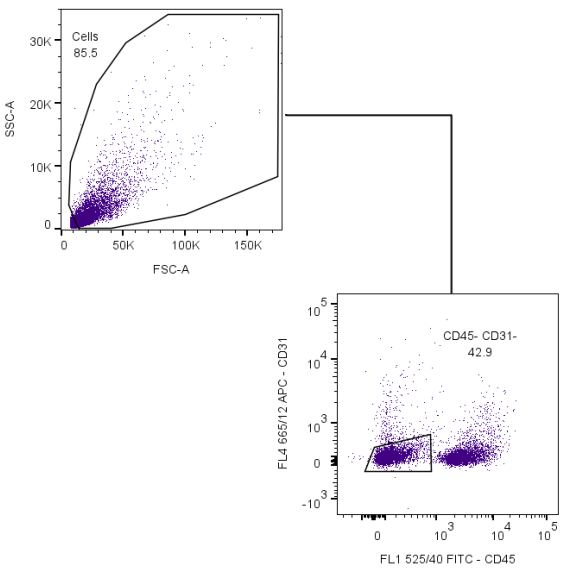

Figure 4: *Gating strategy for CD45 CD31 negative cells. Cells were identified using FSC-A vs SSC-A, then FITC CD45 and APC CD31 negative cells were selected.*

(a)

Highway1

(b)

Incumbent droplet sorter

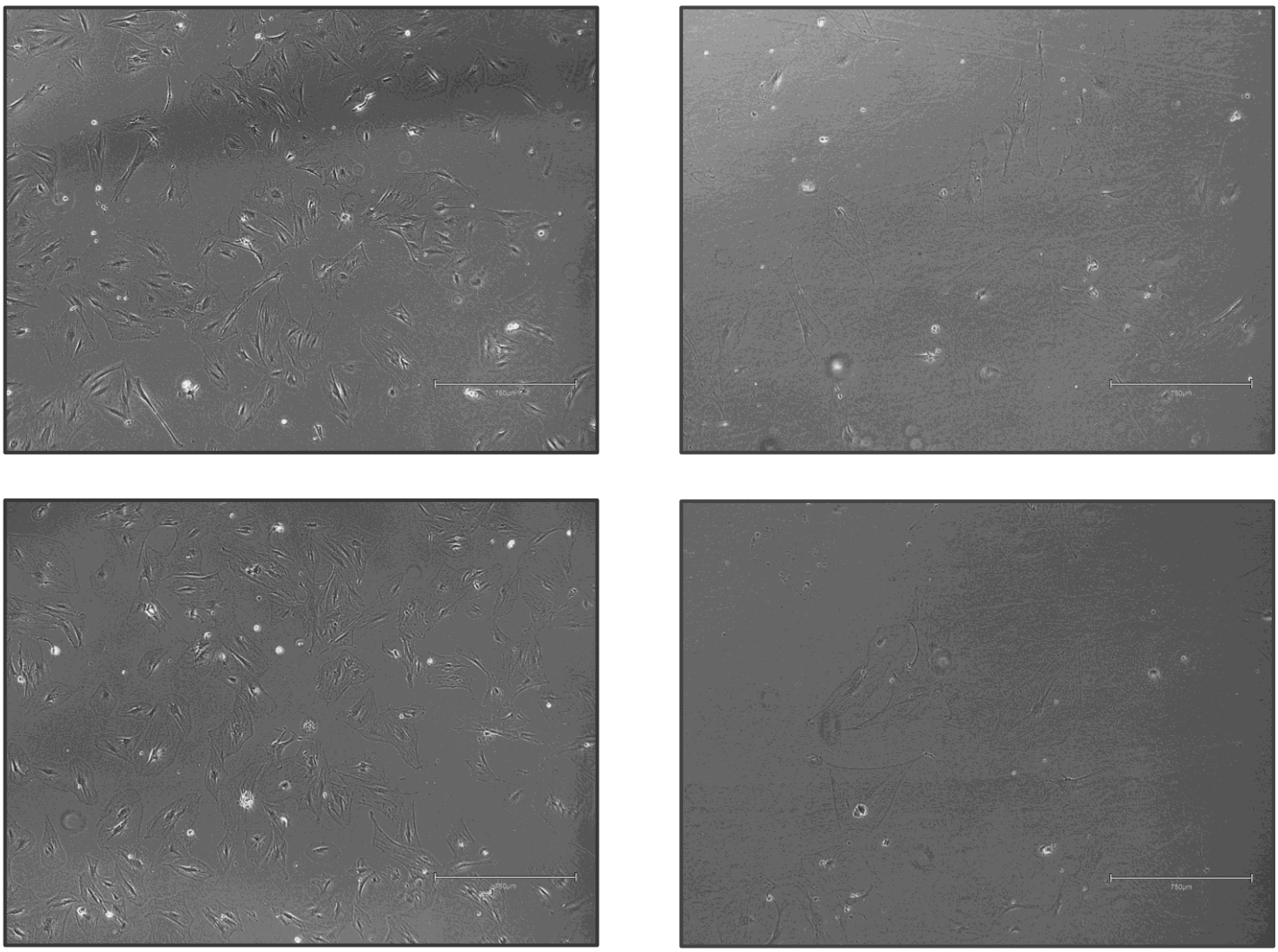

Figure 5: *Outgrowth of cells from tumour microenvironment sorted on Highway1 (a) vs incumbent droplet sorter (b).*

Following sorting, cells were placed in culture. Post-sort it was observed that the cells sorted on the Highway1 recovered much more quickly and were actively dividing. The cells sorted from the incumbent droplet sorter could only be passaged twice and then became senescent, whereas the cells from Highway1 were passaged eight times and continued to grow as of writing this (Figure 5).

Whilst it's unknown exactly what causes cell stress post-sorting, here we show the Highway1 is the ideal candidate for sorting sensitive cells in a fast and sterile manner using its patented VACS technology.

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Summary

- The Highway1 containing VACS technology is the fastest, gentle microfluidic sorter on the market, making it ideal for sorting cells from the tumour microenvironment
- Various cells including endothelial cells, pericytes and fibroblasts were sorted on the Highway1 successfully
- CD45 and CD31 double negative cells sorted on the Highway1 performed far better in terms of viability and growth compared to the incumbent droplet sorter