

Label-free isolation of single cells for IVD cell therapy using impedance flow cytometry

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Summary: Impedance flow cytometry (IFC) is an emerging label-free, high-throughput sorting method that reads cell-specific electrical signatures across frequencies. This work demonstrates the feasibility of isolating presumptive nucleus pulposus progenitor cells (NPPC) and benchmarks its performance to FACS. Results suggest that IFC accurately identifies and sorts NPPCs, representing a significant advancement for cell-based therapies targeting intervertebral disc degeneration.

Keywords: Label-free sorting, progenitor cells, impedance flow cytometry, microfluidics, intervertebral discs

Introduction

Intervertebral disc (IVD) degeneration is a primary cause of low back pain, a global health issue affecting around 7% of the population [1] and costing billions to society [2]. Existing interventions, such as tissue resection and spinal fusion fail to regenerate disc tissue. Population aging and limitations of current treatments motivate the development of alternative therapies.

While nucleus pulposus cells (NPC), which reside within the inner region of IVD, exhibit limited regenerative capacity, their progenitor cells (NPPC) are believed to play a pivotal role in developing novel therapies [3], provided they can be effectively isolated. NPPC differ from mature NPC by expressing the Angiopoietin-1 receptor, also called Tie2.

Single-cell isolation is essential in biology, biotechnology, precision medicine [4]. While fluorescence-activated (FACS) and magnetic-activated cell sorting (MACS) are well established, high-throughput sorting methods, they present significant drawbacks: expensive reagents, time-consuming labeling protocols, and the absence of specific markers for certain cell types [5], limiting their applicability in cell therapy [6]. Several label-free alternatives are under development, including impedance flow cytometry (IFC), which discriminates cells based on electrical properties across multiple frequencies.

This study assesses the potential of IFC for NPPC isolation compared to FACS, evaluating the impact of both technologies on cell viability and phenotype.

Methods

A previously developed impedance flow cytometer [7], utilizing a transimpedance amplifier, FPGA, and co-planar electrodes, was coupled with a piezo-based sorting mechanism [8, 9]

to enable single-cell sorting based on the measured electrical characteristics of cells.

Bovine coccygeal tails obtained from a local abattoir provided nucleus pulposus tissue from proximal discs, which was minced, sequentially digested with pronase and collagenase II, then filtered and washed with PBS. The resulting NPC were then suspended in 1x PBS at a concentration of 1M/mL. Electrical characteristics were measured at 1 and 10 MHz with a 1.2 V peak-to-peak excitation. Initially, Tie2⁺ and Tie2⁻ populations were isolated via FACS using the antibody bs-1300R-BF488 (Bioss), and their electrical signatures were compared to raw bovine NPCs (Fig. 1, 2). Subsequently, the impacts of FACS, IFC, and the handling after the IFC (dilution, centrifugation) on the sorted populations were investigated with a FACS (Table 1) and the distributions compared to the initial FACS. Finally, the IFC system sorted bovine NPCs, and the resulting populations were analyzed by FACS. These latter results are currently under evaluation and will be presented at the conference.

Results

Impedance analysis of bovine NPCs sorted by FACS revealed that both Tie2⁺ and Tie2⁻ cells form clusters that partially overlap (Fig. 1). However, with the data of both frequencies, a quadratic support vector machine (SVM) achieves an identification accuracy exceeding 90% (Fig. 2). Furthermore, both Tie2⁺ and Tie2⁻ clusters were discernible within the unsorted unstained population, providing strong evidence that these subpopulations can be resolved via IFC.

Analysis of the IFC sorter's impact (Table 1) indicated minimal effects on the cell population: post-sorting, the proportion of Tie2⁺ cells decreased by approximately 9.3%, with 7.5% attributable to sample preparation artefacts (di-

Tab. 1: Changes of the distribution of Tie2- / Tie2+

Process	Tie2-	Tie2+
FACS	+34.9%	-28.0%
IFC	+10.8%	-1.8%
Handling after IFC	-16.4%	-7.5%

lution, centrifugation, pipetting) or temperature changes.

Conversely, FACS exhibited more pronounced effects: from an initial Tie2+ percentage of 38% following the first sort, only 10% were recovered upon re-analysis. It is suspected that some of the stained cells adhered to the tube or were lost during preparation.

These results suggest that IFC can effectively sort label-free nucleus pulposus progenitor cells with less cell loss than FACS. Although currently lower-throughput than FACS, IFC enables label-free isolation that facilitates downstream analyses and accelerates cell therapy adoption.

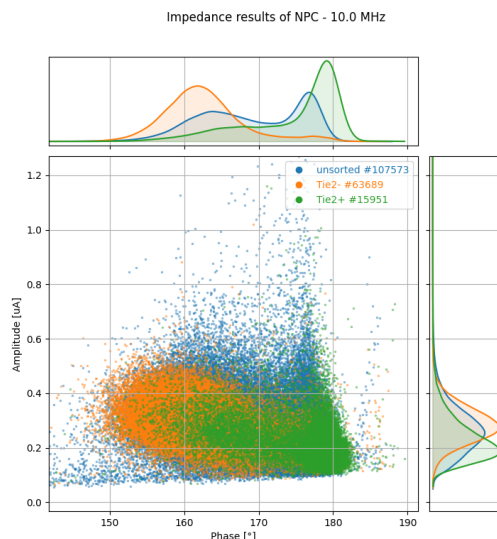


Fig. 1: Scatter plot of cell impedances at 10 MHz of unsorted bovine NPC and sorted Tie2-/Tie2+ bovine NPC. Cells were obtained from four different animals. (#nb of cells)

References

- [1] A. Wu, L. March, X. Zheng, J. Huang, X. Wang, J. Zhao, F. M. Blyth, E. Smith, R. Buchbinder, and D. Hoy, "Global low back pain prevalence and years lived with disability from 1990 to 2017: Estimates from the global burden of disease study 2017," *Annals of Translational Medicine*, vol. 8, p. 299–299, Mar 2020.
- [2] D. Chang, A. Lui, A. Matsoyan, M. M. Safaee, H. Aryan, and C. Ames, "Comparative review of the socioeconomic burden of lower back

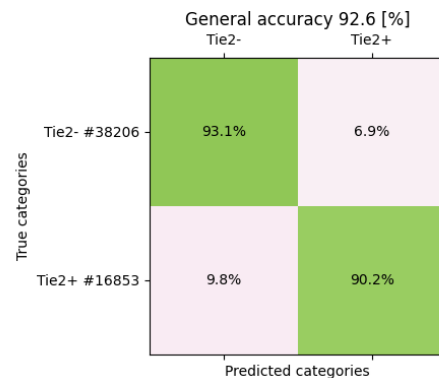


Fig. 2: Confusion matrix given by a quadratic SVM algorithm. Cells were obtained from two different animals. (#nb of cells)

pain in the united states and globally," *Neurospine*, vol. 21, p. 487–501, Jun 2024.

- [3] J. Schol, L. Ambrosio, C. Ruiz-Fernandez, L. Schlagenhof, C. Voskamp, L. T. Laagland, E. Matsushita, H. Soma, T. Warita, G. Vadalà, and et al., "Tie2-positive cells in the nucleus pulposus with a purpose: The who, what and why," *Journal of Biomedical Science*, vol. 33, Mar 2026.
- [4] C. Wyatt Shields IV, C. D. Reyes, and G. P. López, "Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation," *Lab Chip*, vol. 15, pp. 1230–1249, 2015.
- [5] M. Xavier, R. O. Oreffo, and H. Morgan, "Skeletal stem cell isolation: A review on the state-of-the-art microfluidic label-free sorting techniques," *Biotechnology Advances*, vol. 34, no. 5, pp. 908–923, 2016.
- [6] C. A. Lazarski and P. J. Hanley, "Review of flow cytometry as a tool for cell and gene therapy," *Cytotherapy*, vol. 26, pp. 103–112, Feb. 2024.
- [7] M. Favrod-Coune, O. Guenat, and P. Eberle, "Sensitive cell identification in impedance flow cytometry using optimized dielectric buffer properties and low cost microchannels," in *EUROSENSORS XXXVII*, 2025.
- [8] S. Sakuma, Y. Kasai, T. Hayakawa, and F. Arai, "On-chip cell sorting by high-speed local-flow control using dual membrane pumps," *Lab Chip*, vol. 17, pp. 2760–2767, 2017.
- [9] K. Zhang, Z. Xia, Y. Wang, L. Zheng, B. Li, and J. Chu, "Label-free high-throughput impedance-activated cell sorting," *Lab on a Chip*, vol. 24, no. 20, p. 4918–4929, 2024.