PROGRAM N°362 B224

Impedance-based Pipetting of Rare Cells at Single-Cell Resolution and Minimal Loss: From FACS to DispenCell

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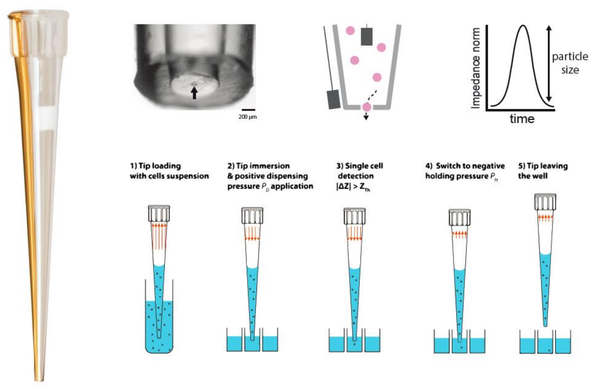
INTRODUCTION

Single cell isolation is a key enabling step in many applications in Life Sciences. Yet, it is a bottleneck when the cells of interest are isolated from scarce tissue samples. A large number of rare cells are usually needed when using current isolation process based on cell sorters. Moreover, it is often not possible to sort different sub-populations at the same time during a cloning sort procedure. The only solution is to go through two consecutive sorts which can cause significant cell loss. In addition, droplet cell sorting does not allow to know with certainty whether a well contains a cell or not. A colorimetric method[1] can be used to verify whether a well has received a single sorted droplet or not, but this does not guarantee that a cell is actually present at the bottom of the well. Here, we tested a new solution for traceable single cell isolation called DispenCell from Seed Biosciences. This new solution consist of an impedance-based pipetting robot for automatic single cell traceable dispensing, a disposable tip to avoid contamination and a software (LIMS) for post-processing single cell data and QC.

Figure 1: DispenCell

IMPEDANCE-BASED TIP

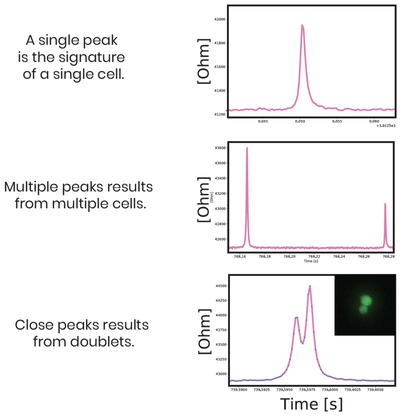
The robot hosts an impedance analyzer connected to a computer and a micro-pump, coupled with a sterile disposable sensing tip that acts as a Coulter counter. The sensing tip features an external and an internal electrode. A 30 µm-diameter aperture in its center seals the lower end of the tip.



Altogether the device senses and records every single particle or cell that flows through the aperture of the tip. A feedback is sent as soon as a particles passes through the tip, in order to stop dispensing and pass to the next well.

SYSTEM PERFORMANCE VALIDATION

In a first assay, we used the FACS ARIA™ FUSION, to sort 500 beads (10 µm, green fluorescent polystyrene beads) into an Eppendorf tube with 30µl of loading solution. Then, we tested the capability of the DispenCell to dispense single beads in a 384-well plate.

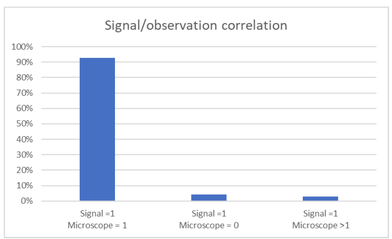


Single particles were dispensed and each impedance profile is magnified and examined in detail.

A Single and sharp peak is the signature of a single particle whereas multiple peaks result from doublets or multiple particles.

After dispensing, the plate was centrifuged at 3500 rpm for 5' and each positive well was then examined under a microscope for the presence of a fluorescent bead. Results from the imager and DispenCell software were compared.

		Bead assay Number of cases
	Not checked	104
		268 (93%)
	Empty well	12 (4,3%)
		8 (2,7%)



The plate filling rate was the 75% (288 / 384 wells). 104 wells were rejected by DispenCell because they contain more than one cell, these wells have not been checked by microscopy. From the 288 wells containing a single cell: 93% of the wells were plated with 1 cell, ~4% were empty and ~3% were filled with 2 cells.

REFERENCES

1 : A rapid method to verify single-cell deposition setup for cell sorters. Rodrigues OR, Monard S. Cytometry A. 2016 Jun;89(6):594-600

WORKFLOW

In a second assay, we sorted four times 500 CHO-GFP+ cells into four different Eppendorf tubes containing 30µl of loading solution. Then, we tested the capability of the DispenCell to dispense single CHO-GFP+ presorted cells in four 96-well plates.

For every plate the DispenCell software gives a clear visual representation of the wells that will be monoclonal (one single cell; in green) or that have to be discarded (either 0 cell or more than one; in red)

Figure 3: DispenCell software plate display.

96 Plate	Plate Filling Rate %
1	77
2	73
3	76
4	71

74.5% of wells contained only one cell. However, the parameters used were the same as those used with the beads and these parameters should be adjusted for each cell line in order to increase this number.

All clones isolated with DispenCell showed high cell density and viability after a few days of culture.

CONCLUSIONS

These experiments showed that impedance-based dispensing DispenCell is able to plate single cells from different kind of samples with minimal cell loss. DispenCell combined with cell sorting, or cell enrichment technology to retrieve a subpopulation of rare cells of interest, provides a complete workflow for single cell plating.

DispenCell software provides an immediate, robust, visible and traceable proof of single cell isolation that can be used for other downstream applications

ACKNOWLEDGMENTS

We thank J.Pontis (Prof Trono Lab) for providing the cells used in these experiments. We also thank all the team from SEED Biosciences SA for all the support.

DB and PR have financial interests in SEED Biosciences SA, a company commercializing DispenCell.