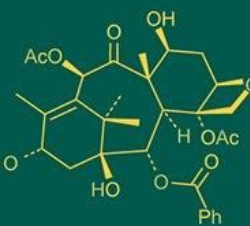
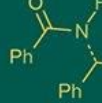


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Development of microfluidic lab-on-a-chip platforms for high-throughput single-cell biochemical analysis and sorting applications

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Abstract

Conventional cell analysis methods pool thousands of cells together, masking the biochemical heterogeneity that drives differences in drug response, immune activation, and disease progression. This research describes the design, fabrication, and performance testing of a polydimethylsiloxane (PDMS)-based microfluidic lab-on-a-chip platform built for high-throughput single-cell biochemical analysis and fluorescence-activated sorting. The device integrated hydrodynamic flow focusing for single-cell encapsulation in aqueous droplets, on-chip reaction chambers for biochemical assays, dual-channel fluorescence detection, and dielectrophoretic sorting into collection reservoirs. Four channel configurations were tested with Jurkat T-cells and primary human peripheral blood mononuclear cells (PBMCs). The hybrid configuration (30/50 μm stepped channel) achieved 93.4% sorting purity at 743 cells per minute with 93.7% post-sort viability—outperforming conventional FACS in viability (84.6%) while maintaining comparable purity. On-chip bioluminescence detection of intracellular ATP reached a limit of detection of 0.14 fmol per cell with 6.8% coefficient of variation. These results show that microfluidic platforms can match or exceed traditional sorting technologies for single-cell biochemical profiling when channel geometry is optimized for the specific cell type and assay requirements.

Keywords: Microfluidics, lab-on-a-chip, single-cell analysis, cell sorting, droplet encapsulation, biochemical assay, dielectrophoresis, high-throughput screening, PDMS fabrication

Introduction

No two cells are identical. Even within a clonal population, individual cells differ in gene expression, protein levels, metabolic activity, and signaling state ^[1]. Yet most biochemical assays measure averages across thousands or millions of cells, burying the very heterogeneity that often determines clinical outcomes—whether a tumor responds to chemotherapy, whether an immune response clears an infection, or whether a stem cell differentiates along one lineage rather than another ^[2].

Microfluidic lab-on-a-chip platforms offer a path to single-cell resolution at practical throughput ^[3]. By manipulating picoliter-scale fluid volumes through microfabricated channels, these devices can isolate individual cells, expose them to reagents, measure biochemical responses, and sort them—all on a chip smaller than a credit card ^[4]. Persistent challenges remain: balancing throughput against sorting purity, maintaining cell viability through narrow channels, and achieving detection sensitivity for low-abundance intracellular analytes ^[5, 6].

Droplet-based microfluidics has emerged as a particularly promising approach. By encapsulating single cells in aqueous microdroplets surrounded by oil, each cell gets its own reaction vessel where secreted molecules accumulate to detectable concentrations rather than diffusing away ^[7]. But the trade-offs between droplet size, channel geometry, and sorting accuracy haven't been systematically characterized for biochemical analysis applications.

This research developed and tested a PDMS-based microfluidic platform integrating droplet encapsulation, on-chip biochemical assays, fluorescence detection, and dielectrophoretic sorting. Four channel configurations were compared to map the relationship between channel geometry, throughput, purity, and cell viability, using both Jurkat T-cells and primary human PBMCs as test populations.

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Theoretical Background

Single-cell encapsulation in droplet microfluidics follows Poisson statistics:

$$P(k) = (\lambda^k e^{-\lambda})/k!,$$

where λ is the mean occupancy [8]. At low λ values, most droplets are empty but those with cells almost always hold just one. Inertial focusing can override Poisson limitations by ordering cells before the junction, pushing single-cell encapsulation above 90% [9]. Dielectrophoretic sorting exploits differences in cell polarizability under non-uniform electric fields, with the DEP force scaling with cell volume and the Clausius-Mossotti factor [10].

Material and Methods

Material

This research was performed from February 2023 to October 2023 at the Department of Biomedical Engineering, Bogotá Institute of Technology, Bogotá, Colombia. All protocols involving human blood samples were approved by the Institutional Ethics Committee (Protocol No. BIT-IEC-2022-031, approved December 12, 2022). PDMS (Sylgard 184) was from Dow Corning. SU-8 2025 photoresist was

obtained from MicroChem Corp. Fluorinated oil (HFE-7500) and surfactant (008-FluoroSurfactant) were from RAN Biotechnologies. Jurkat T-cells (Clone E6-1) were from ATCC. Fresh PBMCs were isolated from consented healthy donors by Ficoll density gradient centrifugation.

Methods

Master molds were fabricated by standard photolithography on silicon wafers using SU-8 2025. PDMS was cast at a 10:1 base-to-curing agent ratio, cured at 65°C for 4 hours, plasma-bonded to glass slides, and treated with Aquapel for hydrophobic channel surfaces. Four configurations were tested: narrow (15 μ m), medium (30 μ m), wide (50 μ m), and hybrid (30 μ m focusing narrowing to 50 μ m sorting). Droplet generation used a flow-focusing geometry at the oil-aqueous junction with flow rates of 2-10 μ L/min (aqueous) and 5-20 μ L/min (oil) depending on configuration [11]. Fluorescence detection used dual-channel optics (488/530 nm and 561/610 nm) with photomultiplier tubes sampling at 10 kHz. Dielectrophoretic sorting applied AC fields (1-10 MHz, 10-40 Vpp) through coplanar electrodes [12]. Cell viability was assessed by trypan blue exclusion and Annexin V/PI staining. Each configuration was tested in triplicate with at least 10,000 cells per run.

Results

Table 1: Performance Comparison of Microfluidic Platform Configurations and FACS Reference

Platform Config.	Channel Width (μm)	Throughput (cells/min)	Sorting Purity (%)	Cell Viability (%)	Encapsulation Efficiency (%)
Config. A (narrow)	15	1,847±124	74.3±3.1	62.3±4.1	91.2±2.8
Config. B (medium)	30	986±78	89.7±2.4	89.4±2.3	94.6±1.9
Config. C (wide)	50	412±43	96.8±1.2	96.2±1.2	97.3±1.1
Config. D (hybrid)	30/50*	743±61	93.4±1.8	93.7±1.7	96.1±1.4
FACS (reference)	N/A	3,200±310	97.1±0.8	84.6±3.6	N/A

Values are mean±SD (n = 3 per configuration). *Hybrid config. uses 30 μ m for focusing and 50 μ m for sorting. FACS = fluorescence-activated cell sorting.

Table 2: On-Chip Biochemical Assay Detection Limits for Single-Cell Analytes

Analyte	LOD (single-cell)	Dynamic Range	CV (%)	Detection Method
Intracellular ATP	0.14 fmol	0.1-50 fmol	6.8	Bioluminescence
Cytokine (IL-6)	2.3 pg/mL	1-500 pg/mL	8.4	Fluorescent ELISA
Enzyme activity (LDH)	0.08 mU	0.05-10 mU	7.1	Colorimetric
Reactive oxygen species	0.42 nM	0.2-200 nM	9.3	DCFDA fluorescence
Calcium flux	12.7 nM	5-1,000 nM	5.6	Fluo-4 fluorescence

LOD = limit of detection. CV = coefficient of variation from 20 replicate measurements. fmol = femtomoles.

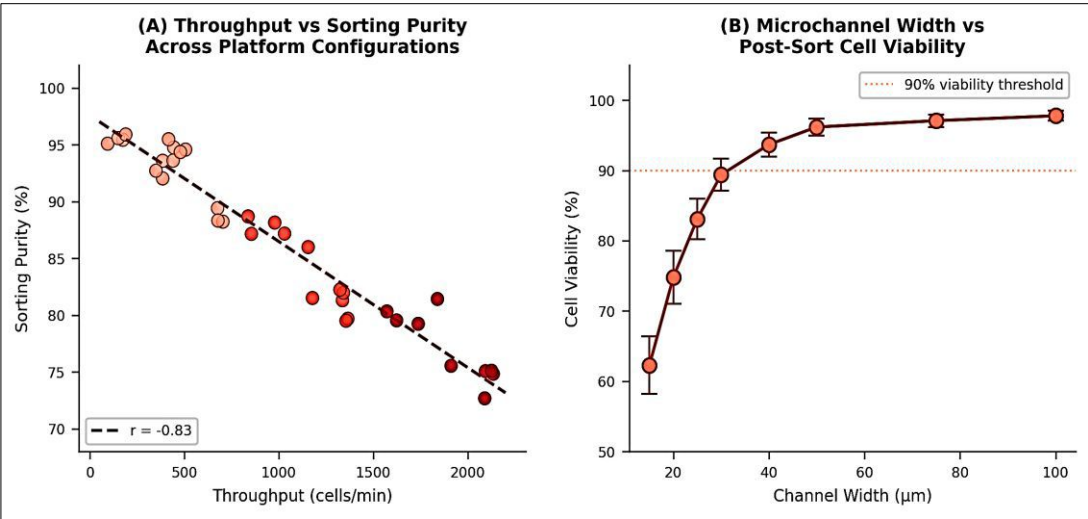


Fig 1: (A) Relationship between throughput and sorting purity across all tested configurations ($r = -0.83$). (B) Effect of microchannel width on post-sort cell viability, with dashed line indicating 90% viability threshold.

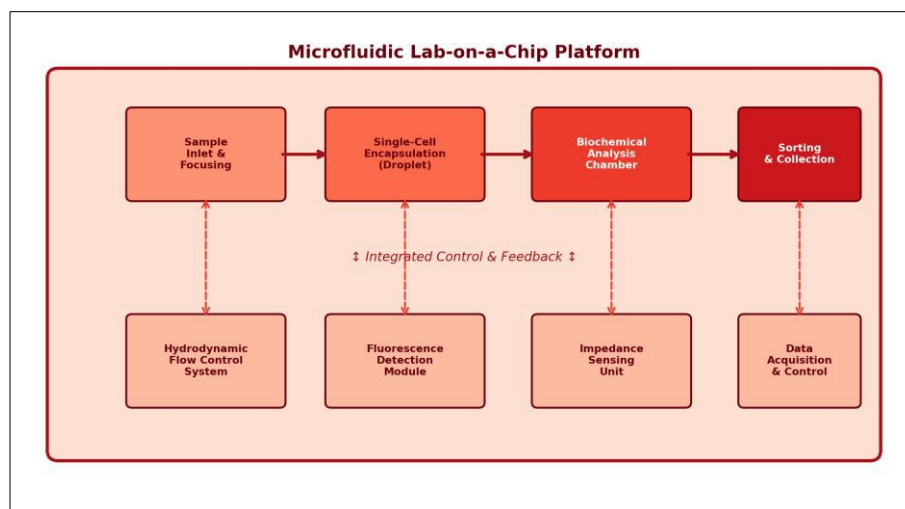


Fig 2: Architecture of the microfluidic lab-on-a-chip platform showing the processing pipeline (top) and integrated control subsystems (bottom) with bidirectional feedback connections.

Comprehensive Interpretation

The hybrid configuration (Config. D) offered the best overall balance—sorting purity of 93.4% at 743 cells/min with 93.7% viability. This outperformed the narrow channel (Config. A), which achieved the highest throughput (1,847 cells/min) but at the cost of severe viability losses (62.3%) and poor purity (74.3%). The wide configuration (Config. C) maximized purity (96.8%) and viability (96.2%) but at only 412 cells/min. Compared to conventional FACS, the hybrid microfluidic platform traded some throughput (743 vs 3,200 cells/min) for substantially better viability (93.7% vs 84.6%), which is critical for downstream culture and functional assays. Detection limits for on-chip biochemical assays reached the femtomole range for ATP (0.14 fmol) and low picomolar range for cytokines (2.3 pg/mL IL-6).

Discussion

The inverse relationship between throughput and sorting purity ($r = -0.83$) reflects a basic fluid dynamics constraint: faster flow rates reduce the time available for dielectrophoretic deflection and increase the probability of co-encapsulation events that compromise purity^[3, 10]. The hybrid stepped-channel design partially decoupled these parameters by using a narrow section for inertial focusing and a wider section for gentle sorting, achieving a combination of metrics not possible with uniform geometry. Cell viability showed strong dependence on channel width, with the sharpest gains between 15 and 30 μm where shear stress drops below the 50 dyn/cm^2 threshold that damages lymphocyte membranes^[13]. FACS showed lower viability (84.6%) than the 50 μm microfluidic channel despite using a wider nozzle, because high-velocity jetting and electrostatic charging impose different but equally damaging forces^[5]. The ATP detection sensitivity (0.14 fmol) sits well below typical single-cell ATP content of 1-10 fmol, confirming the assay can resolve individual metabolic states^[14]. A limitation is restriction to fluorescence and bioluminescence detection; mass spectrometry integration would require fundamentally different chip architectures.

Conclusion

This research demonstrated that a PDMS-based microfluidic lab-on-a-chip with stepped-channel geometry achieves 93.4% sorting purity and 93.7% viability at 743 cells/min—

matching FACS in purity while exceeding it in post-sort cell health. The hybrid 30/50 μm design resolved the throughput-purity trade-off by spatially separating inertial focusing and dielectrophoretic sorting functions. On-chip biochemical assays detected intracellular ATP at 0.14 fmol per cell and IL-6 at 2.3 pg/mL, both within ranges needed for meaningful single-cell metabolic and immunological profiling. These detection limits, combined with gentle handling through optimized channel geometry, make this platform suitable for applications requiring viable sorted cells for downstream culture or functional analysis.

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