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TRƯỜNG ĐẠI HỌC CÔNG NGHỆ**



NGUYỄN THU HẰNG

**DANH MỤC CÔNG TRÌNH KHOA HỌC CỦA TÁC GIẢ
LIÊN QUAN ĐẾN LUẬN ÁN**

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DANH MỤC CÔNG TRÌNH KHOA HỌC CỦA TÁC GIẢ LIÊN QUAN ĐẾN LUẬN ÁN

1. Thu Hang Nguyen, Ngoc Anh Thi Nguyen, Tung Thanh Le, Hanh Van Nguyen, Hang Thu Bui, Hoang Anh Phan, Tung Thanh Bui, Trinh Chu Duc, and Loc Quang Do, “*FEMI: Deep learning-assisted analysis of single-oocyte trapping and maturation for enhanced on-chip IVM*”, *Biomicrofluidics* 1 May 2026; 20(3): 034102. <https://doi.org/10.1063/5.0328820> (Editor's Pick, AIP Scilight).
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FEMI: Deep learning-assisted analysis of single-oocyte trapping and maturation for enhanced on-chip IVM

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ABSTRACT

Traditional *in vitro* maturation (IVM) involves manual handling and static culture conditions and lacks automated assessment. These limitations increase variability, introduce stress that may affect oocyte quality, and fail to recapitulate the dynamic *in vivo* microenvironment. This study presents a system that streamlines, automates, and potentially enhances IVM procedures through the capture, dynamic on-chip cultivation, and evaluation of single oocytes on-chip, termed FemaleTech Enhanced Maturation Intelligence (FEMI). The platform employs a novel microfluidic chip featuring hexagonal micropillars that efficiently trap individual oocytes across a broad size range while inherently excluding low-quality oocytes, ensuring stable positioning throughout culture. Numerical simulations and experimental validation confirmed the trapping principle and high capture efficiency of the proposed design. FEMI supports continuous microfluidic culture under controlled flow conditions, allowing partial recapitulation of physiological transport and mechanical cues while maintaining oocyte immobilization. By integrating deep learning-based image segmentation, the system automatically quantifies cumulus cell expansion, providing single-cell-level insights into oocyte development. The results suggest that FEMI may improve IVM efficiency and provide a useful platform for assisted reproductive technologies.

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I. INTRODUCTION

According to the World Health Organization, in 2023, approximately 17.5% of the global adult population, or one in six people, experienced infertility, indicating a significant need for increased access to affordable and high-quality fertility care.¹ Assisted Reproductive Technologies (ARTs) such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), *in vitro* maturation (IVM), and cryopreservation have revolutionized fertility treatments. These technologies enable millions of people to conceive while also advancing medical knowledge in genetics and reproductive health.² IVM is a technique in which oocytes are retrieved from the antral follicles of unstimulated or minimally stimulated ovaries for further maturation outside the body.³

Although significant advancements have been made, IVM, like other reproductive technologies, remains constrained by several limitations. First, the procedures of oocyte handling and culture remains largely manual, lacking automation. This leads to variability and dependence on operator expertise.⁴ Additionally, the *in vitro* environment fails to fully mimic the dynamic physiological conditions of *in vivo* growth. As a result, oocytes show lower developmental competence and reduced fertilization, implantation, and live birth rates.⁵

Recent progress in microfluidic technology has led to the development of devices capable of automatically trapping, manipulating, and culturing single oocytes. Microfluidic platforms offer dynamic and precise control and enable continuous media

exchange and the introduction of physiological cues such as fluid shear stress. These factors are critical for optimal oocyte development. Han *et al.* presented a microwell-structured microfluidic device that integrates single-oocyte trapping, fertilization, and subsequent embryo culture.⁶ This device utilizes a microwell array to capture and hold individual oocytes during processes such as oocyte and sperm loading, medium substitution, and debris cleaning.⁶ In another study, a microfluidic device is designed with a microchamber array that facilitates the trapping and maturation of individual oocytes within a controlled microenvironment.⁷ This design allows for precise regulation of maturation conditions, potentially enhancing oocyte developmental competence.⁷ A recent work introduced OoTrap, a microfluidic device developed to enhance oocyte collection and maturation by reducing manual handling and replicating *in vivo* dynamic conditions.⁴ The device improves IVM rates, maintains spindle integrity, and enables incubation-free IVM.⁴ Although promising results have been achieved, these microfluidic structures are mostly based on chamber-like designs for single-oocyte trapping, making it challenging to remove debris from the culture environment. This can negatively impact oocyte maturation and obstruct clear visualization of oocytes during culture.⁴ A simple chamber structure may cause flow stagnation, non-uniform shear stress, and nutrient depletion.⁸ Additionally, in the IVM process, oocytes are typically cultured with cumulus cells, forming a cumulus–oocyte complex (COC).^{9,10} The size of the COC is heterogeneous with uniformly sized chambers may result in low trapping efficiency. Furthermore, monitoring individual oocyte progression is important in IVM. Single-oocyte analysis allows continuous and non-invasive assessment, improving automation, reproducibility, and selection accuracy, thus enhancing ART outcomes.^{11,12} Current studies are still limited in terms of real-time and automated oocyte assessment during the developmental process.

In this research, we present the FEMI—FemaleTech Enhanced Maturation Intelligence system, a platform that utilizes intelligent image-assisted analysis to monitor and evaluate the single oocyte trapped and matured on-chip (Fig. 1). A microfluidic chip structure consisting of hexagonal micropillars trapping (HMT) units was proposed to effectively trap and culture single COCs of varying sizes based on hydrodynamic principles, making it highly suitable for IVM. The chip design aims to enhance the interaction between oocytes and the culture medium by increasing the contact area and improve nutrient exchange and maturation outcomes. Numerical simulations were conducted to analyze mechanical stress and velocity distribution and to validate the trapping principle within the HMT chip. The proposed HMT structure was successfully fabricated and demonstrated high trapping efficiency and maturation rate in experimental validation. By integrating microfluidic-based single-oocyte trapping and cultivation with deep learning-driven image segmentation, our platform enables the automated and objective quantification of cumulus cell expansion and morphological changes. This allows for the monitoring and assessment of single-oocyte maturation on-chip. This combination of precise microfluidic culture conditions and AI-assisted assessment may serve as a useful platform for optimizing IVM, while potentially contributing to improved maturation outcomes and the gradual advancement of automation in ART.

II. MATERIALS AND METHODS

A. Microfluidic chip design and principles

Our FEMI system is specifically designed for trapping single oocytes and subsequently maintaining them in culture. Unlike the existing single-cell trapping platforms, this system integrates a size-dependent trapping mechanism that ensures efficient capture of oocytes with varying sizes. The oocyte trapping mechanism operates via hydrodynamic flow control. Upon entering the trapping region, oocytes experience local velocity variations induced by the micropillar array, creating low-flow zones within the trapping compartments. The progressively narrowing trapping unit size ensures that larger oocytes are retained within the larger upstream traps, while smaller oocytes continue migrating downstream until encountering a unit that matches their size.

The selection of a hexagonal geometry for the micropillars was guided by established microfluidic principles and refined through preliminary experimental testing. Conventional U-shaped or chamber-based traps may create flow stagnation, limiting continuous nutrient exchange.^{8,13} These designs can cause non-uniform shear stress and reduced cell-medium contact,¹⁴ whereas square or inverted trapezoidal grooves, as well as triangular pillar geometries pillars, can create high localized shear stress at sharp corners.^{14,15} Although circular pillars provide smooth flow profiles, our initial experiments revealed a significant limitation in retaining highly deformable single cells.¹⁶ The curvature of the circular pillars only partially confined the oocytes at the tangential point, making them prone to slipping through when flow rates slightly increased. In contrast, the hexagonal geometry features flat, sloped surfaces. This design provides a longer restriction zone, enhancing the structural holding capacity and preventing the oocyte from escaping under dynamic flow variations, while maintaining low-shear streamlines. The five-pillar array was carefully sized to form a spacious pocket, allowing the oocyte to shift gently under the continuous culture flow. This architectural feature is advantageous and essential for our platform, as it facilitates seamless, uninterrupted real-time tracking and automated morphological evaluation of the cumulus expansion using the YOLOv8 deep learning module.

The microfluidic chip is designed with an overall length of 28 mm (from inlet to outlet) and a width of 5 mm. The trap region extends over a length of 12 000 μm and is systematically divided into four zones. Each zone features a uniform trap unit size, progressively decreasing to enhance cell trapping efficiency across different cell sizes (Table I). Figure 2 offers a simplified schematic of the trapping architecture. While the figure highlights the structural hierarchy, the actual trap layout is denser. To maintain uniform fluid distribution and stable trapping performance, the spacing between consecutive trap rows is consistently set at 400 μm . Micropillars have a diameter of 200 μm . In region H1, the trap units possess the largest width of 250 μm , offering a broad trapping area that accommodates large cells or multicellular aggregates. The wide design in this region helps reduce flow resistance and facilitates initial coarse filtration. Transitioning to H2, the trap width is reduced to 200 μm to create a more confined geometry conducive and capture medium-to-large single cells. In region H3, the design becomes more refined with trap widths of 150 μm . This

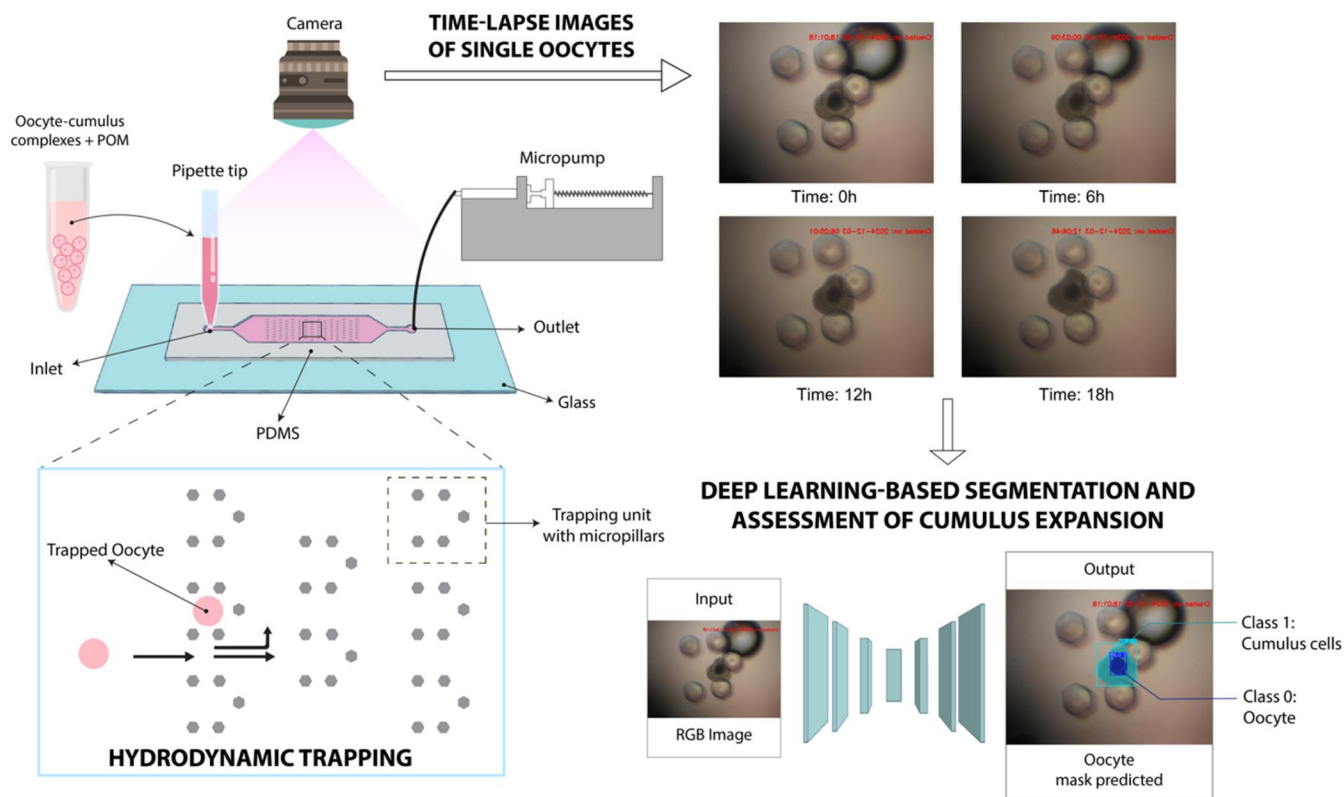


FIG. 1. Schematic and functionality of the proposed FEMI system.

dimension aligns closely with the experimentally observed dominant cell size range of 150–210 μm , which accounts for the majority of cells processed in typical experimental settings. Accordingly, H3 was intentionally designed with the highest density of trap units, making it the most critical zone for high-efficiency capture. The trap was designed to be 150 μm wide, but the actual width could be up to 180 μm due to fabrication differences. Finally, region H4 contains the smallest trap units, measuring 80 μm in width. This region acts as a fine filter designed for trapping small-

sized cells or residual particles that bypassed previous zones. This systematic variation in trap unit size across the four zones establishes a gradient-based trapping mechanism, allowing for selective size-dependent cell capture while preserving a stable and controlled microfluidic environment.

This design not only facilitates the entrapment of oocytes of different sizes but also serves as a passive quality filtering mechanism, as oocytes with a diameter below 100 μm , which are often developmentally compromised or damaged, are excluded.¹⁷ This size-selective trapping strategy may offer potential utility for oocyte screening in ART, particularly in IVM and IVF. Since oocyte size is a critical determinant of developmental competence, this microfluidic platform enables automated and reproducible oocyte selection, reducing the reliance on manual microscopic evaluation. Additionally, the transparent microchannel design allows for real-time observation, facilitating studies on oocyte positioning, morphological dynamics, and response to different culture conditions.

B. Theoretical modeling

In this study, the simulation software COMSOL MULTIPHYSICS version 6.0 was used to analyze computational fluid dynamics (CFD) using the physical parameters listed in

TABLE I. Geometry parameters of the proposed microfluidic chip.

Name	Description	Value
L	Length of the microfluidic chip's trap region	28 mm
W	Width of the microfluidic chip	5000 μm
D	Distance between two consecutive rows of traps	400 μm
r_1	Width of the trap unit in the H1 region	250 μm
r_2	Width of the trap unit in the H2 region	200 μm
r_3	Width of the trap unit in the H3 region	150 μm
r_4	Width of the trap unit in the H4 region	80 μm
ϕ_p	Diameter of the micropillar	200 μm

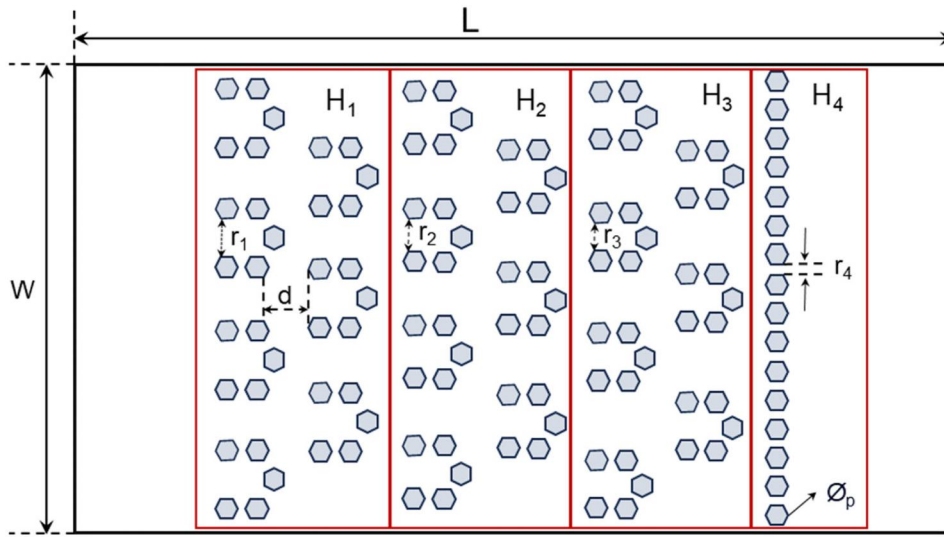


FIG. 2. Refinement of the trapping region design in the microfluidic chip.

Table II and referenced from previous studies.^{18–21} The governing equations for fluid flows are expressed by the incompressible Navier–Stokes equations, formulated as follows:²²

$$\rho \left[\frac{\partial \mathbf{u}_f}{\partial t} + \mathbf{u}_f \cdot \nabla \mathbf{u}_f \right] = \nabla \cdot [-p\mathbf{I} + \mathbf{K}] - 12 \frac{\mu \mathbf{u}_f}{d_z^2} + \mathbf{F}, \quad (1)$$

$$\nabla \cdot [-p\mathbf{I} + \mathbf{K}] + \mathbf{F} = 0. \quad (2)$$

Here, ρ denotes the fluid density (kg/m^3), $\mathbf{u}_f = (u_f, v_f, w_f)$ represents the fluid velocity vector (m/s). The term $\mathbf{I}$ refers to the identity matrix, while $\mathbf{F}$ denotes the volume force affecting the fluid (N/m^3 , or N/m^2 for a 2D model). The factor $\frac{\mu \mathbf{u}_f}{d_z^2}$ indicates a drag or friction force proportional to the fluid's velocity and inversely proportional to the square of the channel thickness (m). This term accounts for the resistive forces in such confined flows, the unsteady inertia force (N/m^3). $\mathbf{K}$ represents the shear stress tensor, which depends on the velocity gradients and reflects the viscous effects in the fluid. The fluid is assumed to be Newtonian, with its

constitutive equation expressed as follows:

$$\mathbf{K} = \mu \left(\nabla \mathbf{u}_f + (\nabla \mathbf{u}_f)^T \right), \quad (3)$$

$$\rho \nabla \cdot \mathbf{u}_f = 0. \quad (4)$$

Here, the notation T indicates transposition and μ stands for fluid dynamic viscosity (Pa s).

The dimensionless quantity employed to quantify the ratio of advective forces to viscous forces is denoted as the Reynolds number (Re), which is determined by the following relation:

$$Re = \frac{\rho UL}{\mu}. \quad (5)$$

In the context of microfluidic devices, the Reynolds number (Re) tends to be very low ($Re \ll 1$). Consequently, the flow maintains a predominantly laminar character across a significant portion of the area.

In the realm of fluids, the shear stress τ is contingent upon the rate of strain $d\gamma/dt$. Viscosity is the inherent property of a fluid to impede the progression of shear deformation. The specific form of the correlation between shear stress and rate of strain varies across fluids, and a majority of common fluids adhere to Newton's law of viscosity. According to this law, the shear stress is directly proportional to the strain rate,²³

$$\tau = \mu \frac{d\gamma}{dt} = \mu \frac{du}{dy}, \quad (6)$$

where τ is the shear stress, μ is the dynamic viscosity, and $d\gamma/dt$ is the strain rate.

Solid mechanics is a fundamental field in physics that examines the deformation and motion of continuous solids under

TABLE II. Physical parameters.

Name	Parameter
Fluid density	10^3 kg/m^3
Fluid viscosity	10^{-3} Pa s
Oocyte density	1150 kg/m^3
Oocyte Young's modulus	300 MPa
Oocyte Poisson's ratio	0.3
Polydimethylsiloxane (PDMS) density	970 kg/m^3
PDMS Young's modulus	300 kPa
PDMS Poisson's ratio	0.49

external forces, displacements, and accelerations. In this study, the solid phase is assumed to exhibit isotropic linear elasticity under small deformations and low loads. Applying solid mechanics principles enables accurate modelling of cellular responses to mechanical stress and fluidic interactions. Finite element modeling (FEM) in fluid–solid interaction (FSI) simulations provides insights into individual cell mechanics, facilitating a computationally efficient representation of cellular dynamics. The governing equations of linear elastodynamics²⁴ describe displacement and deformation,

$$\varepsilon_s = \frac{1}{2} \left[(\nabla \mathbf{u}_s)^T + \nabla \mathbf{u}_s + (\nabla \mathbf{u}_s)^T (\nabla \mathbf{u}_s) \right], \quad (7)$$

$$\nabla \cdot \sigma_s + \mathbf{F}_s = \rho_s \frac{\partial^2 \mathbf{u}_s}{\partial t^2}, \quad (8)$$

$$\sigma_s = C \varepsilon_s. \quad (9)$$

Here, ε_s represents the strain tensor, while $\mathbf{u}_s = (u_s, v_s, w_s)$ denotes the displacement field. Equation (8) corresponds to Newton's equation of motion, with σ_s being the Cauchy stress tensor and $\mathbf{F}_s$ being the body force per unit volume. The material follows an isotropic linear elastic model, where the stress–strain relationship is expressed as $\sigma_s = C \varepsilon_s$, with C being the stiffness matrix.

Fluid–solid interaction (FSI) couples fluid dynamics with structural mechanics. The interaction is governed by

$$\mathbf{F}_A = [-\rho \mathbf{I} + K] \cdot \mathbf{n}, \quad (10)$$

$$\mathbf{u}_{tr} = \frac{\partial \mathbf{u}_s}{\partial t}, \quad (11)$$

where $\mathbf{F}_A$ represents the total force due to fluid pressure and viscous effects on the solid boundary, K is from Eq. (3), and $\mathbf{n}$ is the outward normal vector at the boundary. Equation (11) defines $\mathbf{u}_{tr}$ as the displacement rate of the solid, which matches the fluid velocity $\mathbf{u}_f$ at the liquid–solid interface, ensuring a non-slip boundary condition.

C. Materials and fabrications

The HMT was fabricated using polydimethylsiloxane (PDMS) soft lithography. The process began with the preparation of a master mold by spin-coating SU-8 50 negative photoresists (MicroChem Corp., Newton, MA, USA) onto a silicon wafer. The spin-coating process included an initial step at 500 rpm for 10 s, followed by a second step at 1000 rpm for 30 s to achieve uniform thickness. After spin-coating, the wafer underwent a soft bake, which not only improved adhesion but also stabilized the SU-8 surface, enhanced photosensitivity, and reduced noise, ensuring high-resolution patterning. The baked wafer was then exposed to UV light through a photomask to define the microstructures, followed by post-exposure baking to complete cross-linking. Development was carried out using SU-8 Developer (MicroChem Corp., Newton, MA, USA) to remove the unexposed regions, yielding the final master mold. The SU-8 features had a measured height of 220 μm , as determined by a Dektak ST System Profilometer (Veeco Instruments Inc., Plainview, NY, USA). Once

the master mold was prepared, the PDMS replica was fabricated using a Sylgard-184 Silicone Elastomer Kit (Dow Corning, Midland, MI, USA). The prepolymer was mixed with the curing agent at a 10:1 (w/w) ratio, thoroughly stirred, and poured over the SU-8 master. To eliminate air bubbles, the mixture was degassed utilizing a vacuum chamber for 30 min before being thermally cured at 65 °C for 2 h. After curing, the PDMS layer was carefully peeled off from the master mold, and inlet and outlet ports (2 mm in diameter) were created using a biopsy punch. The surfaces of both the PDMS and the glass slide were treated with oxygen plasma to increase surface energy and enhance adhesion. The processed PDMS layer was then carefully aligned and bonded to a 75 $\times$ 25 mm² glass slide, ensuring a hermetically sealed microfluidic structure. The fabrication of the proposed HMT chip was completed, making it ready for experimental applications.

D. Oocyte preparation and IVM

For the initial biological validation of the microfluidic system, porcine oocytes were selected due to their established relevance in reproductive biology and their similarities to human oocytes. Porcine oocytes share important anatomical and physiological features with human oocytes, including a similar oocyte diameter, comparable timeframes, and proteomic expression patterns across different stages of oocyte maturation, as well as similarities in early embryonic developmental characteristics and lipid content.^{25–29} Additionally, using porcine oocytes helped address ethical and practical constraints, as human oocytes are limited in availability and primarily reserved for clinical treatments. While the ultimate goal of this research is to apply the platform in human clinical settings, the porcine model provides a biologically relevant and widely accepted approach for proof-of-concept studies in reproductive research.^{25,30,31}

Porcine ovaries were collected from prepubertal crossbred sows at the slaughterhouse and transported to the laboratory at 35 °C in Dulbecco's phosphate buffer solution (Gibco, USA) supplemented with 1% antibiotics (penicillin–streptomycin, Thermo, USA). The ovaries were washed several times in phosphate-buffered saline (PBS), and follicles (3–6 mm in diameter) were excised with a No. 21 scalpel. The follicular fluid containing oocytes was allowed to settle in a 15 ml Falcon tube (SPL Life Sciences, Korea) in a 38.5 °C incubator for 15 min. The COCs were collected into 90 mm Petri dishes (SPL Life Sciences, Korea); then, only COCs with multiple layers of cumulus cells were collected and used for the subsequent experiment. After collection, the COCs were transferred to a four-well culture disk (SPL Lifescience, Korea) before being loaded into the microfluidic chip and then placed in an incubator (38.5 °C, 5% CO₂, 100% humidity). Subsequently, the culture medium is prepared using a porcine oocyte maturation (POM) medium as the base,³² with the addition of several essential components to support oocyte growth and maturation. These include 1 mM cyclic AMP (cAMP), which facilitates nuclear maturation; 10 $\mu\text{g}/\text{ml}$ epidermal growth factor (EGF) to promote cellular growth; 10 $\mu\text{l}/\text{ml}$ insulin–transferrin–selenium (ITS) to enhance metabolic function; 10 IU/ml follicle-stimulating hormone (FSH); and 10 IU/ml human chorionic gonadotropin (hCG), both of which stimulate oocyte maturation. To ensure

optimal conditions for oocyte development, the prepared culture medium is equilibrated in an incubator at 38.5 °C with 5% CO₂ for at least 12 h. This step stabilizes the pH and facilitates the establishment of an environment conducive to successful oocyte maturation.

E. Experimental setup of on-chip single-oocyte trapping and culture

The experimental setup is designed for the single-cell trapping of oocytes within a controlled microenvironment. Before the experiment, the microfluidic chip is sterilized and environmentally balanced in an incubator at 38.5 °C with 5% CO₂ to ensure optimal conditions. Oocytes suspended in culture medium are introduced into the microchannel through the inlet using a pipet tip. The outlet is connected to a micropump operating in the withdrawal mode. The system is mounted on a microscope equipped with a high-speed camera, enabling continuous real-time monitoring and precise manipulation throughout the experiment.

All experiments were conducted using five independent biological replicates ($n=5$). Quantitative data are presented as mean $\pm$ standard deviation (SD). Statistical comparisons between the static culture (control group) and the on-chip culture (FEMI group) were performed using a paired one-tailed Student's *t*-test, based on the *a priori* hypothesis that the on-chip system improves oocyte maturation outcomes. A *p*-value <0.05 was considered statistically significant.

F. Deep learning-based evaluation of cumulus expansion

Artificial intelligence (AI) is increasingly being applied to IVM, offering advanced methodologies for assessing oocyte development.³³ Traditional evaluation relies on manual inspection by embryologists, which is inherently subjective, time-consuming, and prone to variability. This study explores the application of deep learning techniques for oocyte image segmentation and precise examination of cumulus cell expansion, a critical parameter in monitoring oocyte maturation.^{34–36} The expansion of cumulus cells is often indicative of the maturation stage of the oocyte, with increased cell area correlating with enhanced oocyte quality and potential for successful fertilization.³⁷ A recent study has shown that abnormalities in cumulus cell expansion can lead to poor oocyte quality and affect embryo development and implantation success.³⁸ Analyzing temporal changes in the cumulus cell area provides valuable insights into the developmental process, contributing to advancements in IVM optimization. The dataset used for training the model consists of time-lapse images capturing individual oocytes on-chip over a 24 h period. These images are then uploaded to Roboflow for expert manual annotation to generate the ground-truth masks, with two defined classes: oocytes and cumulus cells.³⁹ After the annotation process, the dataset is used to train a segmentation model based on YOLOv8, which has been effectively utilized for object segmentation with high precision.^{40,41} After training, the model is deployed to automatically segment oocyte and cumulus cells from input images as shown in Fig. 1. From the coordinates of the generated masks, camera parameters, and the height of the imaging system, the actual size of the oocyte and cumulus cells can be calculated.

III. RESULTS AND DISCUSSION

A. Fabrication of microfluidic device

The microfluidic chip featuring HMT units was successfully fabricated via soft lithography. Figure 3(a) shows the final HMT chip bonded to a glass slide, demonstrating its compact size compared to a 5-cent coin. Scanning electron microscopy (SEM) was employed to evaluate the micropillar arrangement and fabrication quality, as illustrated in Fig. 3(b). The channel height measurements were conducted using a Dektak ST System Profilometer (Veeco Instruments Inc., Plainview, NY, USA). The recorded values ranged from approximately 220 to 230 μm , in close agreement with the proposed design parameters. These fabrication results confirm both the reproducibility and accuracy of the soft lithography process. However, as observed from optical and SEM characterization, geometric deviations exist between the fabricated PDMS structures and the idealized 3D CAD models, particularly the rounding of hexagonal corners. These differences arise naturally from standard SU-8 photolithography and PDMS bonding processes. While the corners are rounded, the main bodies of the pillars retain sufficient angularity to generate the necessary flow resistance and maintain the structural trap units. The rounding of sharp corners leads to smoother flow streamlines compared to idealized geometries, which may result in lower shear stress. The fabrication and bonding process causes a slight height variation between the micropillars and channel, preventing full confinement of the oocyte along vertical walls and allowing medium circulation both laterally and vertically.^{42,43} These conditions may be favorable for long-term cultivation. However, the fabrication errors can slightly reduce oocyte confinement, making them more prone to slipping at higher flow rates, which may affect trapping performance.

B. Modeling of flow field, shear stress, and trapping process

Figure 4 presents a comprehensive computational analysis of fluid velocity distribution and mechanical stress during the trapping of an oocyte in a microfluidic channel. The inlet velocity is set at 400 $\mu\text{m/s}$. The velocity field is illustrated at four time points: 0, 1, 1.5, and 3 s. The velocity field is illustrated at four time points: 0, 1, 1.5, and 3 s, an oocyte proceeds directly toward the trapping chamber. By $t=1.5$ s, the first oocyte has completely entered the trap unit, leading to a local decrease in velocity within the unit due to increased hydrodynamic resistance. Simultaneously, the velocity in the two lateral interstitial gaps adjacent to the trap increases; velocity peaks in the narrow interstitial gaps adjacent to the trap reach up to 1500 $\mu\text{m/s}$, forming new flow paths. This redistribution of the flow field establishes a condition where the second incoming oocyte is in a higher velocity region (lower resistance). As a result, the second oocyte tends to follow these high-velocity paths, effectively bypassing the already occupied trapping unit. At $t=3$ s, this bypass behavior becomes more evident, with the second oocyte diverted around the filled trap and continuing downstream. This mechanism is a fundamental aspect of hydrodynamic trapping, in which cells follow the paths of least resistance, dictated by the surrounding velocity field. In addition, the staggered arrangement of traps ensures that even bypassed oocytes are eventually collected by downstream units, thereby

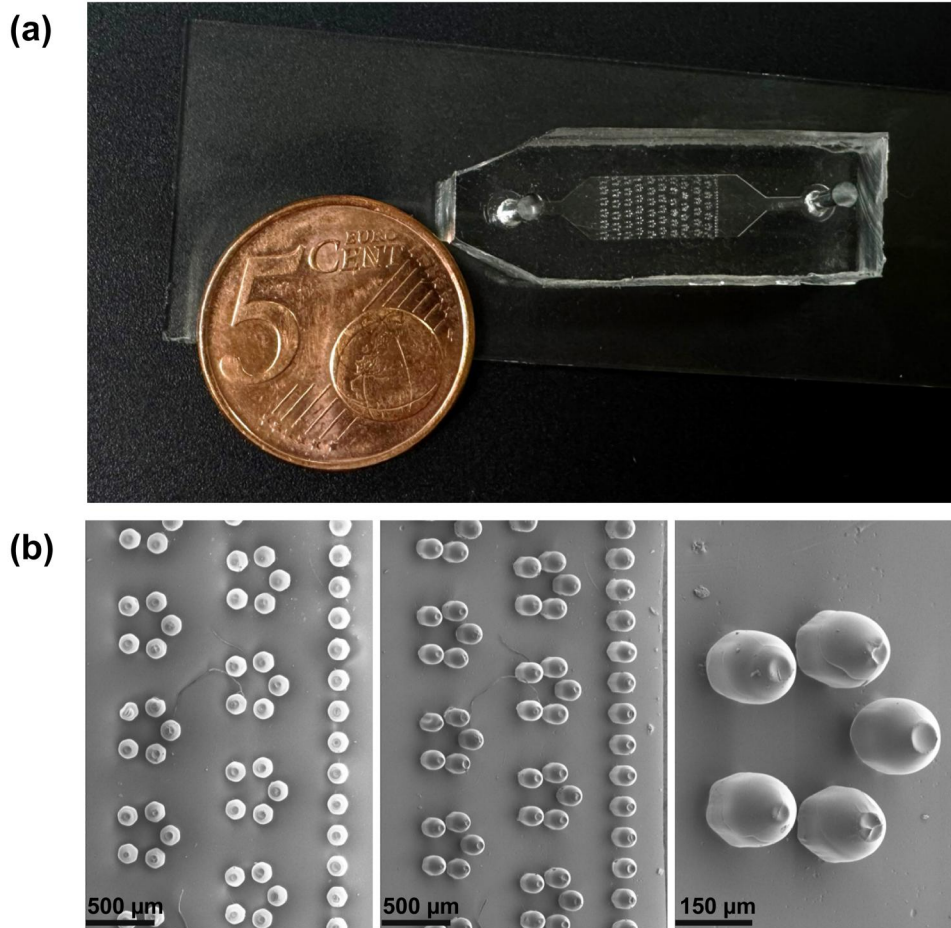


FIG. 3. (a) Fabricated microfluidic chip and (b) SEM characterization of the chip.

enhancing capture efficiency. The simulation also shows that the hexagonal pillars act as a hydrodynamic shield, deflecting the main flow and creating a low-velocity region that stabilizes the trapped oocyte through a gentle pressure gradient.

In addition to analyzing the velocity distribution, mechanical stresses acting on the oocytes were examined, specifically shear stress and von Mises stress. Shear stress arises from fluid-induced friction on the oocyte surface, whereas von Mises stress quantifies the equivalent stress from multiple components under complex loading conditions. The simulation results indicate that both the von Mises stress and the shear stress imposed on the oocyte within the microfluidic platform are significantly lower than the established mechanical thresholds known to cause cellular damage. In particular, the peak von Mises stress observed (~ 0.14 Pa) and the maximum shear stress at the cell–fluid interface (~ 0.018 Pa) are both well below harmful levels such as the 1.2 dyne/cm^2 threshold (~ 0.12 Pa) previously associated with apoptosis induction in early stage embryos.⁴ Previous study shows that even under transient localized flow surges approaching 70 dyne/cm^2 , no morphological deformation or membrane rupture was observed.⁴⁴ Additionally, numerous studies have

reported that the Young modulus of oocytes typically falls within a broad range of $0.1\text{--}10 \text{ kPa}$,⁴⁵ depending on factors such as species, maturation stage, and measurement conditions. This parameter reflects the mechanical stiffness of the oocyte, influencing its response to external forces, including shear stress and deformation during microfluidic manipulation or fertilization procedures. In comparison, simulation results indicate that the shear stress and von Mises stress experienced by the oocyte in the given system are relatively lower than this range. These findings indicate that the design maintains low mechanical stress while enabling stable trapping and long-term culture. To further validate the biological safety of the FEMI system, experiments on on-chip oocyte maturation were conducted, and the results are presented below.

C. Single-oocyte trapping efficiency

In the experiment, a relatively high flow rate of $200\text{--}300 \mu\text{l/min}$ is applied during the initial 30 s to generate sufficient driving force for introducing the oocytes totally into the microchannel. Once the oocytes have entered the main

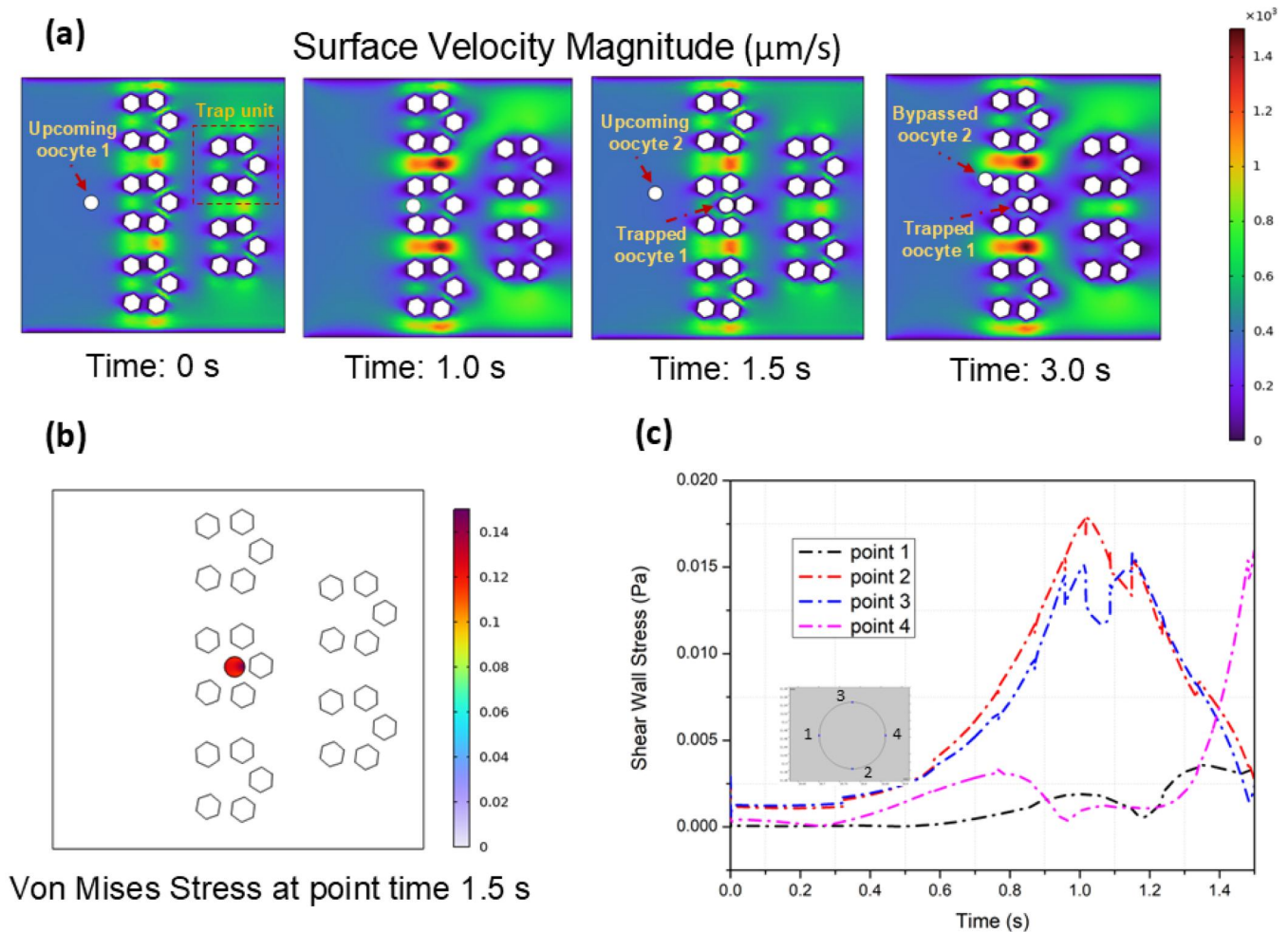


FIG. 4. Computational fluid dynamics (CFD) simulation of the single-oocyte trapping mechanism in our FEM system. (a) Surface velocity magnitude showing flow distribution over time; the color gradient from blue (low) to red (high) indicates fluid velocity. Oocyte migration toward an empty trap unit is shown, as well as the bypass mechanism when an oocyte approaches an occupied trap. (b) Von Mises stress distribution at 1.5 s, illustrating the mechanical environment around the trapped oocyte. (c) Temporal evolution of shear stress arises from fluid-induced friction at selected points on the oocyte surface from beginning to 1.5 s.

trapping region, the flow rate is then reduced to 50–70 $\mu\text{L}/\text{min}$. This lower rate ensures gentle hydrodynamic conditions that promote controlled movement and the individual positioning of oocytes into trap units while minimizing mechanical stress that could compromise cellular integrity. Figure 5 shows the ability of our fabricated HMT to capture single oocytes. However, oocytes tend to aggregate into clusters due to the secretions from cumulus cells, which is rich in hyaluronic acid and promotes cell adhesion.⁴⁶ The formation of these clusters poses several challenges, including difficulties in introducing oocytes into the microfluidic channel and properly positioning them within the trap units. Clumped oocytes may obstruct the channels, disrupt the intended single-oocyte trapping mechanism, or result in multiple oocytes being captured in a single trap unit, which is not optimal for oocyte development.

To assess the trapping performance of the proposed HMT, trapping efficiency is calculated. The trapping rate is defined as the proportion of oocytes successfully retained within the designated trapping units relative to the total number of oocytes introduced into the system. The experimental results reveal an average trapping rate of 88.37% and a standard error of about 1.5%, demonstrating the high efficiency of the microfluidic trapping system. The ratio of the number of oocytes that were not successfully trapped to the total number of oocytes introduced into the chip is approximately 11.63%. While some of these oocytes bypassed the trapping sites due to the variations in flow dynamics or trajectory, a significant portion exited the trapping region entirely. Notably, these escaped oocytes were predominantly smaller in size, with diameters ranging from 90 to 110 μm , including the surrounding cumulus cells. Such oocytes are generally considered to be of

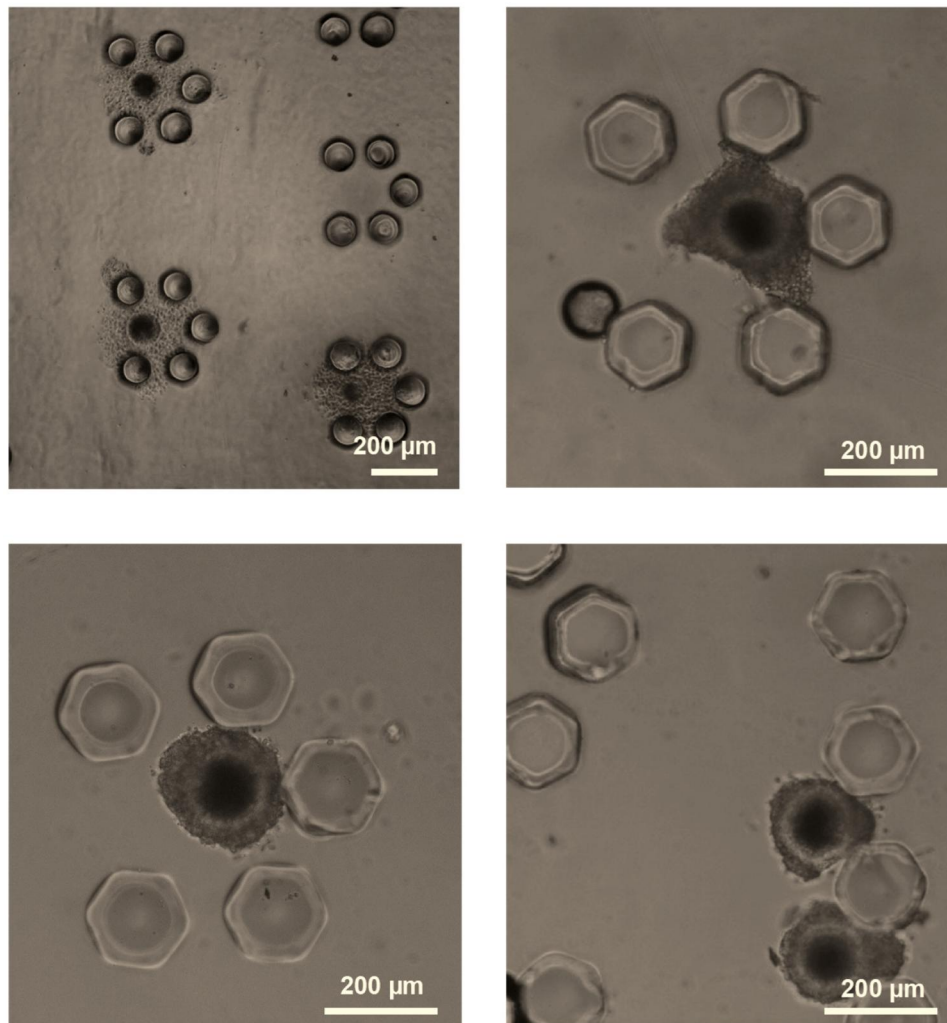


FIG. 5. Single oocytes trapped within the trap units.

relatively lower quality, as their small size poses challenges for further culture and ART research.¹⁷ This suggests that the system inherently performs size-based selection mechanism, wherein the progressive reduction in trap dimensions facilitates a form of oocyte screening. Additionally, the FEMI chip enables automated single-oocyte handling with high precision. Its integrated filter structure also effectively removes debris, overcoming the common issue of debris accumulation found in chamber-based single-oocyte trapping designs.^{4,6}

D. Single-oocyte maturation on chip

To more closely recapitulate the physiological *in vivo* micro-environment, the HMT system was operated under dynamic perfusion conditions and systematically evaluated for oocyte culture. Continuous microfluidic perfusion at a flow rate of 20 nl/min was employed to provide controlled mass transport, thereby enhancing

nutrient and gas delivery while facilitating the removal of metabolic by-products surrounding the oocytes.⁴⁷ In parallel, conventional static microdrop culture on Petri dishes was used as a reference condition. After the 2-day on-chip culture, the COCs are collected and denuded for the evaluation of maturation rate. The presence of the first polar body (PB1) in oocytes was examined, as its extrusion signifies the completion of the first meiotic division, indicating oocyte maturation and suitability for fertilization.⁴⁸ By directly comparing oocyte viability and developmental outcomes between dynamic perfusion within the HMT platform and standard static culture, this study highlights the advantages of microfluidic-based dynamic culture in supporting oocyte maintenance and developmental competence. The experimental outcomes obtained under dynamic microfluidic perfusion and conventional static culture conditions are summarized in Table III.

As shown in Table III, dynamic on-chip culture using the HMT platform showed advantages over conventional static

TABLE III. Comparison of oocyte survival, degeneration, and maturation rates between on-chip dynamic culture and conventional static culture. Boldface values represent the overall statistics for each culture system, expressed as mean $\pm$ SD across the five experimental replicates.

Culture system	Experiment	Survival rate (%)	Degeneration rate (%)	Maturation rate (%)
On-chip culture	1	82.61	17.39	43.48
	2	96.67	3.33	76.67
	3	100	0.00	80.00
	4	97.44	2.56	89.74
	5	97.30	2.7	78.38
	Mean $\pm$ SD	94.8 $\pm$ 6.93	5.2 $\pm$ 3.1	73.65 $\pm$ 7.88
Conventional static culture (control)	1	51.46	48.54	40.78
	2	95.00	5.00	70.00
	3	97.88	2.12	68.09
	4	94.29	5.71	88.57
	5	95.83	4.17	77.08
	Mean $\pm$ SD	86.89 $\pm$ 8.88	13.11 $\pm$ 8.88	68.9 $\pm$ 7.9

culture. Figure 6 shows that the overall distribution patterns of maturation rates are comparable between the control and on-chip groups, with variability observed across experimental replicates. This variation is evident in both conditions, with maturation rates ranging from 43.48%–89.74% (on-chip) and 40.78%–88.57% (control), suggesting an influence of batch-to-batch differences in oocyte quality. When examined in a paired manner, maturation rates between control and on-chip groups follow matched trends across experiments. For example, experiments with higher maturation in the control group (e.g., ~88.57% in experiment 4) also show correspondingly high values on-chip (~89.74%), while lower-performing batches (e.g., ~40%–43% in experiment 1) remain low under both conditions.

Oocytes cultured on-chip exhibited a higher survival rate (94.8 $\pm$ 6.93%) compared with those maintained under

conventional static conditions (86.89 $\pm$ 8.88%), accompanied by a lower degeneration rate (5.2 $\pm$ 3.1% vs 13.11 $\pm$ 8.88%). In addition, dynamic on-chip culture achieved a higher maturation rate (73.65 $\pm$ 7.88%) than conventional static culture (68.9 $\pm$ 7.9%) ($p = 0.04$, paired t -test). Together, these findings demonstrate that the HMT-based dynamic culture system more effectively supports oocyte survival and maturation while reducing degeneration relative to traditional static culture methods. Moreover, the micropillar-based trapping architecture of the HMT platform plays a critical role in stabilizing oocyte positioning during dynamic culture. By preventing oocyte displacement and uncontrolled movement under flow, the system minimizes mechanical stress and ensures uniform exposure to culture conditions. These findings suggest that the HMT platform improves oocyte viability and maturation under dynamic culture conditions,

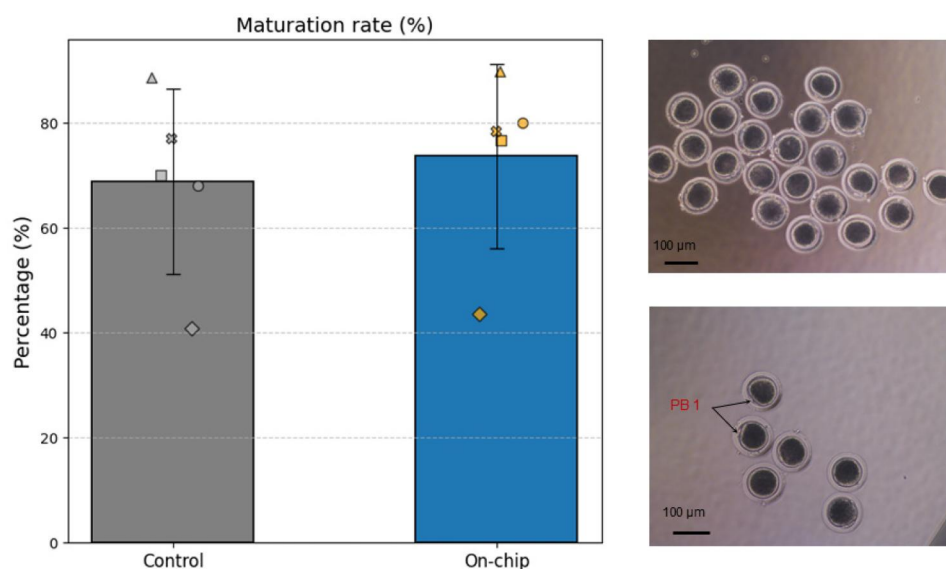


FIG. 6. Distribution of oocyte maturation rates under static and dynamic on-chip culture conditions, with representative images of matured oocytes on the HMT platform.

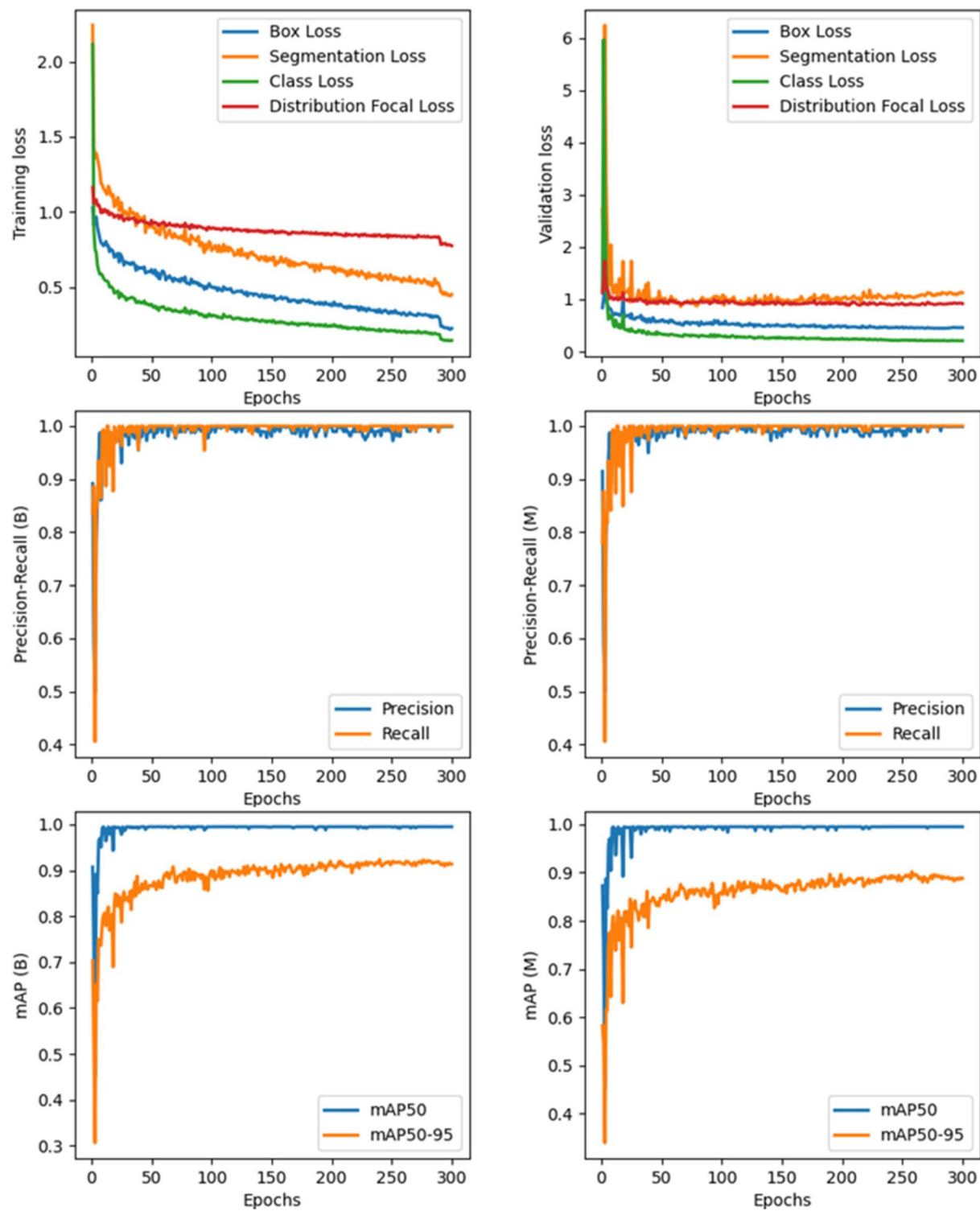


FIG. 7. The model's training result after 300 epochs, with a batchsize of 16 and a dropout of -0.1 . The label "B" refers to the metrics for the bounding box segmentation, while "M" represents the metrics for the mask segmentation.

likely by partially recapitulating key mechanical and transport features of the *in vivo* microenvironment. In addition, the ability of the platform to maintain oocytes at well-defined positions may facilitate its integration with AI-based image analysis for continuous assessment of oocyte status throughout culture, suggesting its potential utility as a versatile platform for experimental studies in IVM. Given the similarities in oocyte size and cellular characteristics between porcine and human oocytes, the hydrodynamic trapping mechanism and shear stress tolerances identified in this study are expected to be applicable to human ART. Future work will focus on validating the FEMI platform using human clinical samples to further confirm its efficacy and safety.

E. Deep learning-assisted analysis of single oocytes

The deep learning model used for segmenting oocyte and cumulus cells was trained on a dataset of approximately 480 images and validated using 120 images. Although 480 training images may appear limited, under the highly controlled microfluidic imaging conditions used in this study, with fixed trapping structures, stable illumination, and low background variability, this dataset size was sufficient for the model to achieve effective segmentation performance. Figure 7 illustrates that the loss function for the training dataset converges rapidly within the first 50 epochs, indicating that the model is learning quickly and effectively on this dataset. A similar trend is observed for the loss function of the validation dataset. However, the relatively high segmentation and focal losses indicate that additional training data are needed to improve performance. Regarding the average precision and recall values, they reach almost perfect levels, and a similar trend is observed for mAP50, demonstrating the model's strong capability in localizing masks for the target objects. In addition, an average mAP50–95 close to 90% indicates that the model

achieves relatively good segmentation performance, which is sufficient to meet the requirements of this study. Further improvement in segmentation accuracy for more complex objects will remain a focus of future work.

Deep learning has been used to automatically assess the size distribution of COCs trapped within the HMT structure. The size distribution histogram, as shown in Fig. 8, illustrates the size distribution of COCs captured in the microfluidic chip. The introduced oocyte population was randomly distributed without pre-selection based on size. The distribution reveals that most trapped oocytes fall within the 150–210 μm range, accounting for approximately 57.56% of the total. This suggests that the 150–210 μm range is the most representative oocyte size.

The proportion of trapped oocytes decreases for smaller size ranges, such as 100–120 μm and 120–150 μm , while the occurrence of larger oocytes, 250–350 μm , is minimal, at around 3.18%. The results demonstrate the microfluidic chip's ability to trap oocytes of various sizes, highlighting its suitability for IVM applications. The culture system was integrated with a real-time monitoring system to track oocyte development over time. Time-lapse images were collected and utilized as a dataset for a deep learning model, which segmented the oocytes and their surrounding cumulus cells to assess the expansion of cumulus cells during the developmental process. The segmented images were then analyzed to quantify the cumulus area based on pixel measurements. For example, Fig. 9 illustrates the morphological changes of an oocyte over 18 h, captured by the time-lapse system and the corresponding segmented images. The prediction model segmented the cumulus cells and provided area measurements. The overall trend indicates an increase in the cumulus cell area over the 18 h period, with an expansion of nearly 25% compared to the initial state, indicating oocyte maturation facilitated by nutrient exchange and reduced shear stress. The experimental results demonstrate the capability of the FEMI system in analyzing and extracting information on the cumulus cell area, as well as its growth during development. This parameter is considered the most reliable for evaluating cumulus cell expansion and strongly associated with oocyte growth and the potential for embryonic growth.³³

Overall, the integration of the YOLOv8 deep learning model enables automated morphological analysis of cumulus expansion. By applying a trained model, the system provides consistent segmentation and area quantification of cumulus cells, offering a more objective and reproducible metric for oocyte assessment. Future improvements to the model could involve increasing the number of training images and implementing fine-tuning strategies to enhance segmentation accuracy for cumulus cells. In additions, we will explore dynamic culture conditions with controlled fluidic flow and further develop the platform towards an organ-on-chip system. Additional experiments will be conducted to culture and collect time-lapse data from a variety of oocytes under different scenarios. These data will be used to evaluate the quantitative relationship between cumulus cell expansion and oocyte competency, fertilization potential, and embryo development by using advanced deep learning models.

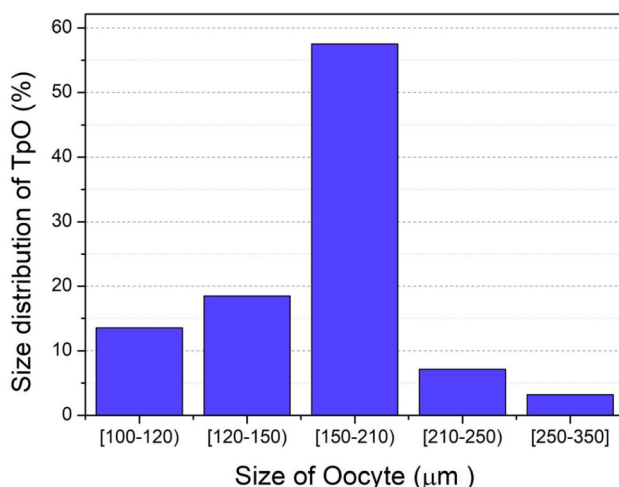


FIG. 8. Size distribution of trapped COCs.

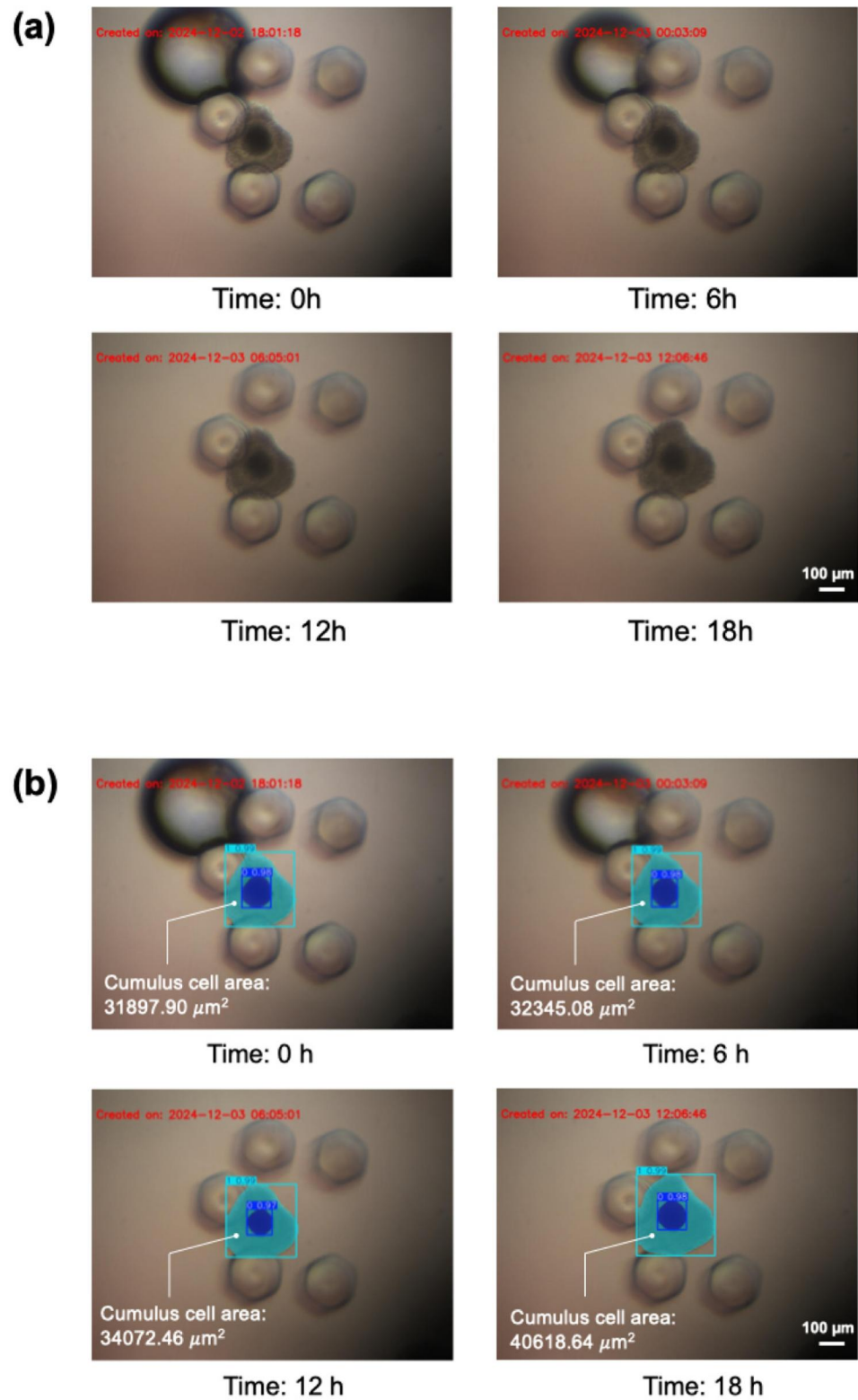


FIG. 9. (a) An example of the oocyte maturation process over 18 h. The time-lapse images are then utilized as input data for model prediction. (b) Output images after the prediction step with class 0 stands for the oocyte and class 1 stands for the cumulus cells.

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IV. CONCLUSION

In this study, we propose the FEMI system, which integrates a microfluidic platform for single-oocyte trapping with automated real-time assessment by deep learning models. The microfluidic chip features a hydrodynamic trapping structure based on hexagonal micropillars that efficiently captures single oocytes of diverse sizes, while simultaneously filtering out small oocytes that are unsuitable for culture. This design ensures high trapping efficiency and optimal selection, laying the groundwork for a controlled oocyte culture environment. The FEMI platform showed a statistically significant improvement in maturation rates and cumulus expansion ($p < 0.05$). Compared to static culture, the dynamic flow within the chip provides more consistent media exchange, which may improve nutrient delivery and waste removal around the oocytes. Furthermore, by integrating deep learning technology, the system enables real-time monitoring and automated analysis of cumulus cell expansion and oocyte development during culture. The contributions of the FEMI system lie in its interdisciplinary integration, combining a biologically optimized microfluidic platform for precise oocyte handling with real-time deep learning-based assessment. The integration of microfluidic trapping, culture, and AI-assisted evaluation of single oocytes may improve workflow efficiency and support automation, suggesting its potential as a useful platform for future ART applications. Although the FEMI platform demonstrates automated oocyte trapping and may contribute to improvements in porcine IVM, its direct translation to human clinical settings requires more extensive investigation. Future research should include the evaluation of the platform with human clinical samples, along with assessment of downstream developmental outcomes, including fertilization success and blastocyst formation rates, to further explore its potential application in ART.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Ethics Approval

This study has Dinh Tien Hoang Institute of Medicine Ethics Committee approval (Ref No.: IRB-A-2408).

Author Contributions

Thu Hang Nguyen: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal). **Ngoc Anh Thi Nguyen:** Formal analysis (equal); Investigation (equal); Writing – original draft (equal). **Tung Thanh Le:** Formal analysis (equal); Investigation (equal). **Hanh Van Nguyen:** Formal analysis (equal); Investigation (equal); Validation (equal). **Hang Thu Bui:** Formal analysis (equal); Investigation (equal); Methodology (equal). **Hoang Anh Phan:** Conceptualization (equal); Formal analysis (equal); Investigation

(equal); Methodology (equal); Software (equal). **Tung Thanh Bui:** Methodology (equal); Supervision (equal). **Trinh Chu Duc:** Conceptualization (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal). **Loc Quang Do:** Methodology (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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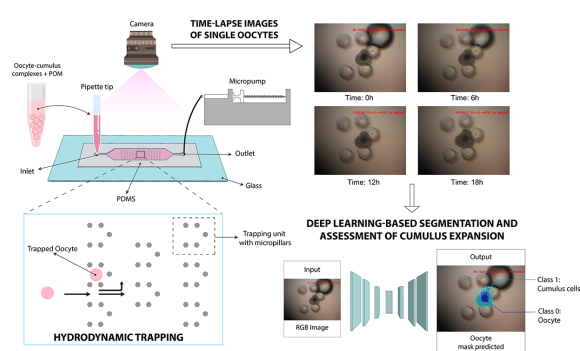
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5 June 2026

Enhancing *in vitro* maturation with microfluidics and artificial intelligence

Chris Patrick

Trapping and growing immature eggs on-chip increased maturation rate compared to traditional culture methods.



In vitro maturation develops immature eggs, or oocytes, in the lab instead of the body. This procedure involves static culture conditions, manual handling, and subjective assessment – limitations that can degrade oocyte quality.

Nguyen et al. present a novel system that combines microfluidics and artificial intelligence-driven image analysis to improve *in vitro* maturation. Simulation and experiment showed that a specifically designed microfluidic chip with hexagonal micropillars efficiently trapped individual oocytes of various sizes while filtering out smaller oocytes, which are often lower quality.

The chip's dynamic flow more closely mimics conditions in the body than static cultures, improving nutrient delivery and waste removal around the oocytes. The team demonstrated that it had a higher maturation rate compared to traditional culture methods, suggesting that the chip may provide a more favorable environment for oocyte development.

In addition, image analysis based on a deep learning model allowed the system to automatically evaluate oocyte development at the single-cell level in real time.

"The study demonstrates that combining AI with microfluidic technologies may transform biomedical research by enabling more precise, automated, and physiologically relevant cell culture and analysis systems," said author Thu Hang Nguyen. "I think this platform could play an important role in optimizing *in vitro* maturation and advancing the next generation of assisted reproductive technologies."

Next, the authors plan to optimize the system and validate its performance on animal models before it is translated to human applications.

"Our research team collaborated closely with clinicians and biomedical research institutes, which enabled us to work towards practical and human-centered applications in assisted reproductive health," Nguyen said.

Source: "FEMI: deep learning - Assisted analysis of single oocytes trapping and maturation for enhanced on-chip IVM," Thu Hang Nguyen, Tung Thanh Le, Hanh Van Nguyen, Hang Thu Bui, Hoang Anh Phan, Tung Thanh Bui, Trinh Chu Duc, and Loc Quang Do, *Biomicrofluidics* (2026). The article can be accessed at <https://doi.org/10.1063/5.0328820>.

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REVIEW

Emerging Bioengineering Technologies in Female Reproduction: Preclinical Advances, Translational Challenges, and Future Outlook

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ABSTRACT

Female infertility, which affects millions of couples globally, has been a highly focused research field owing to the importance of reproduction in humans. Emerging bioengineering technologies, including tissue engineering, microfluidic chips, imaging techniques, personalized medicine, gene editing tools, and artificial intelligence, have the potential to revolutionize the existing assisted reproductive technology. These technologies have enabled creating artificial biomimetic systems for the culture of oocytes and embryos; changed the way they develop; and enhanced their competence evaluation in an automatic manner. However, the implementation and potential integration of these technologies have been a long-entrenched challenge due to the lack of standardized protocols and precise control over reproductive cycles. This review article summarizes recent advances in these innovative approaches, with an emphasis on tissue engineering and microfluidic technology. Their convergence is discussed as a potential pathway toward more integrated, precise, and personalized reproductive systems for next-generation assisted reproductive technology. In this context, key challenges related to ethics, standardization, cross-technology integration, and clinical translation are further discussed.

1 | Introduction

Reproductive or endocrine failure is considered a global health issue [1]. Millions of people, regardless of whether they are from high-, middle-, or low-income countries, suffer from infertility, which also impacts their families and communities. An estimated one in six individuals globally who are of reproductive age will at some point in their lives become infertile [2], showing an urgent need to do in-depth research on the reproductive systems and provide access to high-quality, reasonably priced fertility care. In the female reproductive system, infertility can result from a variety of abnormalities affecting the endocrine system, fallopian tubes, uterus, and ovaries, among other organs

[2]. Despite the need for research on female reproduction, the direct study of the human reproductive system is hindered by multiple factors, including ethical concerns for maternal and fetal safety [3, 4]. Such reproductive experiments may harm the individual, as medications for fetal benefit must be administered through the body [5, 6]. Even though animal models can be used as a substitute, these models may fail to replicate human responses. For example, within the mouse model, despite having been used extensively and approved for its relevance to human reproductive development, there still exist anatomic and developmental endocrinologic differences that could affect the results [7]. Other animals, including rats, cows, buffalo, ewes, mares, etc., and especially nonhuman primates, are more closely related

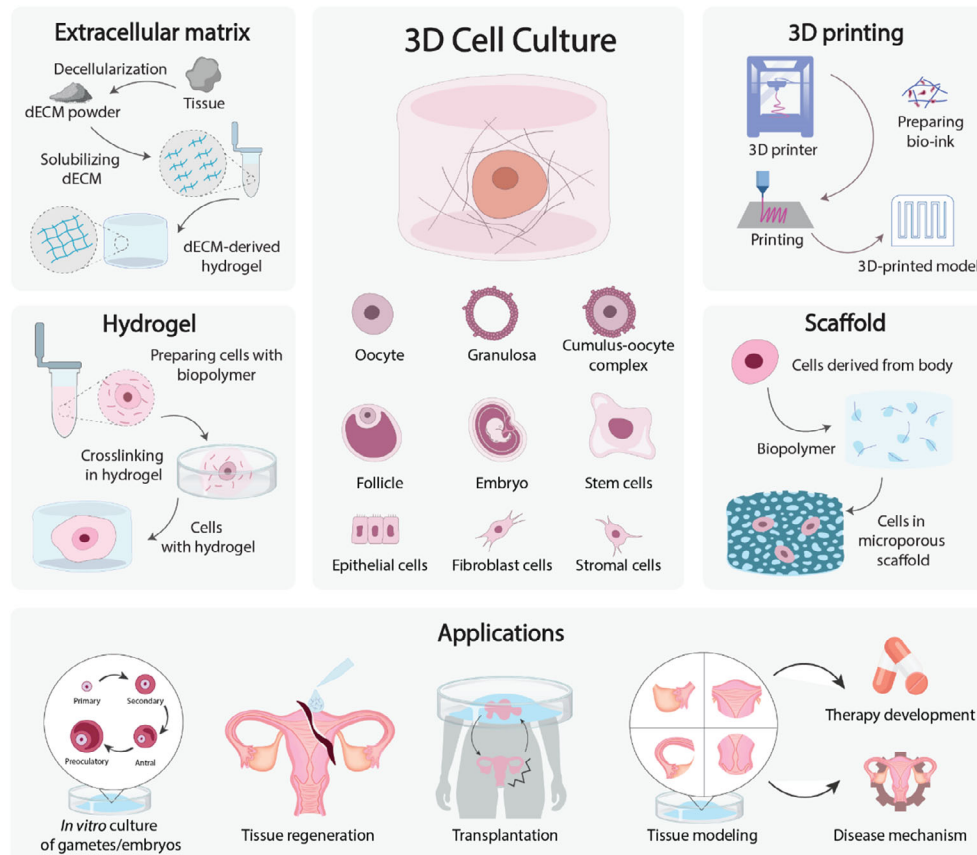


FIGURE 1 | Three-dimensional cell culture platforms for reproductive bioengineering and ART. The schematic illustrates representative 3D culture and tissue engineering strategies used to model female reproductive tissues, organs, and developmental structures. Reproductive cells and structures, follicular-supporting cells, somatic stromal/epithelial cells, and stem cells can be cultured in biomimetic environments generated from decellularized extracellular matrix (dECM), hydrogels, porous scaffolds, or 3D-printed constructs. These platforms provide structural support, ECM cues, cell–cell and cell–matrix interactions that are difficult to reproduce in conventional 2D culture. Collectively, these 3D model strategies are used for in vitro culture of gametes and embryos, follicle development, ovarian or uterine tissue regeneration, transplantation studies, reproductive tissue modeling, therapy development, and investigation of disease mechanisms. By recreating key aspects of the native reproductive microenvironment, these platforms provide preclinical tools for studying reproductive physiology and developing bioengineered approaches for ART.

to humans anatomically and physiologically; however, there are still differences among them in terms of functional morphology, physiology, reproductive senescence, and reproductive aging [8–12]. Therefore, there is an increasing demand to establish and develop new models that are relevant to human reproduction.

As a solution to this worldwide predicament, assisted reproductive technology (ART) has been developed to meet the urgent demand, including all infertility treatments in which either oocytes or embryos are handled [13–15]. ART procedures generally require surgically retrieving oocytes from a woman’s ovaries, fusing them with sperm in the laboratory, and transferring them to the woman’s body or giving them to another woman. Since the early development of reproductive tissue transplantation and in vitro culture, the field has advanced substantially, marked by key milestones such as the first human birth from in vitro fertilization (IVF) in 1978, birth from a frozen embryo in 1984, ovarian tissue transplantation in 2005, in vitro follicle growth using biomaterials in 2006, in vitro maturation of human metaphase-II oocytes in 2015, and the development of the EVATAR reproductive tract model in 2017 [16–24]. Recently, emerging bioengineering methods to study the female reproductive system, including

tissue engineering, microfluidic techniques, imaging techniques, personalized medicine, gene-editing tools, and artificial intelligence (AI), have accomplished remarkable achievements. These techniques support the development of advanced female reproductive models and may improve ART by enabling personalized biomimetic systems, better oocyte and embryo assessment, and automated embryo selection using time-lapse imaging. These systems have emerged as a potential replacement for the traditional in vitro culture of oocytes and embryos in a petri dish system.

Several reviews have examined bioengineering technologies in female reproductive medicine, including biomaterials, organoids, microfluidics, 3D printing, OoC models, and engineered reproductive tissues for modeling reproductive organs, embryo implantation, placental function, and reproductive diseases [18, 25, 26]. Although these studies have highlighted important advances in biomimetic modeling and reproductive tissue engineering, they have mainly focused on individual technologies. A broader discussion is still needed on how these approaches can be combined with advanced imaging, gene-editing tools, AI, and precision medicine to build more integrated and clinically relevant ART platforms.

This review first outlines recent progress in emerging bioengineering approaches for ART, including biomaterials, tissue engineering, microfluidics, imaging technologies, precision medicine, CRISPR–Cas9, and AI, in Section 2. These technologies are examined as complementary components of more integrated, biomimetic, and clinically adaptable ART systems. Current challenges related to technology integration, standardization, ethics, and clinical translation are further discussed in Sections 3 and 4, together with future perspectives for next-generation bioengineered reproductive systems.

2 | Emerging Technologies and Integrative Interfaces in ART Systems

2.1 | Tissue Engineering

2.1.1 | Why 3D Cultures?

In ARTs, 2D (two-dimensional) culture of cells and tissues has been routinely performed in numerous hospitals and laboratories worldwide. However, traditional 2D culture has been inadequate for the development of cells and tissues in vitro [27, 28]. 2D culture fails to reproduce the chemical and physical characteristics of the physiological environment in the body [29, 30]. As a result, 2D culture may inadequately recapitulate the native microenvironment required for oocyte and embryo growth, including cell–cell and cell–matrix interactions [28]. Therefore, the development of a novel approach that better mimics in vivo environments and improves culture efficacy is necessary [31, 32]. Recently, researchers have attempted tirelessly to create artificial tissue, such as ovaries and the uterus, to provide a 3D environment based on 3D biomaterials [33–35]. The biomaterials introduced in scaffold-based 3D culture systems should permit oxygen, nutrients, waste exchange, and support interactions between multiple cell types to recreate functional reproductive systems [36]. When considering biomaterials for 3D culture, chemical and physical properties are the most important parameters [37, 38]. Specifically, low cytotoxicity is undoubtedly one of the key parameters for long-term in vitro culture. The matrix must be noncytotoxic to keep the oocytes/embryos and surrounding cells alive. Additionally, a cell-friendly, biocompatible matrix (ideally antibacterial) that can reabsorb to create space for new tissues and shows connective porosity to cell penetration is prioritized to avoid unwanted interactions between cells and matrix [39, 40]. Permeability and porosity are necessary physical properties for nutrient and gas transportation to promote cell growth, as they directly affect cell behaviors. Figure 1 provides an overview of how 3D culture strategies are applied in reproductive tissue engineering and ART-related research. These systems create partially biomimetic microenvironments that support spatial organization and structure, cell–cell communication, and cell–matrix interactions, which are difficult to reproduce in conventional 2D culture. As illustrated in the figure, 3D culture platforms can be constructed using decellularized extracellular matrix (dECM), natural or synthetic hydrogels, porous scaffolds, and 3D-printed constructs. Their applications range from improving gamete and embryo culture to modeling reproductive tissues, supporting tissue regeneration, and developing transplantation-oriented approaches. The specific biomaterials, tissue reconstruction strategies, and 3D printing technologies are discussed in detail in the following subsections.

2.1.2 | Materials for Enhancing In Vitro Culture of Gametes/Embryos

Several researchers have provided compelling evidence that 3D culture offers a promising approach for gamete maturation improvement, embryo development, and enhanced assisted reproductive technologies. These advances in biomaterials have enabled the creation of artificial biomaterials mimicking the in vivo environment for embryo implantation and development. For example, Govindasamy et al. have created a biomimetic system which recapitulates decidualized endometrial stroma, first observing the implantation process on mouse model in the laboratory [41]. The artificial culture environment, based on synthetic hydrogels (methacrylated dextran or polyethylene glycol (PEG)) crosslinking with commercial cleavable peptide sequences, harbored the cellular implantation mechanism as well as provided important features including biodegradability, decidual-like stiffness, and integrin adhesion. Although the model reproduced aspects of trophoblast invasion and early embryo–maternal vascular interactions, the somatic tissue compartment remained less functional than in vivo tissues, which may restrict long-term embryo culture and developmental progression. In 2019, Li's research group proposed an extracellular matrix (ECM) that contains 2D in vitro culture medium (IVC1 and IVC2), comprising mostly of advanced DMEM/F12 [42] and Matrigel for embryonic culture in a 96-well plate at culturing conditions of 37.2°C, 6% CO₂, and saturated humidity [43]. This system successfully grew human blastocysts until 14 days post-fertilization and mimicked key developmental events in vivo before gastrulation, including embryonic disc, amniotic membrane, basement membrane, secondary yolk sac, anterior–posterior polarity formation, and primitive streak anlage. Based on culture systems integrating IVC1, IVC2, and matrigel, 3D in vitro models for human and non-human primate embryo culture have demonstrated improved performance compared with conventional 2D platforms [44–47]. Among them, a model applying time restriction to matrigel exposure, which enhanced the nutrient diffusion into embryonic tissue through the prevention of trophoblast attachment, was shown to support 3D embryonic morphogenesis and create maternal interaction. It was reported to cultivate monkey embryos 25 days post-fertilization, the longest ex vivo culture period of primate embryos to date [48]. The system provided valuable insights into embryogenesis through advanced gastrulation and early neurulation. Although still preliminary, these systems pave the way for creating artificial maternal environments for embryo development, which could be further combined with an “artificial womb” to grow embryos at multiple developmental stages [49, 50].

Regarding immature oocyte culture, results have shown that 3D culture can overcome several 2D culture detriments and yield better outcomes of the cultivation process: cell phenotypes, nuclear maturation, oocyte growth, fertilization rate, and embryo quality [23, 51–53]. Torre et al. designed a follicle-mimicking 3D coculture structure [54]. Denuded germinal vesicle-stage oocytes cultured in microdroplets were compared with those cocultured with granulosa cells encapsulated in barium alginate capsules. After 48 h, the percentage of mature oocytes reached 90.3% in co-culture model, while in micro drop culture, this figure was only 52%. Also, using alginate-based matrices, Kreeger et al. examined the mouse follicle development in the 3D culture

system modified with ECM molecules for follicles, with an oocyte in the center and layers of granulosa and theca cells surrounding, in two-layered secondary and multilayered secondary stages. The 3D culture model preserved the in vivo follicle morphology and maintained the follicle development. Interestingly, the hydrogel assisted cell–cell and cell–matrix interactions, depending on the follicle development stages and matrix components [55].

2.1.3 | Tissue Engineering for Reconstruction and Transplantation of Reproductive Organs

In recent decades, organ and tissue transplantation have been significantly beneficial to patients suffering end-stage organ diseases, including female reproductive organ failure [56]. Recently, researchers have reported the first woman in the world to give birth whose ovarian tissue was frozen before puberty due to suffering from thalassemia [57]. Cryopreserved ovarian tissue autotransplantation, having emerged as a potential approach for rare genetic diseases and cancer, can reinstate lost ovarian endocrine function and fertility of many prepubescent girls and young patients who experience precocious ovarian insufficiency caused by treatments. Autotransplantation of ovarian tissue without the need for repeated cycles of hormone stimulation, thus reducing the risk of ovarian hyperstimulation syndrome and avoiding delays in chemotherapy treatments. Despite promising outcomes, risks include the possible reintroduction of cancer cells, particularly in patients with ovarian cancer or highly metastatic cancers, as well as loss of the follicular pool due to tissue ischemia and inconsistent graft quality [58, 59]. In addition, organ or tissue transplantation has several severe limitations, including donor shortage, surgery-related risks, side effects, and complications faced by both the living donor and the recipient after a long time of surgery [60]. Therefore, an increasing demand for the development of artificial tissue while avoiding intact tissue usage has arisen. The artificial tissue is helpful not only for cancer patients who need fertility preservation but also for patients having an abnormal or missing uterus, requiring artificial uterine tissue transplantation. 3D culture using proper biomaterial, with the ability to adjust the properties to mimic in vivo environment, served as a good candidate for in vitro cell culture and transplantation.

Tissue transplantation requires specific preservation conditions and applying a method to enhance preservation time as well as the quality of tissues. A recent study developed artificial ovarian tissue using poly(ethylene glycol) vinyl sulfone (PEG-VS) hydrogels crosslinked with functional peptides to investigate follicle development and the effects of ischemia during preservation and transplantation in mice [61]. Modified PEG hydrogels, with remodeling and neovascularization capacity, can mimic the stiffness of soft stromal tissue, physically support oocyte–somatic cell connections and follicular structure, promote follicular expansion, and prolong ovarian tissue longevity. The preserved follicle growth process was consistent with naturally nourished mice. In addition, the appearance of blood cells in the vessels and blood vessel density after 30 and 60 days of implantation showed that PEG hydrogels are capable of graft remodeling and vascularization [61]. Fibrin-based materials, which enhance cell adhesion [62] and support ovarian stromal cell survival and

proliferation [63], have been investigated in numerous studies for the construction of standardized artificial ovaries. Kniazeva et al. transplanted follicles encapsulated in these biomaterial-based grafts into adult infertile mice. Fibrin and its combination with a second component of nondegradable alginate or more slowly degradable collagen provided a supportive environment for follicle organization. All mice exhibited cyclicity after transplantation, subsequently mated with or without the use of vascular endothelial growth factor (VEGF). Surprisingly, live birth was achieved when using VEGF-loaded fibrin beads [64]. Another study evaluated fibrin-based matrices for artificial ovary construction by autografting isolated murine preantral follicles and ovarian cells encapsulated in two fibrin formulations. One week after transplantation, recovered follicles remained largely viable and showed signs of growth, while ovarian cells survived and proliferated within the scaffold. Vascular structures in both formulations suggest early graft integration [65]. Another material utilized for synthetic ovary-related topics is ECM. Most recently, the development of an injectable ovarian-specific ECM hydrogel has made it possible to transfer follicles and create an in situ ovary. dECM, containing a tissue-specific milieu of proteins and proteoglycans, underwent dynamic remodeling to support tissue function and morphological mechanisms for cell proliferation and steroidogenesis. ECM-resuspended follicles were microinjected into the ovarian cortex and were viable for at least 90 days post-transplantation. This work showed sustainable follicle engraftment, spontaneous pregnancy, and live birth after intraovarian microinjection of isolated exogenous follicles into chemotherapy-treated mice [66].

Regarding artificial uterine tissue, this strategy remains challenging for clinical use. Nonetheless, initial experiments have been carried out on animal models such as rats, mice, rabbits, and large animals, e.g., sheep [67–74]. Some have successfully transplanted the artificial uterine tissue in vivo and obtained encouraging results [70–74]. Mats et al. designed decellularized uterine scaffolds to repair a fractionally defective uterus in a rat model [70]. The rat mated 6 weeks after transplantation with a supported pregnancy; fetal development and fetal growth over the transplanted areas were observed. In another work using the rat model, collagen scaffolds were transplanted with bone marrow mesenchymal stem cells seeded into a severe full-thickness injury uterine [71]. The grafts increased the proliferative abilities of the host uterine endometrial and muscular cells, facilitated the regeneration of microvasculature, and the function of the endometrium to receive the embryo rehabilitated for at least 8 weeks. Initial promising results have been observed on the sheep model [67]. Remarkably, significant regeneration was observed in several of the most successful cases, resembling natural uterine tissue after 6 weeks following transplantation. To the best of our knowledge, until now, there has been no published work on the in vivo transplantation of a bioengineered uterus using larger animals or human models. However, advances in this field showed promising results of culturing human cells in bioengineered endometrium tissue [75–78] and cervix tissue [79] in vitro. Most successful in vivo transplantation cases utilized a decellularized matrix, which has also been applied in many other tissues [80]: colon [81], urethra [82], heart [83, 84], and vessel [85]. Decellularized biomaterial refers to the removal of cells and their components from the natural ECM, remaining the specific architecture and preserved properties [86]. The ECM was first

decellularized by chemical, biological, or physical methods and then in vivo or in vitro re-cellularized with cells, mostly host cells. The dECM possesses the advantages of a low immune response [87] and preservation of important components. Thus, dECM has become a useful tool for the regeneration and transplantation of multiple organ tissues, which includes the female reproductive system.

2.1.4 | 3D Printing Technology for Tissue Engineering

Recent advances in 3D hydrogel-based culture for assisted reproduction highlight the importance of simple and reliable microfabrication and encapsulation procedures. The detailed procedures for the hydrogel preparation, fabrication, and applications in cell culture have been presented in the previous paper [88, 89]. 3D bioprinting technology, which enables the creation of accurate artificial structures from biomaterials, has been applied in 3D culture. First, bio-ink containing hydrogel and cells has been printed in precise shapes at 3D positions. For example, Mastrorocco et al. introduced an automated one-step bioprinting method to create alginate hydrogel microbeads enclosing sheep oocyte-cumulus complexes [90]. The size and shape of the alginate hydrogel were controlled by using an open-source tool for the Sphyga bioprinter, allowing homogeneous generation and targeted concentration as expected. Subsequent in vitro culture of the printed spherical microbeads within 24 h witnessed a significantly higher percentage of oocytes reaching the mature (MII) stage than the 2D culture model, allowed full cumulus expansion, and limited the growth of meiosis abnormalities while maintaining the expression of maternal effect genes.

Second, 3D printing has been used to fabricate hydrogel scaffolds with specific 3D architecture for subsequent cell seeding and maturation. A bioprosthetic ovary was developed using 3D-printed, partially crosslinked gelatin scaffolds with a follicle-loaded microporous architecture optimized to provide sufficient depth and multiple contact sites for the in vitro survival and differentiation of murine follicles [91]. Most notably, the surgically sterilized mice with implanted artificial ovaries restored ovarian function in vivo and produced pups through natural mating without stimulation of angiogenic growth factors and hormones, as well as embryo transfer. A similar approach of 3D printing technology has also been proven to be a potential solution for uterine endometrium repair [92]. A mixture of human-induced mesenchymal stem cells (hiMSCs), gelatin, and sodium alginate was utilized as the ink for 3D printing of the cell-loaded hydrogel scaffold, which was later implanted into a deep endometrial injury model. Using hiMSCs to avoid the immune response, the authors failed their main purpose due to an over 1-month acute immune response, even though an immunosuppressant was taken advantage. Although failure in complete restoration, the 3D printed scaffold enhanced endometrial receptivity functional indicators and encouraged the restoration of endometrial histomorphology as well as the regeneration of endometrial and endothelial cells. Another example is a 3D-printed placenta model developed for studying trophoblast migration in preeclampsia, a serious phenomenon in pregnancy [93]. In this work, a 3D bio-engineered model using 5% gelatin methacrylate hydrogel enabled the reconstitution of the spiral

shape of maternal vasculature and the biophysical properties of the placenta, including the native tissue's stiffness and controlled gradient of diffused chemokines and nutrients. This placenta model with embedded cells, growth factors, and ECM showed promising results in the evaluation of trophoblast and human mesenchymal stem cell migration, suggesting potential solutions for preeclampsia direct and indirect treatments.

To produce better compatible artificial organ structures, 3D printing technology could combine with dECM materials. Zheng et al. fabricated porous cylindrical-shaped ovary structures using porcine dECM encapsulating primary ovarian cells based on the 3D printing technique [94]. The scaffold made of bio-ink containing dECM solution mixed with a seaweed gelatin blend solution could maintain its original shape as well as keep 90% of cells viable after a 14-day culture. The proposed artificial ovary was transplanted to repair the mouse ovary without triggering an immune reaction and witnessed neovascularization in transplanted tissues after 4 weeks of implantation, showing better performance in comparison to 3D-printed scaffolds without embedded primary cells in terms of sex hormone secretion and primary ovarian cell proliferation.

The sophistication of 3D cultures is further enhanced by integrating microfluidic technologies, which facilitate the precise control of the microenvironment. This integration allows the dynamic simulation of bodily functions, providing a more accurate model of human reproductive organs. Microfluidic systems, with their precise fluid handling capabilities, offer an ideal platform for implementing the complex tissue architectures developed through tissue engineering.

2.1.5 | Comparative Analysis and Design Principles for Reproductive Biomaterials

Biomaterials are central to reproductive tissue engineering because their mechanical, biochemical, and degradation properties directly influence cell behavior, oocyte and follicle maturation, and tissue function. As summarized in Table 1, each class of biomaterial presents distinct advantages and limitations. Natural biomaterials, such as collagen, fibrin, and dECM, generally provide high bioactivity and support cell-matrix interactions, whereas synthetic hydrogels, particularly PEG-based systems, offer greater control over stiffness, degradation, and structural stability when appropriately engineered. For early ART-related processes, including in vitro maturation and follicle culture, materials with well-defined mechanical properties are preferred to preserve the spatial organization of oocyte-surrounding cells, reduce mechanical stress, and provide a stable 3D microenvironment. Although matrix stiffness contributes to the structural regulation of follicle architecture and expansion, material bioactivity is also critical for delivering biochemical signals that recapitulate the ovarian niche and promote cell-cell and cell-matrix communication. Accordingly, bioinert or weakly bioactive materials may require functionalization with adhesive peptides, ECM-derived motifs, or other bioactive factors to improve their biological performance. In ovarian tissue transplantation, ischemic injury, delayed revascularization, immune or inflammatory responses, and limited graft longevity remain major barriers; however, advanced biomaterials are increasingly being designed to improve

TABLE 1 | Comparison between biomaterial properties.

Application	Material	Type	Mechanical properties	Stiffness tunability	Degradation rate	Bioactivity	References
Embryo implantation, Follicle encapsulation, Artificial ovary	PEG	Synthetic hydrogel	Tunable	High	Low to tunable depending on degradable segments or crosslinkers	Bioinert, enhanced by biofunctionalization	[61, 95–98]
Embryo implantation, Endometrial model	Matrigel	ECM-derived hydrogel	Soft, ECM-rich but poorly defined	Low	Moderate	High	[99–103]
Follicle culture, Embryo culture, Endometrium regeneration	Alginate	Natural hydrogel	Supports spherical structure, preserves follicle integrity	Moderate, formulation-dependent	Low, tunable if modified	Low-Moderate if functionalized	[90, 95, 104–106]
Follicle culture, Endometrial regeneration, Ovarian tissue	Collagen	Natural ECM protein	Soft, flexible	Moderate	Moderate–High; enzymatically degradable	High	[71, 107–109]
Follicle culture, Intrauterine adhesion treatment, Endometrial regeneration	Chitosan	Natural polymer	Soft, structurally supportive	Moderate; formulation-dependent	Low–Moderate; formulation-dependent	Moderate	[110–112]
Artificial ovary, Intrauterine adhesion treatment	Fibrin	Natural hydrogel	Soft, porous network enabling cell infiltration	Low–Moderate; formulation-dependent	High	High	[62, 63, 113, 114]
Endometrial repair, Intrauterine adhesion treatment	Hyaluronic acid	Natural ECM-derived polysaccharide hydrogel	Hydrated and viscoelastic; injectable as hydrogel	Moderate; modification-dependent	Tunable	Moderate-High	[115–117]
Follicle culture, Artificial ovary	Fibrin + Alginate	Composite (hybrid)	Improved structural stability with bioactive cues	Moderate–High; composition-dependent	Tunable	Moderate-High	[66, 118, 119]
Follicle culture, Artificial ovary	PEG-VS hydrogel	Synthetic hydrogel	Tunable	High	Tunable	Low-Moderate	[61]
Ovarian/uterine regeneration, reproductive tissue bioinks	dECM	dECM	Tissue-specific and physiologically relevant	Low, Tunable	Variable	High	[87, 120, 121]

graft survival and functional outcomes. Engineered hydrogels and ECM-mimetic scaffolds have been explored to support follicle survival, promote vascularization, and provide a protective microenvironment for ovarian graft integration and functional recovery. In intrauterine adhesion treatment, biomaterials are increasingly shifting from passive anti-adhesion barriers toward regeneration of a functional endometrial microenvironment. These materials are typically designed to be biocompatible, biodegradable, and injectable or in situ-forming, thereby sup-

porting angiogenesis and endometrial regeneration. Applications related to experimental embryo development, implantation modeling, and uterine tissue engineering often benefit from natural or ECM-derived materials that provide tissue-relevant biochemical cues. However, the limited tunability and batch-to-batch variability of some natural matrices may affect reproducibility. To address these trade-offs, composite biomaterials have increasingly been developed to combine the bioactivity of natural matrices with the physicochemical control of synthetic systems.

2.2 | Microfluidic Systems

2.2.1 | From 3D Culture to Organ-on-a-chip

Although the growth of 3D culture systems has much improved the potential of ARTs, the precise fluid circulation and mechanical/structural integrity of the human body have not been preserved. Key aspects of the *in vivo* microenvironment, including spatial tissue arrangement, multicellular interactions, biochemical gradients, hormone exposure, and biomechanical forces, require more precisely controlled culture platforms. This requirement has promoted the development of microfluidic systems that can integrate 3D culture models with controlled perfusion, compartmentalized culture chambers, and dynamic microenvironmental stimulation. As illustrated in Figure 2, the transition from conventional 2D culture to 3D culture and then to microfluidic platforms provides a stepwise strategy for improving the physiological relevance of *in vitro* reproductive models [29]. Specifically, Figure 2 summarizes how 3D culture components can be combined with microfluidic channels to support dynamic culture, cell–cell interactions, biomimetic stimulation, and hormone-related regulation. These integrated platforms further provide the foundation for reproductive organ-on-a-chip (OoC) systems, including ovary-on-a-chip, oviduct-on-a-chip, decidualization models, embryo implantation models, and placenta-on-a-chip systems, which can be applied to drug screening, reproductive toxicology, disease modeling, and investigation of biological mechanisms. The emergence of OoC technologies since the early 2000s, following advances in biomimetic microfluidic devices for diverse biological functions, has therefore introduced a new class of *in vitro* models capable of partially recapitulating key organ-level features of the human body, including the complex and hormonally regulated female reproductive system [122–128]. Taking advantage of its ability to coculture different cell types and mimic interconnection through intricate endocrine pathways and communication mechanisms, OoC has attracted enormous attention from ART researchers. After several years of development, OoCs have proven to be capable of mimicking various stages of sexual reproduction (Figure 3). As illustrated in Figure 3, representative reproductive OoC models can be organized according to prefertilization, fertilization, and postfertilization stages, with each platform corresponding to specific organs and developmental processes in ART-related research. The recapitulation of reproductive organs helps to increase our understanding of the underlying mechanisms of *in vivo* phenomena and diseases [129, 130]. In addition, the physiological models based on the microfluidic platform offer a powerful paradigm for drug screening and toxicology testing. Recent advances in biosensors and bioelectronics integrated in microfluidic platforms have enabled real-time monitoring and precise control of the biomimetic systems [131, 132].

2.2.2 | Organ-on-a-chip for Hormone Recapitulation

Microfluidic chips, with their ability to manipulate multiple fluidic channels at the micro-scale, have been a powerful tool to mimic hormone stimulations and other factors such as nutrients, drugs, or toxicants. Most female reproduction stages are stimulated by hormones: primordial follicles (dormant follicles) in the ovaries are stimulated by follicle-stimulating hormones (FSH)

secreted by the anterior pituitary gland at puberty to become primary follicles; follicular fluid bring hormones for follicle development; fertilization starts with the ovulation process which is triggered by a luteinizing hormone from the anterior pituitary gland; the fetal growth depends on hormones of the mother, fetus and placenta [133, 134]. The *in vivo* hormone regulation is created on-chip by accurate control of fluid flows inside a biochip. There are several methods to generate dynamic culture platforms, such as gravity-driven flow, syringe pump, or piezo-Braille pin actuation [135]. When using dynamic culture, the flow rate, flow pattern, channel volume, and fluid recirculation need to be considered to have beneficial impacts on embryos [136]. It is noted that induced shear stress greater than 1.2 dyn cm^{-2} may cause harm to blastomeres and lead to embryo degeneration [135].

Ferraz et al. introduced the oviduct-on-chip to enhance cell growth and differentiation under dynamic culture in a PDMS channel. The oviduct undergoes circulating hormone changes during the peri-ovulation period, which is critical for bovine oviduct epithelial cells growth and embryo development [137]. This factor was integrated into an oviduct-on-a-chip device to support a better reproduction of physiological conditions than in conventional IVF [138]. The chip combines two independent perfusable compartments separated by a porous membrane binding bovine oviduct epithelial cells. The apical compartment consists of pillars to trap oocytes and/or embryos while permitting the continuous apical perfusion of the oviduct epithelial cell layer. By using a perfusion system, the results indicate that epithelial cells' development and differentiation on the proposed device was similar to *in vivo* in terms of gene expression, structure, and morphology in response to different hormone stimulations. This directly supported *in vitro* fertilization to produce more *in vivo*-like zygotes in terms of global DNA methylation levels and transcriptome when compared with conventional *in vitro* zygotes. In the following work, the authors proposed a microfluidic chip culturing dog oviductal epithelia and on-chip gene editing by CRISPR–Cas9 to mimic serous tubal intraepithelial carcinoma (STIC), the most common ovarian cancer, which originates from secretory epithelial cells within the fallopian tube [139, 140]. The early detection of STIC and study of STIC progression to HGSC are essential for early diagnosis and proper treatment. For this purpose, the chip contained two chambers (basolateral chamber and apical chamber to create air–liquid interface) separated by a porous membrane to culture cells. The nonedited epithelial cells matured like *in vivo* in this microfluidic chip. The authors used CRISPR–Cas9 to disable canine *p53* gene. *p53* mutant is well known as a marker for tumors. The gene-edited cells grown on this platform showed flattened, reduced ciliation, loss of polarization, and enhanced proliferation. The oviduct-on-chip has potential for the detection of future HGSC biomarkers. Another work proposed a microfluidic chip to study the escaping behavior of sperm by creating the bioengineered oviductal surface [141]. The microfluidic device permitted the generation of a gradient of progesterone, which closely mimics the oviduct milieu *in vivo*.

Recently, a complex fluidic system combining dynamic and 3D culture called EVATAR to recapitulate the human 28-day menstrual cycle hormone profile was engineered [24]. The micro-scale EVATAR system consists of five organs: the ovary, fallopian tube, uterus, cervix, and liver to investigate the ovarian

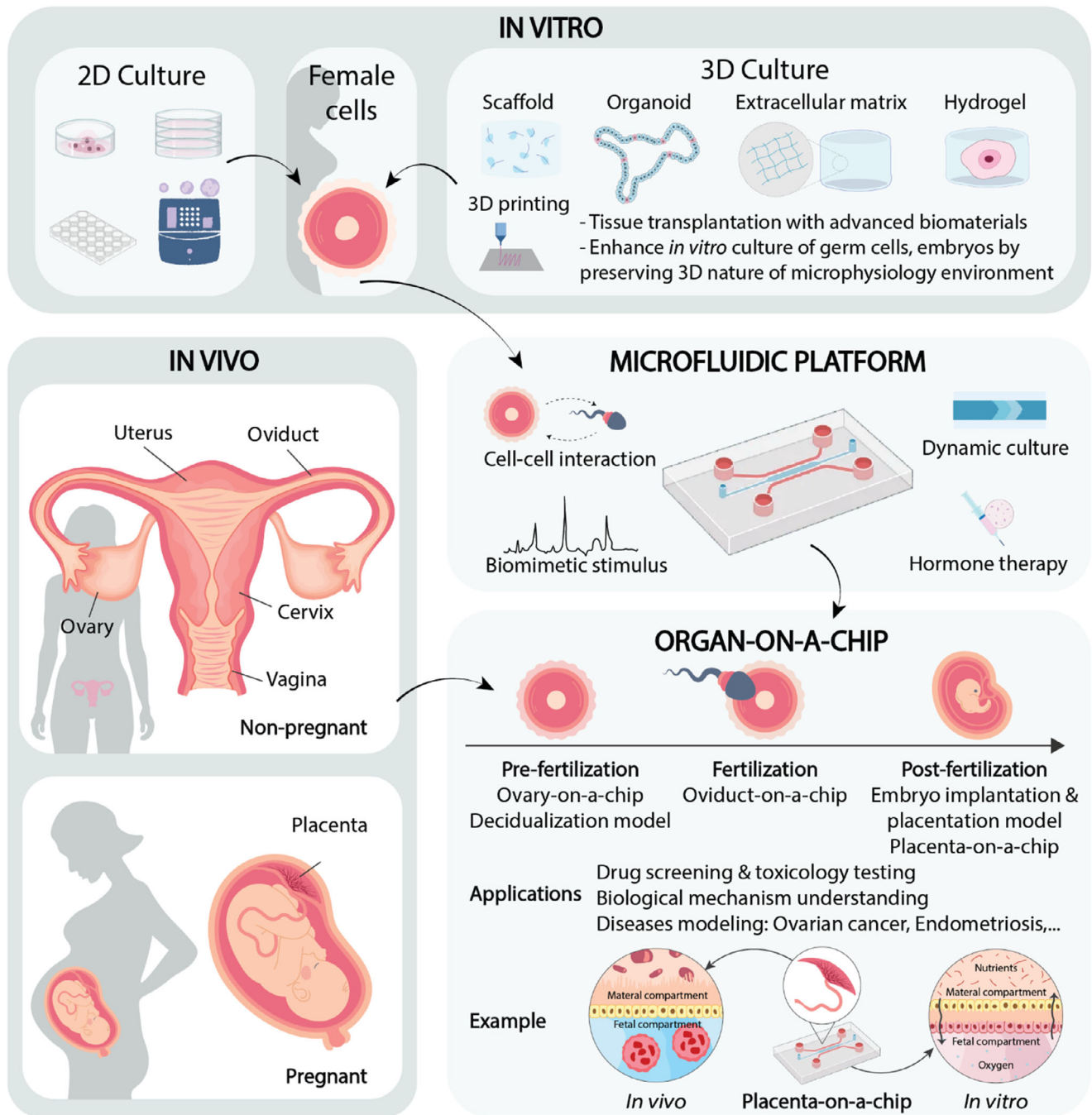


FIGURE 2 | From 2D and 3D culture to their integration into the microfluidic platform and the development of organ-on-a-chip technology replicating the mechanism of the female reproductive system. The schematic illustrates the progression from conventional 2D culture to 3D culture, microfluidic platforms, and reproductive OoC systems. 3D culture strategies, including scaffolds, organoids, extracellular matrix-based materials, hydrogels, and 3D printing, provide more biomimetic environments for female reproductive cells by supporting cell–cell and cell–matrix interactions. When integrated with microfluidic channels, these systems enable dynamic culture, controlled flow, biomimetic stimulation, and hormone-related regulation. Reproductive OoC platforms can be organized according to reproductive stage, including pre-fertilization models such as ovary-on-a-chip and decidualization models, fertilization-related models such as oviduct-on-a-chip, and postfertilization models such as embryo implantation and placenta-on-a-chip. These platforms support applications in drug screening, reproductive toxicology, biological mechanism studies, and disease modeling.

hormonal control of the downstream human female reproductive tract and peripheral tissues. This system is operated using electromagnetically-controlled micropumps to individually control 60 actuators, creating accurate flow and eliminating the need for individual airlines to each channel. To provide similar conditions as *in vivo*, follicles were cultured in a microfluidic

platform with follicle-stimulating hormone added, preserving follicular architecture and spatial cell arrangement. The occurrence of ovulation in the microfluidic indicated that not only individual ovarian follicle growth but also maturation, ovulation, and granulosa cell luteinization were supported by the system.

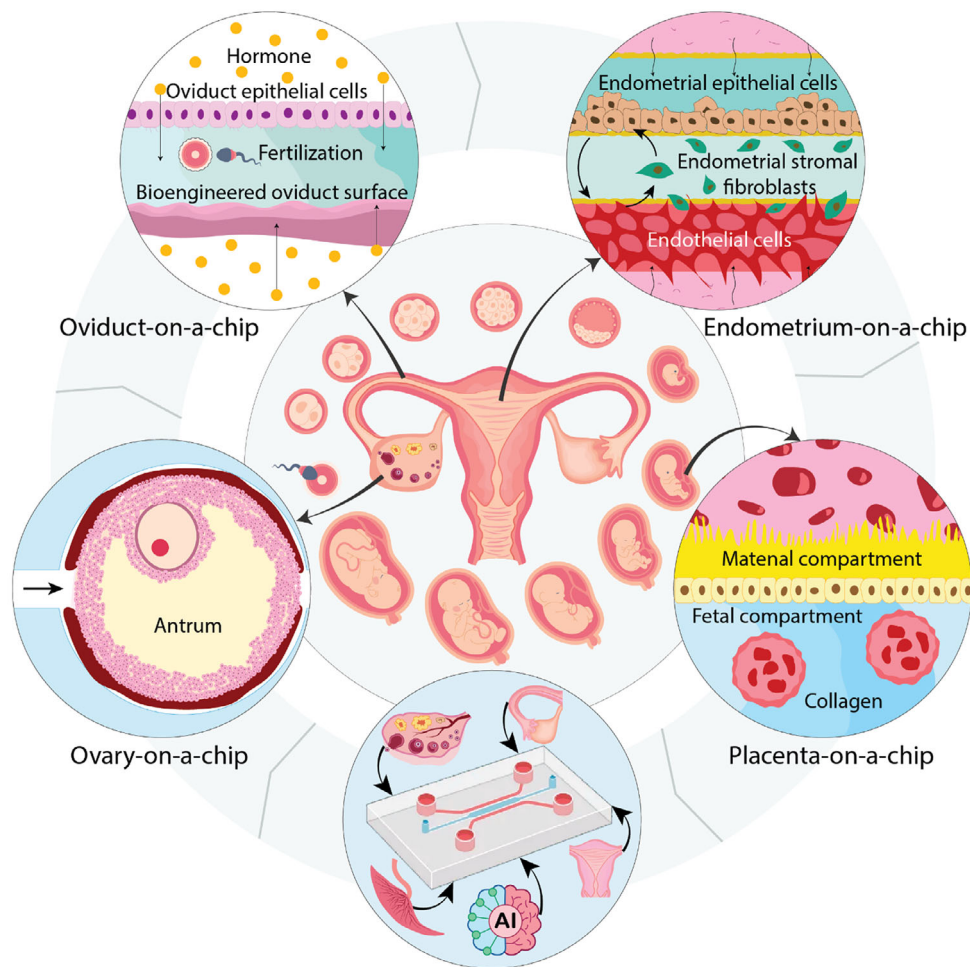


FIGURE 3 | The footage of female reproductive organ-on-a-chip technology on the growth from oocyte to fetus and their integration to build up female reproductive system-on-a-chip in ART. The schematic shows representative OoC models of the female reproductive system. These models correspond to major reproductive organs and developmental stages. Ovary-on-a-chip platforms are used to model follicle growth and oocyte maturation. Oviduct-on-a-chip systems recapitulate gamete transport and fertilization. Endometrium-on-a-chip models support the study of decidualization and embryo–endometrial interactions. Placenta-on-a-chip platforms are designed to investigate maternal–fetal exchange and placental barrier function. Each model incorporates organ-relevant cell types, ECM components, microfluidic structures, and dynamic biochemical or hormonal cues to partially recapitulate reproductive physiology. These individual OoC modules may be further connected to develop integrated female reproductive multiorgan-on-a-chip platforms, enabling studies of tissue–tissue communication, hormone circulation, reproductive toxicology, disease mechanisms, implantation, and other ART-related processes.

One of the first important events for a healthy pregnancy is the formation of decidualization, which refers to the considerable changes in the functional and morphological of the endometrium (uterine line tissue) for preparing embryo implantation [142]. Although this process occurs in the presence of an embryo in most mammals, humans undergo decidualization in each menstrual cycle as a response to the hormonal levels [143]. The differentiation of endometrial stromal fibroblasts into decidual cells has been successfully illustrated in vitro in a bioengineer model coculturing human endometrial stromal and endothelial cells and controls the hormone cycle inside channels [144]. The chip consists of two compartments separated by a 2- μ m porous membrane for a cell co-culture model and molecule diffusion. In this biomimetic environment, the authors found that the morphological changes of stromal cells from fibroblasts to cuboidal shape are similar to those in vivo. This work promises to be a useful tool for extensively understanding

complex decidual processes and reproductive system diseases such as endometriosis. The in vivo proliferative and secretory phases of the menstrual cycle, displayed by endometrial vasculo-angiogenesis and hormonal responses, have been mimicked in an endometrium-on-chip [145]. The multilayer chip recapitulated oestradiol and progesterone levels in the menstrual cycle and investigated the interconnections between stromal fibroblast and endothelial cells. This model was used to assess the impact of the emergency contraceptive medication levonorgestrel, which showed dose-dependent increases in endometrial permeability and blood vessel regression. To model the human peritoneum endometriosis, Chen et al. examined the interactions between endometrial stromal cells (ESCs) and human peritoneal mesothelial cells (HPMCs) on microfluidic channels [146]. First, the ESCs and HPMCs were separately cultured in two microchannels created by PDMS stamps. After incubation, the PDMS stamps were removed, the two cell types migrated freely to communicate

with each other. To investigate the pathogenesis of endometriosis, four cell groups, including ESCs and HPMCs from control and endometriosis patients. The observations indicated that HPMCs from healthy people can resist the invasion of ESCs. However, interestingly, the HPMCs from endometriosis individuals were incapable of preventing ESCs invasion from both groups. These key results showed that endometriosis not only comes from endometrial abnormality but also from the targeted sites.

2.2.3 | Organ-on-a-chip for Mimicking Cell–Cell Interaction

Not only do they create precise and suitable fluid flows, but the microfluidic chips also reproduce the 3D arrangement of multiple cell types, enabling coculture in multiple compartments separated by a thin porous membrane. Cell–cell interactions and interconnection through intricate endocrine pathways are key aspects of in vivo microenvironments. For example, Irma et al. indicated that the immature human oocytes cocultured with surrounding cumulus cells from mature oocytes showed higher maturation rates and enhanced gene expressions compared to those conventionally matured without cumulus cells, especially in genes involved in transcriptional regulation, embryogenesis, epigenetics, and development [147]. Numerous microfluidic chips have coculture systems to study communications between different cell lines, such as oocyte-cumulus-granulosa cell complex in follicle development [52, 53], human endometrial stromal and endothelial cells in the decidual process [144], embryos and fallopian tube cells, blastocyst and endometrial stromal cells in early-stage embryonic development, fetal extravillous trophoblasts and maternal uterine cells during implantation [148, 149]. Promising results have been obtained using these coculture platforms. The bioengineer model successfully mimicked the differentiation of stromal cells in decidualization [144]. In another study, the same research group cocultured human endometrial stromal cells with uterine microvascular endothelial cells in an OoC platform to explore the dependence of stroma differentiation on endometrial vasculature [150]. The microfluidic system creates hemodynamic forces that stimulate endothelial cells, resulting in a considerable enhancement in stromal decidualization. Regarding embryo development, the coculture platform combined with dynamic culture enhanced the blastocyst development rate from 21.40% (off-chip conditions) and 37.50% (on-chip conditions) [151]. An implantation-on-chip has been successfully created to replicate the crucial implantation process of an embryo during pregnancy [148]. The chip is composed of three parallel compartments, reconstructing the in vivo-like extravillous trophoblasts' directed migration toward a maternal artery as well as their interactions with the endothelium that are essential for vascular remodeling. By using a coculture platform, the implantation-on-chip demonstrated the unexplored impacts of maternal immune cells and decidualized stromal cells on the extravillous trophoblast migration and invasion.

Positive outcomes have been obtained when utilizing a coculture platform between trophoblasts (maternal side) and endothelial cells (fetal side) to create an artificial maternal-fetal barrier [152]. The chip, featuring a matrigel microchannel sandwiched between two parallel cell channels, successfully mimicked the morphology of the barrier. In addition, the appearance of a tight junction

of VE-cadherin, an essential molecule in the endothelial cell-specific adhesion junction complex, suggested that there was a full confluence in both trophoblasts and endothelial cells. When TiO₂ nanoparticles were introduced into the maternal channel, the disrupted permeability of the barrier led to various malfunctions in the placenta. Even though the TiO₂ with low concentration had an insignificant impact on oxidative stress and cell motility, the disrupted barrier integrity and reduced immune cells were observed, indicating the potential dangers of nanoparticles on pregnant women. A similar concept was used to model the multistep inflammatory responses of the placental barrier to *Escherichia coli* [153]. Two cell types were cultured in two compartments separated by a porous membrane. Maternal and fetal bloodstreams were filled in the two channels. The introduction of *E. coli* to the maternal channel led to significant cell deaths in both cell types, triggering the secretion of inflammatory cytokines and activation of maternal macrophages. The placental barrier on the chip showed the communication between trophoblasts and endothelial cells, which is evidenced by the secretion of inflammatory cytokines in the fetal site. Based on the concept of coculture of trophoblasts and endothelial cells in a 3D culture platform, placental barrier-on-a-chip models, featuring key properties of the dynamic in vivo maternal-fetal interface, have been standardized, validated, and enhanced throughput for placental pathology research, especially preeclampsia [154]. These OoC models could contribute significantly to the development of more potent treatments in the future and improve our understanding of placental diseases.

2.2.4 | Organ-on-a-chip for Modelling Biophysical Cues

Besides the coculture and dynamic culture platform, many researchers have made attempts to model the in vivo biophysical cues. The importance of contractions in the human body is undeniable in ART [155]. During the transport of embryos from the oviduct to the uterus, muscle contraction and cilia activity in the oviduct play a distinctive role [156]. To investigate the influence of muscle contraction on embryonic development, a microfluidic system utilizing a partially constricted channel mimicking the peristaltic muscle contraction in an oviduct was demonstrated by Kim et al. [157]. Each channel included 2 side reservoirs, an inlet, and an outlet. Three microchannel models were examined to determine the most appropriate structures for embryo development: a straight microchannel and constricted microchannels with 150 and 160 µm constriction widths, respectively. Subsequently, twenty fertilized embryos were loaded into each microchannel placed on a tilting machine, and cultivated for 3 days. The number of embryos that reached the 8-cell stage accounted for 23.9% and 56.7% in the straight channel and 160 µm constriction width channel, respectively. It can then be deduced that the early growth of bovine embryos is enhanced by mechanical stimulation, thus reinstating the importance of muscle movement.

Alongside the capability to mimic the physiological environments within our body, microfluidic technology is also applied in numerous crucial steps in ARTs and research into the reproductive system [158–160]. For oocytes, researchers have leveraged the potential of microfluidics in oocyte selection and screening, oocyte characterization, and manipulation. Hagiwara

et al. introduced an on-chip robot for oocyte manipulations [161]. The oocyte rotation, pick and place, and cutting have been performed on a microfluidic chip by using a microrobot actuated by a permanent magnet. The novelty of this work is the utilization of piezoelectric ceramic to enhance the accuracy, speed, and output force, making it a promising tool for high-accuracy, high-throughput automatic oocyte manipulation. The precise evaluation of oocyte permeability is crucial in the oocyte cryopreservation and ART fields [162]. Microfluidic chips offer a nondestructive, efficient, and high-throughput approach for oocyte membrane permeability measurement [163]. The chip design includes micropillars for capturing individual oocytes, micromixers to generate distinct cryoprotectant concentrations, and multichannels to improve throughput. This allows for the assessment of the effects of varying chemical concentrations on oocyte permeability. Additionally, oocyte denudation, which refers to the removal of cumulus cells from oocyte-cumulus complexes, could also be automatically performed by a microfluidic platform [164, 165]. A jagged-surface constriction microchannel has been proposed to take off cumulus cells from oocyte-cumulus complexes, which provides an inexpensive and simple platform for a critical step in ARTs. Moreover, based on the sedimentation rate differences in a sucrose buffer, good and poor-quality oocytes can also be classified in a microfluidic chip [166]. The biochip has two inlets: a lower one where the better oocytes settle more quickly and are collected, and an upper one where poor-quality oocytes are gathered. To sum up, the microfluidic platforms integrated with microactuators and biosensors have paved the way toward the complete automation and enhancement of oocyte handling. Combining with various microfluidic chips for sperm sorting based on their motility, DNA integrity, morphology, and functioning [167–170], microfluidics holds great promise to revolutionize female reproductive research and optimize the outcomes of ARTs.

2.2.5 | Design Principles for Reproductive Devices

OoC technologies, especially microfluidics, play a key role in enhancing ART research. Although several approaches have been discussed above, their design principles can be classified into four categories. First of all, the modular system facilitates the integration of various reproductive components and replicates systemic endocrine dynamics, thus offering a more biologically relevant model of inter-organ signaling, notably the EVATAR system. This high level of integration is accompanied by increased system complexity, facing challenges in maintaining long-term operational stability, as well as limitations regarding scalability for routine or high-throughput applications. Following that, compartmentalized or multilayered designs enable the control of cell–cell and tissue–tissue interactions through spatial separation and selective mechanisms for molecular exchange, exemplified by endometrium-on-chip, implantation-on-chip, and placental barrier models. This architecture facilitates the investigation of specific biological interactions with superior experimental precision. However, such systems may oversimplify the complexity of the overall physiological environment, thereby running the risk of overlooking system-wide feedback processes as well as long-range signaling effects. An important aspect is the fluidic control system, such as oviduct-on-a-chip and other perfusion systems. This application allows for the meticulous regulation of hormone

gradients, nutrient delivery, and waste removal, thereby faithfully recapitulating the dynamic environments occurring in vivo. However, these systems depend on stability and sensitivity since changes in shear stress or perfusion rates can negatively impact cell viability and embryonic development. Finally, function-specific platforms, including systems designed for reproductive cell and embryo manipulation, oocyte denudation, and sorting, provide notable advantages in throughput, reproducibility, and operational simplicity, thereby supporting their potential clinical and translational applications. However, these devices are generally designed to execute specific, discrete tasks that only recapitulate a limited range of the biological processes occurring in vivo, thereby diminishing their efficacy in the investigation of complex physiological processes. In particular, these design approaches emphasize the necessity of balancing physiological relevance with controllability, establishing a foundation for the future integration of more comprehensive and scalable reproductive systems.

2.3 | Personalized Medicine and CRISPR–Cas9 Genome Editing for Early Human Embryo

Beyond their role as culture and manipulation platforms, microfluidic and OoC systems also provide controllable microenvironments for personalized reproductive modeling. By integrating patient-derived cells, organoids, or engineered tissues, these platforms can be used to examine individualized tissue responses, embryo–maternal interactions, and disease-associated phenotypes under dynamic culture conditions. This creates a conceptual interface with personalized medicine and genome-editing approaches, in which CRISPR–Cas9-modified cells or organoid models may be combined with OoC systems to investigate gene function, disease mechanisms, and potential therapeutic responses in a physiologically relevant setting [171, 172].

This framework is particularly relevant to reproductive medicine, where genetic risk assessment and embryo selection are important for couples carrying inherited disorders. Prenatal diagnosis tests, which are essential for couples with genetic problems, are not viable in some cases [173]. For example, amniocentesis, a common invasive prenatal diagnosis, should not be performed for pregnant women having Hepatitis B or HIV due to the transmission of infections from mother to fetus [174]. The chorionic villus sampling (CVS) or noninvasive prenatal diagnostic test (NIPT) should not be carried out before 10 weeks of pregnancy [175, 176]. The in vitro culture of embryos offers the possibility for parents with genetic disorders to screen and have a healthy baby. Genetic screening of preimplantation embryos provides information on the chromosomal status of all 23 chromosome pairs and enables the detection of genetic abnormalities that could, in principle, be targeted by gene-editing tools. The genome editing tool—CRISPR–Cas9, which is a breakthrough in medicine, holds vast potential in adjusting the pathogenic gene of early human embryos [177]. A recent work conducted by Shoukhrat Mitalipov's research group reported successful repair of the heterozygous MYBPC3 mutation causing myocardial disease in human preimplantation embryos [178]. The experiments were carried out in zygotes formed by heterozygous, carrier sperm and healthy eggs with wild-type alleles. This study found that the CRISPR–Cas9-induced double-strand breaks were

primarily reconstructed utilizing the maternal gene as a template. Mosaicism in embryos was reduced by optimizing the timing of coinjection of sperm and CRISPR–Cas9 system into MII oocytes. The proposed method reached 72.4% of embryos carrying homozygous wild-type MYBPC3^{WT/WT}, which was considerably higher than in the control group (47.4%) and only one mosaic embryo existed. Despite these findings being optimistic, they need both more theoretical and more experimental evidence. Another paper published by Cell journal in 2020 reported different outcomes when using the same experimental setup but for a mutation that causes blindness [179]. The authors used sperm from blind patients fertilized with oocytes from healthy individuals. Sperm and Cas9 ribonucleoprotein (RNP) were coinjected into the MII oocytes. The effective end-joining repair pathway happens, reducing embryo mosaicism. However, severe off-target effects, including indels and chromosome elimination, occur in approximately 50% of embryos. In several cells, the whole chromosome 6 was lost. Off-target effects have also been reported by Alanis-Lobato et al. when using CRISPR–Cas9 for genome editing of human embryos to investigate the function of the pluripotency factor OCT4 (encoded by the POU5F1 gene on the p-arm of chromosome 6) [180]. By using novel computational pipelines for evaluation, the authors indicated that several edited embryos experienced loss of heterozygosity in the span regions beyond the POU5F1 on-target locus, as well as segmental loss and abnormality of chromosome 6. This evidence points to the conclusion that more research is needed to fully understand what will happen and how to manage the unintended on-target and off-target effects [181]. The use of CRISPR–Cas9 for embryos should still be thoroughly examined before clinical trials. To do that, the development of biosensor platforms for assessing the CRISPR–Cas9 editing tool activities in embryos is absolutely crucial. At present, heritable genome editing for reproductive purposes remains clinically unsafe and ethically controversial. However, CRISPR–Cas9-mediated correction of disease-causing mutations remains a subject of considerable interest in personalized ART, particularly for its potential to prevent the transmission of heritable genetic disorders.

2.4 | Imaging Technology

In vivo imaging techniques such as ultrasound, optical imaging, magnetic resonance imaging (MRI), and micro-computed tomography (micro-CT) have been recently reported with significant advancements in their applications to reproductive organs. Well-known for the nonharmful effect on oocytes, embryos, and fetuses, ultrasound's continuous development from 2D to 4D permits a comprehensive diagnosis of the normal and pathologic ovary, uterus, and fallopian tubes in a noninvasive manner [182]. Moreover, better observation provided by real-time ultrasound monitoring can maximize the success rate of ART treatments and surgical procedures, including oocyte retrieval, embryo transfer, and uterine septum, myoma, or synechiae resection [182]. AI-aided ultrasound, though recently appeared in the field, is currently used in clinics for follicular monitoring, optimal timing for transplantation, and pregnancy outcome prediction, holding promising applications for female reproductive function assessment [183]. Besides, the assessment of female reproductive disorders can also be conducted using MRI, thanks to its excellent contrast of soft tissues [184, 185]. Optical coherence tomography,

an approach for hidden cross-sectional images, has also been built to capture the intrinsic morphological and morphokinetic mechanisms of reproductive organs, suggesting a deeper understanding of functional analysis [186–188].

Despite recent advances in technologies supporting reproductive systems in vivo, the development and assessment of embryos in conventional culture dishes or biomimetic platforms, such as OoC systems, remain highly promising for ART research. The application of CRISPR–Cas9, 3D culture systems, organoids, tissue-engineered constructs, and OoC platforms requires reliable methods to evaluate downstream biological and functional outcomes. Imaging technologies provide a common assessment interface across these models. However, these advanced culture systems also require optical techniques that are high-resolution, compact, portable, and cost-effective to meet the specific demands of specialized in vitro and microfluidic environments. Most embryo assessment criteria are based on morphological features; the continuous and real-time tracking of oocytes and embryos is vital in in vitro ARTs [189]. In traditional in vitro ARTs, the embryos are observed at several time points outside the incubators under a bulk light microscope, which may lead to instability of the culture environment [190]. A time-lapse system has been developed and widely used in clinics to continuously and flexibly track the embryos without changing the environment [191]. In a time-lapse system, the compact optical microscope and camera can be integrated into the incubator (benchtop design) or inside the box incubator. Understanding the advantage brought by the time-lapse system, various models have been commercialized for different purposes of usage. Not only does it help the embryologists to evaluate the whole development process of embryos via the detailed morphology and cell division kinetics, but the time-lapse system also provides quantitative and qualitative information on embryos, automatic, time-saving, and unbiased solutions for embryologists. Taking advantage of the huge dataset collected by time-lapse microscopy, algorithms have been introduced to predict related clinical endpoints such as embryo implantation, blastocyst formation, or euploid status of the embryo, providing a better selection of preimplantation embryos for a more successful chance of IVF [192–195].

With the advancement of integrated and automated microfluidic systems for ART, particularly in the development of single-step media that ensure an uninterrupted environment, time-lapse imaging has become an essential tool for real-time, multiparameter tracking of embryo culture. This technique leverages the precision, scalability, and low-volume handling capabilities of microfluidic platforms, which provide an optimal and stable microenvironment for embryo growth while minimizing external disturbances and human interaction [136, 196–199]. By combining the sophisticated microfluidic control with the ability of time-lapse technology to continuously capture high-resolution images, researchers and clinicians can monitor the dynamic changes in embryo morphology and cell kinetics over time. This continuous imaging offers a more detailed understanding of critical developmental milestones, such as cleavage patterns, blastocyst formation, and cellular differentiation, leading to more accurate assessments of embryo quality. Besides widely used techniques like compact microscopic systems, several novel imaging techniques could have potential to be integrated into small systems for individual embryo tracking [200–202].

2.5 | Artificial Intelligence

In recent years, AI, which harnesses the power of advanced electronics and computing to solve problems by mimicking human intelligence have shown huge potential in the biomedical field, especially in diagnosis and treatment [203–205]. Imaging technologies also provide the data foundation for AI-assisted analysis in ART, particularly in embryo assessment. Time-lapse imaging and related microscopy-based approaches can generate large-scale spatial and temporal datasets describing embryo morphology, cleavage dynamics, blastocyst formation, and morphokinetic developmental patterns. AI and deep learning models can then be trained to extract quantitative features, recognize developmental trajectories, support embryo quality assessment, and noninvasively estimate the risk of chromosomal abnormalities such as aneuploidy. Imaging and AI should be considered complementary technologies, with imaging serving as the source of embryo phenotypic and developmental data, and AI providing scalable interpretation, automated assessment, and predictive modeling. These imaging-derived datasets may also support the monitoring of embryo development in advanced culture systems, including 3D and OoC-based platforms. The integration of AI with reproductive OoC and 3D culture systems remains largely conceptual but represents a promising future direction [206, 207]. Current AI studies in ART, mainly involving semen analysis, sperm selection, oocyte/embryo grading and assessment, provide a solid methodological foundation for future integration into complex systems, such as OoC and 3D reproductive culture platforms.

AI has been extensively employed to enhance the success of ARTs by assisting doctors in selecting high-quality oocytes, sperms, and embryos [208]. Traditional automatic image processing tools such as FERTECH [209] or Integrated Visual Optical System (IVOS) [210] for sperm analysis based on WHO criteria have delivered reliable results. However, these platforms are based on WHO criteria only and dependent on staining methods, which may have an influence on expert assessments [211]. Meanwhile, machine learning methods have held great promise in assessing sperm quality based on their morphology, motility, DNA integrity, and other features [211, 212]. Multiple algorithms, including K-Nearest Neighbor (KNN), Support Vector Machine (SVM), Decision Trees, and Naive Bayes, have been used to separate normal sperm from abnormal ones based on images of sperm heads [213–215]. Input images first underwent a feature extraction phase to generate shape-based descriptors, including Hu moments, Zernike moments, and Fourier descriptors [213]. Subsequently, these hand-crafted features were utilized to train the machine learning algorithms. Recently, thanks to the significant advancements of computing resources, deep learning approaches have gradually emerged as a favorable choice in complex image processing tasks, replacing traditional machine learning algorithms. Instead of relying on hand-crafted features, deep learning models boost the performance further by simultaneously extracting features and learning from input images [216–218]. Javadi et al. proposed a model for the identification of morphological irregularities in different segments of sperm, including the head, acrosome, and vacuole [218]. This work obtained an accuracy $F_{0.5}$ scores of 84.74%, 83.86%, and 94.65% in acrosome, head, and vacuole deformity detection, respectively. Notably, this approach showed outstanding results even with nonstained and low-quality images. Thanks to innovative smartphone technologies, recent work

has proposed an at-home semen analysis that features low cost, portability, and simplicity of usage [219]. The device is composed of a smartphone-based semen evaluation for sperm image analysis, an attached microfluidic chip for semen sample preprocessing, and an optical attachment. A convolutional neural network (CNN) has been integrated into this proposed platform to predict the percentage of normal sperm in the semen sample with a sensitivity and specificity of 80% and 92%, opening up an opportunity for inexpensive point-of-care testing [220]. Besides morphology, sperm DNA integrity and motility can also be predicted using AI. DNA integrity evaluation at the single sperm level, which was previously limited when using traditional methods, can now be obtained by employing machine learning algorithms [211]. For example, McCallum et al. developed a CNN to anticipate the sperm DNA quality based on brightfield images within under 10 ms [221]. The predicted DNA integrity relating to live birth outcomes could assist doctors in selecting high-quality sperm for ARTs. Sperm motility, which plays an important role in fertility outcome [222], can also be predicted by machine learning algorithms [223–227]. The sperm motility patterns can be classified based on features obtained from CASA systems, such as lateral head displacement, average path, and straight-line speed [225]; or beating frequency and amplitude of the tail [228]. Goodson et al. classified five sperm motility patterns, including progressive, intermediate, hyperactivated, slow, and weakly motile, by using an SVM-based decision tree algorithm using CASA obtained features with an accuracy of 89.9% [225]. Another work employed machine learning to efficiently analyze sperm videos, combined with patient data to indicate male fertility [224]. These promising results have shown the potential of a new AI algorithm that combines multimetrics such as morphology, DNA integrity, and motility for totally assessing sperm.

The embryo selection for transfer plays a key role in a successful IVF or ICSI. The current methods for embryo selection are based mainly on visual inspection of the morphology and development rate, which may lead to uncertain, variable, and subjective results. AI models have been developed to automatically and quickly evaluate embryo quality at multiple stages such as pronuclear, cleavage, and blastocyst stages [229]. To confirm the successful fertilization, Dimitriadis et al. have used an AI model to discriminate between normal (2PN) and abnormal (non-2PN) embryos at 18 h after insemination [230]. The proposed CNN was trained using embryo images and obtained a sensitivity of higher than 90%. Thanks to the time-lapse imaging technique, several studies have leveraged machine-learning algorithms for embryo assessment based on morphological features [231–236]. STORK, a deep learning model based on Google's InceptionNet, has been developed to choose the best quality blastocyst utilizing about 50,000 time-lapse images [234]. When comparing with embryologists, STORK has outperformed individual embryologists in embryo evaluation. Another work assessed the development potential of embryos at the cleavage stage into the blastocyst stage using a CNN combined with a genetic algorithm [237]. The obtained results showed that the AI method was superior to the highly-trained embryologists in detecting the highest development potential embryos.

In addition, AI-based systems offer the capacity for noninvasive aneuploidy screening, which may overcome the disadvantages of invasiveness, high cost, and mosaicism of the current approach,

namely preimplantation genetic testing for aneuploidy (PGT-A) [238–241]. ERICA (Embryo Ranking Intelligent Classification Algorithm), a system based on deep learning, has been used to predict euploidy utilizing 1231 blastocyst images [238]. ERICA reached an accuracy of 70% with a positive predictive value of 0.79 for predicting euploidy with a ranking time below 25 s for four embryos, outperforming the two senior embryologists. Another AI model, STORK-A for aneuploid screening, was trained and tested on a large dataset of 10,378 embryos (11 h after ICSI) and showed encouraging outcomes [242]. In recent work, AI has been used to detect the abnormalities in chromosomes 21 and 16 associated with morphological properties with an accuracy higher than 70%, showing its potential to be an alternative method to the traditional PGT-A [240].

Although promising results have been obtained, the application of AI in time-lapse systems is hindered by model interpretability, insufficient and nonstandardized datasets, inherent biological variability of embryos, data privacy constraints, and the lack of large-scale external validation and regulatory approval [243]. Moreover, the “black box” nature of many AI models and dataset-dependent bias may reduce clinical trust, transparency, accountability, and generalizability across ART laboratories [244]. Given its potential influence on oocyte and embryo selection, future AI models should not only demonstrate high predictive accuracy but also provide biologically interpretable explanations that can be evaluated by embryologists and clinicians. In addition, current AI-based methodologies in ART remain largely constrained to two-dimensional image analysis to predict the best oocytes and embryos [245]. Emerging time-lapse imaging, 3D culture systems, and OoC platforms can generate high-dimensional datasets that include temporal, three-dimensional spatial, morphological, functional, and microenvironmental information [246]. In OoC-based ART platforms, such data may further include dynamic flow conditions, oxygen and nutrient gradients, cell–cell interactions, embryo–microenvironment responses, impedance-based signals, metabolic readouts, and other sensor-derived parameters. These complex data types are not yet effectively incorporated into most existing AI models because they require advanced methods for 3D time-lapse processing, spatial–temporal feature extraction, and integration of heterogeneous biological readouts [247, 248]. Future AI-assisted ART systems should therefore prioritize explainable and robust models, privacy-preserving data sharing, and multimodal data fusion that integrates embryo imaging, morphokinetic dynamics, 3D/OoC-derived functional readouts, clinical variables, and potentially multiomics data [249]. Besides oocyte and embryo quality, there are several other factors, such as laboratory conditions and human operation could have considerable impacts on infertility treatment outcomes. For the best prediction of final treatment outcome, these factors should be considered in prediction models.

3 | Challenges and Outlooks

3.1 | Outlooks

Besides the innovative IVF, in vitro stem cell-differentiated gametogenesis has also been developed, which allows researchers to generate embryos in laboratories [250]. Specifically, this approach refers to the creation of germ cells (sperm and egg cells) by

reprogramming adult cells and possesses positive initial results in mice and monkeys [251–253]. Zhou et al. presented the successful production of haploid spermatid-like cells by differentiation of embryonic stem cells [251]. These sperm-like cells were fertilized by ICSI and produced healthy, fertile offspring. In another work, researchers are capable of producing MII oocytes from pluripotent stem cells in vitro, which could be fertilized by IVF and generate offspring. These achievements have shown the promise of in vitro gametogenesis application in humans. This technique helps scientists to deeply understand the cellular and molecular mechanisms, offers hope for same-sex couples to have their own babies or fertility preservation of prepubertal children experiencing cancer treatments, just to name a few. In vitro gametogenesis in humans might open up new possibilities in the reproductive, cellular, and molecular fields, but still encounters regulatory and ethical problems that need to be resolved [250].

The future of microfluidic technology in bioengineered female reproductive systems promises revolutionary advancements in personalized medicine and reproductive health by controlling personal hormone settings accurately. Technological innovations have enabled high-throughput high-resolution preimplantation embryo genetic screening and aneuploidy detection, which plays an important role in selecting good-quality embryos [254, 255]. In some cases of gene abnormalities where all of the created embryos are influenced by the gene defect from parents, CRISPR–Cas9 could be applied to modify the gene of chosen embryos, towards “personalized” disease treatments, especially gynecological cancer. The embryos could be cultured in a “personalized” microphysiological system, which may use the maternal cells to develop organoids and OoC systems to grow individual embryos. The genetic profile of the embryo and information about the maternal in vivo environment, such as hormone regulation, could be valuable for a tailored reproductive treatment. Microfluidics is considered to become a cornerstone technology, and the precision control of biological environments facilitates the development of more sophisticated models of the female reproductive tract. The integration of microfluidics with emerging technologies such as CRISPR enables modeling complex genetic diseases and in vivo organ and organ–organ interactions. Combining with imaging techniques and AI for predictive analytics will likely open new avenues for personalized reproductive healthcare solutions, tailored to individual physiological needs.

Microfluidic technology not only recapitulates intricate bodily environments, but when combined with biosensors and micro-robot techniques, it enables automated, accurate, and rapid manipulation, analysis, and screening of oocytes and embryos within that microenvironment. Together, these platforms create an intelligent and multifunctional system that transforms the ARTs and contributes significantly to the study of the physiology and pathology of the reproductive system. Besides microfluidic OoC systems, such as artificial ovaries and artificial wombs, to assist early embryo growth outside the mother’s body on a microscale. However, these microsystems might be inapplicable to a fetus, which is much larger than the microscale. Using the same concept, researchers have developed a biomimetic system to nurture an unborn fetus outside of the womb, called an “Artificial womb” or ectogenesis [256]. The concept of the artificial womb system dates back to the early 1950s when Emanuel M.

Greenberg invented an artificial uterus to develop premature babies (“US2723660A—Artificial uterus—Google Patents”). A work published in the journal *Nature* presented a special culture platform that successfully developed post-implantation mouse embryos from before gastrulation to late organogenesis (organ and limb formation) [258]. Regarding larger animals, very interesting results have been observed in the lamb [50]. Partridge et al. have introduced an artificial womb system called “Biobag” with a pumpless oxygenator circuit connected with a lamb fetus via an umbilical cord interface within a closed “amniotic fluid” circuit. The extremely premature lambs have been supported for up to 4 weeks and shown normal somatic growth, lung maturation, brain growth, myelination, and their eyes have even opened. These successful experiments on animals have given hope for human artificial wombs, which would have valuable contributions to women with abnormal uteri and queer people having a “child wish”. Emerging technologies such as AI, automation, and integrated sensors could be employed in such systems to continuously track and analyze the fetus’s health status and development progress. Not only does it reduce the mortality rate among extremely premature fetuses, but an artificial womb may also facilitate fetal treatments such as surgical operations because these treatments occur outside the body, decreasing risks like surgical complications for the mother [259, 260]. Ectogenesis offers unique opportunities to control and optimize all surrounding environments, including temperature, oxygen concentration, sterility, etc., in culture media. In addition, the artificial environment might minimize the natural environment’s toxicants, which have negative impacts on fetal health, such as alcohol, tobacco smoke, air pollution, and lead. Other harmful effects from mothers, in case they suffer from stress or substance abuse, could be avoided in an artificial womb.

3.2 | Challenges

3.2.1 | Ethical Challenges

There are still several major challenges before microfluidics and other emerging technologies can be reliably applied to study the reproductive tract and grow healthy embryos. Beyond technical barriers, bioethical frameworks should be systematically examined, including the principles of autonomy, beneficence, nonmaleficence, and justice, rather than as isolated concerns. One of the fundamental ethical dilemmas is the distinction between therapeutic and enhancement applications in embryo gene editing. Research on human oocytes and especially embryos raises ethical questions about who will have access to it. He Jiankui, the Chinese scientist who created the first two CRISPR gene-edited babies, has been subjected to severe criticism from most ethical commentators and was put in prison for 3 years [261]. Even though the gene-editing technique has been successfully and regularly used in other species [262, 263], embryonic gene editing, which would produce long-lasting and unpredictable effects on even future generations, is currently deemed too risky [264, 265]. Although gene-editing tools hold potential for research involving preimplantation embryos, the embryos’ responses to this tool need to be investigated more carefully to minimize the undesired impacts [266]. Therapeutic embryo genome editing aimed at preventing serious monogenic diseases may be viewed as more ethically defensible than enhancement-

oriented applications; however, current guidelines emphasize that heritable genome editing remains scientifically and ethically unresolved and should not be clinically applied without robust evidence of safety, efficacy, oversight, and long-term follow-up. Enhancement-oriented modifications raise greater ethical concerns regarding “designer babies,” eugenics, social inequality, and the selection of nonmedical traits [267–269]. The heritable repair of human embryos is prohibited in several nations, such as the US, or can be used with strict control in the UK [270]. Moreover, current international guidelines, particularly those from the ISSCR, provide a comprehensive ethical framework governing research involving human oocytes, embryos, and related reproductive materials. These guidelines emphasize rigorous scientific and ethical oversight, informed consent for the use of human gametes and embryos, transparency, and careful review of research categories according to their ethical sensitivity. Importantly, the procurement and use of human oocytes or embryos for research should be based on voluntary informed consent, appropriate ethical review, and authorized oversight [271]. The creation of embryos or the retrieval of additional oocytes for research purposes requires careful scientific and ethical justification, with priority given to patient welfare, risk minimization, and the prevention of undue inducement or exploitation. Research on human embryos may be considered ethically permissible in some jurisdictions when performed under strict oversight and in accordance with applicable laws, but this does not imply unrestricted embryo creation or clinical use [272, 273]. Similarly, emerging technologies such as in vitro gametogenesis and ectogenesis raise unresolved ethical, legal, and social questions regarding consent, parenthood, embryo/fetal moral status, reproductive autonomy, and intergenerational responsibility [274].

In addition, microfluidic chips, especially OoC models, still experienced ethical issues when acquiring mother and fetal cells or tissues [126]. These issues should be considered within core bioethical principles. From the perspective of autonomy, the collection and use of human reproductive materials require voluntary informed consent, clear explanation of research purposes, and appropriate oversight regarding future use, storage, sharing, or commercialization of biological samples. The ISSCR guidelines emphasize that the procurement of human cells and tissues should avoid undue inducement, unacceptable influence, and exploitation of vulnerable individuals or populations [271]. The principle of nonmaleficence is also highly relevant, as inappropriate sourcing, excessive tissue collection, or inadequate handling of biological materials may pose physical or psychological risks to donors and raise concerns about exploitation [275]. Ethical considerations also arise from the technical limitations of reproductive OoC systems. Because of the complex nature of human organs, much more effort is being made to accurately produce the physiological environment. Although these platforms can model key reproductive interactions but still face challenges in accurately reproducing endocrine dynamics, tissue complexity, and long-term physiological responses [276]. If these models are insufficiently validated, they may generate misleading biological conclusions or inappropriate translational expectations. Therefore, ethical use of OoC platforms requires transparency regarding model limitations, careful validation against human physiological data, and avoidance of overinterpretation when applying in vitro findings to clinical reproductive medicine.

The increasing use of AI in ART introduces another layer of ethical concern. AI-assisted systems can support embryo assessment, image-based analysis, and decision support. However, they rely on sensitive reproductive and health-related data, raising issues of privacy, data ownership, consent for secondary data use, and cybersecurity. Data privacy, algorithmic bias, fairness, transparency, explainability, responsibility, and justice are central ethical issues for the responsible implementation of AI in ART [277, 278]. In addition, algorithmic bias may occur if AI models are trained on datasets that are not representative of diverse patient populations, embryo culture conditions, imaging systems, or clinical practices, potentially leading to unequal or unreliable recommendations across patient groups. The over-reliance on machines to make decisions and the personal health data have also raised ethical problems and the question remains: to what extent should the doctors' decisions depend on the machine algorithm? Surveys have pointed out that about 5% of adults in high-income developed countries undergo diagnostic errors in outpatient clinics annually. More than half of these mistakes resulted in severe harm being inflicted on the patient [279, 280]. Even so, these figures are still much higher in low- and middle-income countries [279]. Therefore, the potential of machine algorithms in assisted reproductive techniques is undeniable; however, at this time and in the near future, AI cannot completely replace clinicians in diagnosis. Even when the computer algorithms are intelligent enough, their use without doctors might still face ethical and clinical issues. Thus, AI systems in ART should be implemented as clinician-guided decision-support tools, with transparent validation, explainable outputs where possible, continuous performance monitoring, protection of patient data, and clear responsibility for final clinical decisions.

3.2.2 | Standardization Challenges

The second major challenge is the standardization of the protocols across emerging research in OoC systems. Despite considerable advancements, inconsistencies persist in key aspects, including cell sources, fluidic control, gene-editing reproducibility, and AI data analysis. These variations hinder reproducibility, limit cross-platform comparability, and inadvertently create barriers to practical clinical translation. Addressing these issues requires a more systematic framework for standardization.

Initially, the lack of cell sources—the most important aspect of OoC technique, especially the commercial cell lines, has led to difficulties in mimicking the *in vivo* reproductive organs and standardizing cell culture protocols [126]. The development and standardization of reproductive OoC models are further complicated by the limited availability of suitable cells and the ethical concerns associated with obtaining them through clinical procedures, such as IVF or surgery. Furthermore, these cells exhibit substantial heterogeneity depending on donor-specific factors, such as age, hormonal status, and underlying pathologies, which can lead to inconsistent experimental results [281–284]. On the other hand, maintaining stable phenotypes in the *in vitro* environment remains a challenge, as many types of reproductive cells rapidly lose their functional characteristics, including hormone responsiveness and gene expression profiles, while culturing [285]. To address these challenges, recent initiatives have concentrated on establishing standardized cell source frameworks to ensure more

stable and repeatable biological inputs [286]. In particular, a fully annotated master cell bank, including detailed donor data such as age, hormone status, and medical history, can be proposed in the near future to significantly improve comparability across studies. Moreover, the maintenance of physiological phenotypes is further improved by the advancements in 3D culture systems and co-culture strategies, enhancing the replication of natural cell–cell and cell–matrix interactions.

In addition, even though the standard procedures for pumping fluid flows are currently used, the proper control of multifluidic channels inside a multiorgan chip system to create hormone circulation is still complicated and needs to be standardized. In particular, reproducing hormone circulation requires coordinated temporal and spatial control of flow rates, concentration gradients, and recirculation loops [287]. However, differences in device fabrication, channel geometry, hydrodynamic resistance, and interconnection interfaces can lead to discrepancies between intended and actual flow conditions, thereby limiting the reproducibility of physiologically relevant hormone profiles. Regarding these, platform researchers should implement postintegration calibration frameworks to quantify the discrepancies between nominal and *in situ* flow rates in the presence of varying hydrodynamic resistances in order to resolve this constraint. Such efforts should be aligned with existing microfluidic standardization initiatives, such as ISO 22916:2022 [288] for device dimensions, connections, and initial classification and ISO/TS 6417:2025 [289] for pump symbols and performance reporting.

Regarding gene-repairing technologies, as discussed previously, the two research groups conducted experiments to eliminate mutations in early embryos using the same concept but observed different results [178, 179]. These discrepancies may partly reflect the lack of standardization in experimental conditions, embryo characteristics, editing protocols, and outcome assessment. To strengthen the cross-study comparability, it is crucial to establish standardization in several key aspects, including the origin and developmental stage of embryos, the type of mutation and target locus, delivery method, and dosage of gene-editing components, as well as the criteria for evaluating outcomes based on sequencing methods. In addition, unified definitions for repair efficiency, mosaicism, and embryo viability are required to ensure consistent interpretation of results across studies [290–292].

Finally, as outlined earlier, AI has emerged as a compelling supportive tool for improving the accuracy and efficiency of gamete and embryo selection. However, when using AI, the results are considerably dependent on the trained data sets, which often consist of embryo images, time-lapse videos, morphokinetic parameters, and associated clinical outcomes. Several properties of data, including size, quality, objectivity, and representativeness, could result in a biased model [211, 293]. Such bias may arise when datasets are collected from a limited number of clinics, imaging systems, patient populations, embryo culture protocols, or annotation practices, thereby reducing model generalizability across different ART laboratories. Ongoing efforts have been made to enhance the accuracy and efficiency of AI models; for example, through the usage of big data analytics [293]. However, before regulatory approval and widespread clinical implementation, AI-based ART tools require standardized protocols for image acquisition, embryo annotation, outcome definition, data repro-

cessing, model training, and performance reporting. Moreover, human errors in labeling and outcome assessment should be minimized through expert consensus, quality control procedures, and transparent validation frameworks.

3.2.3 | Clinical Challenges and Practical Gaps

Despite significant progress, certain pathologies persist that require a level of intensive clinical intervention that is difficult to fully replicate or execute in an *in vitro* environment. These conditions are associated with intricate biological mechanisms, including local immune responses, interaction among the stroma, epithelium, and endocrine system, as well as tissue regeneration processes. One of the most representative examples is recurrent implantation failure (RIF), which refers to more than three failed implantations of good-quality embryos by IVF [294]. Factors related to uterine conditions, such as advanced maternal age, immunology, endometrial receptivity, or external causes, including smoking status, high body mass index, stress levels, and male factors, contribute unlikely among patients [295]. RIF treatment needs to be evaluated and tailored appropriately to each individual through the process of sperm analysis, preimplantation genetic testing, IVF protocols, and endometrial receptivity assessment. Refractory endometrial injury represents another major clinical challenge closely associated with impaired receptivity, RIF, and pregnancy loss. A clinically relevant example is Asherman syndrome, which is pregnancy loss attributable to refractory endometrial injury, is also considered immensely dependent on clinical intervention. Patients who experienced curettage of a gravid uterus to address miscarriage, postpartum bleeding, retained products of conception, or pregnancy termination might have the potential for intrauterine scar tissue or adhesions, which is the major cause of Asherman syndrome [296]. Treatment relies on hysteroscopic surgery to remove intrauterine adhesions and restore the normal anatomical structure; however, even with moderate cases, the success rate still remains inferior [297]. Recent postoperative studies have yielded encouraging indications for the recurrence prevention, such as hormonal medications, hydrogel barriers, or novel stem cell therapy [298].

Therefore, a better understanding of the mechanisms underlying endometrial repair, including epithelial regeneration, stromal remodeling, angiogenesis, immune regulation, ECM reconstruction, and decidualization, is essential for developing effective therapeutic strategies. In recent years, various bioengineering and tissue engineering approaches, such as biomaterial-based scaffolds, hydrogels, 3D bioprinting, stem cell-loaded matrices, endometrial organoids, and endometrium-on-a-chip platforms, have shown promising potential for modeling endometrial dysfunction, promoting tissue regeneration, and restoring receptivity-related features in preclinical studies [67, 92, 144, 145, 299, 300]. These findings suggest that artificial or bioengineered alternatives may provide valuable tools for investigating embryo–endometrial interactions and for developing regenerative strategies for damaged endometrium. However, despite these encouraging advances, clinical translation remains challenging. Complete endometrial restoration requires not only structural repair but also recovery of hormonal responsiveness, vascular remodeling, immune tolerance, decidualization, and embryo-supportive function. Although current bioengineered constructs

can partially improve endometrial morphology and angiogenesis in preclinical models, their ability to fully restore the dynamic functions of the human endometrium remains uncertain. In addition, large-scale clinical manufacturing remains a major barrier, as reproductive bioengineering products must satisfy multiple clinical-grade production requirements, for example, GMP compliance [301], with strict control of sterility, endotoxin, mycoplasma, residual reagents, mechanical integrity, and biological activity. Surgical applicability is another important consideration, as the delivery, positioning, retention, and integration of scaffolds or regenerative matrices within the uterine cavity may strongly influence therapeutic outcomes. Moreover, because these technologies may affect gametes, embryos, implantation, pregnancy progression, and potentially offspring health, their evaluation requires a higher level of reproductive safety assessment than many other regenerative applications. Long-term follow-up studies are needed to examine pregnancy outcomes, placental development, fetal growth, neonatal health, epigenetic stability, germline integrity, and possible intergenerational effects. Finally, high production costs and the lack of large-scale randomized clinical trials further limit their adoption. Therefore, although bioengineered platforms offer a promising direction for addressing refractory endometrial injury, further validation, standardized protocols, harmonized regulatory frameworks, and long-term reproductive safety data are required before these approaches can be reliably translated into clinical practice for patients with recurrent implantation failure or pregnancy loss.

Concurrently, overall fertility potential has been pronounced reduce by the global phenomena including declining fertility rates, population aging, and delayed childbearing. Consequently, the preservation and improvement of gamete quality in advanced-age individuals, including both females and males, is considered a promising strategy. To date, gamete preservation as well as embryo preservation are highly committed to the cryo-process [302]. In addition, cryopreservation can also apply for ovarian tissue; however, the method's physical effects can impair oocyte viability and affect the integrity of follicular cells and stromal preservation [303]. Within today's technologies, cultivation processes can be applied post-defrost to control the quality of gametes before fertilization or embryos before implantation. On the other hand, aging significantly affects oocyte quality. Research on enhancing the quality of oocytes is considered an urgent issue [304]. Chromosomal cohesion deterioration, misaligned chromosomes, telomere shortening, DNA damage and related genetic mutations, oxidative stress, mitochondrial dysfunction, and epigenetic modifications are phenomena that occur with aging oocytes. Senotherapies by antioxidants against reproductive aging and mitochondrial therapy are documented as an effective method. Moreover, pluripotent stem cells or somatic cells can rejuvenate functional oocytes and ovaries [305]. In addition, tissue engineering and OoC-based platforms have emerged as promising tools for female fertility preservation and reproductive aging research [306, 307]. Artificial ovary systems, biomaterial-based ovarian scaffolds, and hydrogel-supported follicle culture have been explored to preserve ovarian function, support oocyte survival, restore endocrine activity, and model ovarian microenvironmental changes [308]. However, most available tissue engineering and OoC studies primarily focus on fertility preservation, follicle culture optimization, ovarian function modeling rather than direct improvement of aged oocyte

quality. Therefore, improving gamete quality in advanced-age women remains an important but insufficiently explored research direction in reproductive bioengineering. Future studies should determine whether these approaches can reduce age-related ovarian dysfunction and support oocyte competence, embryo development, and reproductive outcomes in advanced-age women. Importantly, these approaches require rigorous validation of safety, reproducibility, pregnancy outcomes, and potential effects on offspring health before clinical translation can be considered.

3.2.4 | Technology Integration Challenges

Last but not least, how to harness the strength of all these emerging technologies in a system is a remaining challenge. Microfluidics excels at small-scale manipulation, while one of the main goals of tissue engineering is to generate functional tissues at a scale suitable for clinical applications. The scale-up of microfluidic systems to support the growth of larger tissue constructs is a significant challenge. In addition, the creation of the dynamic microenvironment that provides the right biochemical and physical cues for multiple cell types and the ECM in tissue engineering is complex. Producing microfluidic devices for tissue engineering can be costly, especially when precision and custom designs are required. The combination of microfluidics and imaging techniques poses challenges due to the fast-flowing or dynamic nature of the fluidic system. Microfluidic systems often have deep channels or multilayer structures and complex fluids, such as blood or cell suspensions, requiring imaging across varying depths. The integration of a gene editing tool into microfluidics can be complicated. Delivering CRISPR-Cas components into targeted cells using microfluidic systems can be difficult due to the complexity of gene-editing machinery, which could lead to off-target effects.

One of the significant challenges in integrating multiple technologies, such as combining time-lapse imaging with AI in OoC models, is the technical complexity of synchronizing real-time imaging with dynamic, 3D microenvironments. In terms of engineering, overcoming this challenge requires advancements in both imaging resolution and AI algorithms specifically designed to process data from complex 3D cultures. The suggested development of specialized imaging techniques, such as high-resolution, multimodal imaging systems, that can operate within the confined spaces of microfluidic devices, coupled with AI models trained on large datasets from these environments, to enhance predictive accuracy. Additionally, the challenge of ensuring precise and targeted gene editing in embryos while minimizing off-target effects can be addressed by refining CRISPR-Cas9 engineering. This includes optimizing the design of guide RNAs and integrating real-time monitoring systems to detect and correct any unintended edits using molecular probes. For integrative system monitoring and accurate control, advances in biosensors and bioelectronics could be extensively applied. Biosensors and bioelectronics facilitate the standardization, validation, and testing of integrative systems for fulfilling their potential in disease modeling, drug discovery, and personalized treatments. High-speed cameras, advanced algorithms for real-time image processing, and state-of-the-art optical techniques are essential. In addition, downstream analysis, with “omics” in general, might be a useful approach for assessment of integrative system performance.

4 | Conclusions

In summary, this paper illustrated the emerging technologies in the female-assisted reproductive field. Innovative biotechnologies such as OoC, tissue engineering, gene-editing tools, automation, and AI have shown enormous potential in revolutionizing the way oocytes and embryos are created: how to assess and select the best embryo, how embryos are developed outside our body until offspring are born. A whole system that combines these above-mentioned techniques promises to transform the field of reproductive treatments. However, to make this possible, there are several major ongoing challenges to be faced. To bridge this gap, much more extensive investigations need to be carried out to validate these techniques before regulatory approval for use and standardization of the protocols. Ongoing efforts are being made to continuously monitor and accurately control the integrated systems. In the future, each patient may receive a precision treatment, and their high-quality embryos may grow in a “tailored” biomimetic environment with an automatic monitoring and manipulating system. As such, healthy babies will be born with fewer harmful effects on patient health.

Author Contributions

Thu Hang Nguyen, Hung Anh Nguyen: conceptualization, writing – original draft, data curation, investigation. **Y-Van Tran Thi, Hoang Trung Nguyen:** writing – original draft, investigation, formal analysis. **Amber Cao:** writing – original draft. **Quang Loc Do:** writing – review and editing, funding acquisition. **Thanh Tung Bui:** writing – review and editing. **Duc Trinh Chu, Hung Cao:** conceptualization, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Abstract:	• Research question: Conventional oocyte denudation in assisted reproductive technology remains labor-intensive, time-consuming, and highly dependent on skilled embryologists. Can microfluidic technology enable automated, high-throughput, and efficient cumulus cell removal while reducing manual handling and operator dependence? • Design: In this study, we present the DENO chip (Denudation On-Chip), a novel microfluidic platform incorporating a quadrilateral-shaped micropillar array designed to achieve efficient, high-throughput oocyte denudation under continuous-flow conditions. • Results: This cost-effective chip-based system enables automated oocyte processing, thereby improving reproducibility, ease of operation, and overall accessibility in ART. Experimental and numerical investigations using porcine oocytes demonstrate that the device can process 100 oocytes within 90 seconds with high denudation efficiency (around 96%), substantially enhancing throughput and performance. Embryo developmental outcomes following in vitro fertilization from on-chip denuded oocytes were at least equivalent to those achieved using conventional denudation approaches. • Conclusions: Given the demonstrated efficiency in oocyte denudation, the proposed method can be extended to effective, high-throughput separation applications, including the isolation of rare cells from heterogeneous cellular or biological complexes.

DENO: A High-throughput Microfluidic Platform for On-chip Oocyte Denudation

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Abstract

- **Research question:** Conventional oocyte denudation in assisted reproductive technology remains labor-intensive, time-consuming, and highly dependent on skilled embryologists. Can microfluidic technology enable automated, high-throughput, and efficient cumulus cell removal while reducing manual handling and operator dependence?
- **Design:** In this study, we present the DENO chip (Denudation On-Chip), a novel microfluidic platform incorporating a quadrilateral-shaped micropillar array designed to achieve efficient, high-throughput oocyte denudation under continuous-flow conditions.
- **Results:** This cost-effective chip-based system enables automated oocyte processing, thereby improving reproducibility, ease of operation, and overall accessibility in ART. Experimental and numerical investigations using porcine oocytes demonstrate that the device can process 100 oocytes within 90 seconds with high denudation efficiency (around 96%), substantially enhancing throughput and performance. Embryo developmental outcomes following *in vitro* fertilization from on-chip denuded oocytes were comparable to those achieved using conventional denudation approaches.
- **Conclusions:** Given the demonstrated efficiency in oocyte denudation, the proposed method can be extended to effective, high-throughput separation applications, including the isolation of rare cells from heterogeneous cellular or biological complexes.

Keywords: microfluidics, high-throughput, oocyte denudation, assisted reproductive technologies

1. Introduction

With rapid advances in reproductive medicine, assisted reproductive technology (ART) offers hope for those unable to conceive and enables genetic screening and selection (Graham et al., 2023). ART, including conventional IVF (Elder, 2001) and ICSI (Palermo et al., 2017), requires precise and controlled handling of gametes and embryos. Despite substantial advances in ART, several laboratory procedures still rely on manual handling by skilled embryologists. Current good-practice recommendations emphasize minimizing unnecessary handling of oocytes and embryos and reducing their exposure to mechanical and shear stress during laboratory procedures (Group et al., 2026). These processes are also labour-intensive and time-consuming, requiring skilled embryologists to perform delicate manipulations. Their operator-dependent nature may introduce variability in procedural consistency and can present challenges for scalability, particularly in settings with limited access to trained personnel (Smith and Takayama, 2017). Microfluidic and lab-on-a-chip technologies have emerged as promising approaches for improving the automation and standardization of laboratory procedures through precise manipulation of fluids and biological samples at the microscale (Bodas, 2025; Yeo et al., 2011). In ART, microfluidic technologies have been increasingly investigated for different stages of the reproductive workflow (Pensabene et al., 2026; Sequeira et al., 2020). Examples include oocyte trapping and cultivation (Franko and de Almeida Monteiro Melo Ferraz, 2025), analysis of semen for the procurement of sperm (Knowlton et al., 2015), fertilization (Han et al., 2010; Ma et al., 2011; Suh et al., 2006), oocyte characterization (Wacogne et al., 2008), etc. However, the degree of clinical translation varies considerably among these applications. Clinical adoption has been most evident for microfluidic sperm sorting and selection, whereas approaches for oocyte processing and fertilization remain largely at the experimental or preclinical stage.



OPEN Numerical study on a facing electrode configuration dielectrophoresis microfluidic system for efficient biological cell separation

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Circulating tumor cell separation has been the focus of numerous studies owing to its importance in the diagnosis, prognosis, and therapy of cancer. This study reports a highly efficient microfluidic device that integrates a specialized dielectrophoresis configuration, namely the facing-electrode configuration dielectrophoresis (FEC-DEP) structure, to isolate circulating tumor cells (CTCs) from various blood components, including red blood cells, white blood cells, and platelets. The FEC-DEP design features a bottom-slanted electrode array positioned parallel to a basic rectangular top electrode. A non-homogeneous electric field is produced between these parallel electrodes, generating dielectrophoretic forces acting on cells. Consequently, when the FEC-DEP is integrated into a flow, it can direct various biological objects in the flow along separate trajectories. As a result, cells with comparable characteristics might move together within a similar path. This configuration may simplify the microfabrication process and lessen dependency on particle position within the microchannel. The separation process was numerically analyzed using the finite element method, and device parameters were optimized to obtain high-efficiency and high-purity cell separation. The simulations show that the microfluidic device may effectively enrich tumor cells in a label-free and non-invasive manner, with a high-efficiency rate of almost 80%.

Keywords Dielectrophoresis, Microfluidic chip, Cell separation, Cancer cell analysis

Many biochemical and biomedical applications necessitate cellular manipulations and separations to facilitate the identification, isolation, and investigation of phenotypes or samples of interest. For example, the process of isolating blood cells in a sample is crucial due to the distinct functions that each blood component serves, especially in the case of cancer patients^{1,2}. During the pre-metastatic stage, the presence of cancer cells in the bloodstream and lymphatic system offers valuable clinical information for diagnosing the medical condition and determining appropriate treatment plans for patients^{3–5}. In regenerative medicine, the separation and purification of stem cells play an important role to study the heterogeneity of stem cell populations and assure the safety and efficiency of treatment^{6,7}. In reproductive biology and assisted reproductive technologies, the first steps to better treatments for infertility are to separate, and select high-quality reproductive cells in a lab setting. These cells include motile sperm and highly competent oocytes^{8,9}. In addition, the aim of prenatal diagnosis has shifted to non-invasive methods using fetal cells circulating in maternal peripheral blood in order to collect entire fetal genetic information while minimizing dangers to the mother and fetus¹⁰. The creation of separation methods with great efficiency is required to extract the rare fetal cells from the mother's blood¹⁰. Thanks to the ability to precisely control fluids in micro-scale channels, microfluidic platforms have been widely used for cell manipulation and separation in blood samples. Microfluidic cell separation is a cutting-edge technology with numerous applications in both research and clinical settings such as rare cell separation from blood sample¹¹, stem cell sorting and isolation⁷, reproductive cell manipulation, sorting and separation^{12,13}, etc. These platforms

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provide many benefits, such as reduced sample volume, enhanced patient safety, and expedited analysis¹⁴. Microfluidics-based label-free approaches for cell isolation are primarily categorized into passive, active, and hybrid techniques based on differences in cell properties. Active approaches such as dielectrophoresis, magnetophoresis, acoustophoresis, etc. employ additional forces in the microfluidic chips^{15–20}. Although enhancing the separation efficacy by adding another control level of cell trajectories, active approaches may lead to cell damage and equipment requirements²¹. In contrast, passive technologies utilize hydrodynamic force and chip geometry to distinguish cancer cells from other cell types. This approach, which does not need any additional equipment, presents a more simplified operating procedure. Nevertheless, it often experiences an extended period of analysis²¹.

Dielectrophoresis (DEP) is a modern, non-invasive technology used for determining the electrical properties of biological objects, enabling further analysis and manipulation of target cells within the sample^{22,23}. DEP is a method that involves the application of a force on a particle that is neutral but polarized when it encounters a non-homogeneous electric field^{24,25}. DEP have been proven to effectively manipulate and separate various biological cells including cancer cells²⁶, stem cells²⁷, blood cells¹⁵, motile sperms²⁸, dead and viable cells²⁹, etc. In the field of oncology research, the detection and separation of CTCs from various blood constituents, including white blood cells, red blood cells, platelets, and plasma, pose significant challenges due to the extremely low number of CTCs present in the blood sample and the biophysical heterogeneity among the CTC population³⁰. Varmazyari et al. proposed a DEP-based microfluidic system, that used parallel sidewall electrodes. This system had encouraging outcomes, achieving a separation efficiency of 90% for CTCs³¹. However, the fabrication process is complex for setting up electrodes at the sidewall.

Additionally, several research groups have proposed microfluidic chips with embedded electrodes on only a single side of the microchannel in order to facilitate cell separation^{32,33}. A recent paper designed and simulated a microfluidic chip that contained coplanar electrodes to separate CTCs (MDA-MB-231 cell line) and RBCs and obtained an efficiency of more than 90% under optimal conditions³². In another study, Wu et al. introduced a biochip including coplanar electrodes positioned only at the bottom of the microchannel to trap yeast cells and polystyrene microbeads suspended in diverse aqueous solutions, each exhibiting a specific conductivity³³. Results were promising, with a single yeast cell occupancy of over 72% achievable. Nevertheless, this technique necessitates a significant concentration of the electric field around the electrode area, thus compromising resolution due to the positional dependence of the DEP force.

Recently, numerous research investigations utilized a microfluidic chip to separate different cell lines based on DEP and the findings demonstrated good matches between numerical and experimental results^{34–36}. As an example, recent work suggested the use of a zigzag shape microfluidic channel to accomplish the separation of platelets by DEP forces generated by a non-uniform electric field that influenced cell movement³⁴. The authors used COMSOL Multiphysics software to optimize the device design and operating settings, simulate cell trajectories under various conditions, and investigate the effects of significant factors on separation efficiency. At a voltage of 20 V and a velocity ratio of 1:4, the microfluidic chip that was fabricated with the ideal structure demonstrated a separation efficiency of 99.4%.

Our work aims to apply DEP methods to isolate CTCs from cancer patients' blood cells. The proposed microfluidic system is illustrated in Fig. 1. The proposed microfluidic system consists of two inlets and four outlets, and an integrated DEP structure, namely the facing-electrode configuration dielectrophoresis (FEC-DEP) structure. The FEC-DEP structure comprises a bottom-slanted electrode array positioned parallel to a basic rectangular top electrode. These facing electrodes with FEC-DEP design generate a non-uniform electric field causing DEP forces.

In our previous study, a microfluidic device was introduced to enrich CTCs from a mixture of red blood cells (RBCs) and white blood cells (WBCs) featuring three distinct output configurations³⁷. In the current study, we have further optimized this system to efficiently separate CTCs from all major cell types found in processed blood samples, including RBCs, WBCs, and platelets (PLTs). In this study, HT-29 is used to represent CTCs in blood stream. HT-29 is a type of human colon adenocarcinoma cell line commonly utilized in biomedical research, especially for studies into cancer biology, drug development, and cancer therapy³⁸. Prior to introduction into the microfluidic chip, blood samples undergo preprocessing steps to retain four types of cells including CTCs, WBCs, RBCs, and PLTs, and are then diluted in phosphate-buffered saline (PBS) to achieve an appropriate concentration. Following this preparation, a stream of blood cells – comprising RBCs, WBCs, and PLTs – is directed into the chip's inlets. Once inside, the cells encounter a non-uniform electric field, due to the distinguishable electrical properties and sizes of blood cells and tumor cells, which subjects them to different DEP forces. The differential application of force causes the cell types to follow distinct trajectories inside the microchannel and concentrate at different exits of the channel.

The proposed microfluidic device is expected to provide a potentially effective, small-scale, simply integrated, and affordable solution for CTC separating.

Theoretical analysis

In microfluidic systems, laminar flow occurs when the Reynolds number (Re) is low, typically less than one, indicating that inertial forces are negligible compared to viscous forces. The fluid flow behavior is described by the Navier-Stokes, viscous forces dominate, and inertial forces can be disregarded. Torsional flow, also known as Stokes flow, is prevalent in systems with high viscosity or small geometric length scales. Fluids in microfluidic systems can be either compressible or incompressible, and exhibit Newtonian or non-Newtonian behavior. In the analysis of such flows, momentum balance and mass conservation equations are simplified due to the dominance of viscous forces, characteristic of Stokes flow³⁹.

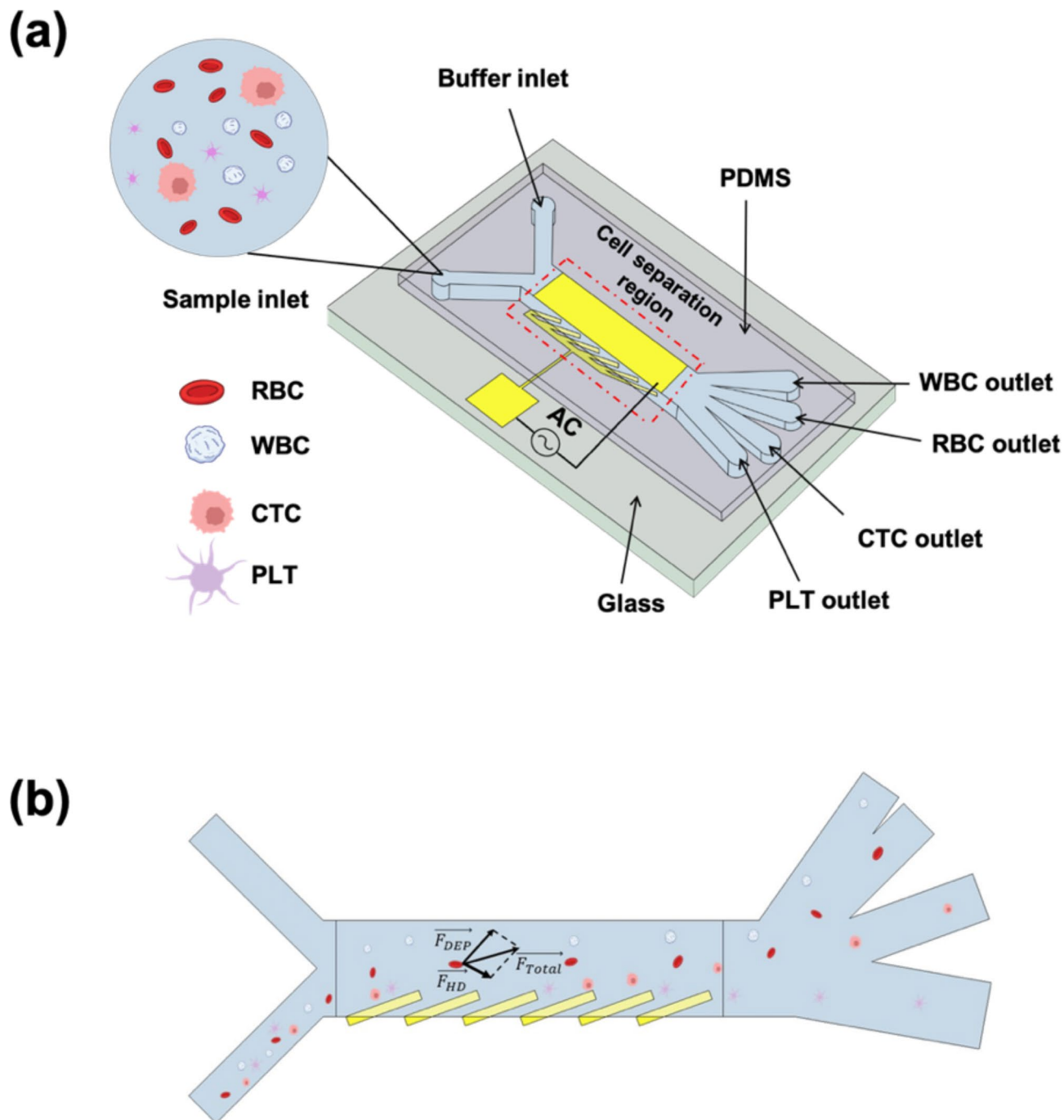


Fig. 1. (a) Design of the proposed microfluidic chip with integrated facing-electrode configuration dielectrophoresis (FEC-DEP) structure for separating CTCs from blood constituents, (b) concept of the FEC-DEP-based device: top-view.

$$\nabla \cdot [-p \vec{I} + \vec{K}] + \vec{F} = 0 \quad (1)$$

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{v}_m) = 0 \quad (2)$$

Here, p is the pressure, $\vec{K}$ is the viscous stress tensor, $\vec{F}$ is the volume force vector, ρ is the fluid density, and $\vec{v}_m$ is the fluid velocity.

Hydrodynamic force on a (single) suspended (rigid) particle is governed by Stokes' law in a microfluidic channel:

$$\overrightarrow{F_{HD}} = 6\pi \eta R_p (\overrightarrow{v_m} - \overrightarrow{v_p}) \quad (3)$$

Here, R_p is the radius of the bioparticle, η is the dynamic viscosity coefficient of the medium and $\overrightarrow{v_m}$ is the velocity of the flow.

The electric field distributed within the proposed FEC-DEP device is governed by the current conservation based on Ohm's law⁴⁰:

$$\nabla \cdot \overrightarrow{J} = Q_{j,v} - \overrightarrow{J} = \sigma \overrightarrow{E} + \overrightarrow{J}_e \quad (4)$$

in which $\overrightarrow{J}$ denotes current density, $Q_{j,v}$ is the volumetric source of current, σ represents electrical conductivity, and $\overrightarrow{J}_e$ is an externally generated current density.

The electric potential V is calculated by the relationship with the electric field $\overrightarrow{E}$:

$$\overrightarrow{E} = -\nabla V \quad (5)$$

The motion of particles induced by DEP commences when a non-uniform electric field is applied, creating a polarization gradient between the cells and the surrounding medium. DEP mechanism relies on the size and electrical properties of particles, as well as the binding characteristics of proteins and particle surfaces, to produce specific particle deflection⁴¹. When particles encounter this non-homogeneous electric field, two distinct forces are exerted between them and the surrounding medium, resulting in a net force. The direction of particle movement can be influenced by either positive DEP (p-DEP) or negative DEP (n-DEP) effects, determined by the relative polarizability of the particle and the suspension medium. p-DEP happens as particles migrate towards regions of high electric field gradients, whereas n-DEP occurs when particles move towards areas of decreasing electric field gradients. Both effects are influenced by the Clausius-Mossotti relation. The DEP force driving the particle is calculated by the equation⁴²:

$$F_{DEP} = 2\pi R_p^3 \varepsilon_f \text{Re}(K_{CM}) \nabla |\overrightarrow{E}|^2 \quad (6)$$

where R_p is the cell radius, ε_f is the permittivity of the medium. K_{CM} or Clausius-Mossotti (CM) factor of particle is expressed by this equation:

$$K_{CM} = \frac{\varepsilon_c^* - \varepsilon_f^*}{\varepsilon_c^* + 2\varepsilon_f^*} \quad (7)$$

The complex permittivity ε^* of both particle (or cells in this study (c)) and the fluid (f) are calculated as follows:

$$\varepsilon^* = \varepsilon - j \frac{\sigma}{\omega} \quad (8)$$

The polarizability of the particles is measured by the real part of CM factor which is determined by permittivity ε and the electrical conductivity σ ; $\omega = 2\pi f$ is the angular frequency of driving AC voltage, and j is the imaginary part defined as $j^2 = -1$. The $\text{Re}\{CM\}$ can be adjusted within the range of -0.5 to 1 by altering the frequency of the voltage, leading to negative DEP (n-DEP) or positive DEP (p-DEP). At the crossover frequency, the term $\text{Re}\{CM\}$ vanishes. The magnitude of the $(\nabla |\overrightarrow{E}|^2)$ is another important factor influencing the interaction with a particle which is dependent on the driving voltage. From Eq. 5, the gradient of electric field magnitude $|\overrightarrow{E}|^2$

depends on the gradient of the squared gradient of the potential (voltage) changes over space. The design of the fluidic chip-integrated electrode is required to increase $\nabla |\overrightarrow{E}|^2$, and to regulate other electrokinetic forces⁴³.

The corresponding relative complex dielectric constant of a cell, encompassing the cell membrane and interior, is substituted for the complex dielectric constant of the particle when calculating the DEP force (Fig. 2). The single-shell model describes the complicated permittivity of the cell ε_c^* as follows⁴⁴:

$$\varepsilon_c^* = \varepsilon_{mem}^* \frac{\left(\frac{R}{R-d_{mem}}\right)^3 + 2 \frac{\varepsilon_{cyt}^* - \varepsilon_{mem}^*}{\varepsilon_{cyt}^* + 2\varepsilon_{mem}^*}}{\left(\frac{R}{R-d_{mem}}\right)^3 - \frac{\varepsilon_{cyt}^* - \varepsilon_{mem}^*}{\varepsilon_{cyt}^* + 2\varepsilon_{mem}^*}} \quad (9)$$

Biological cells in the microchannel undergo the DEP force as they follow the streamlines of fluid flow. The DEP force ($\overrightarrow{F_{DEP}}$), which is directed perpendicular to the flow, and the hydrodynamic force ($\overrightarrow{F_{HD}}$) are the primary factors influencing cell movement. Minor affected forces such as gravity and Brownian motion are generally ignored as the cell radius is greater than 1 μm as indicated in Table 1. Newton's second law, which accounts for the combined vector forces of DEP and hydrodynamics, determines the velocity and trajectory of the cell:

$$\overrightarrow{F_{HD}} + \overrightarrow{F_{DEP}} = m_p \frac{d\overrightarrow{v_p}}{dt} \quad (10)$$

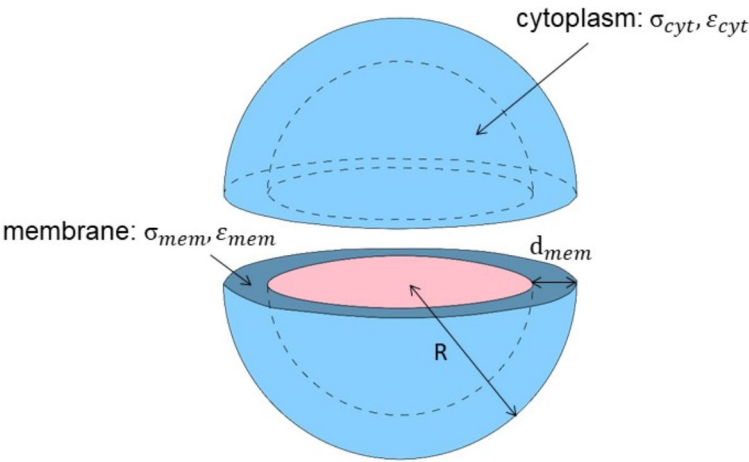


Fig. 2. Single-shell cell model.

Parameter	HT-29	RBC	WBC	PLT
Density (g/l)	1077	1110	1080	1064.5
Diameter (μm)	11	6	14	2.5
Relative permittivity	120	59	150	50
Electrical conductivity (S/m)	0.72	1.31	0.76	0.25
Shell thickness (nm)	4	8	7	7
Shell relative permittivity	11.1	4.44	6.01	6
Shell electrical conductivity (S/m)	34×10^{-6}	1×10^{-6}	24×10^{-6}	1×10^{-6}

Table 1. Electrical properties of each cell type^{15,45–47}.

where $\vec{v}_p$ represents the velocity and m_p stands for the mass of the cell, respectively.

Design of microfluidic device and simulation model

To produce the DEP effect and separate CTCs from blood cells, FEC-DEP has been used with a parallel electrode on top of a microfluidic system and a slanted electrode array embedded in the bottom (Fig. 3). The blood sample containing PLTs, RBCs, WBCs, and HT-29 tumor cells, as well as the buffer solution, are injected through two inlets on the biochip platform. The FEC-DEP design is placed in the center of the microchannel as the separation region, and four different outlets are positioned at the end to collect four types of cells. The ground electrode at the top of the microchannel is set as ground while and herringbone-patterned electrode array at the bottom is configured as positive polarity. The geometrical parameters of the chip are shown in Fig. 3. The electrode length of 80 μm was determined based on a combination of factors including manufacturability, the scale of the biological samples, and electrical field requirements for effective dielectrophoretic manipulation. The inlets are tilted at angles of 45° and the outlets are inclined at angles of 35°, 45°, 70° and 110° to the horizontal axis for the WBC, RBC, HT-29, and PLT outlets, respectively. The tilted angles and geometrical parameters of outlets are optimized based on the trajectories of each cell type. The biochip parameters could be further adjusted for other biological object separations with different sizes and electrical characteristics.

At the beginning of the separation process, the diluted blood sample which includes RBCs, WBCs, PLTs, and CTCs, is injected into the inlet channel at a flow rate of 200 μm/s, while the buffer solution flows at 900 μm/s. The suspending medium is phosphate-buffered saline (PBS), which has a conductivity of 55 mS/m and a relative permittivity of 80⁴⁵. Owing to differences in size and electrical characteristics between various cell types, different DEP effects are delivered to WBCs, RBCs, PLTs, and HT-29 cells, guiding each type of cell along a particular trajectory. Interestingly, the DEP effects on cells along the y-axis intensify as the electrode angle increases. This technique makes it possible to separate CTCs from other blood cells efficiently, providing a potential platform for cancer research.

An inhomogeneous electric field induces dielectrophoresis (DEP) force in all particles, causing them to migrate away from the electrode region and upward. In this biochip, because of the impacts of the buffer flow, HT-29 cells and PLTs tend to deflect toward the sidewall close to the electrodes. Equation 6 illustrates that the strength of the DEP force is proportional to the third-order cell size. As a result, WBCs are subjected to the highest DEP force and forced to outlet 1. In contrast, PLTs undergo the least amount of DEP force and are

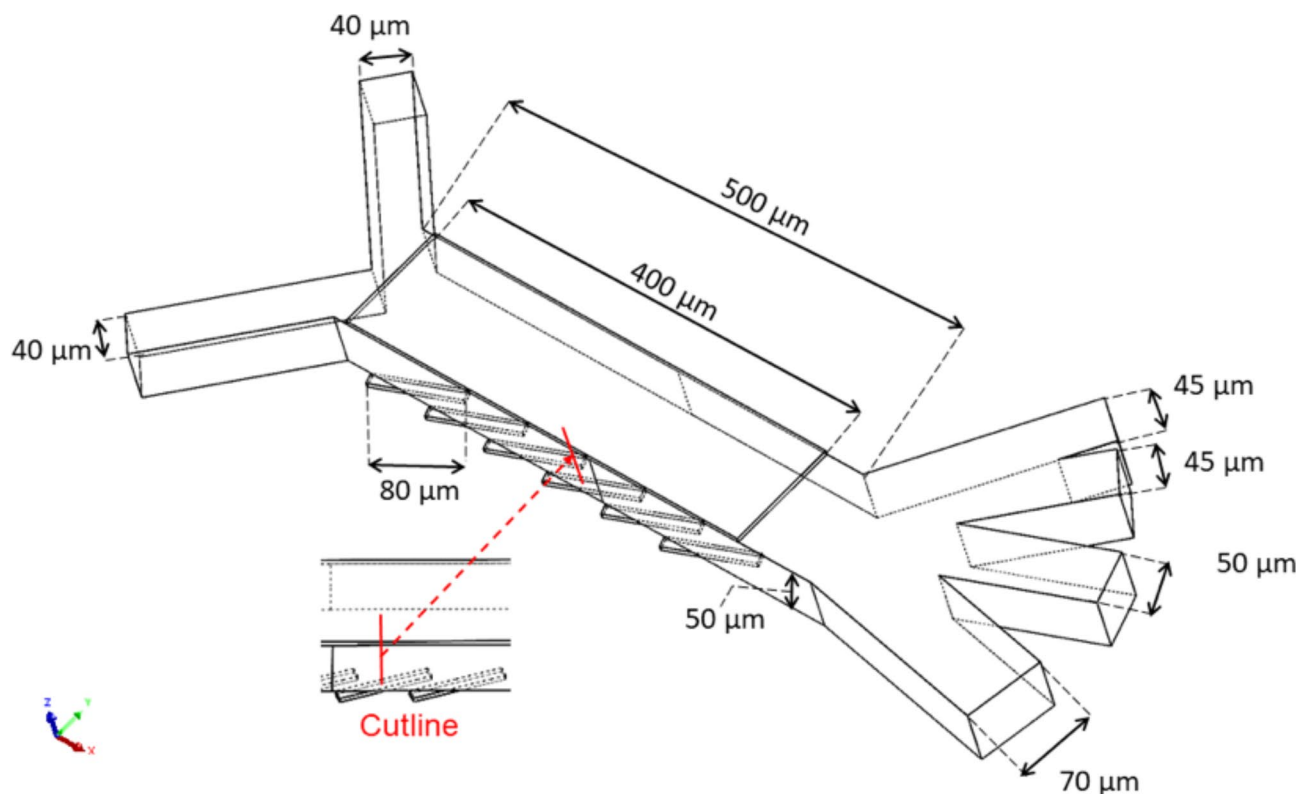


Fig. 3. Illustration of the proposed microfluidic system with dimensions of fluidic channel and FEC-DEP structure.

Mesh type	Maximum element size (μm)	Minimum element size (μm)	Maximum element growth rate	Curvature factor	Number of domain elements	Number of boundary elements
Extra fine	45.6	3.32	1.4	0.4	521,536	50,379
Finer	66.4	8.29	1.45	0.5	207,121	25,241
Fine	82.9	14.9	1.5	0.6	93,356	14,238
Normal	124	23.2	1.6	0.7	31,891	7060
Coarse	158	33.2	1.7	0.8	15,905	4438

Table 2. Mesh independency test: mesh element quality and size of four mesh types.

collected at outlet 4, since they have the smallest diameter. RBCs and HT-29 cells are collected at outlets 2 and 3, respectively. The ratio of blood sample to buffer flow rate is 1:4.5, while the applied voltage is 6 V and the AC voltage frequency is 1 kHz.

Table 1 provides the electrical characteristics of single-shell cells used in simulations^{15,45–47}. WBCs comprise different subtypes such as granulocytes, monocytes, and lymphocytes, each exhibiting distinct characteristics, including size. In this work, to reduce computational complexity for the simulation process, representative parameters for WBCs that have been employed in other related studies within the field are used^{45,46,48}.

COMSOL Multiphysics software has been used to examine the separation mechanism, the efficiency of the microfluidic device, and the impacts of various factors on the cell separation process using a 3D model. To model and simulate this biochip, the Electric Currents, Creeping Flow, and Particle Tracing for Fluid Flow modules were utilized. The Creeping Flow accounts for the fluid behavior and velocity profile across the channel following the Stokes equation and continuity equation. To calculate the frequency-dependent electric field, the Electric Currents solve for Ohm’s law. Finally, the Particle tracing module permits the evaluation of DEP forces exerting on cells, and particle trajectories under total forces. The “no-slip” and “freeze” conditions on the channel walls ensured that there was very little fluid velocity close to the walls and that the location and velocity of the particles were maintained when they collided with the wall. All cells were added to the sample inlet at random. The mesh was meticulously defined with fine-resolution mesh at the electrode regions. The mesh independent test was conducted using four mesh types: extra fine, finer, fine, normal, and coarse with different element sizes and numbers of domain and boundary elements. The mesh qualities are summarized in Table 2. The velocity field and the electric potential distribution in the microchannel have a direct influence on the separation process

and efficiency. Therefore, the mesh independence study was carried out by computing the velocity and electric potential along the cut line near the electrode as shown in Fig. 4.

Considering the computational complexity, resources, and accuracy of the simulation, in this work, the fine mesh was selected. The meshing model and the boundary conditions are shown in Fig. 5.

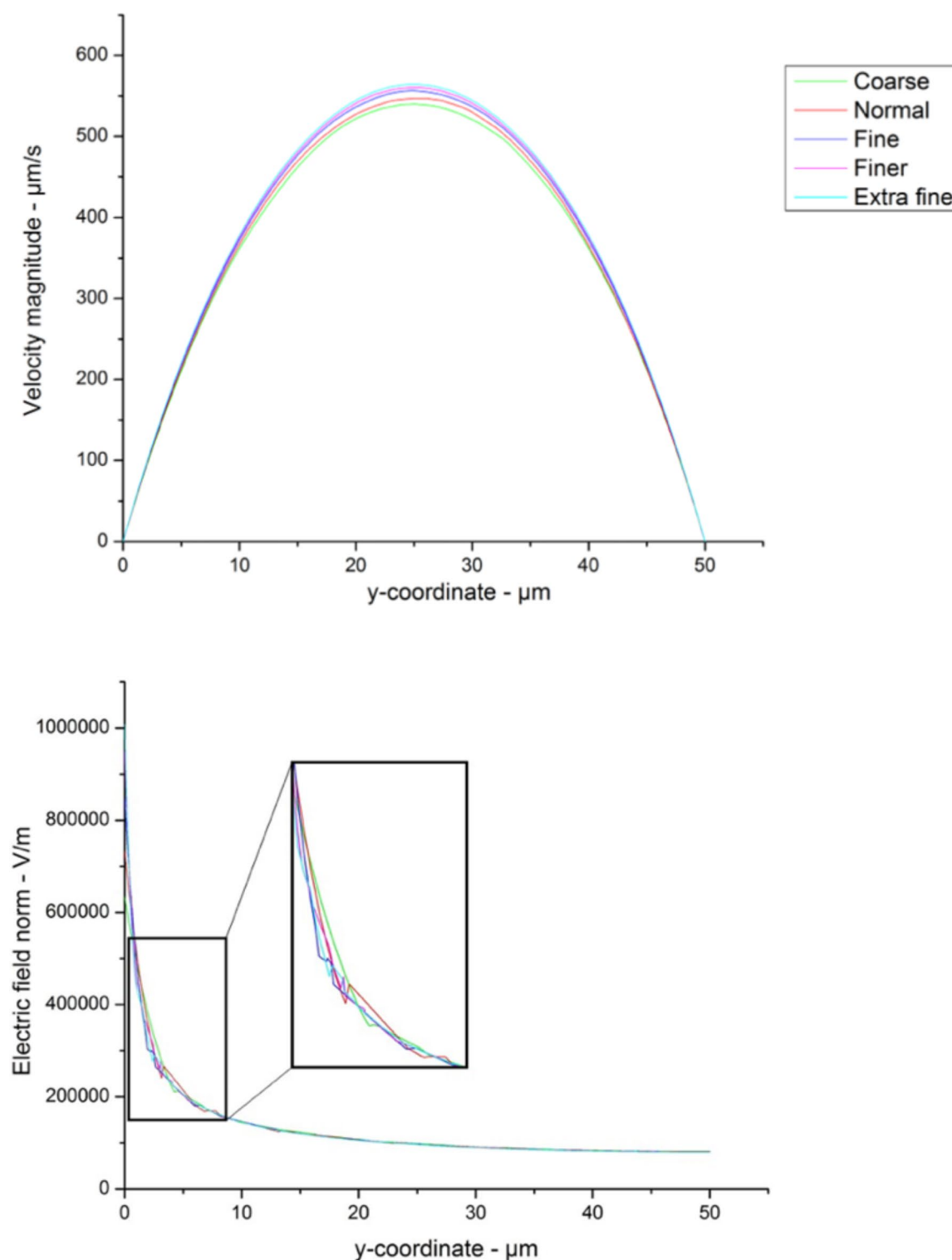


Fig. 4. Velocity magnitude and electric field norm across the cutline.

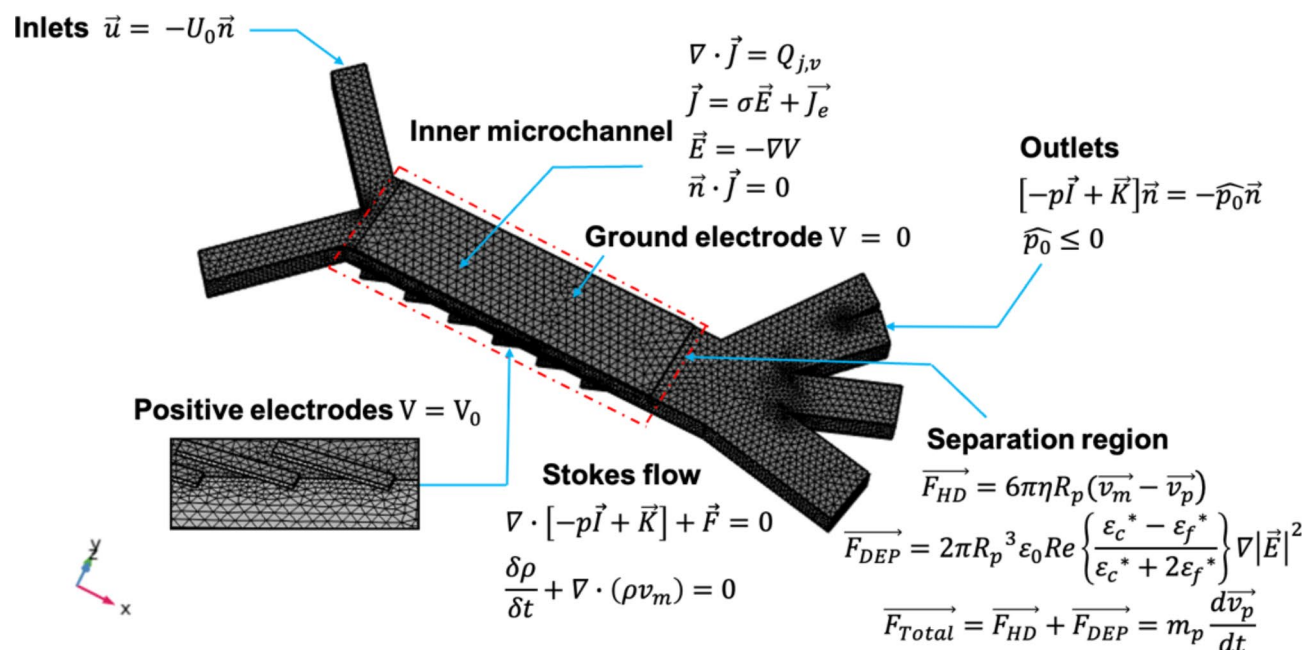


Fig. 5. Meshing model and the boundary conditions of the FEC-DEP-based device.

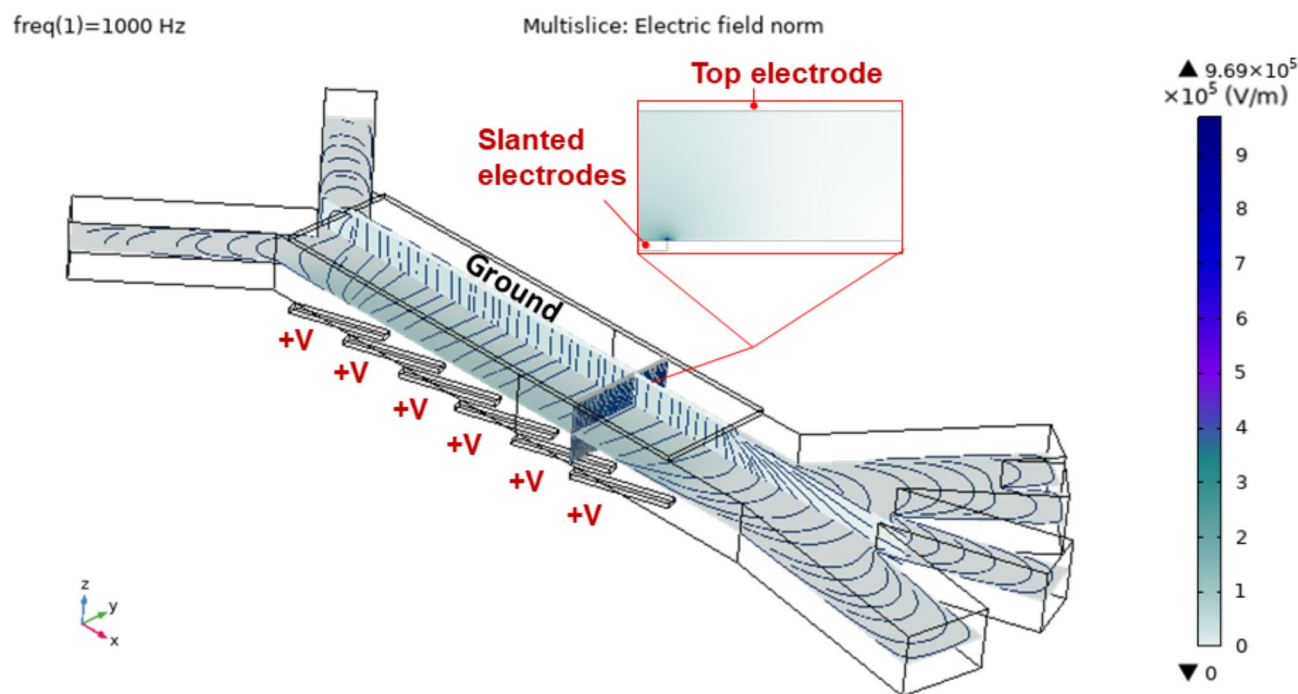


Fig. 6. Distribution of the electric field norm within the microchannel.

Results and discussion

In this section, the electric field, velocity distribution, and cell movements inside the microfluidic chip are reported. The results also indicated the influences of both operating and geometrical factors on cell trajectories including driving voltage, velocity ratio, electrode angle, channel height and the number of electrodes. While investigating the impacts of each factor, other parameters remain constant.

Separation process

Figure 6 depicts the electric field norm distribution inside the microfluidic channel. It can be seen that the regions near the positively polarized electrodes, especially at the electrode edges, feature high electric potential, resulting

in high electric field magnitude. The electric field strength decreases as the distance to the ground electrode reduces below 1×10^5 V/m. The FEC-DEP design generates a non-uniform electric field in the separation part of the microdevice, as shown in Fig. 6, which creates n-DEP effects exerting on cells and the path deflections of those cells, as seen in Fig. 7.

The $\text{Re}(\text{CM})$ values for CTCs and blood cells are plotted on a graph in Fig. 6, which covers the frequency range of 100 Hz to 10 GHz. The $\text{Re}(\text{CM})$ factor of the CTCs, RBCs, and WBCs was negative at the selected frequency of 1 kHz. The HT-29 cells, WBCs, RBCs, and PLTs exposed to varying amounts of nDEP force migrated farther away from the electrodes in both vertical and horizontal directions, or the region of low electric field gradient. Even although the $\text{Re}(\text{CM})$ value of WBCs was lower than that of RBCs and PLTs, the WBCs experienced the greatest deflection, indicating that the $\text{Re}(\text{CM})$ factor could not accurately indicate the magnitude of DEP force. According to the DEP equation, the DEP force is influenced by several factors, including the cell's electrical conductivity, permittivity, and diameter. The 1 kHz is chosen to create nDEP effects acting on all cells. In this case, the unexpected effects of electrical currents on cells are reduced since all cells moved to the top of the microchannel, which has a decreased electric field, when they entered the separation area.

The cell flow paths and electric potential cross the microchannel are illustrated in Fig. 8. The velocity steadily drops from its maximum value at the center of the channel to the lowest value at the channel side wall. The electric potential reaches its highest value at the electrode edges and decreases to zero at the ground electrode. In this simulation, a velocity ratio of 1:4.5 was maintained between the sample and buffer flows. Figure 8a shows the cell trajectories in the absence of DEP force. Simulation results show that only hydrodynamic forces from the buffer flow would force all cells toward the channel wall near the bottom electrodes. All cells migrated in the same direction from the inlet to a single outlet. As a result, the outlet would contain cells randomly arranged according to the cell distribution at the inlet. In the case of applying DEP separation, numerical computations were conducted using an excitation AC voltage of 6 V at 1 kHz. Firstly, four distinct cell types were introduced into inlet 2, while the buffer flow was pumped into inlet 1 to drive those cells towards the electrode part. Under the effects of the applied DEP force, all cell trajectories were deflected in both horizontal and vertical directions

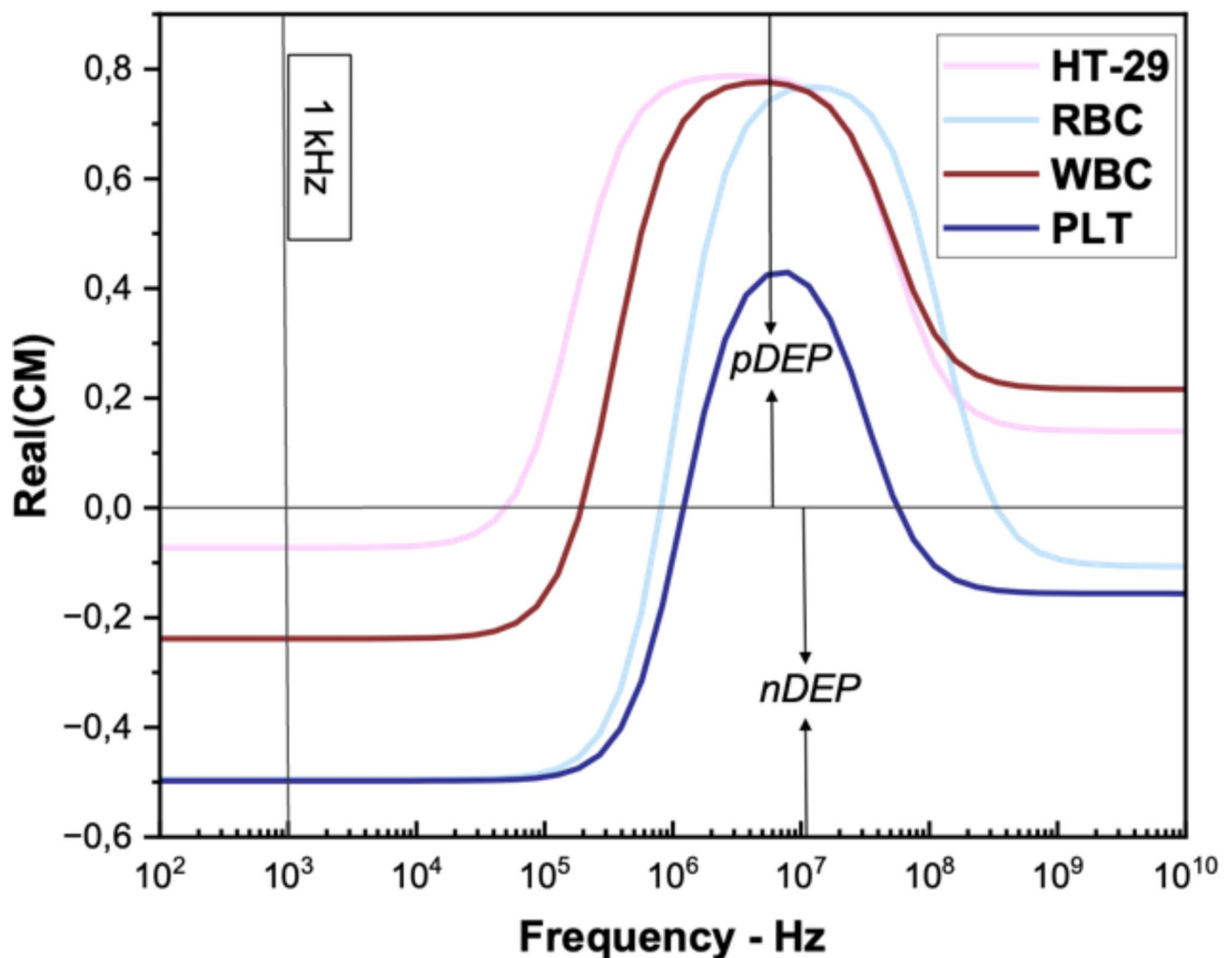


Fig. 7. The real component of the Clausius-Mossotti factor indicates that all cell types suffer nDEP at the specified frequency of 1 kHz.

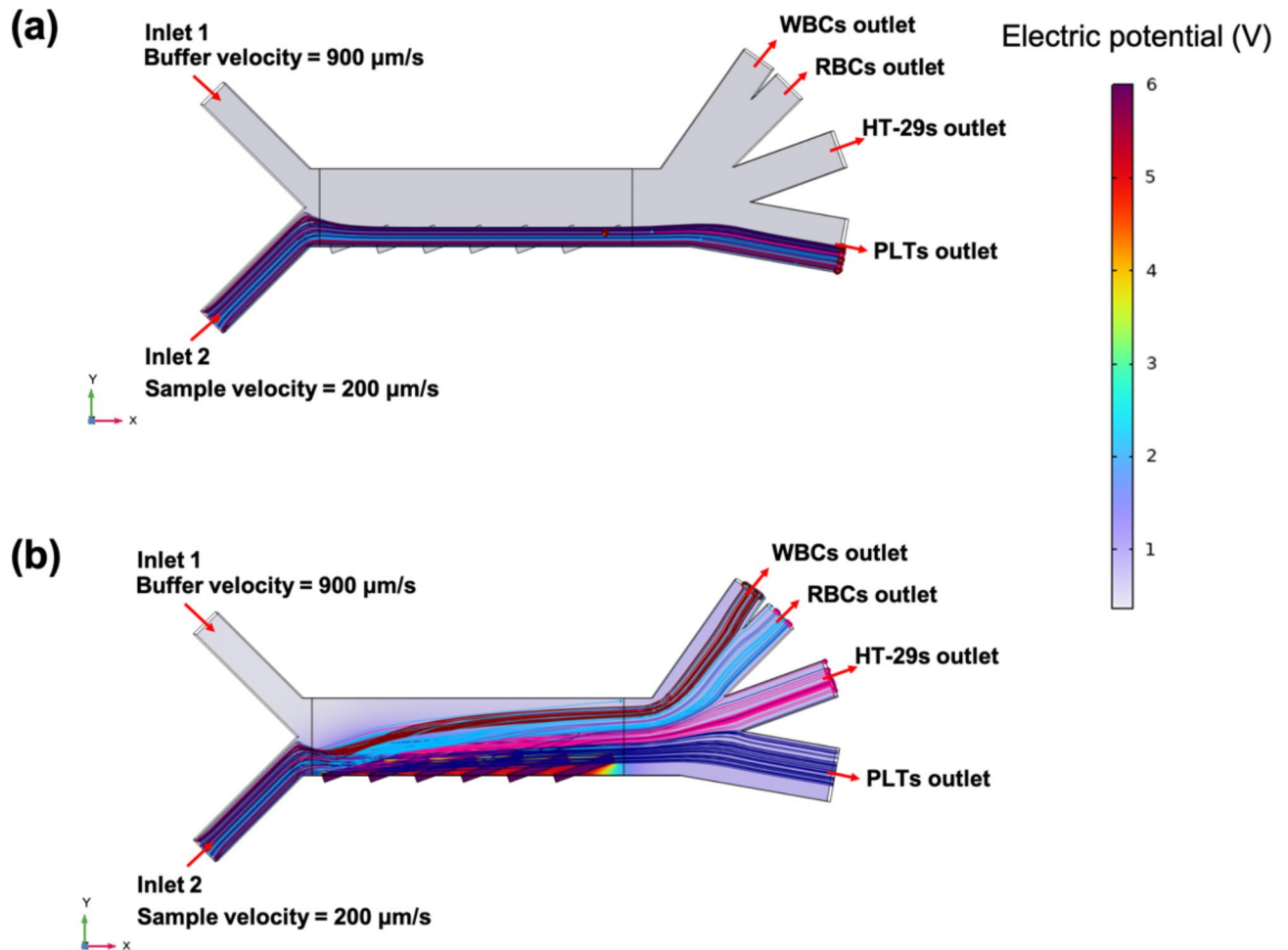


Fig. 8. Separation of CTCs from RBCs, WBCs, and PLTs in the microfluidic device without (a) and with (b) DEP force applied; electric potential within the microfluidic chip (color bar in the x-y plane).

away from the electrode region. As the WBCs, RBCs, HT-29 cells, and PLTs have their own size range and electrical properties, these cells distinctively respond to DEP effects and enrich at specified outlets (outlets 1, 2, 3, and 4 respectively).

To evaluate the FEC-DEP-based device's performance, two parameters were used: purity for each type of cell and separation efficiency. Separation efficiency, or SE, is the proportion of cells injected into the inlet that flows to the desired outflow. Separation purity, or SP, is the ratio of the number of targeted cells collected at a certain outlet to all cells collected there. The SE and SP are calculated as follows:

$$SE(A) = \frac{\text{Total type A cells found at the outlet of A}}{\text{Total type A cells included at the inlet}} \quad (11)$$

$$SP(A) = \frac{\text{Total type A cells found at the outlet of A}}{\text{Total all cell types found at the outlet of A}} \quad (12)$$

For HT-29 cells, RBCs, WBCs, and platelets, the SE was 80%, 74%, 100%, and 86%, respectively, at the ideal conditions in this study.

In the subsequent sections, the influence of various factors on separation efficiency and purity will be thoroughly analyzed through simulation, including the applied voltage on the electrodes, the velocity ratio between the buffer inlet and the cell inlet, the electrode tilt angle, the microchannel height, and the number of electrodes, all with the aim of optimizing the FEC-DEP microfluidic structure. Each factor affects the separation performance in different ways, and these factors can be considered sequentially to determine the optimal conditions for the proposed FEC-DEP structure, including the optimization of both flow dynamics and the electric field and its gradient within the microchannel. While flow velocity conditions primarily influence the flow dynamics within the microchannel, the remaining factors predominantly affect the electric field and its gradient. The effects of flow conditions within the channel will be presented first.

Influences of velocity ratio

The ratio between the flow rate of the buffer solution and blood sample is a major importance to control the flow field and cell trajectories. Figure 9 depicts the effect of buffer medium flow rate on the separation efficiency. To evaluate the influences of ratio of buffer velocity to cell inlet velocity, this study calculated the separation efficiency with an electric voltage frequency of 1 kHz and a constant driving voltage of 6 V. The cell velocities were fixed at 200 $\mu\text{m/s}$ injected to inlet 2 while the buffer flow rate ranged from 700 $\mu\text{m/s}$ to 1000 $\mu\text{m/s}$, corresponding to the ratio of 1:3.5 to 1:5. Lower buffer velocity decreases the drag force acting on particles, the DEP forces are dominant. As a result, all cells deflect towards the upper outlets. In this case, the WBC efficiency is high at 100% because WBCs concentrated at the first outlet while the other cell efficiencies are low. By contrast, higher buffer velocity increases the hydrodynamic force that particles experience, pushing all cells downward and reducing the deflection in cell trajectories. In this case, the velocity ratio was 1:5 which means the hydrodynamic force was dominant. Consequently, particles were tended to be forced to the lower outlets. The efficiency and purity of cells are low since they are collected at inappropriate outlets. When the buffer velocity was 1000 $\mu\text{m/s}$, i.e., the ratio of the blood sample speed to the buffer speed was 1:5, the SP of HT-29 cells, RBCs, WBCs, and PLTs were approximately 59%, 72%, 80%, and 98%. Successful separation of cancer cells and blood cells occurred at optimal buffer velocity of 900 $\mu\text{m/s}$ or a blood sample to buffer speed ratio of 1:4.5. An HT-29 separation efficiency of 80% was attained, accompanied by a roughly 80% purity level.

Influences of the electrode tilt angle

When considering factors affecting the electrical field along the separation regions, four parameters, including electrode tilt angle, applied voltage, channel height, and electrode numbers are investigated. The electrode angle varies the repetitive electric field profile along the channel, while the channel height influences the electric field

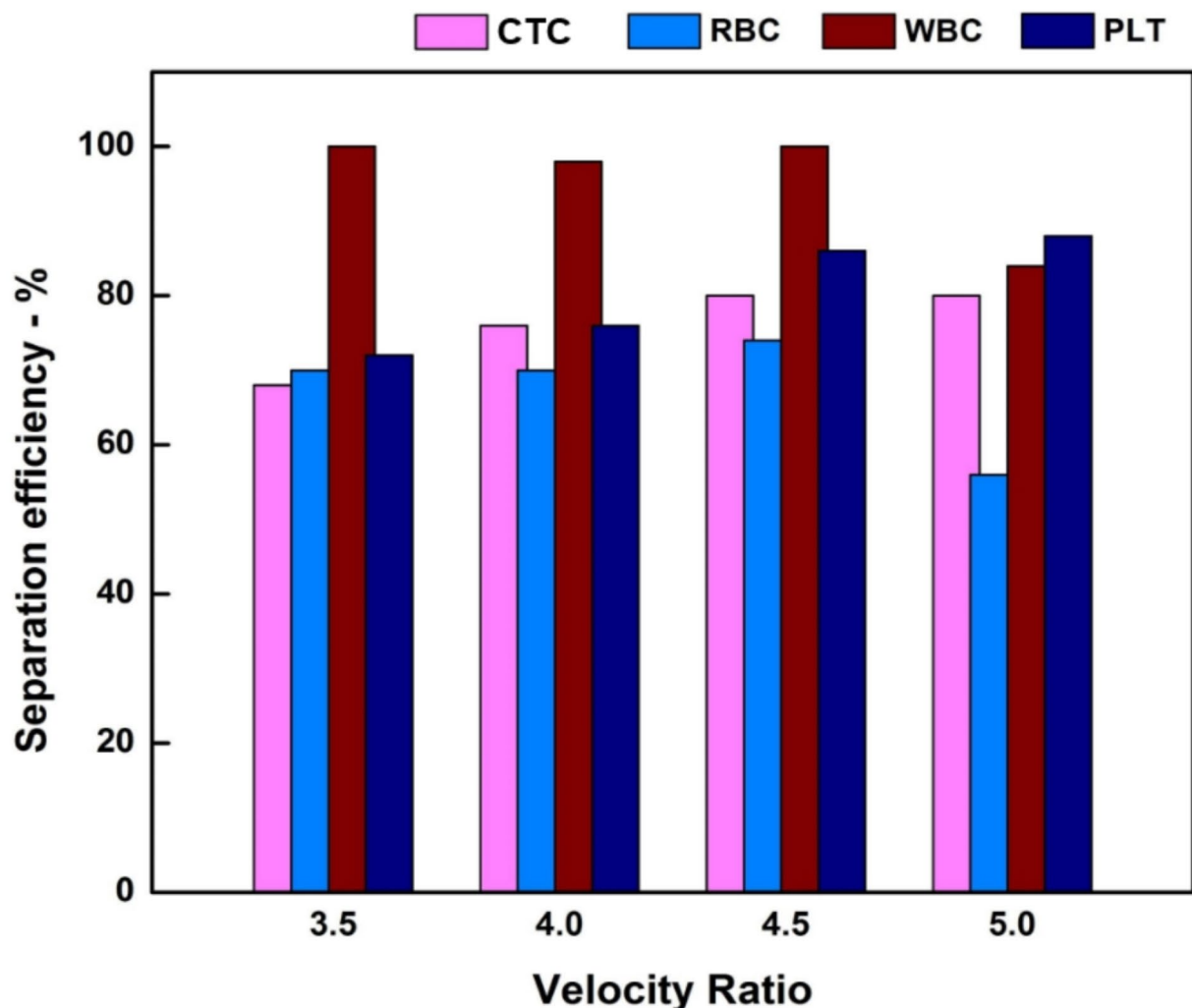


Fig. 9. Effects of the velocity ratio between the buffer inlet and the sample inlet on separation efficiency.

gradient. Meanwhile, other parameters such as applied voltage and the number of electrodes primarily scale the magnitude of the effects within this electric field profile. For these reasons, the subsequent sections will be organized as follows: the impacts of electrode tilt angle, channel height, applied voltage, and finally, the number of electrodes on the separation performance will be discussed.

The electrode tilt angle is an important geometric component with a considerable impact on the separation performance. The cell trajectories for each of the three scenarios are displayed in Fig. 10. To the y-axis, the tilted positive electrode angles were 0° , 35° , and 70° . The electric voltage frequency was always set at 1 kHz, the driving voltage was 6 V and the particle-to-buffer velocity ratio was 1:4.5. The DEP force has little effect on the trajectory of the cells moving through the channel at 0° and there was no separation. In this case, the hydrodynamic force directs all cells to the lower outlet. The electric field gradient increases with increasing electrode incidence angle, which also increases the DEP force applied horizontally to the particles. The DEP force deflected the particles in the channel with the electrodes positioned at a 35° angle, but it was insufficient to direct the particles into the appropriate outlets whereas pressure from buffer flow was dominant and forced them against the channel wall. HT-29 separation was 0% in both cases. At 70° , HT-29 purity was 80% after the most effective separation method.

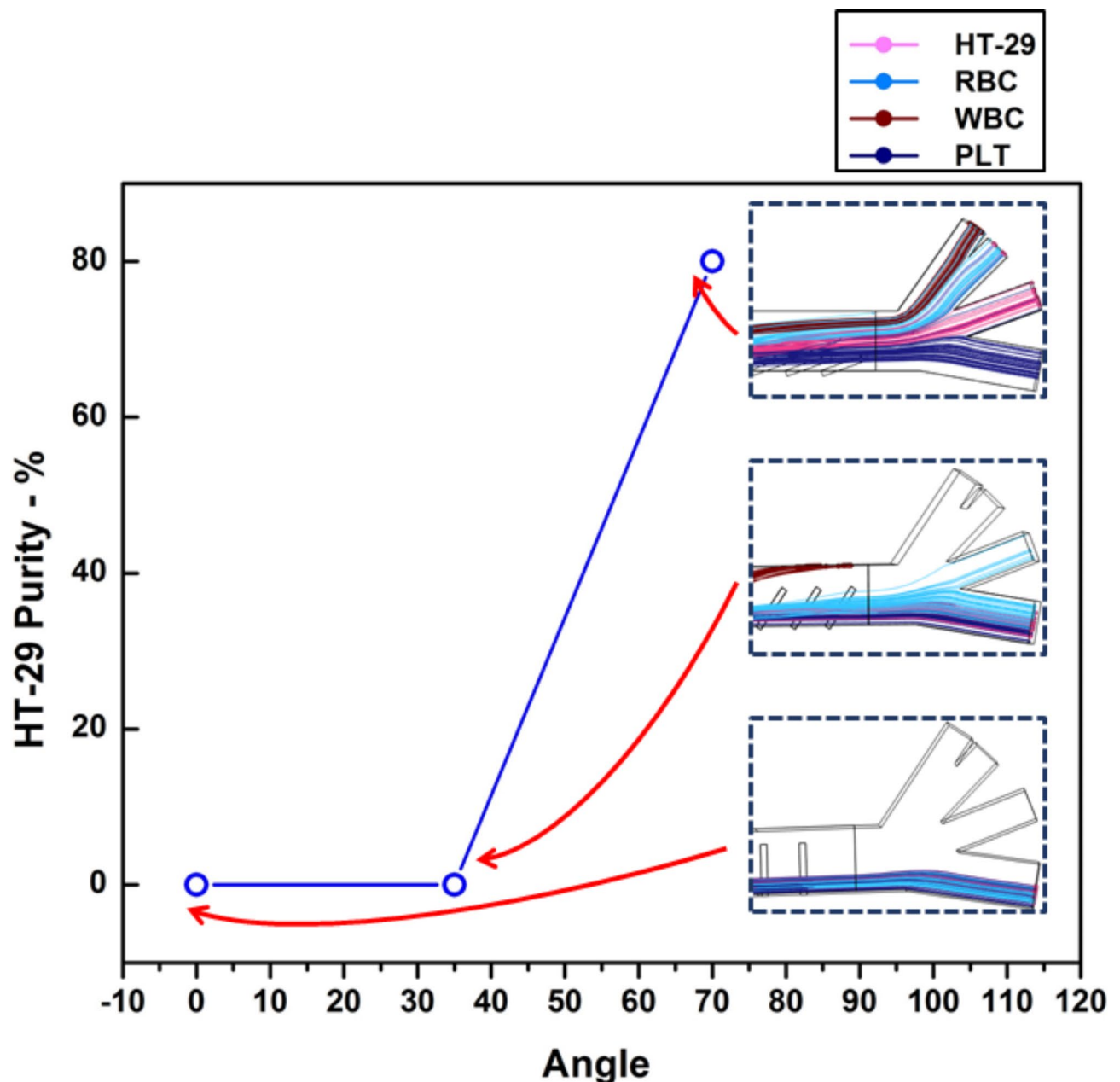


Fig. 10. Effects of the bottom electrodes' slanted angle on the purity of HT-29 purity.

Influences of microchannel height

The height of microchannel is also an important factor that contributes significantly to efficiency as the excitation electrode and the ground electrode are upper and lower structures. Because facing-electrode configuration generates non-uniform electric field all over the separation region of the microchannel, microchannel height would affect the electric field gradient. The shorter the height of the channel, the higher electric field gradient which results in greater DEP forces acting on bioparticles as the electrodes are placed closer. To evaluate the influence of microchannel height on the particle trajectories, our simulation calculated the separation efficiency with an electric voltage of 1 kHz and an applied voltage of 6 V also the velocities of cells and buffer flow ratio is 1:4.5. The channel height was assessed from 25, 50, 75 and 100 μm .

Figure 11 illustrates how lower channel height affects separation efficiency. Further electrodes distance decreases DEP force acting on particles, the drag forces were dominant. Hence, the particle tended to move toward the inner channel wall and to be collected at the downward outlets. In the 75 and 100 μm circumstances,

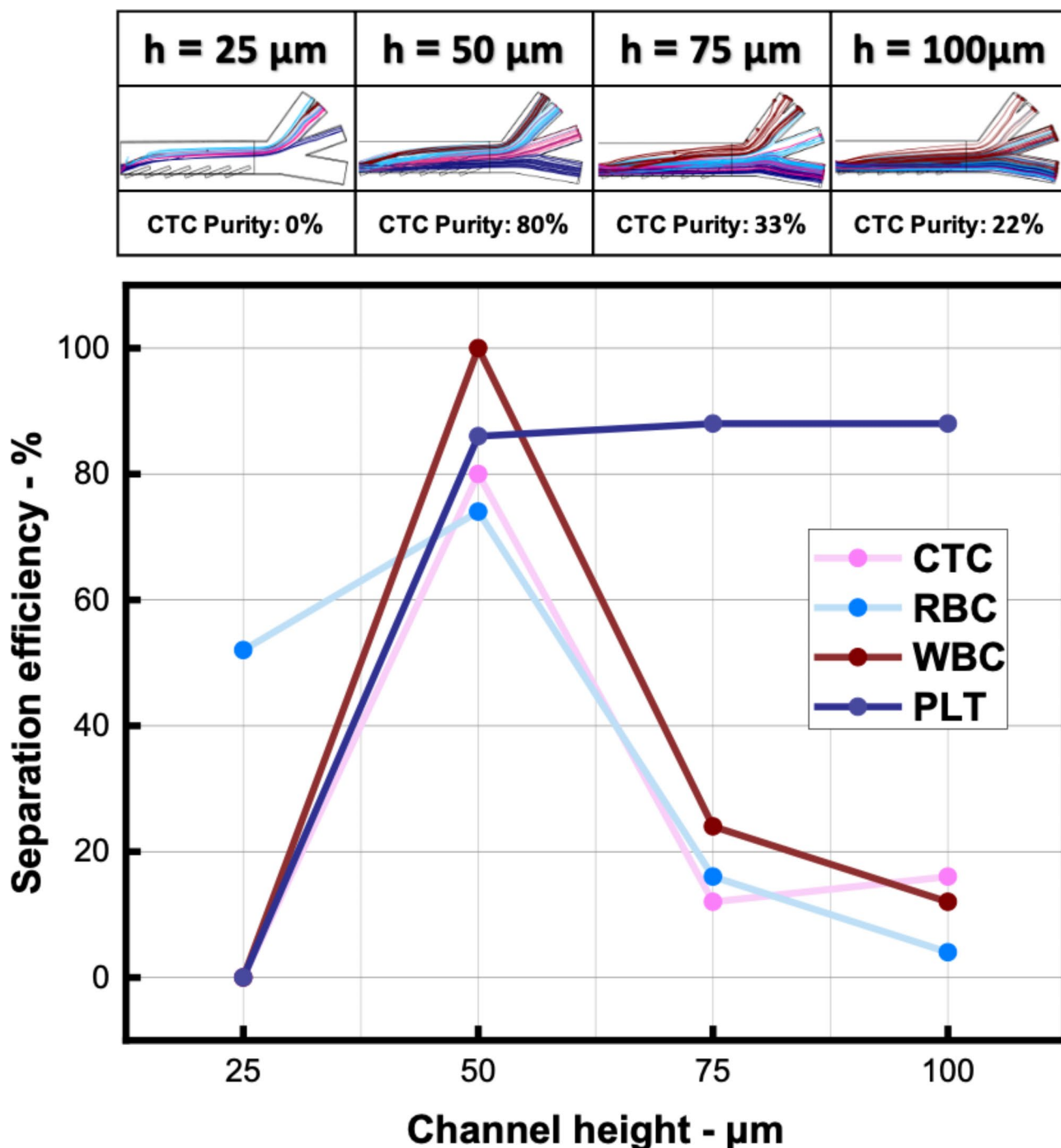


Fig. 11. Effects of the channel height on the separation efficiency.

the PLT efficiency was high because the PLTs concentrated at the lower outlet while the other cell efficiencies were low. In contrast, in the lower microchannel height case, the cells experience a higher DEP force and move toward the upward outlets. Due to the low channel, several cells, when passed through the separation region, are trapped in the upper and lower channel walls. There was no particle found at the PLT outlet and most of the particles concentrated at the RBC and CTC outlets. In our study, at 50 μm channel height the simulation results reached the highest separation efficiency and is feasible for fabrication.

Influences of applied voltage

One of the most significant aspects is the value of the driving AC voltage, which greatly affects the electric field strength and DEP forces acting on bioparticles. The separation efficiency and cell trajectories at driving voltages ranging from 5 to 7 V are shown in Fig. 12. The electric voltage frequency and cell-to-buffer ratio remained at 1 kHz and 1:4.5, respectively. According to the DEP equation, the electric field gradient increases with voltage, which should result in a greater particle deflection as voltage increases. However, the position

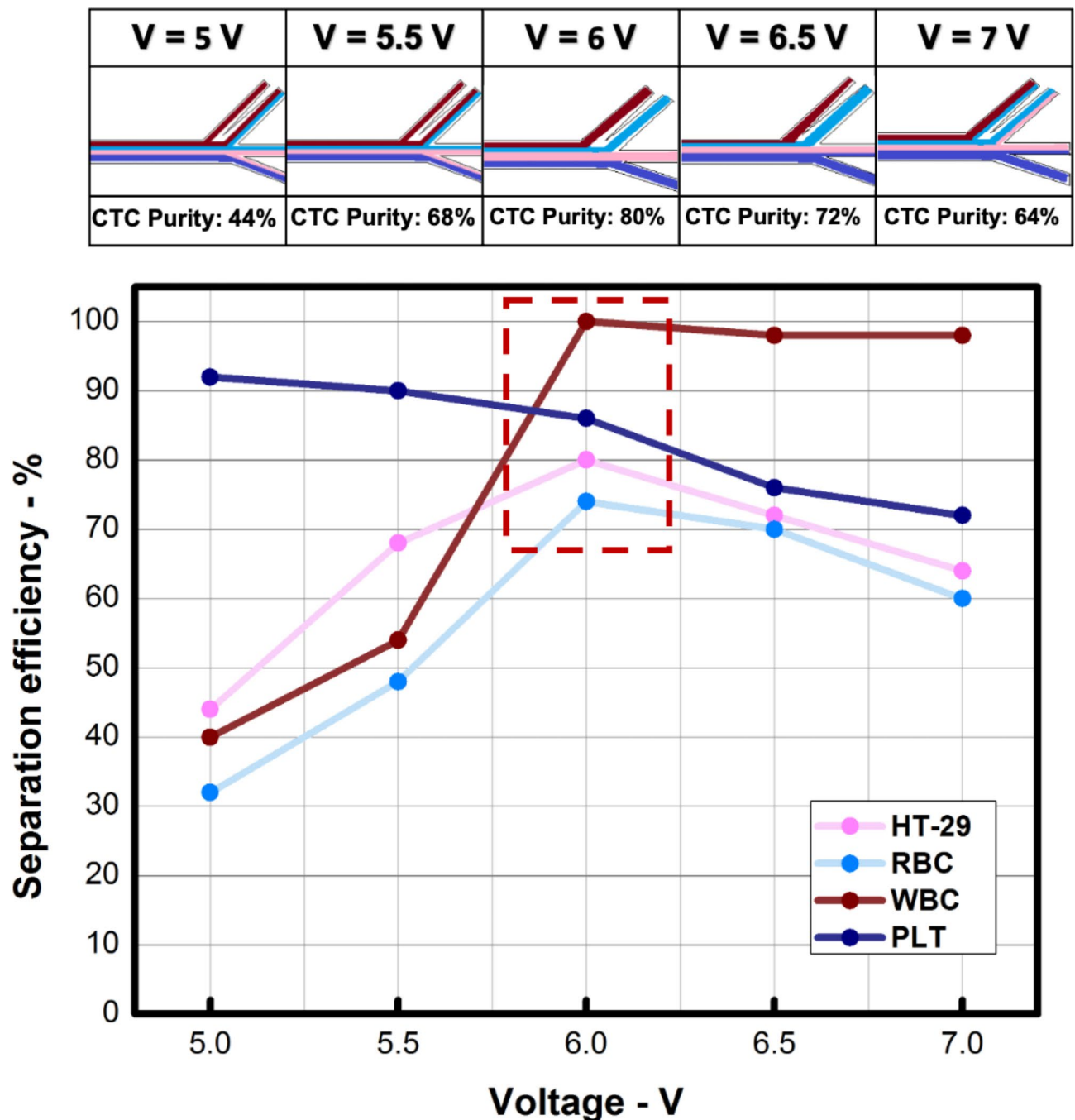


Fig. 12. Impacts of the applied voltage magnitude on separation performance.

of the outlets remains unchanged in all cases. Therefore, when cells move with a higher deflection, they tend to be collected at the upper outlets, which results in lower separation efficiency. Results show that the DEP impact increases considerably at 7 V applied voltage. In this case, particles were deflected away from the positive electrode, oriented toward the WBC outflow. Around 38% of RBCs were found in the WBC outlet, and a notable number of HT-29 cells were directed toward the RBC outlet. Similarly, a portion of PLTs concentrated at the HT-29 outlet. On the other hand, at a voltage of 5 V, the DEP forces were inadequate to result in a distinguishable deflection, the HT-29 cells, WBCs, RBCs, and PLTs followed a slightly deflected path. The cell trajectories were deflected and followed the anticipated path at the ideal voltage of 6 V (Fig. 12), which produced maximum efficiency for all cell types.

Influences of the number of electrodes

Cell separation was facilitated by the non-uniform electric field generated by the FEC-DEP-based device, with the electric field's strength depending on electrode quantity and arrangement. The electric field gradient is higher as the number of electrodes increases. In this simulation, the electrode number ranged from 4 to 7, while the driving voltage was constantly set to 6 V, the electric voltage frequency was 1 kHz, and the cell velocity to buffer ratio was 1:4.5.

Figure 13 illustrates how cutting the electrode number from six to four weakens the electric field and, as a result, lowers the DEP force exerting on the cells, leading to inadequate separation and low purity levels. Using 5 electrodes, HT-29 separation efficiency was only 68% and reached 45% on the purity level.

Moreover, in the case of 4 electrodes, that figure of HT-29 was even poorer with 48% and 27% on separation efficiency and purity level, respectively. Increasing the number of electrodes from six to seven, on the other hand, increases the electric field's intensity and, therefore, the DEP force acting on the cell particles. Because the intended DEP effect was present in both situations, the separation efficiency was pretty equal. On the other hand, while employing six electrodes, the purity level of RBCs is almost 80%, which is slightly greater than when utilizing seven electrodes (roughly 76%). The reason for this is that more HT-29 cells were gathered at the RBC outflow because of their larger deflection angle.

It can be observed that the electrical field strength and the resultant lateral shift of cells change as the change of the applied voltage gradient throughout the channel, whereas the lateral shift of cells may roughly scale as the number of electrodes. Therefore, the lateral shift of cells can be varied in an analogous way by the applied voltage as well as the number of electrodes. In experimental investigations, increasing the applied voltage is preferable to modifying the number of electrodes, as it can be implemented more easily without necessitating changes to the device's design and allows for adjustments during operation.

The process of cell separation has significant importance in a broad range of life sciences applications. Besides cancer cell isolation, the separation of blood components is essential for cell analysis, disease detection, understanding of cell functions and in vivo mechanisms, and precision medicine. Recently, there have been several studies utilizing DEP to separate CTCs; however, these works mostly focused on the isolation of just two specific cell types^{31,32,49–52}. Shamloo and Kamali have suggested a novel DEP-based microfluidic chip with curved outlet which successfully separated PLTs, RBCs, T-lymphocytes, and MD-231 cancer cells⁵³. Nevertheless, this work used sidewall electrode configuration, which may encounter complex fabrication processes. In a previous study, a combination of DEP and magnetophoresis (MAP) in a microfluidic platform was presented and showed its capability to completely separate CTCs, RBCs, WBCs, and PLTs¹⁵. Although achieving high efficiency, the integration of both MAP and DEP into a device presents challenges in terms of complexity and control. Therefore, we presented in this work the FEC-DEP, which utilized a facing electrode configuration device to separate not only CTCs but also other blood components in four outlets. The obtained results have demonstrated the ability of the FEC-DEP to isolate CTCs, RBCs, WBCs, and PLTs with high efficiency and purity in a simpler configuration.

DEP-based biochips have emerged as a powerful tool for the transportation, accumulation, separation, and characterization of micro-/nano-scale bioparticles in microfluidic systems^{54–56}.

The application of our FEC-DEP separation techniques could be extended beyond cancer cells and blood cells, including several biological entities such as stem cells, DNA, algae, yeast, or bacteria. Therefore, this device shows significant potential for a wide range of applications in the field of biomedical and biotechnology, including cell research, oncology, stem cell studies, bacterial infection analysis, DNA research, and more. The proposed FEC-DEP structure is feasible for experimental fabrication based on established techniques. This includes the use of SU-8 patterned opposite ITO electrodes^{57,58}, SU-8 membranes coated on ITO⁵⁹, and PDMS with Chromium/Gold-coated glass slides⁶⁰. These methods have been successfully applied in previous studies, demonstrating the practicality of fabricating the FEC structure.

Conclusions

This study offers an efficient FEC-DEP-based microfluidic chip designed for separating HT-29 cancer cells from RBCs, WBCs, and PLTs. The biochip design performance was confirmed and examined using numerical evaluation. By using the FEC-DEP structure, DEP force is applied to bioparticles, enabling reduced cell position dependence in the channel and simplified fabrication complexity. The effects of the geometric and operational features were examined; under optimal conditions, there was a high separation efficiency and purity for WBCs, RBCs, PLTs, and HT-29. The HT-29 tumor cells were effectively collected at the designated outlet. Results showed how the geometric and physical characteristics of the proposed device influence particle trajectories and separation efficiency. This study laid a strong foundation for further experimental research by proposing and validating the feasibility of the FEC-DEP platform through numerical analysis, with planned experiments set to further assess and refine the device's performance. The FEC-DEP-based system has many benefits, such as being label-free, non-invasive, small in size, low applied voltage, and very efficient. The biochip has the potential

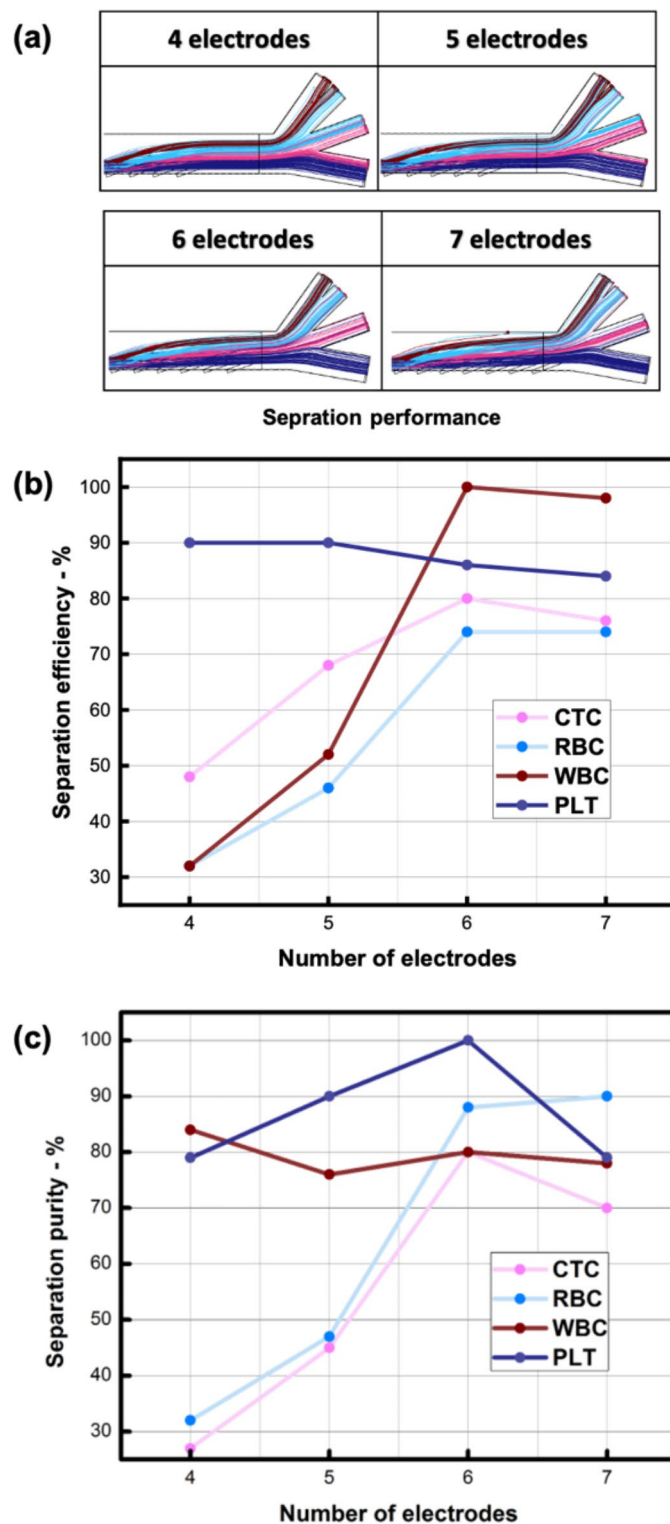


Fig. 13. Impacts of the number of bottom electrodes on separation performance (a), separation efficiency (b), and separation purity (c).

to be used for the manipulation, detection, assessment, and analysis of cancer and could also be used on diverse biological objects, including stem cells, reproductive cells, dead and viable cells, etc.

Data availability

All data generated or analysed during this study are included in this article or from the corresponding authors upon reasonable request.

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Author contributions

Thu Hang Nguyen and Nam Anh Ngo carried out the literature review. Thu Hang Nguyen, Hoang Trung Nguyen, Mai Chi Nguyen, and Hang Bui Thu ran the simulations and analyzed the data. Thu Hang Nguyen and Loc Do Quang presented the data and wrote the manuscript. Jens Ducrée and Tung Thanh Bui reviewed and edited the manuscript. Trinh Chu Duc and Loc Do Quang reviewed, edited the manuscript, and supervised the project.

Declarations

Competing interests

The authors declare no competing interests.

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Consent for publication

All authors have given consent to this publication.

Consent to participate

Not applicable.

Additional information

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Numerical Analysis of Dielectrophoresis-Based Microfluidic Chip with a Facing-Electrode Design for Cell Separation

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Abstract

Purpose Circulating tumor cell separation and analysis have played a critical role in cancer diagnosis, prognosis, and treatment. In this work, we aim to design and investigate a novel biochip that integrates dielectrophoresis, microfluidic technology to separate circulating tumor cells from blood cells. To create a dielectrophoresis-induced non-uniform electric field, a facing-electrode design was proposed and utilized, in which a slanted electrode array and a simple rectangular ground electrode are placed parallel on the top and bottom parts of the microfluidic channel, respectively. This design can reduce the particle position dependence in the microchannel and the complexity of the microfabrication process.

Methods The separation process, efficiency, and optimization of the proposed device were numerically investigated using the finite element method. Parametric research was conducted to comprehensively examine the impact of various operating and design factors on the cell movement and trajectories in the microfluidic device.

Results The results indicated the potential of the proposed biochip to ensure cancer cell separation from blood cells with high efficiency, high purity in a label-free, non-invasive, easy integration, and low-cost manner. Under the optimal conditions, the separation efficiency reached 92%, 88%, and 96% for human colon cancer cells (HT-29), red blood cells, and white blood cells, respectively.

Conclusions In this study, a novel DEP-based microfluidic chip was proposed to separate HT-29 tumor cells from blood cells and numerically investigated to verify the performance of the biochip design. Our findings could provide a foundation for further theoretical and practical investigations. The proposed system can separate cancer cells from red blood cells and white blood cells as well as offers numerous advantages, such as compact size, low voltage, high efficiency, non-invasiveness, and label-free nature. The tumor cell enrichment platform has the potential for application in cancer detection, analysis, and assessment.

Keywords Cancer cell separation · Dielectrophoresis · Microfluidic chip · Finite element method

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Introduction

Cancer-related disease is a leading cause of death globally and causes approximately eight million deaths annually (Bray et al., 2021). The predicted number is expected to rapidly increase in both the near- and long-term future (Thun et al., 2010). Cancer refers to a group of diseases that occurs when aberrant cells divide rapidly and uncontrollably and then invade and spread to other tissues and organs of the body (Martin et al., 2013). Despite tremendous breakthroughs in medical fields, oncologists still cannot absolutely control and treat cancer. More than 90% of cancer-related fatalities are caused by tumor metastases (Wang et al., 1997). The metastasis process commences with cancer cells breaking out from primary cancer cells, then migrating via the blood, lymph nodes, and lymphatic systems, and finally reaching other body tissues to create and establish new tumors. Circulating tumor cells (CTCs) are formed by a subset of cancer cells originating from the original tumor and infiltrating the peripheral blood circulation system (Derakhshan et al., 2021). Compared with primary cancer cells, CTCs have a higher frequency of mutations, accumulation, and more comparable genetic alterations via somatic cell mutations and acquisition of extracirculatory system properties (Zhang et al., 2021). Therefore, early identification and analysis of CTCs have the potential to assess metastatic progression and evaluate cancer treatment efficiency. The quantity of CTCs in the patient's blood sample is specifically known as an indicator of overall survival or progression-free survival prediction (Wakizaka et al., 2004; Zeng et al., 2013; Gao et al., 2020). Nevertheless, the exceedingly small quantity of CTCs, i.e., 1–100 CTCs in approximately 5×10^9 red blood cells (RBCs) and 5×10^6 white blood cells (WBCs), poses difficulties in the identification and separation of CTCs from blood cells at high concentrations and acceptable throughput.

Regarding CTC identification and isolation, various therapy approaches and improved medical prediction standards, such as tumor biopsy, have been developed in recent decades; however, such techniques have failed to attain optimal performance due to tumor heterogeneity (Janku, 2014; Dagogo-Jack & Shaw, 2018; Bedard et al., 2013; Zhu et al., 2021). Other techniques, such as EpCam and cytokeratin, require labeling CTCs before separation and using specific cell surface markers, i.e., fluorescent labeling, prestaining, and attachment, to enable enumeration and visualization at post-separation (Mikolajczyk et al., 2011). Label-free approaches or techniques requiring additional biomarkers could potentially recognize a greater number of cancer cells which are currently undetectable (Antfolk et al., 2017). However, poor recovery or

label-dependent results, which could lead to the loss of CTCs subpopulation during the epithelial-to-mesenchymal transition, have been obtained by those research initiatives. In recent years, the utilization of microfluidics for microscale cell manipulation and enrichment has piqued the interest of many scientists because of its advantageous features, including simplicity, cost-effectiveness, easy integration, and efficiency, among others.

Dielectrophoresis (DEP) is reportedly an effective approach for cell manipulation when processing samples and classifying biological objects (Zhang & Chen, 2020; Hewlin & Edwards, 2023). DEP can be defined as the movement of particles in a non-uniform electric field when the particles and their surroundings have varying polarisations (Hakoda & Shiragami, 2000). DEP-based microfluidic devices have great potential for a variety of applications in the biosystems engineering field including cell research, oncology, stem cell investigation, drug delivery, DNA research, bacterial infection,... Abd Rahman et al. (2017). This technique can be applied to various biological objects such as blood cells, cancer cells, stem cells, DNA, algae, yeast, bacteria,... Abd Rahman et al. (2017). In cancer research, DEP-based biochips selectively separate target cancer cells depending on their size and dielectric properties, which is critical for the detection of early-stage cancer, discrimination between cancer cells depending on aggressiveness, as well as treatment decisions (Chan et al., 2018; Alshareef et al., 2013). Besides cancer cells, DEP has evolved as a promising tool in the research of microalgae biomass and microalgae biotechnology (Kim et al., 2018). DEP can be used for separating microalgae based on their intrinsic properties, since their dielectric properties may significantly change during bioproduction, in particular for lipid-producing species. A variety of DEP-based microfluidic designs and devices were used to detect microalgal species and analyze various properties and responses of microalgae, separate based on their size and intracellular lipid content, as well as for screening applications. Thanks to the ability to manipulate small objects, DEP-based approaches have also been applied to separate targeted DNA from samples (Sonnenberg et al., 2014).

Several studies have utilized DEP for CTC isolation. For instance, a DEP-based microfluidic platform associated with a capacitive sensor was designed to detect rare tumor cells. The device consists of a lollipop-shaped gold microelectrode design integrated into a microchannel. The proposed device successfully detected S-180 cells with high sensitivity (Do et al., 2018). Another recent study proposed a microfluidic system comprising the two stages of hydrodynamic inertial focusing and DEP separation to separate CTCs from blood cells in a label-free, fast, and continuous separation manner. In the first stage, cells were focused on sorting WBCs and CTCs from RBCs and PLTs. Meanwhile, viable CTCs

and WBCs were selected and enriched in the second stage that utilized DEP due to their different electrical properties (Bakhshi et al., 2022). Furthermore, a special DEP-based microfluidic structure was used to isolate MDA-MB-231 tumor cell lines from various WBC subtypes. Sidewall electrodes with different electrode designs were used for the assessment of separation efficacy. The numerical investigations using the finite element method (FEM) showed that semi-circular electrodes have the highest efficiency with a recovery rate of roughly 95%. Although outstanding results were obtained, the fabrication of sidewall electrodes integrated into the microchannel remained much more complex than the coplanar electrode design.

Several recent studies have proposed the DEP-based microfluidic separation of bioparticles by embedding coplanar electrodes at the bottom of channels. Recent literature introduced a planar electrode-configured PDMS channel to separate yeast cells and polystyrene microbeads (Wu et al., 2018). The separation efficiency exceeded 90% for both cells and beads. Another scholar has recently proposed a fully automated microfluidic device for bacterial cell enrichment and separation of a single cell type from a sample (Oshiro et al., 2022). The multi-functional device consisted of two parts consisting of sample pretreatment with hydrodynamic filtration and DEP cell separation. The proposed device performed cell separation and enumeration with a low sample volume (0.5–1 mL) and a rapid process without a sample preparation process. However, all of the abovementioned works used coplanar electrodes at a single side of the microchannel, which could lead to a high concentration of the electric field near the field of the channel bottom only. As particles experience the DEP effect in a non-uniform electric field, their design may result in a particle position dependence problem.

Recently, a number of studies using microfluidic chips to isolate different cell lines based on DEP have been conducted and showed a good agreement between simulation and experimental results. For example, a recent work proposed a zigzag-shaped microchannel to achieve platelet separation by DEP forces created by a non-uniform electric field (Guan et al., 2020). The authors simulated the cell trajectories under different conditions, investigated the influences of important factors on separation efficiency, and optimized the device structure, and operating parameters using COMSOL Multiphysics software. Using the optimal structure, the fabricated microfluidic chip achieved a separation efficiency of 99.4% when using a voltage of 20 V and a velocity ratio of 1:4. Piacentini et al. presented a microfluidic device capable of separating platelets from other blood cells in a continuous flow using DEP field-flow-fractionation (Piacentini et al., 2011). The theoretical cell trajectory has been calculated by numerical simulations of the electrical field and fluid flow. The simulated trajectories overlaid the actual flowing

cells, showing a good agreement between the theory and the experiments. The proposed device obtained high efficiency and high purity of platelet separation. Another study proposed a capillary DEP chip to separate blood cells from a drop of whole blood (approximately 1 μ l) sample using negative DEP force (Liao et al., 2013). The numerical analysis of the electric field helped optimize the electrode design. The highest separation efficiency of approximately 90% within 30 s was achieved using the optimal biochip design.

To address the shortcomings of previous work, in this study, we proposed a microfluidic structure that integrates a facing-electrode design to separate cancer cells from blood cells. The proposed biochip comprises an electrode array at the bottom of the microchannel and a ground electrode on top of the microchannel to create a non-uniform electric field along the whole separation region. The bottom electrodes are tilted at a proper angle to create a DEP force not only in the vertical direction but also in the horizontal direction, resulting in the high-efficiency separation of CTCs from other blood cells. The proposed configuration could overcome the shortcomings of several existing microfluidic chip devices, such as sidewall electrode design issues and particle position dependence. The working mechanism and efficacy of the proposed microfluidic design chip were verified by a simulation method based on the FEM in COMSOL Multiphysics software. The results indicated that under optimal conditions, the efficacy of separating CTCs from the blood can reach 92%, and the purity level is 100% with an applied voltage of only 4 V at 10 kHz, with a 1:3.25 ratio between the blood sample flow and the buffer flow velocities. We also investigated the effects of important factors on the separation process and results, including electrode tilt angle, applied voltage, and velocity ratio. This work reveals insights into the cell trajectories in a DEP-based microfluidic device with various conditions. This study is expected to offer a potentially efficient, portable, easily integrated, fabricated, low-cost biochip device for CTC separation. The design of the proposed biochip could be applied not only to cancer cells and blood cells but also to other biological objects such as algae, yeast, and plant cells,... in biosystem engineering.

Theory

Newton's Second Law

In the microchannel, biological cells follow the streamlines of fluid flow and experience the DEP effect. When calculating the motion of particles in the background fluid, the particles are assumed to be spherical cells. Cell movement is mainly affected by the DEP force (F_{DEP}) and the hydrodynamic force (F_{HD}). Gravitation force could be ignored due to the small size of the cells. Brownian force which

has minor effects as the cells' radius is higher than $1\ \mu\text{m}$ shown in Table 1 is generally ignored (Hao, 2005). The cell motion and trajectory are expressed by Newton's second law, which provides the composition vector forces of DEP and hydrodynamics:

$$\vec{F}_{HD} + \vec{F}_{DEP} = m_p \frac{d\vec{v}_p}{dt} \quad (1)$$

where v_p and m_p are the velocities and the mass of the cell, respectively.

Hydrodynamic Force

The momentum balance and mass conservation were calculated as Stokes flow (Romano, 2012):

$$\nabla \cdot [-p\vec{I} + \vec{K}] + \vec{F} = 0 \quad (2)$$

$$\frac{\delta\rho}{\delta t} + \nabla \cdot (\rho\vec{v}_m) = 0 \quad (3)$$

where p is the pressure, $\vec{K}$ is the viscous stress tensor, $\vec{F}$ is the volume force vector, ρ is the fluid density, and $\vec{v}_m$ is the fluid velocity. Hydrodynamic force can be computed by Navier Stokes's law in a microchannel:

$$\vec{F}_{HD} = 6\pi\eta R_p(\vec{v}_m - \vec{v}_p) \quad (4)$$

where R_p is the radius of the spherical shape, η is the dynamic viscosity coefficient of the medium.

Dielectrophoresis Force

As mentioned above, DEP is the manipulation of polarizable particles in a non-uniform electric field. Consequently, CTCs and blood cells pass through the channel and experience the DEP force while traveling between the electrode region. The DEP force affecting the cell is described by Yao et al. (2018):

$$\vec{F}_{DEP} = 2\pi R_p^3 \epsilon_f \text{Re} \left[\frac{\epsilon_c^* - \epsilon_f^*}{\epsilon_c^* + 2\epsilon_f^*} \right] |\nabla \vec{E}|^2 \quad (5)$$

while R_p is the particle radius, ϵ_f is the permittivity of fluid medium. This study utilized the single-shell model with a core-shell structure to evaluate the Clausius-Mossotti coefficient using the features of electrical properties and the DEP force acting on particles with a thin dielectric shell. When computing the DEP force, the complex dielectric constant of the particle is replaced by the equivalent relative complex dielectric constant of a homogeneous particle, including the shell and the interior of the particle. According to the

single-shell model, the complex permittivity of the cell ϵ_c^* can be described by Huang et al. (1992):

$$\epsilon_c^* = \epsilon_{mem}^* \frac{\left(\frac{R}{R-d_{mem}}\right)^3 + 2 \frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*}}{\left(\frac{R}{R-d_{mem}}\right)^3 - \frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*}} \quad (6)$$

where R and d_{mem} are the radius of the particle and the thickness of the membrane, respectively. Also, ϵ_{mem}^* is the complex permittivity of the cell membrane and ϵ_{cyt}^* is the complex permittivity of the cytoplasm.

The second bracket is given the Clausius-Mossotti (CM) factor describing the dielectric properties of the cells (c) and the fluid (f) in which the complex permittivity is defined as follows:

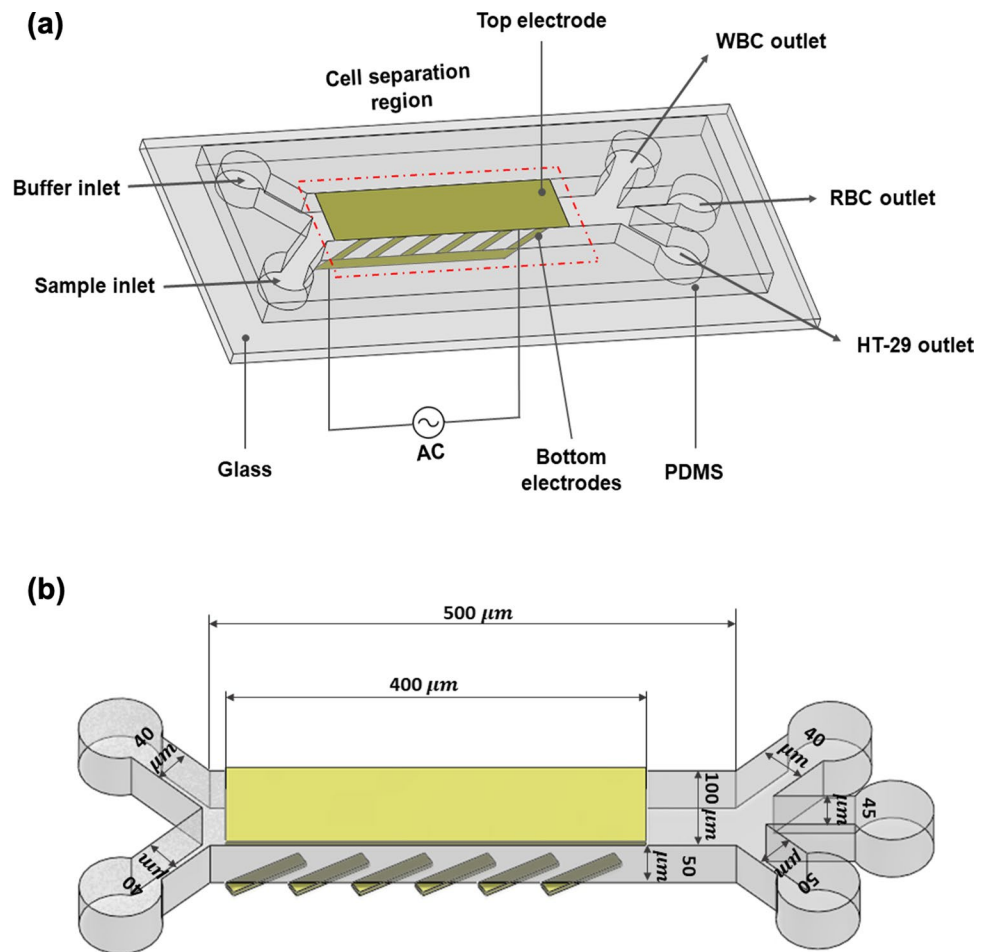
$$\epsilon_c^* = \epsilon - j \frac{\sigma}{\omega} \quad (7)$$

The CM factor measures the polarizability of the particles, which depends on the real part of permittivity ϵ and the electrical conductivity σ , $\omega = 2\pi f$ is the angular frequency of applied oscillating voltage, and j is the imaginary part defined as $j^2 = -1$. The $\text{Re}(\text{CM})$ can be within -0.5 to 1 by changing the frequency of the voltage, resulting in negative DEP (nDEP) that pushes the particles away from the highest electric field gradient and positive DEP (pDEP) with the opposite direction of the movement. In the case of $\text{Re}(\text{CM}) = 0$, the frequency is called the crossover frequency, which is the border of pDEP and nDEP. Also, in the lower frequencies, σ plays an important role in calculating CM whereas ϵ has significant effects in high-frequency regions. The magnitude of the $(|\vec{E}|^2)$ is the second factor that impacts the interaction with a particle which depends on the applied voltage. The design of the fluidic flow and electrode arrangement is crucial, which maximizes the $(|\vec{E}|^2)$ and control other electrokinetic forces (Henriksson et al., 2022).

Design of the Proposed Device and Simulation Model

This study proposes a microfluidic system to separate CTCs from blood cells based on the DEP effect created by the embedded electrode array in a herringbone pattern as shown in Fig. 1. The proposed microfluidic structure includes two inlets, one for the blood sample containing HT-29 tumor cells, RBCs, and WBCs and the other for the buffer solution. In this work, the design of the microfluidic chip was optimized for HT-29 cell line separation. Various cancer cell lines from the lung to the cervical have their own "electrical signature," thus respond differently to the DEP force. When dealing with another type of cancer cells, a similar DEP separation mechanism based on our proposed microfluidic

Fig. 1 Design of the proposed biochip: **a** Illustration of the DEP-based microfluidic chip for cancer cell separation using top-bottom electrode configuration, **b** dimension of microfluidic chip



device can be used; however, the operating parameters and outlet design need to be optimized for better separation and collection of targeted cells. The center part of the microchannel is the electrode region that employs separation and the three distinctive outlets for collecting three cell types. The electrode consists of six identical positive electrodes placed diagonally in herringbone shape on the bottom of the microchannel and a ground electrode placed on top of the microchannel. The top-bottom design helps create an electric field in the separation region to reduce the influence of the cell position in the microchannel. When conducting the experiments, it is important to observe the actual trajectories of CTCs, RBCs, and WBCs under a microscope. The use of the top electrode could hinder this observation. Therefore, one-fifth of the top microchannel length was uncovered to better detect, evaluate, and optimize the cell trajectories inside the microchannel. Blood samples, including RBCs, WBCs, and CTCs, and a buffer solution are introduced with velocity ratios of $200 \mu\text{m/s}$ and $700 \mu\text{m/s}$, respectively. For the experimental setup, the blood samples will be extracted from the cancer patient donor through venipuncture following an approved protocol at the hospital. After that, the collected sample will be put into a vacutainer containing

the anticoagulant citrate dextrose. The blood sample will be preprocessed to remove other components such as plasma to ensure only blood cells and cancer cells are retained. The sample will then be diluted in the PBS buffer with an appropriate volume ratio before being injected into the microfluidic chip. The buffer solution is PBS with a conductivity of 55 mS/m and a relative permittivity of 80 Shamloo et al. (2022). The high velocity at the buffer inlet leads to cell focusing at the beginning of the microchannel. The inclined electrodes generate a non-homogeneous electric field, resulting in the DEP force moving not only in the vertical direction but also in the horizontal direction. Given the different cell sizes and electrical properties of the cell types, the DEP force acting on the HT-29 cells, WBCs, and RBCs can be distinguished, guiding each type to different trajectories. The higher the electrode angle is, the greater the DEP forces on the y-axis exerted on the cells. The findings have shown that under the effect of an uneven electric field, HT-29, RBCs, and WBCs affected by a significant nDEP force are pushed away from the electrode, i.e., a low electric gradient. As the amplitude of the DEP force is directly proportional to the third-order cell size, the WBCs experience the greatest DEP force and move to outlet 1, whereas the RBCs are collected

at outlet 2. The AC voltage is 10 kHz, the applied voltage is 4 V, and the ratio of blood sample velocity to buffer velocity is 1:3.25, the bottom electrode number was 6, and the bottom electrodes tilted at 60°. The electrical properties of the single-shell cells are shown in Table 1. The blood cells and cancer cells diameter and physical properties were directly obtained from previous literature which matched well with the actual cells (Shamloo et al., 2022; Thi et al., 2022; Wu et al., 2012; Low & Kadri, 2016). The number of CTCs introduced into the inlet is smaller than that of RBCs, WBCs. However, the number of cells injected into the inlet is much smaller than the actual cell number in the blood sample. This is because this work mainly focused on the concept of the DEP-based microfluidic for the separation of cancer cells and blood cells. The smaller cell number helped reduce the iteration of computations and made the numerical calculations easier to converge. Even though the simulated blood sample containing RBCs, WBCs, and CTCs could not completely represent the complexity of actual human blood samples, the simulation results could lay a strong foundation for further experimental investigations.

The simulation process included the following steps: the geometrical model creation, selection of a material type and the input parameters, physical setup and boundary conditions, meshing, simulation, and implementations with different conditions and processing of the outcomes. The

Table 1 Electrical properties of each cell type (Thi et al., 2022)

Parameters	HT-29	RBC	WBC
Density (kg/m ³)	1077	1110	1080
Diameter (μm)	11	6	14
Relative permittivity	120	59	150
Electrical conductivity (S/m)	0.72	1.31	0.76
Shell thickness (nm)	4	8	7
Shell relative permittivity	11.1	4.44	6.01
Shell electrical conductivity (S/m)	34×10^{-6}	1×10^{-6}	24×10^{-6}

proposed DEP-based device was 3D modeled using electric currents, creeping flow, and particle tracing for the fluid flow module of COMSOL for calculating the velocity profile, the electric field in the microchannel, DEP effect acting on cells, and particle movements under the impacts of external forces. The condition of the channel walls was set as no-slip and “freeze,” which means that the fluid velocity near the walls was approximately zero, and the particle position and velocity were unchanged when the particles hit the wall. Also, random distribution was used for all cell types at the sample inlet. The model was carefully meshed using variable adjacent triangles (Fig. 2). The complete mesh consisted of 70,066 domain elements, 12,132 boundary elements, and 1211 edge elements.

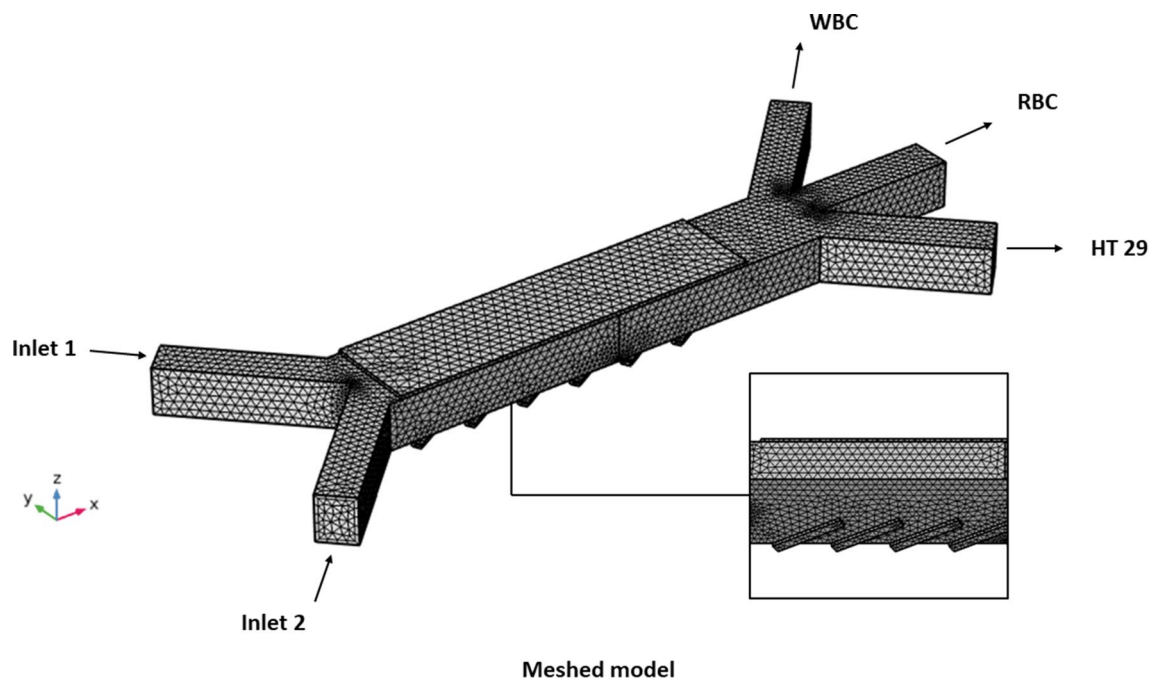


Fig. 2 Meshing model of the device

Results and Discussion

Electric Field, Velocity Distribution, and Cell Separation Process

The electric field distribution in the microfluidic device is illustrated in Fig. 3. As can be seen in the electrode region, the closer the area is to the electrode, the greater the electric potential and hence the higher the electric field. The observed area experienced a much higher square gradient of the electric field, mostly focused on the electrode edges. The arrangement of the slanted positive electrodes at the bottom of the channel and the ground electrodes on top of the channel jointly generated a non-uniform gradient in the whole separation region, which caused a DEP force on the cells in the horizontal and vertical directions.

Figure 4 shows a graph of $\text{Re}(\text{CM})$ values calculated corresponding to the frequency range from 100 Hz to 10 GHz for CTCs and blood cells. At the chosen frequency of 10 kHz, the $\text{Re}(\text{CM})$ factor of the CTCs, RBCs, and WBCs were negative. However, the $\text{Re}(\text{CM})$ factor could not directly reflect the effect of the DEP force, as evidenced by the WBCs with the highest force, leading to a stronger deflection even though its $\text{Re}(\text{CM})$ factor value was smaller than that of RBCs. The reason is that several factors affect the DEP force, comprising the cell diameter, the permittivity, and the electrical conductivity of the cell. The $\text{Re}(\text{CM})$ factor indicated that all cell types

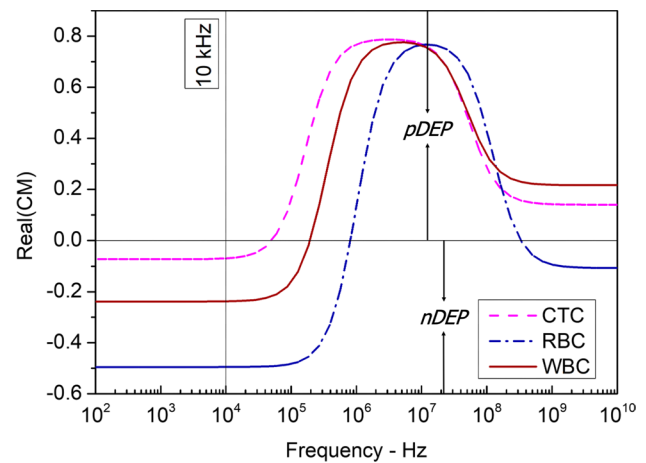


Fig. 4 Real part of Clausius-Mossotti factor indicating that three cell types (HT-29, RBCs, and WBCs) experience nDEP at the chosen 10 kHz frequency

experienced nDEP. Thus, the HT-29 cells, WBCs, and RBCs subjected to nDEP force with different magnitudes moved further away from the electrodes in both vertical and horizontal directions, i.e., the low electric field gradient region.

The cell trajectories and velocity distribution are shown in Fig. 5a, in which the inset presents the fluidic velocity distribution at the cross-section. The velocity reaches its highest value at the center of the channel and gradually decreases

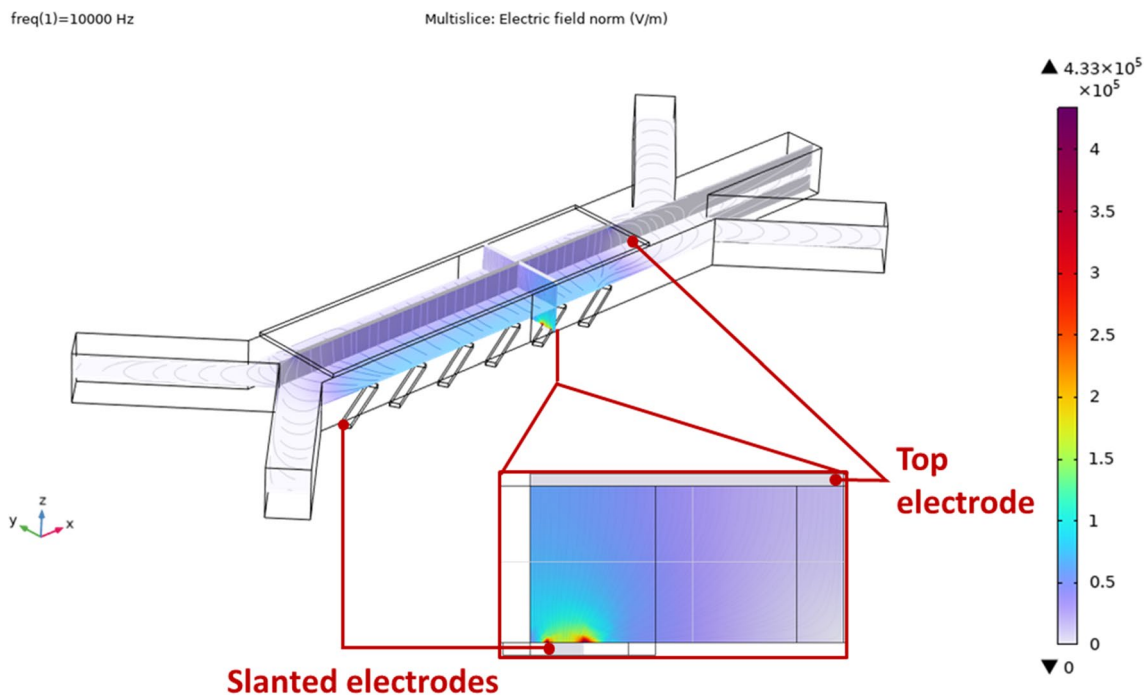
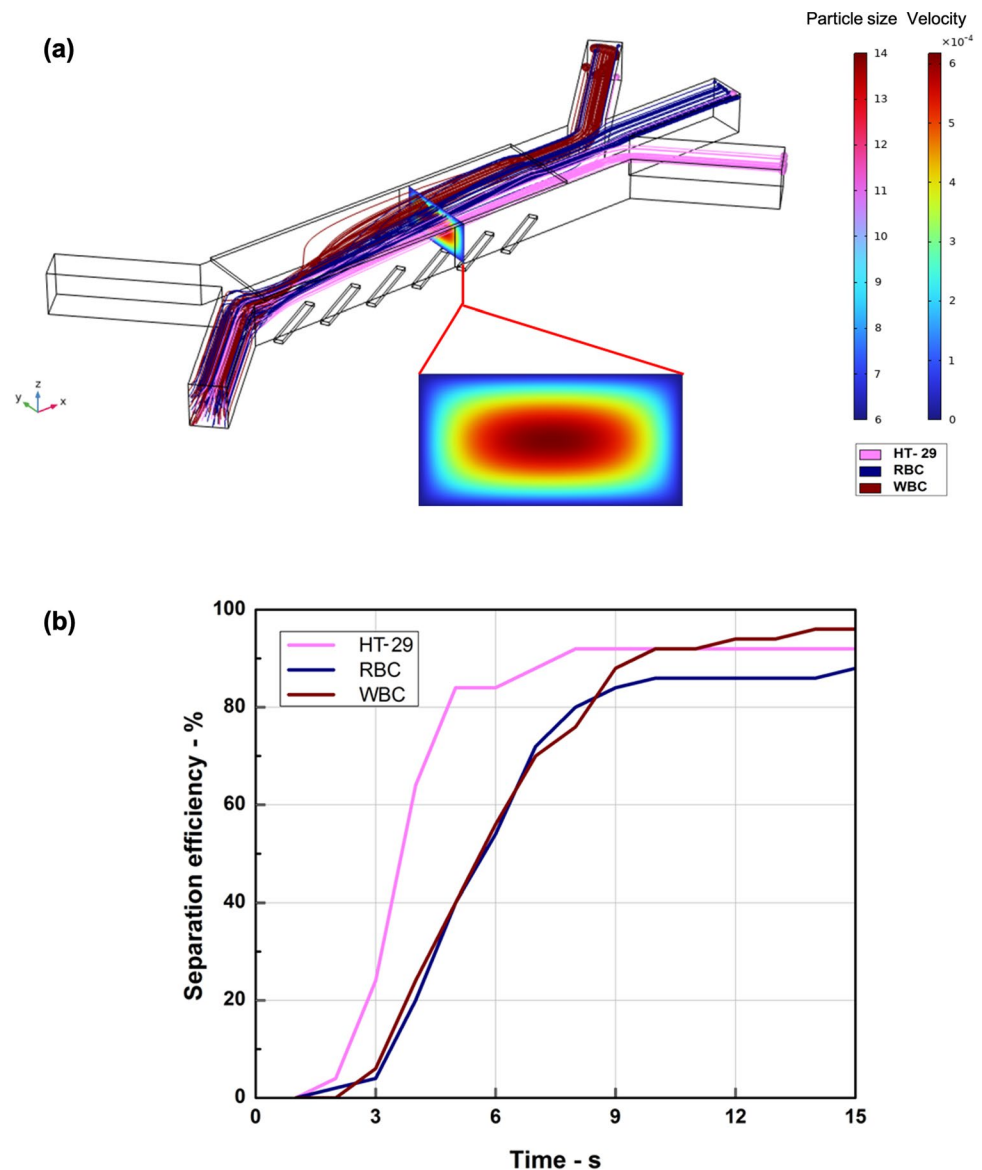


Fig. 3 Electric field norm distribution inside the microchannel

Fig. 5 Separation of CTCs from blood cells in the proposed device: **a** Cell trajectories at $t = 15$ s (inset: velocity magnitude distribution), **b** separation efficiency of HT-29, RBC, and WBC versus time when the AC voltage of 4 V at 10 kHz, velocity ratio between sample flow to buffer flow of 1:3.25, bottom electrode number of 6, the tilted electrode angle of 60° were applied



as it moves to the side of the channel. After examinations, the optimal ratio of sample flow velocity to buffer flow velocity was set at 1:3.25. The excitation AC voltage of 4 V at 10 kHz was utilized to perform the numerical calculation. At first, three cell types were injected into the sample inlet, i.e., inlet 2, and directed toward the electrode region by the influences of the buffer flow injected to inlet 1. As can be seen in Fig. 5a, because all cell types experience nDEP, all these cells were repelled from the electrode region in both horizontal and vertical directions. However, due to the flow-focusing effect of the buffer flow, the cells were focused near the sidewall region. When entering the separation region, all cells traveled to the top of the microchannel, which has an electric field below 0.75×10^5 V/m (Fig. 3). This value is lower than the threshold for cell electroporation (Low & Kadri, 2016). Therefore, this microfluidic chip can ensure

the cell viability. Then, given the distinguishable properties of the three simulated cell types, the WBCs, RBCs, and HT-29 tumor cells would concentrate at designated outlets (outlet numbers 1, 2, and 3, respectively), which accords with their different influences under the DEP force during separation. To assess the DEP-based device's efficiency, two criteria were utilized, namely separation efficiency and purity for each cell type. The separation efficiency is defined as the percentage of the number of cells injected into the inlet flowing to the targeted outlet. The purity is the percentage of the number of targeted cells collected at the proper outlet to the total number of cells concentrated at that outlet. Figure 5b illustrates the separation efficiency of the HT-29 cells, RBCs, and WBCs over time. Here, the separation efficiency of a cell type refers to the ratio of the cell number at the proper outlet to the cell number injected into the inlet.

As time progressed, more target cells were manipulated to the corresponding outlet. Under the optimal conditions as proposed in this study, the separation efficiency reached 92%, 88%, and 96% for HT-29 cells, RBCs, and WBCs, respectively.

Optimization of the Proposed Device

Parametric research was conducted to comprehensively examine the impact of various operating and design factors on the movement and trajectories of the bioparticles. The selected operating parameters included the applied voltage and velocity ratio between the cells and buffer, while the geometric parameters included the angle of electrodes and the number of bottom electrodes.

Figure 6 presents the separation efficiency and cell trajectories with different applied voltages ranging from 3 to 5 V. The electric voltage frequency and the ratio between the particles and buffer were assigned 10 kHz and 1:3.25, respectively. Theoretically, the particle declination will increase with higher voltage, which can be explained by the electric field gradient in the DEP equation directly proportional to the applied voltage. The simulation results demonstrate that an applied voltage of 5 V led to much great DEP effect and a significant amount of HT-29 cells deflected in the RBC outlet. Consequently, 38% of the RBCs were also found in the WBCs outlet. Meanwhile, for an applied voltage of 3 V, the DEP force was insufficient to cause a prominent deflection,

and the HT-29 cells, WBCs, and RBCs flowed in a slightly deflected path. At the optimal voltage of 4 V, the cells were deflected and followed the expected path (Fig. 4a), and the efficiency reached the highest point for all cell types. This low voltage has negligible Joule heating effects acting on cells, which leads to the preservation of cell viability.

Figure 7 illustrates the influence of the buffer solution velocity on the particle trajectory. As random cell distribution led to different positions and trajectories in microchannels, the buffer solution also influenced the particle movement in a certain path before traveling to the separation region. In particular, the inlet velocity was defined in this research as the ratio between the cell sample velocity and buffer velocity. In all cases, the electric voltage frequency was 10 kHz, and the driving voltage was 4 V. Furthermore, the velocity of the cells from inlet 2 was set at 200 $\mu\text{m/s}$. This study solely studied the changes in buffer velocity in inlet 1 that influenced the movement of introduced cells. According to the hydrodynamic force formula, the higher the buffer solution speed is, the greater the drag force (F_{HD}) flow acts on the particles. The particles tended to move toward the direction near the bottom electrode side, avoiding touching and being trapped in the upper wall of the microfluidic channel. When the buffer velocity was 700 $\mu\text{m/s}$, i.e., the ratio of the blood sample speed to the buffer speed was 1:3.5, the cancer cells and blood cells could be effectively separated. In this research, the HT-29 CTC separation efficiency reached 92%, and its purity level was approximately 85%.

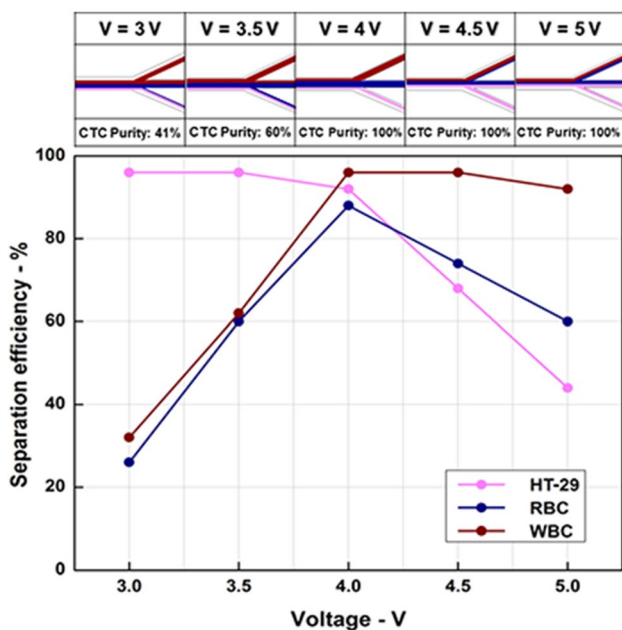


Fig. 6 Dependence of the separation efficiency on the applied voltage magnitude when the voltage frequency remained 10 kHz, velocity ratio was 1:3.25, the bottom electrode number was 6, the bottom electrodes tilted at 60°

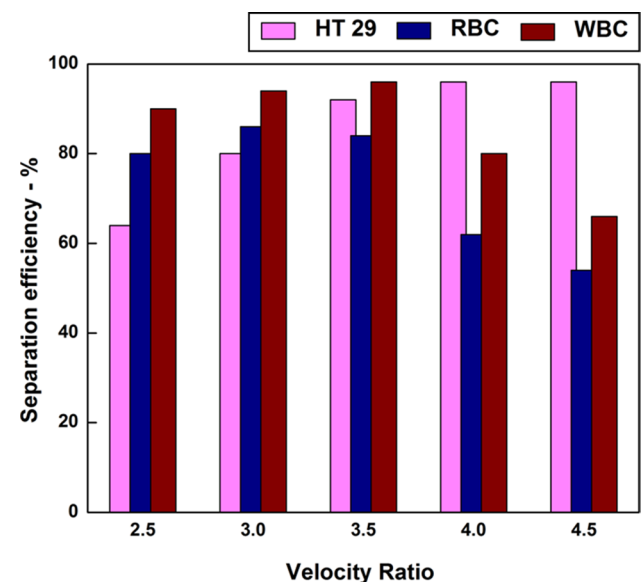


Fig. 7 Impacts of velocity ratio of the buffer inlet to the sample inlet on the separation efficiency when the applied voltage remained 4V at 10 kHz, the bottom electrode number was 6, the bottom electrodes tilted at 60°

The electrode array created a non-uniform electric field that assisted the cell separation. However, the number and position of electrodes would influence the generated electric field. The change in the electric field would also modify the magnitude of the force exerted on the cell, further affecting cell separation performance. In all cases, the electric voltage frequency was 10 kHz, the driving voltage was 4 V, and the optimal ratio of the cell velocity to the buffer velocity was 1:3.25. As shown in Fig. 8, reducing the number of electrodes from 6 to 4 weakened the electric field, thereby reducing the value of DEP force acting on the cells and causing insufficient separation and poor purity level. On the contrary, increasing the number of electrodes from 6 to 7 strengthened the electric field, thereby increasing the magnitude of the DEP force acting on the cell particles. The separation efficiency of both cases was fairly similar because of the desired DEP effect. However, the purity level of RBCs while using 6 electrodes was approximately 98%, which was slightly higher than the configuration of 7 electrodes (around 93%). It is because of the higher number of HT-29 cells collected at RBC outlet due to the higher deflection angle of HT-29 cells.

The electrode angle is another important geometric parameter that significantly affects the acquired performance. Figure 9 shows the cell trajectories for the three different cases. The corresponding electrode angles were 0°, 30°, and 60° to the y-axis. In all cases, the electric voltage frequency was set at 10 kHz, and the velocity

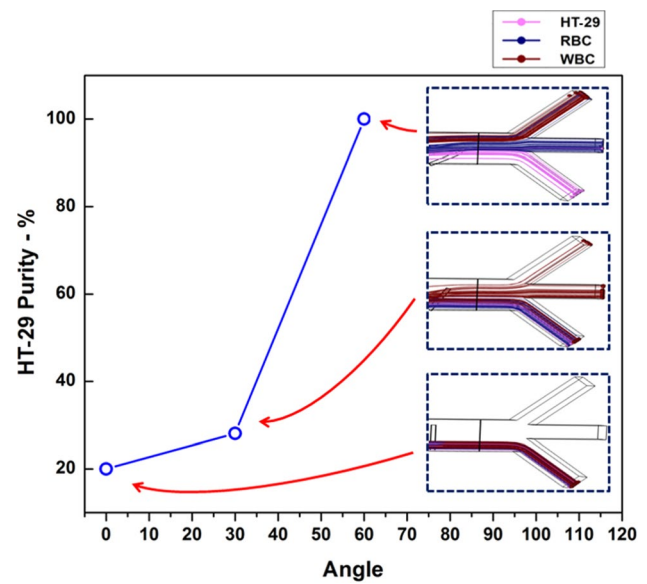
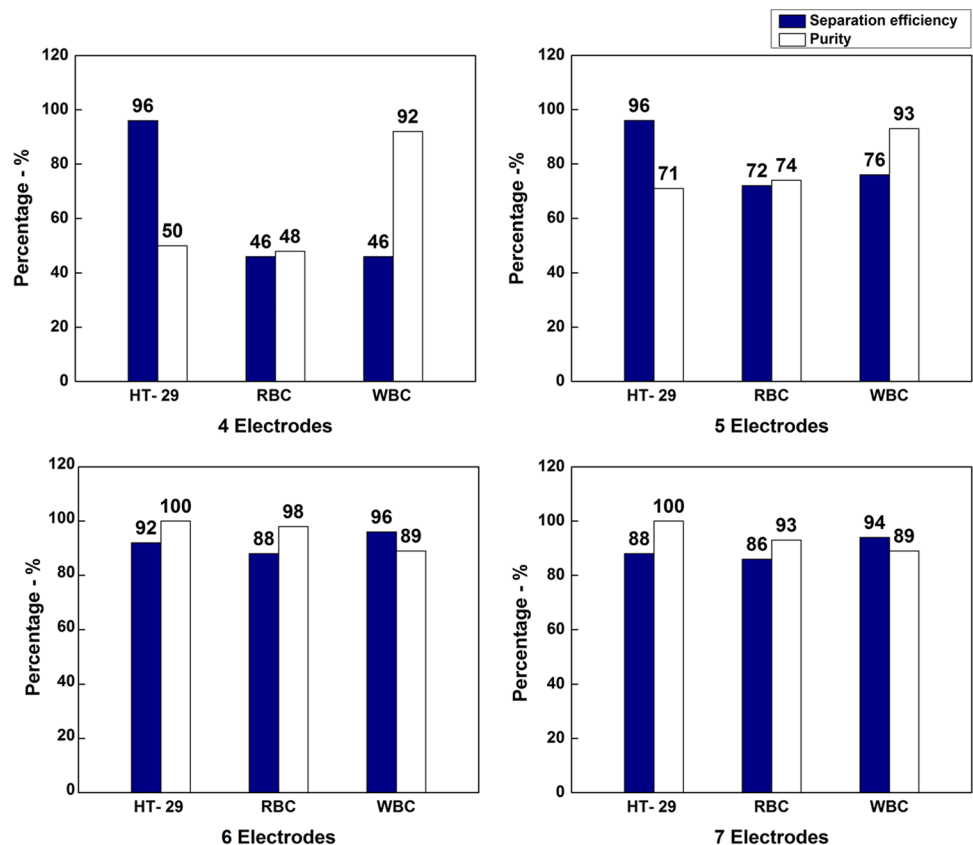


Fig. 9 Influences of the tilted angle of the bottom electrodes on the HT-29 purity time when the AC voltage of 4V at 10 kHz, velocity ratio between sample flow to buffer flow of 1:3.25, bottom electrode number of 6 were applied

ratio of the particles to the buffer was 1:3.25. At 0°, the DEP force was only applied vertically to the particles. No separation

Fig. 8 Effects of bottom electrode configuration on separation efficiency and purity when the AC voltage of 4V at 10 kHz, velocity ratio between sample flow to buffer flow of 1:3.25, electrode tilted angle of 60° were set



occurred, as the outlets were horizontally arranged. The higher the incidence angle of the electrodes, the greater the electric field gradient, further leading to a higher DEP force horizontally on the particles. For the channel with electrodes placed at 30°, the DEP force caused a deflection, but it was not strong enough to deflect the particles in the correct outlets. At 60°, the separation process was the most efficient, with an HT-29 purity of 100%.

Conclusion

In this study, a novel DEP-based microfluidic chip was proposed to separate HT-29 tumor cells from blood cells containing RBCs and WBCs and then numerically investigated to verify the performance of the biochip design. The DEP phenomenon has been created by the slanted electrode array on the bottom of the microchannel and the ground electrode on top of the microchannel. This design facilitates the reduction of the position dependence of the cells in the channel and the complexity of fabrication. Additionally, the impacts of both operating and geometrical parameters were investigated. Under optimal conditions, HT-29, WBCs, and RBCs were separated with high separation efficiencies. The HT-29 tumor cells were enriched at the proper outlet with a high efficiency and purity ratio. The ratio between the flow velocity of the buffer and blood sample and the geometrical and physical parameters considerably influenced the trajectory of the particles. This work has provided a numerical model for studying the separation of biological particles by using the DEP effect and the proposed microfluidic device. Our findings could provide a foundation for further theoretical and practical investigations. The proposed system can separate cancer cells from RBCs and WBCs and offers numerous advantages, such as compact size, low voltage, high efficiency, non-invasiveness, and label-free nature. The tumor cell enrichment platform has the potential for application in cancer detection, analysis, and assessment.

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Data Availability The data and models are described in detail in the manuscript, as well as in referenced publications.

Code Availability Not applicable

Statements and Declarations

Ethics Approval Not applicable

Consent to Participate Not applicable

Consent for Publication All authors have given consent to this publication.

Conflict of Interest The authors declare no competing interests.

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Design and proof-of-concept of a micropillar-based microfluidic chip for trapping and culture of single cells

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Abstract

Single-cell analysis provides a groundbreaking avenue for exploring cell-to-cell variation, the heterogeneity of cell responses to stimuli, and the impact of DNA sequence variations on cell phenotypes. A crucial facet of this analytical approach involves the refinement of techniques for effective single-cell trapping and sustained culture. This study introduces a microfluidic platform based on micropillars for hydrodynamic trapping and prolonged cultivation of individual cells. The proposed biochip design, termed three-micropillars based microfluidic ($3\mu\text{PF}$) structure, incorporates interleaved trap units, each featuring three-micropillars based microfluidic structure strategically designated to trap single cells, enhance the surface area of cells exposed to the culture medium, and enable dynamic culture, continuous waste removal. This configuration aims to mitigate adverse effects associated with bioparticle collisions compared to conventional trap units. The study employs finite element method to conduct a comprehensive numerical investigation into the operational mechanism of the microfluidic device. The simulation results show that the filled trap unit demonstrates a low-velocity magnitude, reducing shear stress on cells and facilitating extended culture. The hydrodynamic single-cell trap mechanism of the proposed device was also verified. The insights derived from this work are pivotal for optimizing the device and guiding future experimental examinations, thus contributing significantly to the progression of single-cell analysis techniques.

Keywords Single-cell trapping · Microfluidic · Micropillar · Long-term cell culture · Single-cell analysis

1 Introduction

Recent years have witnessed the impressive development of microfluidic technology (Aubry et al. 2023). Microfluidics refers to a system that manipulates small amounts of liquid at micro and nanometer scales using channels ranging in size from ten to several hundred micrometers (Zhang et al. 2023). Microfluidic technique has found diverse applications across various fields of research and industry, including material synthesis and discovery, automated

systems, lab-on-a-chip and point-of-care (PoC) devices, and biosensors (Jiang and Korivi 2014). Microfluidic technology is a promising tool for biomedicine applications, especially for single-cell trapping and analysis (Zhou et al. 2021). Single-cell trapping can be defined as the process of immobilizing single cells in devices for further manipulation and analysis. Single-cell trapping techniques offer precise control, enhanced sensitivity, and valuable insights into cellular functions (Sinha et al. 2022). Their ability to focus on individual cells reveals hidden nuances in cellular responses and allows for a thorough exploration of heterogeneity within populations. The single-cell analysis offers a novel approach to study individual cells, the differences in cell responses, and the genetic heterogeneity based on single-cell DNA or RNA sequence analysis (Bakker et al. 2016; Goldman et al. 2019; Muhl et al. 2020). Integrated into advanced platforms, these techniques can be applied in versatile applications across scientific disciplines, from genomics to drug discovery and cancer research (Wen et al. 2022; Van de Sande et al. 2023). Microfluidic devices offer

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several advantages over conventional methods for single-cell trapping and analysis, such as low sample consumption, high spatial and temporal resolution, multiplexing capability, and integration with other analytical techniques (Yin and Marshall 2012; Dusny and Grünberger 2020).

Not only in single-cell research, microfluidics finds extensive application in cell culture for increasing our understanding of cell study, tissue morphology, pathology, drug release and their effects, protein production (Kapałczyńska et al. 2016). Cell culture on a chip is a technique that allows researchers to study cells in a more controlled environment. Microfluidic devices can also create dynamic microenvironments that can mimic physiological conditions or induce cellular responses (Xu et al. 2020; Dusny and Grünberger 2020). The use of cell culture chips presents a significant advantage over traditional culture methods, such as batch culture or dynamic cell culture. The dynamic microenvironments achieved through microfluidic systems allow for more intricate control and modulation of the culture conditions, facilitating the emulation of in vivo-like scenarios. This dynamic control is particularly beneficial for mimicking complex cellular interactions, inducing cell differentiation, and simulating various physiological cues (Kim and Hayward 2012). In essence, cell culture on a chip offers a superior and more versatile platform for studying cells under conditions that closely mirror their natural milieu, providing a valuable tool for advancing our understanding of cellular behavior and responses.

In recent years, the trapping and cultivation of single cells using microfluidic chips have attracted considerable attention from many research groups. Jo et al. designed a single-cell analysis platform based on the microfluidic chip to investigate the replicative aging phenomenon in the budding yeast *Saccharomyces cerevisiae* (Jo et al. 2015a). The biochip contained an array of U-shaped single-cell trapping units in the chamber. The proposed design efficiently trapped up to 8,000 single yeast cells with a trapping efficiency of 96% and enabled long-term culture of trapped cells. Xu et al. designed, numerically examined, and optimized the microfluidic chip for single-cell trapping with inverted trapezoidal trap grooves (Xu et al. 2013a, b). A recent work presented a microfluidic chip for separating plasma, red blood cells, and individual trapping of white blood cells (Kuan et al. 2018). The trapping structure consisted of a triangular pillar and two rectangular pillars, which allowed trapping up to approximately 1800 cells in 20 min. However, the U-shaped trap structure in long-term cell culture presents challenges due to the limited contact area between the cell and the culture media when cells are trapped within the trap units, which could potentially impede the exchange between the culture medium and the cell surface. In case of inverted trapezoidal grooves or triangular pillars, the presence of

corners may result in cell damage due to collisions during the trapping process (Xu et al. 2013a). During the cultivation phase, it can lead to deformations and potential harm to the cells. To address the aforementioned limitations, a new structure has been proposed for both cell trapping and long-term dynamic culture while minimizing cell damage.

This study proposes a micropillar-based microfluidic platform for both hydrodynamic trapping and long-term dynamic culture of single cells, which could be integrated into other platforms such as biosensors and real-time microscopy. The three-micropillars based microfluidic (3 μ PF) structure features interleaved trap units for hydrodynamic cell trapping. Each trap unit was designated with three well-aligned micropillars to enhance the surface area of single-cell exposed to the culture medium and reduce the negative effects when the bioparticles collide with the trap. In this work, the finite element method (FEM) was used to analyze the working principle of the microfluidic device and optimize the biochip structure. The simulation results showed that the proposed design enables trapping and long-term culture of single cells.

2 Theoretical fundamentals

The operating principle of the 3 μ PF was examined through numerical computations using COMSOL Multiphysics 6.0 software. In this study, the fluid flow was considered a creeping flow, calculated by the Navier-Stokes equations, while the simulated cell follow the linear elastic dynamics and Newton's equation of motion. The cell movements are governed by the combination of liquid and solid mechanics, or fluid-solid interaction (FSI). The fundamental concept is that a structural component experiences hydrodynamic forces exerted by a fluid, leading to its subsequent deformation (Peksen 2018). In turn, the deformed shape of the structure imparts velocity to the fluid domain, inducing changes in the flow field. The continual motion and interactions of both the fluid and particles lead to a deformation of the mesh geometry. To capture this dynamic behavior, the Arbitrary Lagrangian-Eulerian (ALE) technique is employed. This technique effectively characterizes the dynamics associated with the deforming geometry and the moving boundaries of the mesh. Its application facilitates the generation of a new mesh, ensuring the preservation of numerical stability and accuracy throughout the computational process (Xu et al. 2013a; Abbas et al. 2022).

2.1 Fluid flow

The fluid flow behaviours are governed by the incompressible Navier-Stokes equations, as shown in Eqs. (1), (2) (Henrik Bruus 2008):

$$\rho \left[\frac{\partial \mathbf{u}_f}{\partial t} + \mathbf{u}_f \cdot \nabla \mathbf{u}_f \right] = \nabla \cdot [-p\mathbf{I} + \mathbf{K}] - 12 \frac{\mu \mathbf{u}_f}{d_z^2} + \mathbf{F} \quad (1)$$

$$\rho \nabla \cdot \mathbf{u}_f = 0 \quad (2)$$

where $\rho, \mathbf{u}_f = (u_f, v_f, w_f) = 0, t, p, \mathbf{I}$, and $\mathbf{F}$ are the fluid density (kg/m^3), the fluid velocity field (m/s, m/s, m/s), the time (s), the pressure (Pa), the identity matrix, is the volume force affecting the fluid (N/m^3 , or N/m^2 for a 2D model), respectively. For a pressure-driven flow without gravitation or other volume forces, $\mathbf{F} = 0$. Moreover, $\rho \frac{\partial \mathbf{u}_f}{\partial t}$ represents the unsteady inertia force (N/m^3). The fluid is assumed Newtonian with the corresponding constitutive equation is written by:

$$\mathbf{K} = \mu \left(\nabla \mathbf{u}_f + (\nabla \mathbf{u}_f)^T \right) \quad (3)$$

where T indicates the transposition, K refers to the shear stress tensor, μ is the fluid dynamic viscosity ($\text{Pa} \cdot \text{s}$).

In this study, 2D simulation models were implemented to simplify the computations and reduce time and resources due to the model symmetry. In microfluidic channels characterized by an approximately rectangular cross-section, where the thickness is considerably smaller than the channel width, basic 2D models often prove inadequate in yielding precise results. This inadequacy arises from the omission of boundaries in these models, a critical factor that significantly influences the flow dynamics (Xu et al. 2013a). To account for the influence of these boundaries, a shallow channel approximation is introduced (Henrik Bruus 2008). This approximation introduces a drag term as a volumetric force in the fluid flow equation. This term signifies the resistance exerted by the parallel boundaries on the flow. In other words, Eq. (1) is an equation from COMSOL Multiphysics that has been adjusted to give a more accurate calculation result. The drag term is calculated by the following equation (Xu et al. 2013a):

$$\mathbf{F}_\mu = -12 \frac{\mu \mathbf{u}_f}{d_z^2} \quad (4)$$

where d_z is the channel thickness (m).

The dimensionless number used to define the ratio of the advective to viscous forces is known as Reynolds number, Re. Reynolds number is given by the following relation:

$$Re = \frac{\rho U L}{\mu} \quad (5)$$

where U is the velocity of the fluid (m/s), L is the characteristic length of the fluid (m).

In case of flow in microfluidics devices, Re becomes very low ($Re \ll 1$). The Strouhal number $St_r = \frac{fL}{U}$ (f is the frequency of vortex shedding) is significant (on the order of 1), and viscosity plays a dominant role in governing the fluid flow, it leads to a collective oscillating motion of the fluid. In this work, the fluid flow is Stokes flow, commonly referred to as creeping flow or creeping motion. Stokes flow represents a type of fluid motion wherein advective inertial forces are notably smaller compared to viscous forces. Therefore, the non-linear inertial force $\rho(\mathbf{u}_f \cdot \nabla) \mathbf{u}_f$ can be neglected.

2.2 Solid mechanics

Solid mechanics is a pivotal discipline in physical science which focuses on the deformation and movement of continuous solids subjected to external forces such as forces, displacements, and accelerations (Wang and Qin 2019). In the case of our solid subject to small deformation and low load, it will have isotropic linear elasticity. By conceptualizing cells as solid structures, this work accurately models and simulates their mechanical traits, including elasticity and deformability. Employing solid mechanics principles facilitates an accurate representation of cellular responses to external forces, deformation under mechanical stress, and interactions within their immediate surroundings. Utilizing FEM to simulate the solid phase in FSI models allows for the intricate modeling of individual cell mechanics, providing nuanced insights into their behavior across diverse fluidic environments. This method harmonizes biological understanding with classical solid mechanics, enhancing the computational representation of cellular dynamics for academic and research purposes. The solid's displacement and deformation follow the governing equations of linear elastodynamics (Bower 2009):

$$\epsilon_s = \frac{1}{2} \left[(\nabla \mathbf{u}_s)^T + \nabla \mathbf{u}_s + (\nabla \mathbf{u}_s)^T (\nabla \mathbf{u}_s) \right], \quad (6)$$

$$\nabla \sigma_s + \mathbf{F}_s = \rho_s \frac{\partial^2 \mathbf{u}_s}{\partial t^2}, \quad (7)$$

$$\sigma_s = C \epsilon_s. \quad (8)$$

Here, Eq. (6) is the strain-displacement (compatibility) equation, with ϵ_s denoting the infinitesimal strain tensor and $\mathbf{u}_s = (u_s, v_s, w_s)$ denoting the solid displacement field (m, m, m).

Equation (7) is Newton's equation of motion, with σ_s the Cauchy stress tensor, F_s is the body force per unit volume (N/m^3) or boundary force per unit area in 2D (N/m^2), and ρ_s denoting the solid density (kg/m^3).

The stress-strain relations for an isotropic, linear elastic solid are repeated below for convenience. Equation (7) is the linear elastic stress-strain law with $C = C(E, \nu)$ as the stiffness matrix given by

$$C = \frac{E}{(1+\nu)(1-2\nu)} \begin{bmatrix} 1-\nu & \nu & \nu & 0 & 0 & 0 \\ \nu & 1-\nu & \nu & 0 & 0 & 0 \\ \nu & \nu & 1-\nu & 0 & 0 & 0 \\ 0 & 0 & 0 & 1-2\nu & 0 & 0 \\ 0 & 0 & 0 & 0 & 1-2\nu & 0 \\ 0 & 0 & 0 & 0 & 0 & 1-2\nu \end{bmatrix} \quad (9)$$

Here, E and ν are Young's modulus (Pa) and Poisson's ratio of the solid.

2.3 Fluid-solid interaction (FSI)

Fluid–solid interaction (FSI) applications involve the coupling of fluid dynamics and structural mechanics disciplines, in general. The interaction equations are determined through:

$$F_A = [-\rho I + K] \cdot n \quad (10)$$

$$u_{tr} = \frac{\partial u_s}{\partial t} \quad (11)$$

In this, F_A represents the total force (caused by the fluid pressure and viscous force) exerted on the solid boundary, K is presented in the Eq. (3) and n is the outward normal to the boundary. From Eq. (11), u_{tr} is the rate of change for the displacement of the solid. In this case, u_{tr} equal to the fluid velocity u_f . In other words, at the liquid–solid interface, a non-slip boundary condition is formed for the liquid domain.

3 Design and setup of numerical simulation

3.1 Design of the microfluidic single-cell trapping device

Figure 1 illustrates the proposed 3μ PF design with interleaved trap units. The microfluidic channel presented in this report is designed with two inlets and one outlet. Experimentally, micropillars within each trap units can be potentially made of polydimethylsiloxane (PDMS), with each trap unit designated with three micropillars to enhance the surface area of single-cell exposed to the culture medium and reduce the negative effects when the bioparticles collide

with the trap, which helps overcome the limitations of other trap unit designs (Xu et al. 2013a; Jo et al. 2015b) (Fig. 1).

Hydrodynamic trapping employs mechanical barriers or obstructions to segregate the target particle from the main flow. Once separated, these target particles are captured and held in hydrodynamic trapping sites, facilitating their utilization for various investigative purposes (Narayana-murthy et al. 2017). Our 3μ PF, constructed from precisely aligned micropillars, enable hydrodynamic cell entrapment. In the main channel, trap units are alternately arranged with suitable spacing to ensure that all cells, carried by the liquid flow into the channel, will be effectively trapped. This design aims to ensure that every simulated cell in the flow path becomes captured due to the repetitive and staggered configuration of the trap arrays. Each trap unit exclusively accommodates a single cell. In this work, human embryonic stem cells (hESCs) were used to verify the working mechanism of the proposed biochip by using simulation methods, with the referenced mechanical parameters being Young's modulus and Poisson's ratio (Kiss et al. 2011; Shin et al. 2016). The design parameters of the 3μ PF device are illustrated in Table 1. Each trap unit is designed to ensure that the pillar position and the trap area are suitable to trap only a single cell while eliminating the existence of more than one cell. Since the cell diameter is $16\mu\text{m}$, the opening of the trap unit T_gap is a little greater, at $23\mu\text{m}$. When considering pillar size, bigger pillars might lead to low cell surface exposed to medium while smaller pillars could encounter the complex fabrication process. The pillar size of $5\text{--}10\mu\text{m}$ was found to be appropriate for stem cells (He et al. 2022; Long et al. 2024) and is compatible with human embryonic stem cell (hESC) size. In this research, micropillars with diameter of $5\mu\text{m}$ were used. Based on the hydrodynamic mechanism, the trap units are interleaved (Carlo et al. 2006); therefore, in this work, the gap between the two neighboring traps on the same row R_w is equal to a trap opening. The gap between two successive rows is required to be greater than the cell diameter so that cells can pass through the filled trap units.

Initially, the fluid traverses the channel, carrying multiple cells. Upon passing through the trap units, each cell is captured in a unit trap, leading to a gradual decrease in velocity to zero at this position. The trapping mechanism is illustrated in the inset of Fig. 1 within the trap method section. A schematic representation illustrates the hydrodynamic resistance of the flow in the proposed 3μ PF under two cases: an empty trap unit (pre-trapping) and post-successful entrapment of a cell. When a cell is caught in a trap unit, the surrounding velocity will approach zero, the cell will tend to move in the direction of greater velocity and avoid the filled trap units. This structure, in addition to ensuring good single-cell trapping performance, can be used for long-term

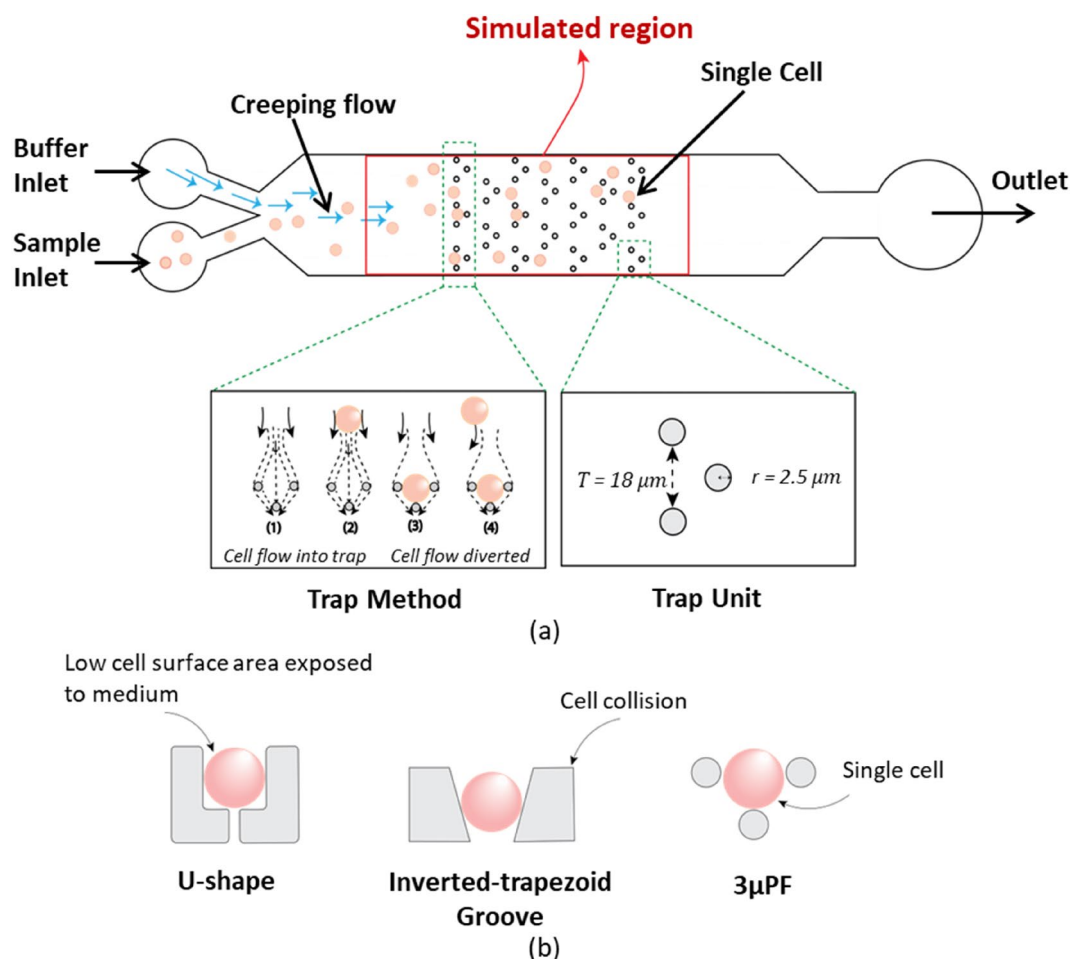


Fig. 1 (a) Design of the proposed microfluidic chip device. The microfluidic chip has two inlets for cell introduction and medium input, and an outlet. The single-cell is trapped based on hydrodynamic mecha-

nism, with each trap unit designed with three micropillars; (b) The comparison between the proposed trap unit with two other trap units, namely U-shape and inverted-trapezoid groove

Table 1 Geometrical parameters

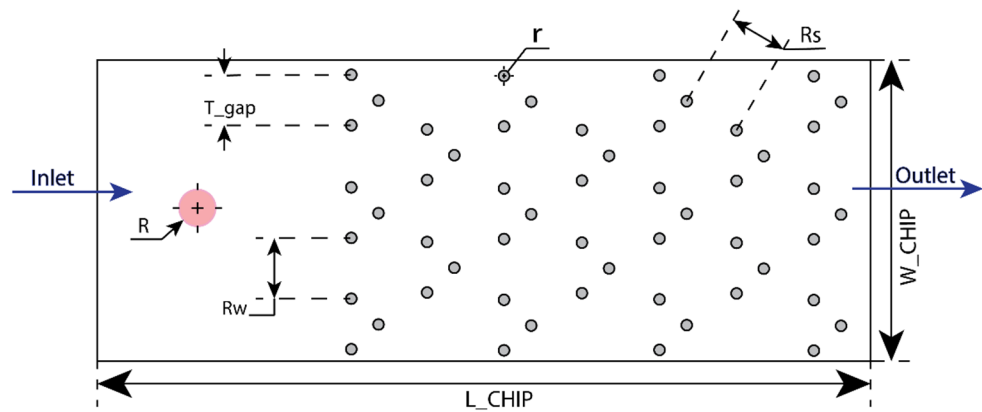
Name	Description	Value
L_CHIP	Chip length	360 μm
W_CHIP	Chip width	140 μm
Rs	Gap between two successive rows	18 μm
Rw	Gap between two neighboring traps on the same row	23 μm
T_gap	Trap gap	23 μm
R	Radius of the simulated cell	8 μm
r	Radius of a micropillar	2.5 μm

single-cell culture for further investigation. The micropillars in each trap unit have the advantage of micrometer size which facilitates the increase in the contact area of the cell with the culture medium or other nutrients—ensuring that the concentration of the medium inside and outside the trap is almost identical. In addition, the microfluidic chip design enables the dynamic culture of single cells, permitting continuous nutrient supply and waste removal.

3.2 Simulation setup

3.2.1 Simulation model

The 2D model of the 3 μ PF is illustrated in Fig. 2. In this work, numerical analysis was used to verify the mechanism of hydrodynamic trapping and the pillar-based concept. Therefore, the simulation model focuses only on the hydrodynamic trapping region. This simulation model consists of an inlet, a trapping area, and an outlet. The simulated cell was inserted into the microfluidic channel to determine the operating principle of the device. It is emphasized that in the equations governing solid mechanics and fluid-solid interaction, only the simulated cells are treated as the relevant “solid” entities while the traps are assumed to remain rigid, serving as fixed elements that enforce the no-slip boundary condition on the fluid. The physical properties of the structure, such as strength, load capacity, or thermal conductivity, are described through mathematical equations and

Fig. 2 2D simulation model of the proposed 3 μ PF structure**Table 2** Physical properties (Kiss et al. 2011; Shin et al. 2016)

Description	Value
Fluid density	1000 kg/m ³
Fluid viscosity	0.001 Pa·s
PDMS density	970 kg/m ³
PDMS Young's Modulus	3×10^9 Pa
PDMS Poisson's Ratio	0.49
The simulated cell density	1050 kg/m ³
The simulated cell Young's Modulus	10 kPa
The simulated cell Poisson's Ratio	0.45

assigned to elements. This method simplifies the geometry and reduces the calculation without changing its hydrodynamic properties. The flow direction is characterized by the movement from regions of high pressure to those of lower pressure. A boundary condition at the outlet was initially imposed, setting the pressure value to zero. In this study, the simulation is initiated at the moment when one or more simulated cell are introduced into the channel after a time t , the simulated cell will be some distance apart from our trap arrays by the fluid flow. The physical parameters of the cells and the biochip materials are shown Table 2.

3.2.2 Boundary and initial conditions and model meshing

Computational fluid dynamics (CFD) simulations serve as a vital tool for analyzing and visualizing the effects of fluidic forces and stresses on cells, while also playing a crucial role in predicting and testing the numerous parameters that influence cell behavior in the future (Huang et al. 2010). The fluid moves from the inlet to the outlet of the channel, propelled by the pressure gradient between these two components. At the inlet, the flow is fully developed laminar characteristics, featuring a parabolic velocity profile with a mean velocity of U_0 (m/s). Meanwhile, at the outlet, the boundary condition is characterized by the absence of viscous stress and a Dirichlet condition on the pressure. Solid walls within the simulation domain, including sidewalls and fixed obstacles, are set as a no-slip wall condition, with

prescribed mesh displacements for these walls set to zero. The specific boundary condition values are clearly depicted in the Fig. 3.

In FSI simulations, ensuring the mesh is compatible with both the fluid and solid domains is crucial. FSI simulations are described using the Finite Element Method (FEM) and the Arbitrary Lagrangian-Eulerian (ALE) method (Hou et al. 2012). COMSOL's ALE solver is customarily tailored for FSI simulations. It functions by iteratively updating the mesh and solving the interconnected fluid-solid equations to represent the dynamic interaction between the two domains accurately. COMSOL's ALE solver employs a segregated solver approach, wherein the fluid and solid domains are solved individually, and relevant information is exchanged between these domains at each time step to accommodate the FSI effects. Sustaining the static and undeformed state of the simulated cell mesh is crucial for upholding the fundamental assumptions essential in achieving accurate simulations of the interaction between a rigid body and a deformable fluid. This approach ensures a faithful representation of the underlying physical phenomena while optimizing computational efficiency and numerical stability. Concurrently, it enables dynamic responsiveness of the fluid domain's mesh, accommodating its deformation in response to system dynamics. This approach also facilitates mesh smoothing and accommodates dynamic simulated cell mobility during simulations, effectively capturing the system's intricate dynamics.

Additionally, mesh smoothing techniques, such as Winslow smoothing and hyperelastic smoothing, are recommended for achieving mesh quality and addressing deformation challenges (Knupp 1999) (Triantafyllidis and Aifantis 1986). Winslow smoothing serves as a well-established method to enhance mesh quality, reducing element distortions and improving overall mesh performance. This method is particularly effective in areas of the simulation domain characterized by minimal to moderate deformation. In contrast, hyperelastic smoothing demonstrates exceptional capabilities in situations where the mesh experiences

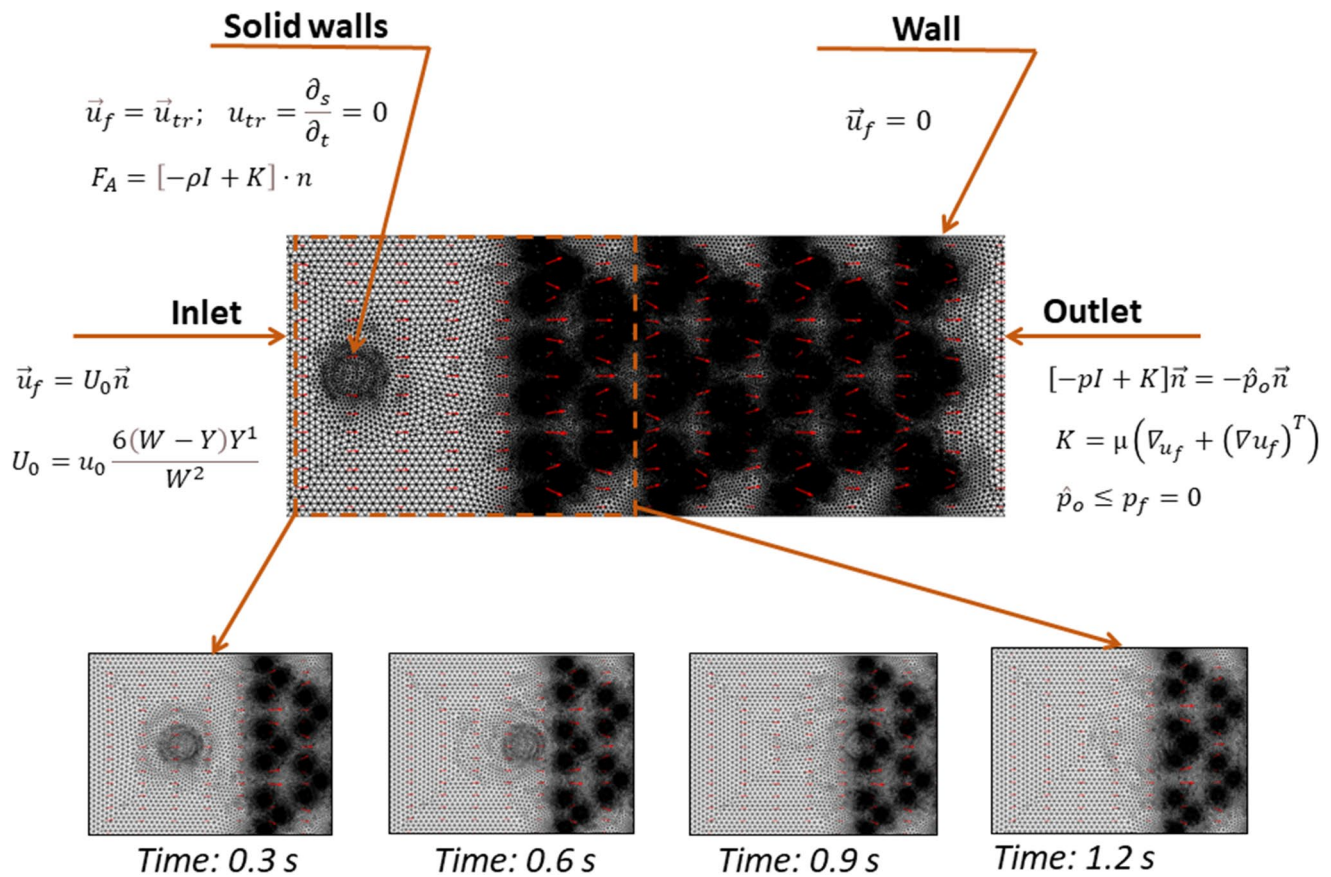


Fig. 3 Meshing model (boundary conditions and moving mesh method)

significant stretching and deformation. This technique is particularly advantageous when simulating solid particles like cells, known for their pronounced elasticity and deformation tendencies. It enables a detailed representation of complex particle behaviors under varying flow conditions. Adapting the selection of mesh smoothing methods to the unique characteristics of the simulation contributes to improved result accuracy and computational efficiency, ultimately enhancing the overall quality and effectiveness of the study. Based on the mentioned advantages, the hyper-elastic smoothing method was used in this work.

Figure 3 illustrates the mesh movement in the direction of the simulated cell's displacement at specific time points: $t=0$ s, 0.3 s, 0.6 s, 0.9 s, and 1.2 s. The mesh exhibits non-uniformity, characterized by denser and smaller elements at the boundaries between the fluid and solid domains and, conversely, looser and larger elements within the fluid domain. However, it's important to note that the mesh maintains a uniform distribution around the single cell. This study opted for a finer mesh resolution to attain convergent results (Frei et al. 2017) (Anderson et al. 2004).

In scenarios where mesh deformation in the Arbitrary Lagrangian-Eulerian (ALE) method becomes substantial, such as when the simulated cell approaches the trap, the

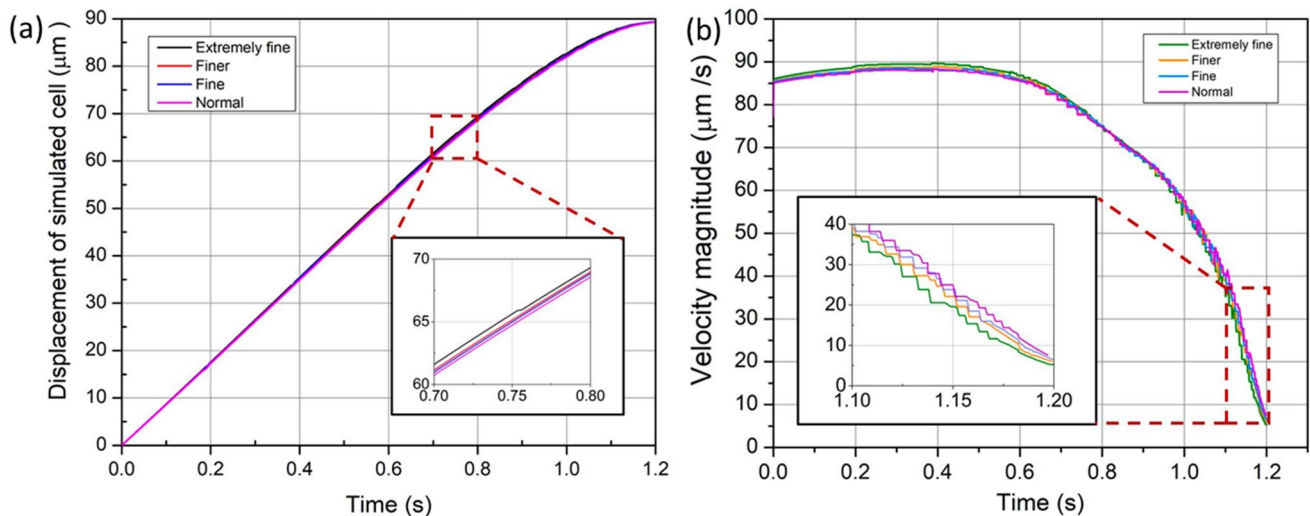
mesh quality, created and maintained by the smoothing function, can deteriorate. This may lead to elements with warped or inverted coordinates. Inverted coordinates, while not indicative of simulation failure, render results from these elements unusable in subsequent iterations. When these elements are not in the immediate region of interest, the simulation's reliability remains generally unaffected. However, a high prevalence of inverted coordinates can reduce solution accuracy and lead to convergence challenges. In this work, a solution has been implemented to solve the above problem, which involves creating a new mesh for the deformed region, allowing the solver to continue. A specified mesh quality threshold, typically less than 0.2 and within the 0 to 1 range, triggers the solver to pause and revert to a prior solution time for re-meshing. Subsequently, the simulation proceeds using the newly generated mesh, ensuring accurate and stable results despite significant mesh deformation (Storti et al. 2008; Hirt et al. 1997).

The mesh independency test was performed using four mesh types: extremely fine, finer, fine, and normal. The mesh qualities are shown in Table 3.

The single-cell trapping is directly related to the cell velocity and displacement. Therefore, the mesh independency verification is performed by calculating the cell

Table 3 Mesh qualities of four mesh types in mesh independence study

Mesh type	Maximum element size (μm)	Minimum element size (μm)	Maximum element growth rate	Curvature factor	Number of domain elements	Number of boundary elements
Extremely fine	0.905	0.0027	1.05	0.2	239,322	2916
Finer	3.78	0.054	1.1	0.25	102,217	1999
Fine	4.73	0.135	1.13	0.3	70,276	1692
Normal	6.08	0.27	1.15	0.3	62,731	1663

**Fig. 4** Mesh independency test: (a) Cell displacement along the x direction versus time at different mesh scales, (b) Cell velocity versus time at different mesh scales

velocity and displacement over time as shown in Fig. 4. The most significant difference exists between results derived using normal mesh and extremely fine mesh. It can be seen that the differences between the cell velocity and displacement for the extremely fine and finer are quite minor. Considering the computational time, resources and to lessen the possibility of convergence fail, in this study, finer mesh was used for following analysis.

4 Simulation results and discussions

We discuss in this part the simulation results, analyze and evaluate the advantages and potentials of this device, and optimize the device geometry. The proposed device initially consists of interleaved micropillars-based trap units, each micropillar has a radius of $2.5 \mu\text{m}$. The single cells with a radius of $8 \mu\text{m}$ follow the flow of fluid introduced into the channel. The simulation model enabled the calculation of flow velocity and pressure field. The velocity and exerted total force of the cells were calculated, the stress on and deformation of the simulated cell was also shown (Xu et al. 2013a). In addition, the cell culture potential will be analyzed by the device through the trap geometry, thanks to the low-velocity field around the trap site when cells are

trapped. In order to investigate the trapping mechanism, we have implemented the situation of introducing two single cells into the channel.

4.1 Flow velocity field and simulated cell displacement

First, simulations are performed in case the input velocity of the inlet is $v = 70 \mu\text{m/s}$. The analysis of fluid velocity within both the main channel and the trap channel is conducted both prior to and following the cell trapping process. Initially, the fluid velocity in the main channel before the cell trap region is observed to be lower than that in the trap unit, as depicted in Fig. 5. However, subsequent to the entrapment of the cell within the trap unit, the flow velocity within the trap channel gradually decreases, eventually reaching approximately $6 \mu\text{m/s}$. The low velocity profile inside the cell culture area in the proposed microfluidic chip is consistent with several recent work performing dynamic culture (Yu et al. 2017; Nocera et al. 2022; Feng et al. 2023). The fluid velocity magnitude around the trapped cell in Fig. 4 is greater than that of about 20% of the body's mass, at approximately $0.1\text{--}2 \mu\text{m/s}$ (Clementi et al. 2018). The reduction in fluid velocity around trapped cells helps decrease the stress acting on cells while still allowing nutrient delivering. The simulation study to

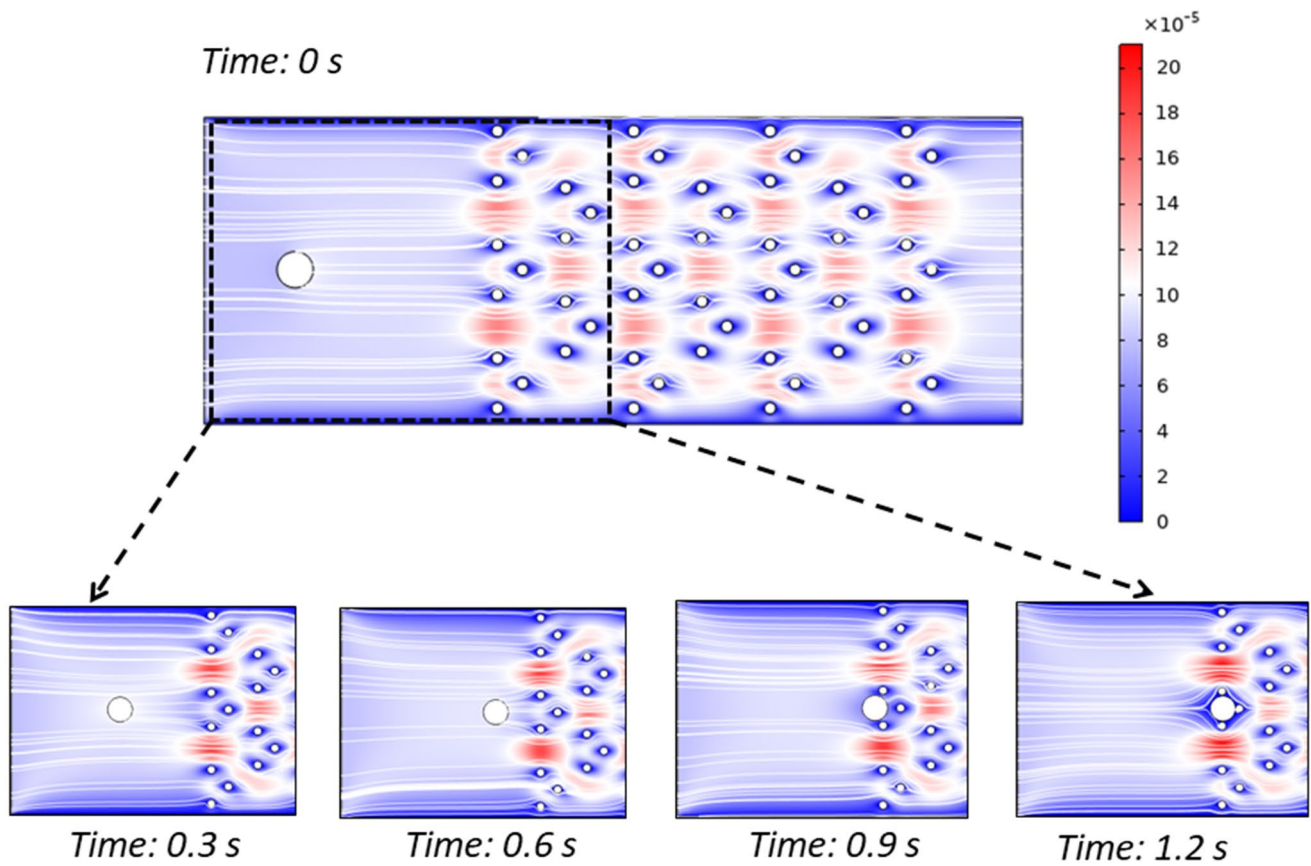


Fig. 5 Velocity magnitude distribution inside the device during the cell trapping at different time points: $t = 0$ s, 0.3 s, 0.6 s, 0.9 s, and 1.2 s

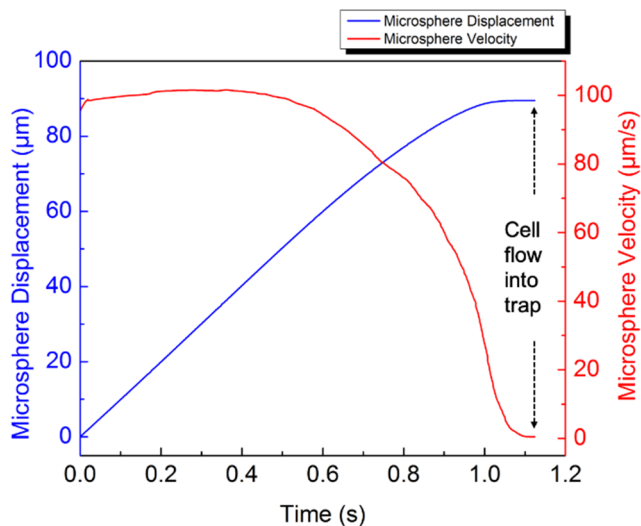


Fig. 6 Simulated cell displacement and velocity magnitude over time (until the cell was trapped)

model the transport of nutrients by means of diffusion by Fick's law and convection coupled with fluid flow shows the interaction between cells and medium.

Figure 6 illustrates the calculated flow velocity field at various time intervals, providing insights into the dynamic

alterations within the system. The graphical representation in Fig. 6 includes a comparative analysis of the correlation between flow velocity and the cell displacement from the inlet. The findings illustrate a gradual reduction in fluid velocity to zero as the cell becomes entrapped within the trap unit, coinciding with an increase in the distance from its original position.

These results show that when cells are trapped in the trap unit, the contact area between the medium and the cells remains relatively large, and the flow velocity is very low. Beginning at the simulation time of 0.9s, when the cell initiates contact with the trap unit, and continuing until its full immobilization at 1.2s, there is a significant reduction in velocity, dropping from approximately 40 $\mu\text{m/s}$ close to 0. This condition is particularly suitable for culturing cells within the trap under dynamic culture conditions. The low flow velocity contributes to a stable culture environment, allowing for the gradual introduction of new culture solutions and the removal of wastes. Importantly, this process can be automated, eliminating the need for manual operation, which proves advantageous during long-term culturing. Furthermore, only the flow near the simulated cell is affected by the motion of the simulated cell. When simulated cell fill a trap, flow is obstructed at the trapping site.

This observation aligns with the principle of hydrodynamic trapping, indicating that when the trapping site is empty, the trapping unit displays lower flow resistance compared to the main channel (Ahmad Khalili et al. 2016). This scenario establishes a flow pathway guiding cells toward the trap unit. Individual trapped cells obstruct the trap units, significantly decreasing liquid velocity within the trap unit. Subsequently, incoming cells are directed towards the bypass pathway, which provides a route with fewer obstructions and reduced resistance. Figure 7 clearly shows this process. The migration process of a simulated cell is considered when the opposite trap unit has been filled by another simulated cell assumed after 1.2 s in the simulation.

The simulation results are consistent with the hydrodynamic trapping theory. When a trap unit is occupied, approaching cells strategically choose to bypass the unit, opting for the path of least resistance to prevent direct entry and potential stacking. The staggered arrangement of trapping units proves effective in capturing nearly all introduced cells without overlap.

4.2 Fluid pressure and boundary load on the simulated cell

In the context of single-cell traps and microfluidic cell culture, mechanical forces generated by flow pressure fields, particularly shear stress, play a central role in influencing diverse cellular responses (Espina et al. 2023); Li et al. 2005). These responses encompass crucial aspects of cell behavior, including gene expression modulation, adhesion, migration, and signaling. Additionally, the pressure fields intricately govern microenvironment dynamics and nutrient distribution within microchannels, significantly impacting cell metabolism (Zhan et al. 2020). This microfluidic holds immense value in tissue engineering and disease modeling, faithfully replicating in vivo-like conditions.

It becomes evident that the pressure field plays an important role in cell culture. The results are calculated at specific time points $t=0$ s, $t=0.3$ s, $t=0.6$ s, $t=0.9$ s, and $t=1.2$ s (Fig. 8). The findings indicate a consistently diminishing magnitude of the pressure field distribution along the

channel, extending from the inlet to the outlet. When the cell is distant from the trap, it experiences the influence of flow pressure and the viscous drag present in its surrounding environment. Initially, at time $t=0$, the pressure field exhibits a range of values from 0 to approximately 1.21 Pa. As the cell initiates movement, this value progressively diminishes, reaching an approximate distribution of 0 to approximately 0.75 Pa. Significantly, at time $t=1.2$ s, corresponding to the point when the simulated cell is positioned within the micropillar-based trap unit, there is a noticeable decrease in fluid pressure.

The simulation results demonstrate a gradual reduction in the pressure field across the entire channel. As the simulated cell consistently approached the trap unit, a progressive rise in resistance coincided with a decrease in drag force, leading to a swift decline in the velocity of the simulated cell. Upon entering the trap unit, both resistance and drag maintained a stable equilibrium, enabling the cell to move at a constant speed. Subsequently, as the resistance exceeded the drag, the simulated cell initiated a deceleration process until it eventually came to a complete stop. The results also indicate that the pressure field within this structure is lower compared to that of the inverted trapezoidal groove structure (Xu et al. 2013a). The microfluidic design with a notably reduced pressure field offers several crucial benefits (Zhu et al. 2012; Liu et al. 2013; Liang Huang, Shengtai Bian, Yinnuo Cheng, Guanya Shi, Peng Liu, Xiongying Ye 2017). First and foremost, this small pressure field allows for precise control of fluid flow and the accurate positioning of cells within the microfluidic channels. This precision proves especially valuable for capturing and placing cells at specific locations and creating controlled gradients of nutrients or signaling molecules for cell culture. Additionally, the reduced pressure field minimizes shear stress on cells, which is vital for maintaining cell viability, particularly for sensitive cell types. This feature helps prevent cell damage or detachment caused by excessive mechanical forces. Moreover, the microfluidic structure's capacity to maintain a small pressure field is conducive to long-term cell culture by ensuring a stable and consistent microenvironment. This stability is crucial for extended cell culture studies. So,

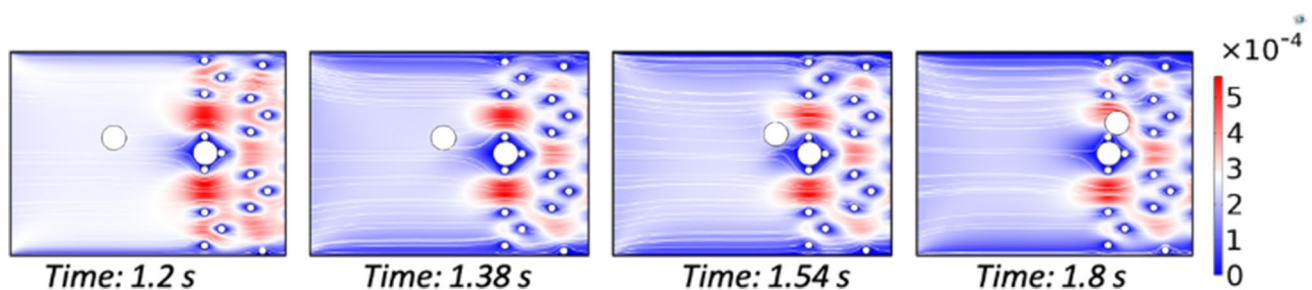


Fig. 7 Cell movement toward a filled trap unit

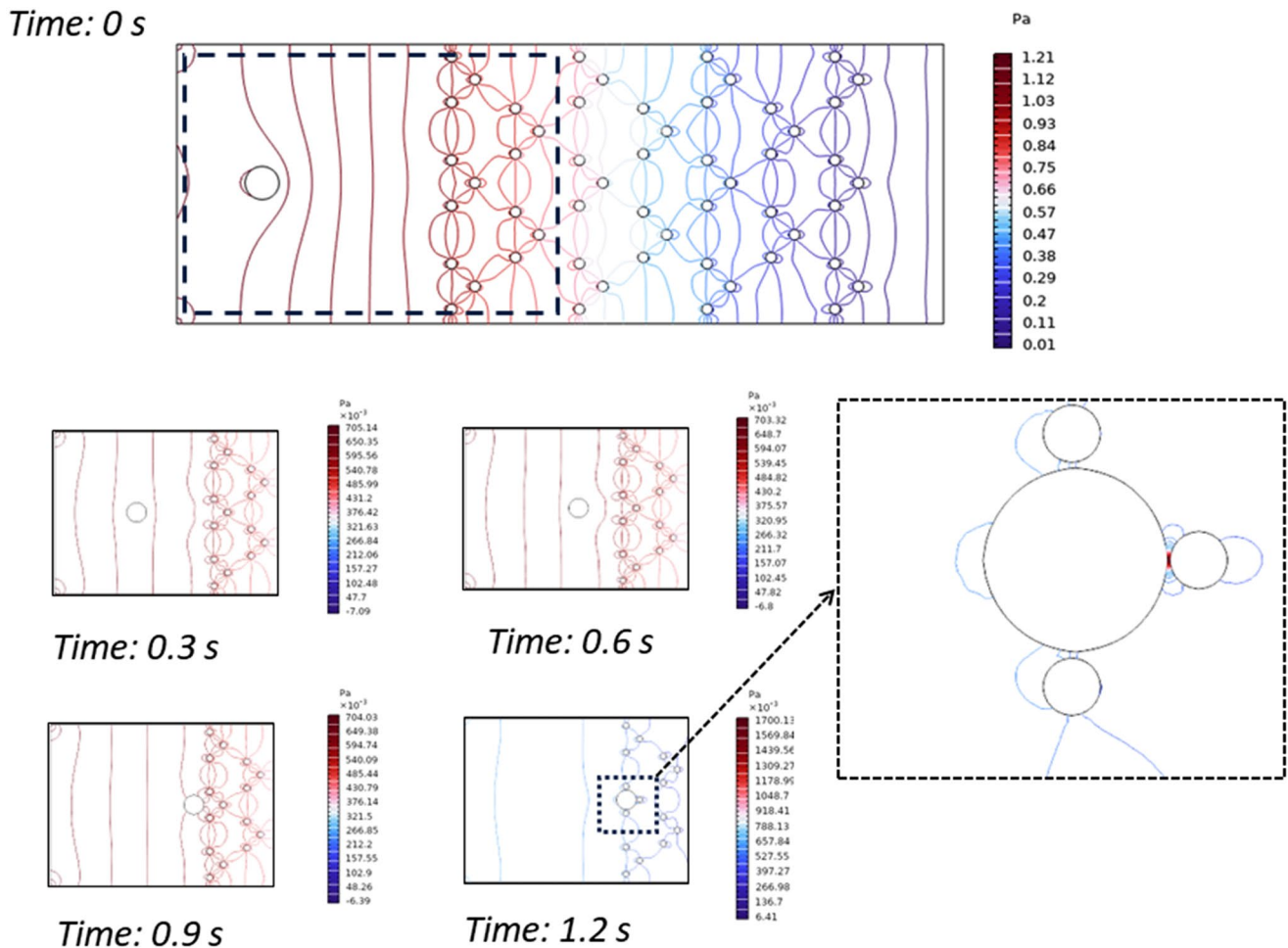


Fig. 8 Visualization of the fluid pressure field extracted during the single-cell trap simulation

this design is well-suited for single-cell investigations and applications requiring the isolation and precise positioning of individual cells, making use of the benefits of the reduced pressure field for high-precision trapping and positioning of single cells.

The relationship between fluid pressure and the total cell boundary load, encompassing both normal and tangential loads, is contingent upon the specific conditions and characteristics of the system under consideration. Within the context of fluid-structure interaction (FSI) simulations, the impact of tangential and normal loads on structures, considered here as simulated cells, due to fluid forces is a critical aspect influencing the behavior of these cell-like entities (Papadakis et al. 2022). These loads, integral components of the total force exerted by the fluid on the simulated cell surface, contribute significantly to the deformation and stress distribution within the simulated cellular structure. Tangential loads, also known as shear loads, lead to twisting or sliding deformation patterns, influenced by the geometry and material properties of the simulated cell. Normal loads, on the other hand, induce stresses perpendicular to the cell

surface, affecting overall deformation, structural response, and integrity. Elevated normal loads may result in compression or tension forces, potentially causing structural failures. In this work, the calculated load value is shown in Fig. 9, highlighting the importance of understanding dynamic behavior in fluid-structure interaction scenarios.

Beginning with the normal load, it is essential to recognize that the pressure of the fluid plays a crucial role in identifying the force exerted perpendicular to the cell boundaries. This relationship adheres to Pascal's law, which stipulates that within a confined fluid at rest, alterations in pressure are uniformly transmitted in all directions. Concomitantly, the tangential load arises from shear stress induced by fluid pressure on the cell boundaries. Shear stress materializes when adjacent layers of fluid undergo sliding motion relative to each other. The magnitude of shear stress is intricately linked to fluid viscosity and the rate of deformation.

From a mathematical standpoint, the aforementioned value is computed by dividing the result of Eq. (10) by the total area unit. The findings reveal that the initial temporal load magnitude falls within the interval of 0.2–1 N/m². This

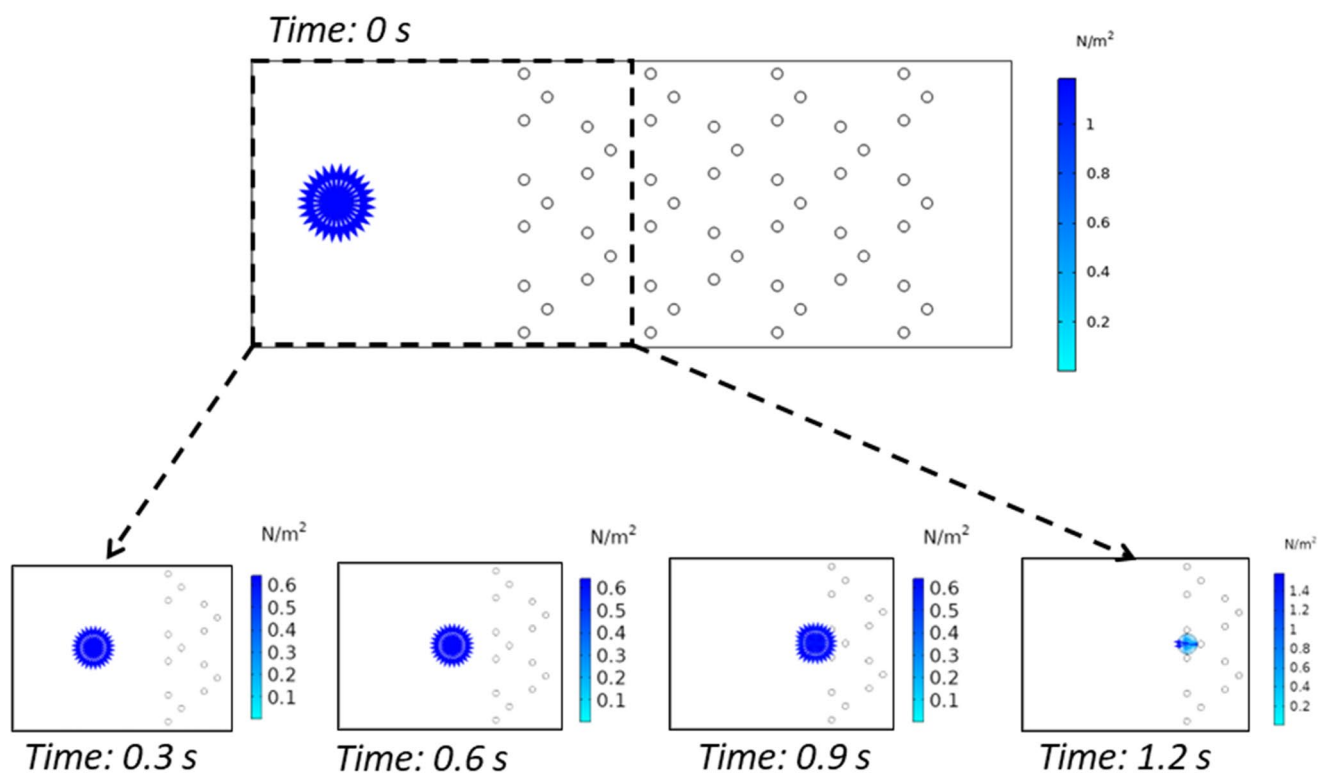


Fig. 9 Total load applied to the simulated cell

manifestation symbolizes the equilibrium influence across all spatial orientations of the the simulated cell structure. At temporal instances 0.3 s and 0.6 s, the boundary load remains in equilibrium across all orientations, albeit exhibiting a gradual reduction in magnitude, presently measuring approximately 0.2–0.6 N/m². Starting from 0.9 s, the value of the boundary load on the cell remains constant. However, a directional discrepancy becomes evident, with the arrow representing the outer edge of the cell (not in contact with the trap) displaying greater thickness compared to the arrow on the side of the cell facing the trap unit. Upon complete entrapment of cells within the trap unit, this value experiences a noteworthy augmentation, spanning within the range of 0–1.4 N/m². Nonetheless, this effect is infrequent and exhibits a pronounced bias towards one side, deviating from the direction of cell movement.

In summary, the total cell boundary load is the amalgamation of both normal and tangential loads. The specific relationship between fluid pressure and these loads hinges on the geometrical attributes of the cell, the orientation of its surfaces, and the rheological properties of the fluid, including factors such as viscosity. A comprehensive understanding of these considerations, coupled with the utilization of pertinent fluid dynamics equations, is indispensable for accurately describing this relationship within a given scenario.

4.3 Von mises stress and on the single-cell

The von Mises stress, often referred to as the von Mises yield criterion, holds significant importance in the fields of solid mechanics and materials science (Xiang et al. 2007). It serves as a valuable tool for quantifying the likelihood of yielding or failure in materials exposed to intricate stress conditions, encompassing both tensile and shear stresses (Budynas and Nisbett 2011).

Within the realm of solid-liquid interactions in microfluidics, particularly in biomedical electronics, the von Mises stress is notably pertinent. It pertains to the mechanical stresses experienced by simulated cell, here representing cells, as they traverse or interact within microfluidic channels and devices. This application is instrumental in understanding the potential mechanical deformation or injury to these sensitive biological entities. Given the inherent fragility of cells, they are highly susceptible to mechanical stresses, including shear forces encountered within microfluidic channels, which can result in deformation or damage. The assessment of von Mises stress stands as a critical determinant of whether these stresses remain within a range supportive of cellular viability (He et al. 2014), (Hartmann et al. 2006). In solid mechanics, Poisson's ratio and Young's modulus are crucial properties used in von Mises stress calculations. Poisson's ratio describes how a material responds to uniaxial stress by relating lateral contraction to axial

extension. Young's modulus represents the stiffness and capability to resist deformation of a material under axial loading. These properties collectively play a pivotal role in understanding how materials react to diverse stress conditions. The simulation results indicate that the von Mises stress impacts cells at different time intervals, specifically at $t=0$ s, $t=0.3$ s, $t=0.6$ s, $t=0.9$ s, and $t=1.2$ s.

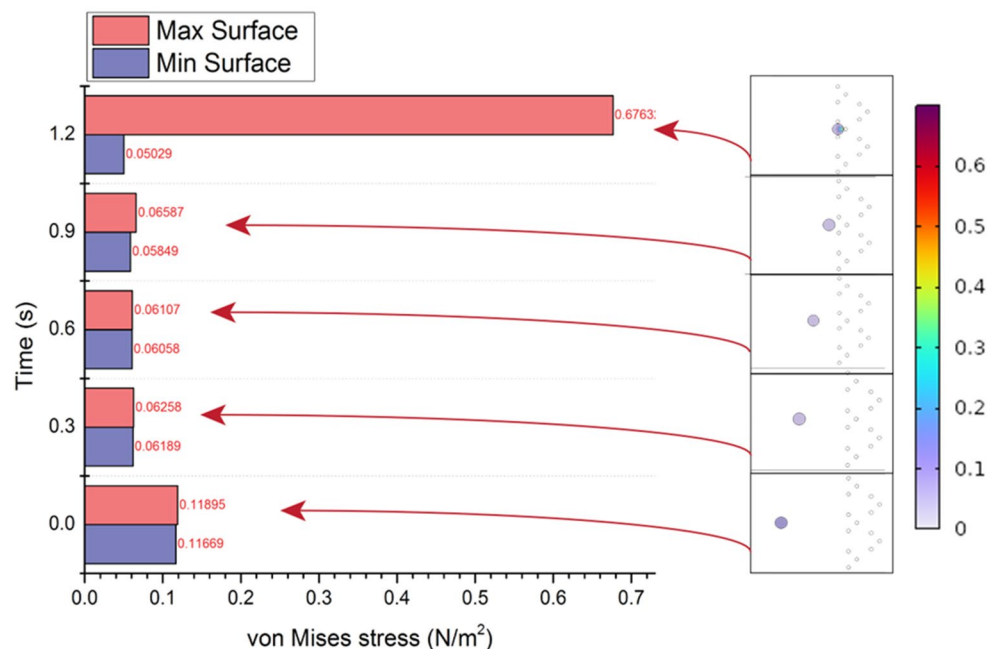
The surface results display the positions of maximum and minimum stress applied to the cell, visualized through varying levels of gradient color intensity (Fig. 10). It is evident that when the cell is not yet trapped within the trap unit, the von Mises stress is almost uniformly distributed throughout the cell and remains of relatively low magnitude. The variation between the highest and lowest stress values is very small. At the initial time, the maximum value reached 0.118 N/m^2 , the minimum value reached 0.116 N/m^2 , the difference is only about 0.002 N/m^2 . A consistent disparity persists at subsequent temporal intervals, specifically at 0.3s and 0.6s, indicating a nearly uniform distribution of von Mises stress throughout the entire cellular surface. As the cell initiates ingress into the trapping unit, a discernible escalation in the observed discrepancy becomes apparent. Specifically, at the temporal juncture of 0.9 s, the minimum von Mises stress value attains an approximate magnitude of 0.058 N/m^2 , while the corresponding maximum value registers at 0.065 N/m^2 , yielding a discernible difference of 0.007 N/m^2 . Subsequently, upon complete entrapment of the cell within the trapping unit at time $t=1.2$ s, the minimum von Mises stress value is recorded at 0.05 N/m^2 , with the maximum value surging to 0.676 N/m^2 , reflecting a ratio exceeding 13.5-fold. The rationale behind this substantial variance lies in the inherent

occurrence of cell-wall collisions as cells enter the trap unit and become ensnared. Moreover, throughout the progression of cell cultivation and maturation within the confines of the trap unit, collisions with the micropillars are an inevitable aspect of the dynamic. Nonetheless, it's crucial to note that hESCs have elasticity values within the range of 0–10 kPa ($1 \text{ Pa} = 1 \text{ N/m}^2$) (Kiss et al. 2011). This implies that even when the von Mises stress values are significantly higher at the time of impact, the results demonstrate that they remain within the permissible range for cell deformation. Because the interplay between a cell's elasticity and Von Mises stress is a critical determinant of its response to external forces (Tan et al. 2012). Elasticity, quantified by the modulus of elasticity, signifies a cell's ability to endure and recover from deformation. Von Mises stress, on the other hand, measures the material's stress under external forces. Consequently, these findings indicate that the results will not lead to hESCs deformation or damage. In general, the elasticity of cells ranges from 0 to 100 kPa (Alonso and Goldmann 2003). Since the proposed device is applicable to hESCs which have elasticity values within the range of 0–10 kPa, this microfluidic structure might be suitable to various cell types.

4.4 Comparison between three- and five-micropillar trap unit

After an in-depth analysis of the trapping and static cultivation capabilities of our initial three-pillar structure, an extended survey on a modified configuration featuring five pillars. Figure 11 compares velocity field distribution between three- and five-micropillar trap unit at time $t=1.2$ s

Fig. 10 Stress on the microsphere during the trapping process



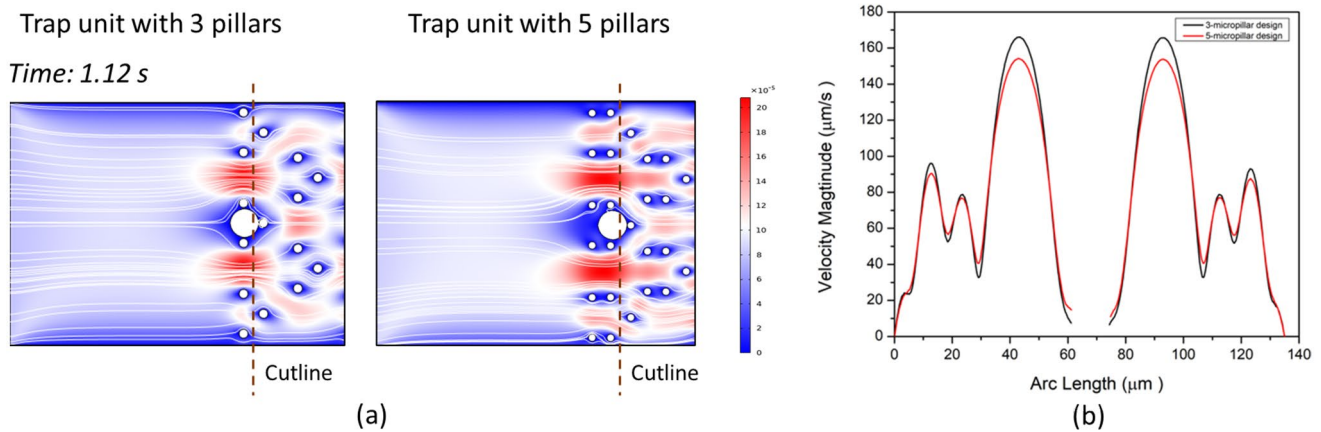
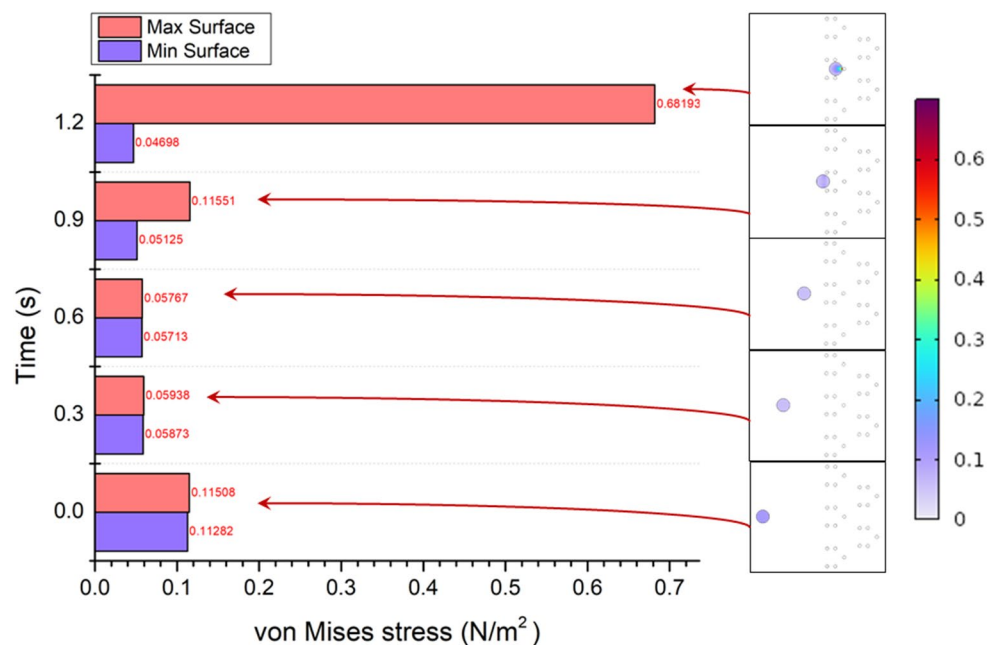


Fig. 11 (a) Velocity field distribution and (b) Velocity magnitude at the cutline of trap unit with three pillars and five pillars

Fig. 12 Stress on the simulated cell during the trapping process of the 5-micropillar structure



(single cell captured at trap). The results revealed that the zero-velocity trapping zone within the trap region of the new structure is significantly larger, indicating an expanded area for ensuring stability in both trapping and static cell cultivation.

Figure 11 shows the velocity magnitude along the cut line at time $t = 1.2$ s, corresponding to fluid velocity field around the trapped cell in the trap unit. In the 5-micropillar design, the fluid velocity in the area near the cell is approximately $10 \mu\text{m/s}$, representing a $4 \mu\text{m/s}$ increase over the velocity observed in the 3-micropillar design. This difference arises from heightened diffusion effects resulting from the greater number of pillar rows inside a trap unit, consequently resulting in a slight increase in flow rate. Figure 12 shows the von Mises stress on the cell at several time points ($t = 0, 0.3, 0.6, 0.9$, and 1.2 s) of the 5 micropillar structure. A comparative

analysis between structures composed of 3 and 5 micropillars reveals negligible differences in the von Mises stress levels, with the maximum stress observed at approximately 0.7 Pa across both configurations. The heightened velocity levels may promote improved nutrient transportation and cell interactions within the culture medium while remaining the stress value. Additionally, the velocity region surrounding the cell within the trap structure featuring 5 micropillars remains a region of low velocity. Notably, this region exhibits a significantly broader extent compared to the configuration with 3-micropillars, contributing to the enhanced stability of the cell culture zone.

5 Conclusions

This work proposes a biochip for single-cell trapping and long-term culture using a passive trapping method based on hydrodynamic principles. The trapping unit designed with micropillars was proved to ensure high-precision single-cell entrapment, interactions between cells and culture medium, stable but dynamic culture environment. The obtained results lay strong foundations for single-cell trapping mechanism understanding as well as further design, optimization, and experiments of the proposed biochip. Our design accommodates on-site cell cultivation, offering flexibility in experimental design. Furthermore, our structure aligns with the trend of dynamic cell cultivation, enhancing utility for high-throughput studies. The micropillars within each trap unit could potentially be fabricated from polydimethylsiloxane (PDMS), an elastomer known for its biocompatibility, exceptional optical, electrical, and mechanical properties, making it well-suited for a variety of biomedical applications. Its seamless integration into automated systems positions it at the forefront of advancements in automation within cell biology. Additionally, its characteristics make it a noteworthy candidate in the development of “Organ-on-a-Chip” technology, supporting both trapping and dynamic cultivation of cells. This multifaceted design, combining simplicity with versatility, stands as a promising tool in the evolving landscape of microfluidic technology and cell biology.

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Author contributions T.H.N, N.A.N.T, and L.D.Q conducted the literature review, ran the simulations, and analyzed the data. T.H.N, N.A.N.T, and H.B.T presented the data and wrote the main manuscript text. T.T.B, T.C.D, and L.D.Q reviewed, edited the manuscript, and supervised the project.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Quang Loc Do report financial support was provided by Vingroup Joint Stock Company, Thu Hang Nguyen report financial support was provided by Vingroup Innovation Foundation - VinIF. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRITICAL REVIEW

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Concepts, electrode configuration, characterization, and data analytics of electric and electrochemical microfluidic platforms: a review

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Microfluidic cytometry (MC) and electrical impedance spectroscopy (EIS) are two important techniques in biomedical engineering. Microfluidic cytometry has been utilized in various fields such as stem cell differentiation and cancer metastasis studies, and provides a simple, label-free, real-time method for characterizing and monitoring cellular fates. The impedance microdevice, including impedance flow cytometry (IFC) and electrical impedance spectroscopy (EIS), is integrated into MC systems. IFC measures the impedance of individual cells as they flow through a microfluidic device, while EIS measures impedance changes during binding events on electrode regions. There have been significant efforts to improve and optimize these devices for both basic research and clinical applications, based on the concepts, electrode configurations, and cell fates. This review outlines the theoretical concepts, electrode engineering, and data analytics of these devices, and highlights future directions for development.

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1. Introduction

The cellular biophysical properties of single cells play a critical role in disease diagnosis, including cancer metastasis^{1–4} and

other diseases, such as arthritis, asthma, elliptocytosis, malaria, sickle cell anemia, inflammation, and spherocytosis.⁵ Understanding the biophysiology of single stem cells also provides valuable insights into regenerative medicine.⁶ The measurement of cellular bioelectrical and biomechanical properties is vital for gaining a deeper understanding of abnormalities during cell growth.⁷ Because of the diverse nature of cell populations, single cell analysis has been extensively used to reveal cell-to-cell variation and heterogeneity of drug response in evaluations of the efficacy of a drug treatment or a therapy.⁸

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For example, the analysis of single cells provides the profile of genetic, epigenetic, proteomic, and lineage information of individual cells. To obtain cellular bioelectrical properties, impedance measurement platforms which analyze cells inside an electric field created by a frequency-dependent voltage are essential.⁹ Therefore, integrating high-throughput impedance analysis into the system can reveal relationships across cellular morphology and modalities, based on dielectric properties and internal components of cells, a fundamental component of impedance measurement.

Bioimpedance is a label-free, non-invasive technique for analyzing cellular biophysics. The biophysical characteristics of cells refer to cell surface mechanics and cell electrical properties that affect subcellular structures, cell deformability, cytoadherence, locomotion, and neoplastic transformation. In recent years, electric impedance flow cytometry has been widely integrated into microfluidic chips for studies of single cells using a Coulter counter.^{10–12} The Coulter counter detects an alteration of low frequency alternating current (AC) impedance signals in the presence of cells as they pass through the sensing area, enabling the recording of the complete cell morphology in a label-free, time-saving, and miniaturized format.

Before conducting experiments on bioparticles, several impedance sensing platforms have been investigated both numerically and experimentally using physical particles, mostly polystyrene. The use of physical particles provided insights into the effects of particle properties and channel geometries on the obtained signals, helping to calibrate and standardize the devices.¹³ For example, polystyrene particles with different diameters have been used to evaluate the sensitivity of impedance measurements to cell size.¹⁴ Other works also evaluated the dependence of impedance signals on particle positions within the microchannel, yielding valuable information for optimizing the device geometry.^{15,16}

Microfluidic integrated impedance technologies, such as impedance flow cytometry (IFC) and electrical impedance

spectroscopy (EIS), are label-free, high throughput methods for electrical measurement at a critical frequency range of objects as they pass through the sensing regions by applying AC voltage to patterned electrodes on the fluidic channel.

Novel electrode designs enhance the performance and technical resolution of microfluidic devices. Subsequently, data analysis allows for the collection of multiparametric features such as size, electrical properties, and mechanical properties of both cell populations and single cells.^{17,18} Interestingly, impedance-based techniques have various advantages, including real-time measurement, non-invasiveness, and rapid responses, and enable high throughput with large cell populations. Zhang *et al.*, for example, used a microchip to monitor single-cell impedance during the dynamic process of cell development and osteogenic differentiation of mesenchymal stem cells under electrical stimulation.¹⁹ As a result, impedance spectroscopy and imaging techniques have become essential tools for different types of cells and tissues. These techniques have improved with innovative fabrication methods and established theoretical models, enabling the studies of cell adhesion, growth, and differentiation. Furthermore, high-density microelectrode arrays (MEAs) also provide high spatially-resolved measurements with subcellular resolution in *ex vivo* brain slices.²⁰

The topic of single-cell impedance flow cytometry has been previously reviewed by Sun and Morgan, who summarized the basic principles from the early work to 2010.²¹ In 2015, Chen *et al.* presented the advancements in microfluidic impedance flow cytometry, including (i) improved sensitivity, (ii) accompanying optical systems for single-cell research, and (iii) portable platforms for point-of-care (PoC) systems.⁹ In 2017, Petchakup *et al.* focused on advancing impedance cytometry and its applications in the biomedical field.¹⁸ A review article in 2019 provides fascinating insights into the applications of impedance characterization of cells for cell adhesion and migration, and



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cancer cell and cytotoxicity screening.²² Honrado *et al.* recently concentrated on raw impedance signal analysis methods to obtain cell characterization and applications.¹⁷

Here, we present a procedure for whole cell impedance sensing. This includes the measurement of cell impedance, analysis of the processing of cell impedance signals, and applications, as shown in Fig. 1. Two techniques, IFC and EIS, mainly used for cell impedance measurement, are described in subsection 2.1. Three typical electrode designs (coplanar electrodes, parallel electrodes, and constriction channels) are illustrated in subsection 2.2. To obtain impedance data the signals can be directly analyzed through the waveform or indirectly using cell models, which are discussed in subsection 2.1: Theoretical concepts. The application of machine learning and other techniques for evaluating cell information is presented in section 4 and throughout section 3. Finally, the biomedical applications of electric and electrochemical microfluidic platforms including cell counting, single-cell profiling, cell layer analysis, and electrochemical biosensors are summarized in section 3. This review focuses on recently updated concepts, electrode configuration, cell characterization, and data analytics of impedance-sensing microfluidic platforms. Recent advances in robust designs and remarkable translations of miniaturized chip-based cytometry regarding portability, simplicity, and characterization of single cells in small populations are introduced.

2. Bioengineering approaches for cell analysis

2.1. Theoretical concepts

From the perspective of electrical engineering, cells can be modeled as either a single-shelled spherical model that rep-

resents the dielectric properties (permittivity, conductivity) of cells in suspension or an electrical circuit composed of a resistor and a capacitor connected in series, which corresponds to the cellular cytoplasm and membrane. To evaluate cell impedance, a frequency-dependent voltage is applied, and the electrical current response of the system containing the cell in suspension is measured. The electrical impedance of the whole system, $\tilde{Z}(j\omega)$, is defined as the ratio of the applied voltage, $\tilde{U}(j\omega)$, and current response, $\tilde{I}(j\omega)$.²³

$$\tilde{Z}(j\omega) = \frac{\tilde{U}(j\omega)}{\tilde{I}(j\omega)} \quad (1)$$

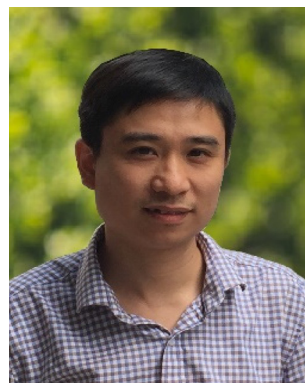
In the single-shelled model, as illustrated in Fig. 2A, the cell consists of the cytoplasm and a single shell with distinct dielectric properties. The dielectric properties of the whole cell suspended in a medium have been reported in a previous paper.²⁴ This model has been expanded to a multi-shell model to better represent the cellular structure.²⁵ In the equivalent circuit model, the cell membrane is modeled as a capacitor representing the phospholipid bilayer in parallel with a resistor representing the transmembrane ion transport proteins. The cell cytoplasm is modeled as a resistor and a capacitor in series. Fig. 2B shows this complete cell electrical model. In many cases, for simplicity, the electrical circuit model consists of a resistor in series with a capacitor that represents the cellular cytoplasm (R_i) and membrane (C_{mem}), while the cell cytoplasm capacitance (C'_i) and membrane resistance (R'_{mem}) are ignored. Thein *et al.* expanded this simplified model by using an area-contact model to investigate the single-cell covered circular surface-modified microelectrode array.²⁶ They determined the key sensing parameters, including the capacitance and resistance of the cell membrane and the averaged cell-substrate separation, to indicate the optimal frequency band. The experimental results were fitted to this model and pro-



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vided information about the electrical characteristics of cell elements and cell–substrate interactions. Another work used finite element methods to model the impedance signal of cell growth on electrodes and compared the results to a lumped equivalent circuit (Fig. 2D).²⁷ The model was visualized in three-dimensional space using FEMLAB.²⁷ The results indicated that the impedance value increased by at least 100% with cell coverage on a majority of the electrode surface. The authors also investigated impedance and phase changes *versus* frequency due to cell metabolism and biosynthesis in the cell growth process. The finite element method is applicable to arbitrary cell shapes, dimensions, and positions in the device, given the non-uniform electric field. Recently, Ablaihan and Chi proposed the first statistical model that suitably modeled the biological diversity among various cell types and bacteria based on membrane elasticity and cell motility mechanisms.²⁸ Fig. 2C depicts this statistical model, a more realistic three-dimensional representation of adherent cells that was proposed using a combination of multiparametric models with different electrical responses from multiple cells. This model was verified by EIS of a human cervical carcinoma cell line (HeLa) on an electrode array and showed good agreement with experimental results.

To measure cell impedance, EIS and IFC are the two most widely used techniques. Both EIS and IFC can be performed on cell populations or single cells to characterize their electrical properties. In principle, EIS measures the electrical properties of static cells over a wide range of frequencies in the electrode region. The full spectrum electrical characteristics provide insight into the properties of biological cells by fitting the cell electrical model. Impedance signals at low frequencies (no greater than 1 MHz) provide information about cell size, while at higher frequencies (on a MHz scale), they provide characteristics of the cell membrane. Very high frequencies reveal the cell cytoplasm or intracellular information.²⁹ Before conducting EIS, cells are usually trapped into the sensing electrodes by several methods such as hydrodynamic trapping,^{30–32} dielectrophoretic trapping,^{33–38} pressure trapping,^{39,40} and

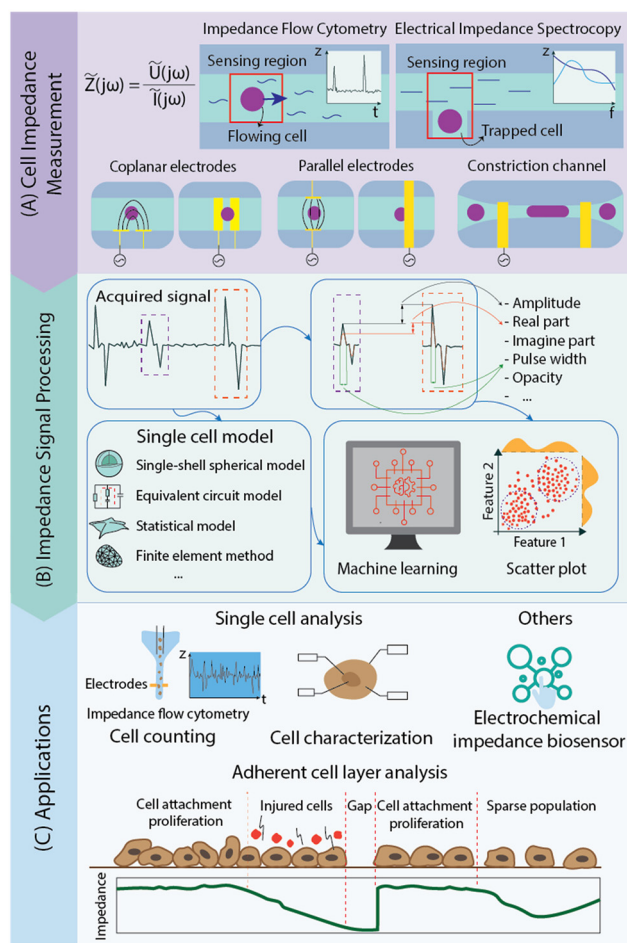


Fig. 1 Cell impedance sensing: from impedance measurement configuration, data analytics to applications; (A) cell impedance measurement by two primary methods (impedance flow cytometry and electrical impedance spectroscopy) and typical electrode configurations, (B) obtained impedance signal processing by directly extracting information from signals (amplitude, the real and imaginary parts of impedance, opacity, pulse width, etc.) or indirectly by applying a cell model and postprocessing, (C) cell impedance sensing applications including single cell analysis, adherent cell layer analysis and electrochemical impedance biosensors.



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surface-modified electrode trapping.²⁶ Hydrodynamic cell trapping, based on the path of least resistance concept in a serpentine microchannel, showed a cell capture rate of over 95%. Tang *et al.* used a homemade EIS for impedance measurement of human breast tumor cells (MCF-7) at frequencies between 1.953 kHz and 1 MHz.³² The impedance changes revealed the trapping process of MCF-7 and showed differences between different cell trapping conditions and sizes. Regarding rare cells, Jen's research group proposed a biochip that combined dielectrophoretic focusing and impedance measurement of a lung cancer cell line using circular electrodes,³³ which enabled cell enrichment with a capture rate of over 90%. The impedance analysis showed a highly linear relationship between the number of A549 cells on the trapping electrode and an increase in magnitude, making this device an ideal sensor for

Single cell model

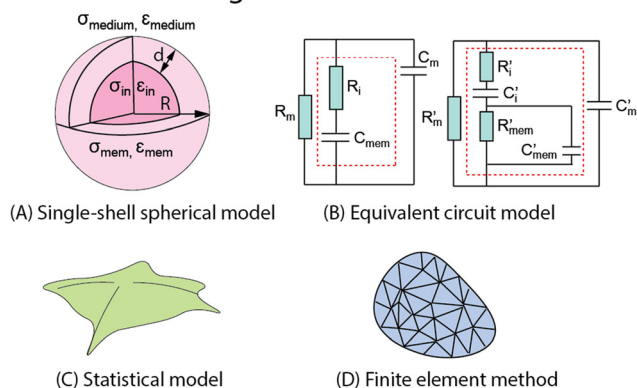


Fig. 2 Single cell models: (A) single-shell spherical model describing the dielectric properties of a cell in a suspension, (B) equivalent circuit model of a single cell in a suspension: (right) complete model, (left) simplified model containing cytoplasm resistor and membrane capacitor, (C) statistical model to model diversity in cellular responses, (D) finite element method used to model the cell impedance signal.

cell number detection with a detection limit of 3 cells at an optimal frequency range. The resolution of the devices relies on surface electrodes modified with materials, such as specific antibodies or aptamer conjugated-carbon nanotubes (CNTs) and gold nanoparticles (AuNPs), to capture cancer cells for impedance sensing. Recently, hybrid electrodes made of indium tin oxide (ITO) were modified by EpCAM antibody-coated CNT multilayers to conduct sensitive and specific sensing of cancer cells. The system was evaluated by challenging the device with a liver cancer cell line (HepG2) undergoing real-time electrical impedance with a HepG2 cell detection limit of 5 cells per mL of blood. As a result, the device can differentiate normal and tumor cells at early stages. Park *et al.* separated normal cells from cancer urothelial cell lines using both EIS over a range of frequencies and real-time EIS at the optimal frequency (119 kHz), where the EIS signals of the two cell types were most different.³⁹ To implement EIS, the microfluidic device contained negative pressure channels to trap cells, leading to tighter contact between captured cells and the sensing electrodes on the channel sidewall.

Impedance flow cytometry (IFC) measures and analyzes the electrical impedance of cells as they pass through sensing electrodes. The first IFC device, the Coulter counter, was introduced by Coulter in 1956 and consisted of two isolated electrical fluid chambers connected by an aperture.^{21,41} However, it had low resolution due to its large size. Currently, the Coulter counter has been miniaturized to increase resolution, and is used in microfluidic technologies such as point-of-care (PoC) systems. For example, Satake *et al.* introduced a micro-fabricated sensor-based Coulter counter using MEMS technology for cell counting.⁴² The electrodes were arranged on both sides of a center aperture to evaluate the resolution of polystyrene latex particles. In 2007, Wood *et al.* made improvements in electrode geometry and electronic tuning of the reflectometer

for signal-to-noise ratio (SNR) enhancement, resulting in a throughput of approximately 5000 beads per s in the micro-Coulter device.⁴³ Likewise, other works performed IFC in microfluidic devices at multiple frequencies to better characterize cells.^{44–46} However, a major drawback of IFC is its limited sensitivity due to the variation of particle position in the sensing area. This is because the different cell positions of cells as they pass through the sensing region correspond to the different impedance signals.^{47,48} The limited sensitivity can be explained through theoretical simulations. Spencer and Morgan conducted numerical investigations (finite element method) and experiments to study the positional dependence of cells in IFC devices.¹⁶ The results showed that the impedance signal can be doubled when the “test” particle flows close to the electrodes because of the stronger electric field at the electrode edges. More interestingly, the impedance magnitude is higher when the particle is passing near the sensing electrodes compared to the particle passing close to reference electrodes. It can be explained by analyzing the direction of current flow through the differential parallel electrodes in microelectrode systems. Although small microchannels can address the issue of impedance signal positional dependence of particles in microfluidic systems, they may simultaneously cause channel clogging. The problem can be addressed through focusing or focusing-free methods.⁴⁹ The principal of focusing methods relies on the sheath, inertial, or dielectrophoretic hydrodynamics to manipulate particles on sensing electrodes.⁴⁹ First, the hydrodynamic sheath flow focusing technique uses an additional fluid stream with high pressure to concentrate the particles into a single stream. Hydrodynamic focusing can be two-dimensional (2D)¹⁴ or three-dimensional (3D).^{50,51} The position of particles is precisely controlled by the velocity ratio between the sheath flow and the cell flow. In this focusing structure, the sheath fluid is required to not disturb the investigated particles. Another technique, inertial focusing, uses hydrodynamic inertial lift forces in a specific fluid regime to drive particles with the same diameter to the equilibrium position in the microchannel. The position of the particles, under the effects of inertial force, changes to equilibrium positions which are dependent on the channel geometry and cell sizes.^{52,53} Inertial focusing is a passive technique that allows for particles to be focused into a single stream without the use of external forces. This makes it a simple and high throughput.⁴⁹ However, it requires an appropriate channel length for particle focusing to occur. Dielectrophoresis, on the other hand, uses a non-homogeneous electric field to manipulate dipole particles.^{54–56} By integrating electrodes into the fluidic channel to generate this field, dielectrophoresis allows for control over the position and movement of particles. In addition to the flow-focusing approach, the focusing-free methods can enhance the sensitivity of IFC devices by improving the uniformity of the electric field through an optimal electrode. A study conducted by Sun *et al.* compared the electric field distribution and sensitivity of two common electrode designs – coplanar and parallel configurations – through numerical analysis.⁵⁷ The results indi-

cated that the coplanar electrode configuration had lower sensitivity due to its more confined uniform electric field.

2.2. Electrode engineering

In this review, we mainly focus on three common electrode configurations: the coplanar design, the facing design, and the constricted channel design. The coplanar electrode design was introduced by Gawad's research group in 2001 for cell counting and separation in diagnostic applications.⁵⁸ This design consists of two-dimensional electrodes placed on the top or bottom of a microfluidic channel. When an AC voltage is applied between the electrodes, a non-homogeneous electric field is generated. Coplanar electrode configurations are widely used due to their easy, low-cost, and reproducible fabrication process, as well as their miniaturization.

The fabrication process of coplanar electrodes only requires a single alignment step to secure the appropriate positions of the microelectrode within the channel. However, the resulting impedance signal can be significantly affected by the location of objectives in the microchannel due to the non-uniform electric field in the sensing region. Additionally, this design often causes high background noise and uneven drift.⁵⁹ As a result, the main drawbacks of the coplanar electrode design are its

position dependence and low sensitivity. The sensitivity of coplanar electrodes can be improved by optimizing the geometrical parameters of the microchannel. A recent study presented a comprehensive study on the design of coplanar electrodes for impedance sensing using both simulation and experiment methods.⁶⁰ The results showed that a short, optimized design (SO) greatly enhances the signal amplitude for centered cells, while a long, optimized design (LO) reduces dependence on particle height. However, this design has a high background noise, which can be reduced by using a three-electrode design for differential impedance measurements. The AC voltage is applied to the working electrode, and the two side electrodes serve as reference electrodes. When an object flows through the sensing region, the impedance characteristics of an object are calculated by taking the differential between the two impedance signals. Fig. 3A illustrates the conceptual design, fabricated device, measurement setup, and data analytics for a typical microfluidic device integrating coplanar electrodes for microalgal cell health assessment.⁶¹ Furthermore, a three-electrode design has been applied to various applications, including analysis of cell size,⁵⁸ characterization of the human lymphoma cell death mechanism,⁶² profiles of T-lymphocyte activation,⁶³ and investigation of

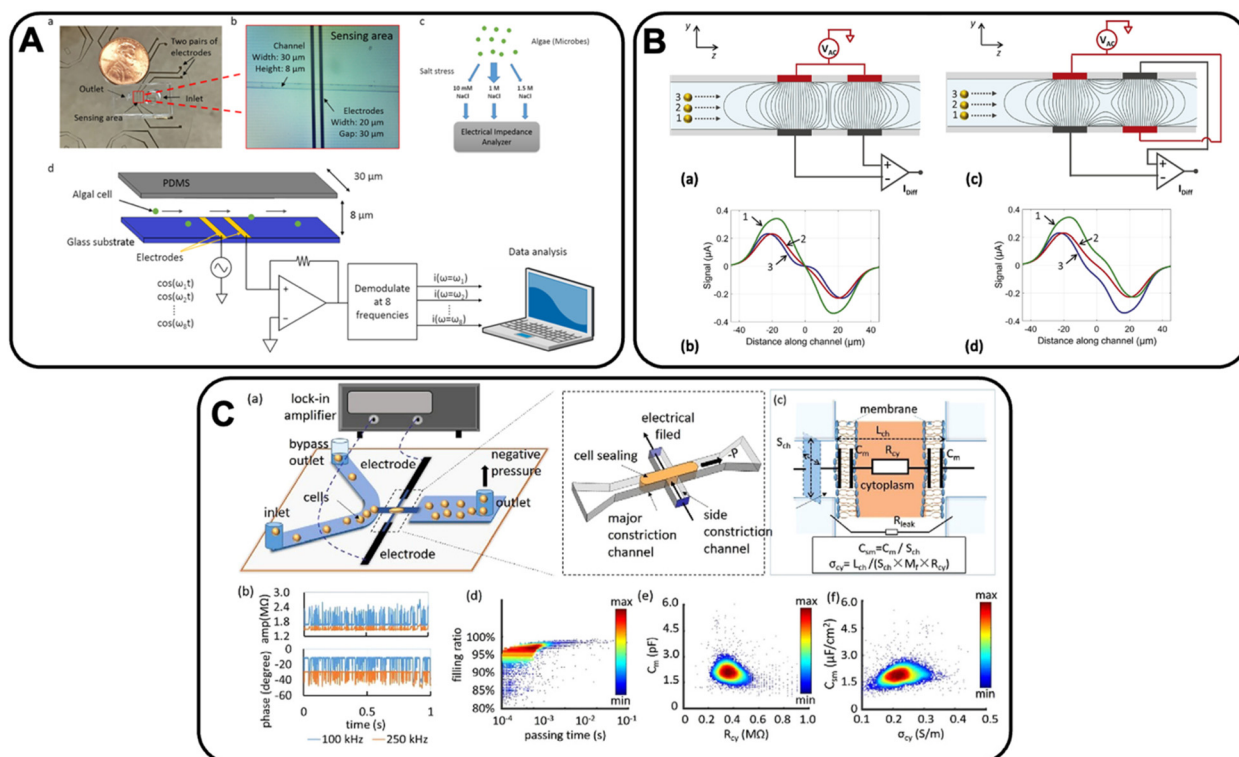


Fig. 3 Three typical electrode designs: (A) coplanar electrode design used in the algal cell phenotype and health sensing: (a) fabricated microfluidic device by the soft-lithography technique, (b) coplanar electrode design, (c) experiment plan, (d) electrical impedance measurement setup and data analysis;⁶¹ (B) (a) and (b) typical differential parallel electrode design generating a differential impedance signal, (c) and (d) a novel wiring scheme to enhance system sensitivity without using the particle focusing technique.⁷⁰ Reprinted from ref. 70, Copyright 2023, with permission from Elsevier; (C) constriction channel applied in the characterization of dielectric properties of different cell types: (a) microfluidic channel design and operation, and cell deformation through the constriction channel, (b) acquired electrical signals, (c) cell equivalent circuit model, and (d–f) scatter plot for cell property characterization. Reproduced with permission from ref. 80. Reprinted from ref. 80, Copyright 2023, with permission from Elsevier.

paclitaxel-treated HeLa cells.⁶⁴ While the three-electrode design significantly improves the sensitivity of impedance measurement, it also doubles differential electrodes with two-step signal differentiation. This allows for high throughput impedance cytometry, even for submicron-precision particle characterization. The two stages of differentiation enhance the signal-to-noise ratio (SNR) by first canceling out phase noise, and then removing electrical noise from the medium and intrinsic noise. The results showed that the SNR increased from 13.98 dB to 32.64 dB compared to the traditional differential configuration, and the limit of detection (LoD) decreased to a biological particle diameter of 0.4 μm .⁶⁵ Additionally, the proposed design eliminates the electrical double-layer effect of traditional differential coplanar electrodes.

The design of parallel electrodes was first introduced by Gawad and his colleagues in 2004.⁴⁷ In this design, the electrodes are aligned at the top and bottom of the microfluidic channel.^{47,66–70} This configuration aims to create a more uniform electric field than coplanar electrodes, thus enhancing the sensitivity of the system.⁵⁷ However, this design also has the problem of particle position dependence in the channel.¹⁶ Although parallel electrodes have advantages such as higher sensitivity and high throughput, their fabrication process is more complex than that of coplanar electrodes. It requires precise patterning of electrodes onto two substrates and precise alignment between the microchannel and the electrode layer.⁵⁹ As a result, parallel electrodes are not as widely used as coplanar electrodes, and it is essential to propose and develop a simple fabrication procedure.

This electrode design can also be improved by using a pair of parallel electrodes to reduce the background noise and analyze biological samples, as shown in Fig. 3B. For example, differential facing electrodes were integrated into a microfluidic device for PoC blood cell analysis.⁷¹ Other works have utilized two pairs of parallel electrodes for differential measurements, such as the analysis of parasitic protozoa,⁶⁹ differentiation of RBCs and ghosts, and RBCs and fixed RBCs,⁷² and assessment of the viability state of *E. coli*,⁷³ all with traditional impedance flow cytometry. This technique has only allowed for cell characterization at one or two simultaneous frequencies. To overcome this limitation, Morgan and Spencer proposed a novel multi-planar electrode design for morphological discrimination of non-spherical cells and bioparticles using multiple frequency impedance measurements in a narrow microchannel region, referred to as a microfluidic constriction channel system.⁷⁴

The constriction channel design refers to an impedance flow cytometry system containing a narrow region, a relaxing region, and a pair of electrodes.^{75–79} The narrow region has dimensions smaller than that of cells. Fig. 3C shows the design, cell equivalent circuit model, electrical signal, and scatter plots for cell dielectric property measurement.⁸⁰ The electric field inside this region changes, causing an increase in impedance signal magnitude. Due to the narrowed sensing region, the constriction channel also has the advantage of self-

focusing, which has been used to measure single-cell cortical tension, specific membrane capacitance, and cytoplasm conductivity.⁸¹ The reduced variation in cell positions enhances the sensing sensitivity and enables continuous characterization, leading to the determination of cellular physiological and pathological states. Although the constriction channel has the disadvantage of clogging and low throughput compared to other electrode design, it still has a potential application in cell characterization, which will be discussed in detail in the next sections.

3. Applications

3.1. Suspended cell analysis

3.1.1. Cell counting. Impedance analysis, a powerful tool for detecting and quantifying targeted cells, is a promising alternative to fluorescence counting. It provides a compact and fully integrated system for PoC applications. The impedance-based method has proved to be effective for counting various cell types, including white blood cells,^{50,71,82,83} red blood cells,^{84,85} and tumor cells.^{86–88} In recent years, it has been widely used for cell enumeration, even at low concentrations in a microfluidic chip. With the advantages of multifunction integration, targeted cells are commonly pre-concentrated in microfluidic chips using labeled techniques such as immunoassay methods or label-free techniques like dielectrophoretic enrichment, magnetophoretic manipulation, and inertial focusing before quantification.^{89,90} IFC has advanced to the point where it can analyze and count thousands of particles per second, comparable to the throughput of fluorescence-activated cell sorting (FACS), the ‘gold-standard’ method for cell sorting and quantification. While FACS requires highly trained personnel and is more expensive with frequent re-alignment of optical instruments,⁹¹ the IFC instrument is small-volume, portable, low-cost, and fully integrated, and also provides information about the cell’s mechanical properties due to the cell deformation in a micro-constriction channel. IFC can also be integrated into other functional platforms such as cell isolation and enrichment platforms, making it useful for rare cell counting where FACS fails to detect cells in low quantities.⁹² However, the main drawback of IFC is its limited capabilities of cell type detection compared to FACS which can characterize various cell types in mixed populations by using different cellular markers.⁹¹

Circulating tumor cells (CTCs) are detected and quantified by impedance analysis, CTCs are rare in blood samples, with numbers usually ranging from 1 to 10 cells per mL of blood, while other cell types such as white blood cells, red blood cells, and platelets are present at concentrations of $5\text{--}10 \times 10^6 \text{ mL}^{-1}$, $5\text{--}9 \times 10^9 \text{ mL}^{-1}$, and $2.5\text{--}4 \times 10^8 \text{ mL}^{-1}$, respectively.⁹³ Despite the very low number of CTCs in blood, their physical and biomechanical properties are distinct from other blood cells.⁹⁴ To improve the detection of CTCs, techniques for CTC enrichment have been developed, including microfiltration, hydrodynamics, and dielectrophoresis. In addition, fabrication

and signal processing techniques to maintain impedance analysis as an affordable, time-saving, and highly sensitive tool for CTCs are also developed.⁹⁵ Impedance cytometry is a label-free method that allows for the post-analysis of target cells without any damage. For example, a recent work used constriction channels to detect and count different tumor cells (breast and prostate) in murine blood samples.⁹⁶ The results showed high accuracy, even when using off-chip electrodes, which has the potential to lower the cost of the device. Other works used IFC to quantify a low number of spiking MCF7 cells in whole blood (1:1000 leukocytes) by erythrocyte removal and cell enrichment with Ep-CAM antibodies.⁸⁶ The sensitivity of the method was 100 MCF7 cells in 1 mL of whole blood.⁸⁶ For other cell types, a rapid, portable, and fully automated platform for counting CD4⁺ T cells in blood samples has also been developed, utilizing electrical impedance spectroscopy and magnetic beads coated with antibodies. The proposed microfluidic chip integrates a part of cell lysis, immunoassay, and impedance sensing on a single chip, enabling CD4⁺ T cell quantification with a limit of 25 cells per μL within only 5 minutes.⁹⁷

In recent years, the development of integrated circuit technology and fabrication techniques have facilitated miniaturized and optimized cell counting systems based on impedance sensing. The proposed systems aim to be fully integrated, low-cost, and user-friendly for the commercialization of PoC health monitoring and diagnosis. The Abbas research group introduced a wearable impedance cytometry platform in the integrated form of a flexible circuit wristband that integrates a polydimethylsiloxane (PDMS) microfluidic chip and a microcontroller for live smartphone readout.⁹⁸ The device showed high accuracy in blood cell counts from a pin-prick blood sample injected into the biochip with a coplanar electrode pair.⁹⁸ The Jen research group proposed a handheld electronic module integrated into a microfluidic chip for counting instead of using a traditional impedance analyser; they used a miniaturized, cost-effective handheld printed circuit board (PCB) module for DEP force generation and impedance sensing, for human lung cancer cell (A549 and MRC-5) count and characterization after DEP enrichment.^{34,35} The above-mentioned works mostly use embedded electrodes, which requires a complicated fabrication process and reuse. To overcome these drawbacks, the Muhammad group proposed a flow cytometry system with integrated microneedles for simple operation, disposable needles, and low-cost cell detection.⁹⁹ The commercial Tungsten needles used as measurement electrodes can be reused and easily washed by ultrasonic cleaning.⁹⁹ This work provided a low-cost, reusable device that maintained the core function of an impedance-sensing device. Sobahi *et al.* proposed a cell counting platform that integrated multiple-outlet counting using only a single pair of staircase-shaped planar electrodes,¹⁰⁰ enabling particle position detection and multiple cell-type quantifications after cell separation into multiple outlets.

3.1.2. Cell characterization. The early work on cell size measurement based on impedance was implemented in 1956

when the Coulter counter measured the DC resistance change caused by particle movement through a small aperture connecting two electric chambers. The magnitude of the response signal indicated the cell size depended on the amount of fluid displaced. In recent years, many research groups have analyzed low frequency impedance signals for cell size estimation either directly through the signal amplitude or indirectly using reference particles.¹⁰¹ Cell size is an important biophysical indicator for cell type discrimination. For example, the measured CTC diameter commonly ranges from 15 to 25 μm , a significantly higher value than that of white blood cells (WBCs), red blood cells (RBCs), and lymphocytes which are from 5 to 13 μm , 8.7 to 9.9 μm , and 7.1 to 10.5 μm , respectively.¹⁰² Recent work reported on impedance cytometry for high throughput cell enumeration, using cell size distributions.⁵³ The results showed a linear relationship between the cell size and impedance signal magnitude, with impedance peaks following a Gaussian distribution with a low coefficient of variation. MCF-7 cells and WBCs were successfully discriminated using an amplitude threshold and enumerated with a throughput of approximately 5000 cells per second.

Besides cell size, the cell shape is also a useful marker of cell states such as division and mitosis.¹⁰³ Shaker *et al.* proposed an IFC device capable of discriminating spherical and elongated particles by tracking shape changes during cell division through two-dimensional sensing with two pairs of liquid electrodes in an orthogonal configuration.¹⁰⁴ Another work differentiated the yeast shape based on the pulse width of the impedance signal in an IFC device.¹⁰⁵ Discrimination of early and late budded and unbudded yeast cells was performed using the pulse width, magnitude, and proportion of width and amplitude of impedance signals.

Several recent reports have shown cell-type classification based on electrical characteristics.^{106–108} Each cell has a cell membrane, a phospholipid bilayer that corresponds to the membrane capacitance, transmembrane ion channels, and cell cytoplasm corresponding to resistance in an electrical model.¹⁰⁹ The cell membrane potential (voltage across the membrane), which is associated with the presence of different ion channels, has been proven to be an important clinical marker for cancer detection and for monitoring cancer cell proliferation and migration.¹¹⁰ Previous studies have shown that breast cancer cell lines exhibit specific dielectric signatures compared to other cancer and healthy cell lines.¹¹¹ Additionally, the dielectric properties, membrane capacitance, and cytoplasm conductivity of cancer cells have been linked to tumorigenic and metastatic potential.^{109,112} To calculate the dielectric properties of a cell from the impedance signals, the signal processing of raw impedance signals starts with the signal conditioning step to acquire electrical signals at single or multiple frequencies and to improve the signal quality using denoising techniques. Next, the electrical pulses that correspond to cell transitions through the sensing region are selected, segmented, and analyzed. The cell dielectric properties are acquired by fitting numerical and analysis models as described in the previous section.²¹ By using these models,

impedance signals can provide information about dielectric properties.^{80,107,113–115}

Impedance spectroscopy enables classifying cells by analyzing the waveform of the received electric signal directly or indirectly determining dielectric properties through numerical models. The resistance change magnitude and percentage of impedance signals at different frequencies are widely used for cell differentiation as the impedance magnitude is linked to cell size. Other factors for cell discrimination can be calculated from these basic parameters, including opacity. Opacity, which is the ratio between the impedance amplitude at two different frequencies, normally a high frequency and a low frequency, is often used as it is considered insensitive to changes in the cell size and position in the sensing region.^{18,47,72,116} This parameter has been confirmed to have a similar opacity spectrum for cells with the same dielectric properties.⁷² It has been utilized in the classification of different cancer cell lines (MCF-7, A549, HeLa, HL 60, GM 12878),¹¹⁷ and in the differentiation between lymphocytes, granulocytes and monocytes.⁸³ The use

of these parameters in cell discrimination is summarized in Table 1.

Mechanical properties provide information about the biochemical and pathophysiological states of cells in diseases.^{118,119} Cancer cells often alter their cytoskeletal structures, making them softer and more deformable than healthy cells, which allows them to detach, move, and invade tissue during metastasis. These alterations in cell stiffness can be measured using a constriction channel that reflects its deformability and surface friction.¹⁰⁹ Previous works have reported that cancer cells with high metastatic potential are much softer and more deformable than benign cells due to complex cell receptors and membrane proteins.¹⁰² The mechanical properties of cells are usually obtained in IFC systems by using constriction channels where the cell is bigger than the narrow detection region. This requires the cell to deform and fill the constricted region as it passes through, the electrode region, allowing its mechanics to be characterized by measuring the time it takes the cell to travel through the channel.^{75,120–122}

Table 1 Applications of impedance sensing for cell type classification

Cell type	Result	Impedance sensing technique			Data analysis		
		Method	Electrode design	Frequency	Properties	Method	Ref.
H1299, HeLa, A549	H1299 and HeLa cell discrimination with a success rate of 90.9% A549 after epithelial–mesenchymal transitions discrimination with a success rate of 76.5%	IFC	Constriction channel, cell populations	100 kHz, 250 kHz	Specific membrane capacitance, cytoplasm conductivity	Neural network	80
Hepatic, oral, and lung cancer cells	Detection of the EpCAM-positive CTCs and classification of the EpCAM-positive CTCs of hepatic, oral, and lung cancer types	IFC	Constriction channel	1 kHz, 100 kHz	Specific membrane capacitance, cytoplasm conductivity	Scatter plot	78
MCF-7 cells and WBCs	Successful discrimination between MCF-7 and WBCs	IFC	Liquid electrodes	No information	Peak magnitude of the impedance signal	Threshold amplitude	53
Normal (SV-HUC-1) and cancer (TCCSUP) human urothelial cell lines	Effective differentiation between SV-HUC-1 and TCCSUP	EIS	Parallel electrodes	10, 50, 100, 500 kHz, and 1 MHz	Amplitude and the phase angle of the electric signal, membrane capacitance, and cytoplasm resistance		106
Cancer cells (lung, ovarian, thyroid, kidney and breast cancer) and normal cells	Discrimination of human-derived, dissociated cancer cells and red RBCs, peripheral blood mononucleated cells (PBMCs) in a sample	EIS	Differential coplanar	200 kHz–250 kHz	Peak-to-peak voltage	Scatter plot	152
MCF7, A549, and WBC	Differentiation of MCF7 cells with an accuracy of 95% and differentiation of A549 cells with an accuracy of 92.3%	IFC	Differential coplanar	500 kHz and 2 MHz	Diameter, opacity, and phase shift	Machine learning	125
8 tumor cell lines (A549, HepG2, SW620, AGS, PANC-1, HeLa, CAL 27, and HL-60), tumor cell subtypes of 293 T, patient tumor cells	Discrimination of: (i) eight tumor cell lines with success rates of 87.6% $\pm$ 9.1%; (ii) tumor cell subtypes of 293 T; and (iii) patient cancer cells with success rates of 96.1% $\pm$ 3.8%	IFC	A main and two lateral constriction channels	100 kHz and 250 kHz	Membrane capacitance, cytoplasm conductivity, and cell size	Scatter plot	107

This passage time can be divided into two mainframes: the entry time and the transit time, corresponding to the deformability of cells and surface friction, respectively.¹²⁰ Adamo *et al.* indicated that stiffer cells have longer transit times, and the travel time strongly depends on the cell size.¹²³ Recent work has provided both the electrical and mechanical properties of MCF-7 cells with a throughput of more than 1000 cells per minute by using a constriction channel and four pairs of coplanar electrodes.¹²⁰ The passage times of red blood cells (RBCs), MCF-7 cells, PMA-modified MCF-7 cells, and fixed MCF-7 cells were analyzed, with the RBCs having the shortest time due to their smaller sizes. PMA-modified MCF-7 is more deformable and has less surface friction compared to MCF-7 cells, resulting in a shorter entry and transit time. On the other hand, fixed MCF-7 cells have cross-linked proteins which makes them less deformable, thus increasing their passage time through the constriction channel. The mechanical characterization is combined with electrical characterization to enhance cell-type discrimination. To clearly show the different properties of various cell types, a scatter plot is a useful representation and is widely used in many articles. In recent years, thanks to significant advances in computer hardware and software algorithms, machine learning has become an innovative and powerful technique for analyzing impedance.^{80,124,125}

The impedance analysis enables the assessment of cell viability and detection of phenotypic changes under specific conditions at single-cell resolution, especially for measuring the efficacy of drugs or cytotoxins,^{113,126–132} and predicting the patient response. This technique does not require staining or labeling and enables subsequent analysis. This also allows for simple and continuous tracking of the drug effects on cells, outperforming the current gold standard ‘snap-shot’ technique.¹³⁰ Impedance spectroscopy can be studied using equivalent circuit models, single or multiple-frequency measurements, and opacity ratio measurements.¹¹³ Several recent works have used cell index (CI) values for impedance analysis as the proportion of the change in cell impedance to the impedance value without the cell. The CI value increases with cell numbers and reflects a correlation between cell types, drug effects, and different types of drugs.^{131,133} The higher drug concentrations have a higher influence on the CI value.

Impedance analysis at different frequencies revealed slight cellular changes, such as cell–cell interactions and severe cell damage, during a toxicity test.¹³⁴ A higher concentration of the toxin Acetaminophen (AP) caused a decline in impedance over time.¹²⁶ The results also showed the different dielectric properties of HepG2/C3A cells after they recovered from AP treatment. A recent work presented a valuable method for evaluating bacterial antibiotic susceptibility using electrical resistance measurements.¹³⁵ The device consisted of a set of microfluidic channels, each with nanoconstriction to trap the bacteria. The bacteria were then incubated in drugs, and the electrical resistance of the microchannel corresponding to the number of bacteria was monitored. Interestingly, the electrical signals not only revealed the growth and death of bacteria but also the

morphological changes during an antibiotic susceptibility test. Ostermann *et al.* presented the use of IFC for nanotoxicity screening. Necrotic and viable human lymphoma cells (U937) were distinguished by IFC measurement, using an optimized signal trigger level, frequency (6 MHz for membrane permeability assessment), and buffer solution (sucrose-based). With these optimal parameters, the results, presented as density scatter plots of the signal phase and amplitude, clearly differentiated between viable and necrotic cell populations at different time intervals after treatment.¹²⁸ The application of impedance sensing techniques in viability and cell status assessment is presented in Table 2.

3.2. Adherent cell layer analysis

Electric cell substrate impedance sensing (ECIS) measures the electrical properties of adherent cell layers growing on electrodes in culture media. The presence of cells with their insulating membrane on the electrode surface leads to a change in impedance. Fig. 4 illustrates the characteristics and behavior of the cell layer, such as cell attachment and spreading, proliferation, cell invasion and migration, cell viability, and responses to stimuli, which can be continuously monitored during the culture time.

Szulcek *et al.* thoroughly described the use of ECIS measurement to study cell attachment, spreading, proliferation, quantification of the cell barrier function, motility, cell–cell and cell–substrate interactions, and cell behavior responses to stimuli.¹³⁶ It was found that the cellular behavior corresponding to electrode coverage can be best observed by using a capacitance value at a frequency greater than 40 kHz. The differentiation between cell attachment, spreading, and proliferation is achieved through a combination of impedance and capacitance values. Cell–cell interactions blocking the current flow are measured by resistance. Continuous impedance measurements have also been successful in monitoring cell migration after electric wounding and thrombin activity. Rapier *et al.* determined the position and the movements of cells in cell culture by using numerous electrode pairs along the channel length in real-time.¹³⁷ The impedance signals revealed the cell confluency levels in the sensing region and evaluated the number of cells inside the device.¹³⁷ Regarding cell migration analysis, ECIS techniques allow for conducting labor-saving, non-invasive, continuous, and real-time measurements. There have been many attempts to conduct wound-healing assays using ECIS techniques to evaluate cell migration.¹³⁸ The cell layers on electrodes were initially dead due to severe electroporation. Afterward, ECIS was used to monitor the migration of healthy cells for wound healing, revealing a noticeable resistance drop (corresponding to cell death) followed by a return to the control level. The wound healing curves are mostly influenced by the cell types. Regarding cancer cell invasion, ECIS has been successfully used to assess the invasion process, and the interaction between the cancer cells and underlying adherent cells on electrodes. A recent study has involved the use of ECIS to study ovarian cancer cell invasion through a monolayer of human

Table 2 Application of impedance sensing for cell assessment

Target cells	Treatment	Results	Impedance sensing technique			Assessment technique		Ref.
			Method	Electrode design	Frequency	Properties	Method	
Human hepatoma cells (HepG2)	Anti-cancer drug (ovarian/breast anti-cancer drug; broad-spectrum anti-cancer drug; liver anti-cancer drug)	3D electric cell/matrigel-substrate impedance sensing (ECMIS) matched excellently with the conventional imaging method	ECMIS sensing + 3D cell culture	Parallel electrodes	100 Hz–100 kHz	CI value		131
Human gastric cancer cells (SGC-7901)	Cell dead	The correlation index increased by 2.4%, and the maximum error of the area-normalized impedance spectrum (ANIS) method was 4% lower than that of the impedance sensing method	Electric cell substrate impedance sensing (ECIS), ANIS	Cell culture on the electrode array	1 kHz–100 kHz	Normalized area of the impedance spectrum		153
HepG2/C3A cells	Acetaminophen (AP)	Minor cellular changes could be detected long before cellular death	EIS	Bipolar interdigitated electrode (IDE) pair	100 Hz–3 MHz	Normalized impedance magnitude and phase of the impedance signal		126
Human lymphoma cells (U937)	Nanomaterials at different concentrations	Clear discrimination between viable and necrotic cells	IFC	Parallel electrodes	6 MHz	Amplitude and phase of the impedance signal	Scatter plot	128
Metastatic prostate cancer cell (PC-3)	Enzalutamide drug	PC-3 cells experiencing death express a distinguishable spectrum from the healthy cells	EIS	Cell culture on the electrode	10 kHz–40 MHz	Amplitude, phase of impedance signal, membrane capacitance, cytoplasmic resistance, and opacity ratio	Principal component analysis	113
Colon cancer cells	Doxorubicin with different concentrations	Wild type (chemo-sensitive) and doxorubicin-resistant of HT-29 cells exhibit different impedance response	Electric cell substrate impedance sensing	Cell culture on interdigitated electrodes	No information	Normalized impedance and phase variation of the impedance signal		154
HeLa cancer cells	Cisplatin	Impedance sensors successfully access the viability of cells with different cisplatin concentrations	Temperature/impedance integrated bio-sensors	Cell culture on electrodes	150 kHz	Temperature and capacitance values of cells		129

umbilical vein endothelial cells.¹³⁹ The invasion process resulted in a rapid drop in electrical resistance for *trans*-endothelial cells. In addition, the cellular biophysical properties were changed due to the invasion of the cancer cells, such as junctional resistance and the average separation between the cell and the substrate. Micromotion could also be tracked by analyzing various modes of frequency.

ECIS can also be used to study cell junctions. In a recent study, stretchable electrodes coupled with an ultra-elastic

PDMS surface were used to perform electrical impedance sensing of a cell monolayer under stretching conditions.¹⁴⁰ The impedance spectra of Madin–Darby canine kidney (MDCK) cell monolayers were recorded and compared to those of A549 cell monolayers under a single-axle mechanical strain of above 20%.¹⁴⁰ The results showed that the changes in impedance magnitude and phase under strain depended on the type of epithelial cells studied and the tightness of the cell junctions.

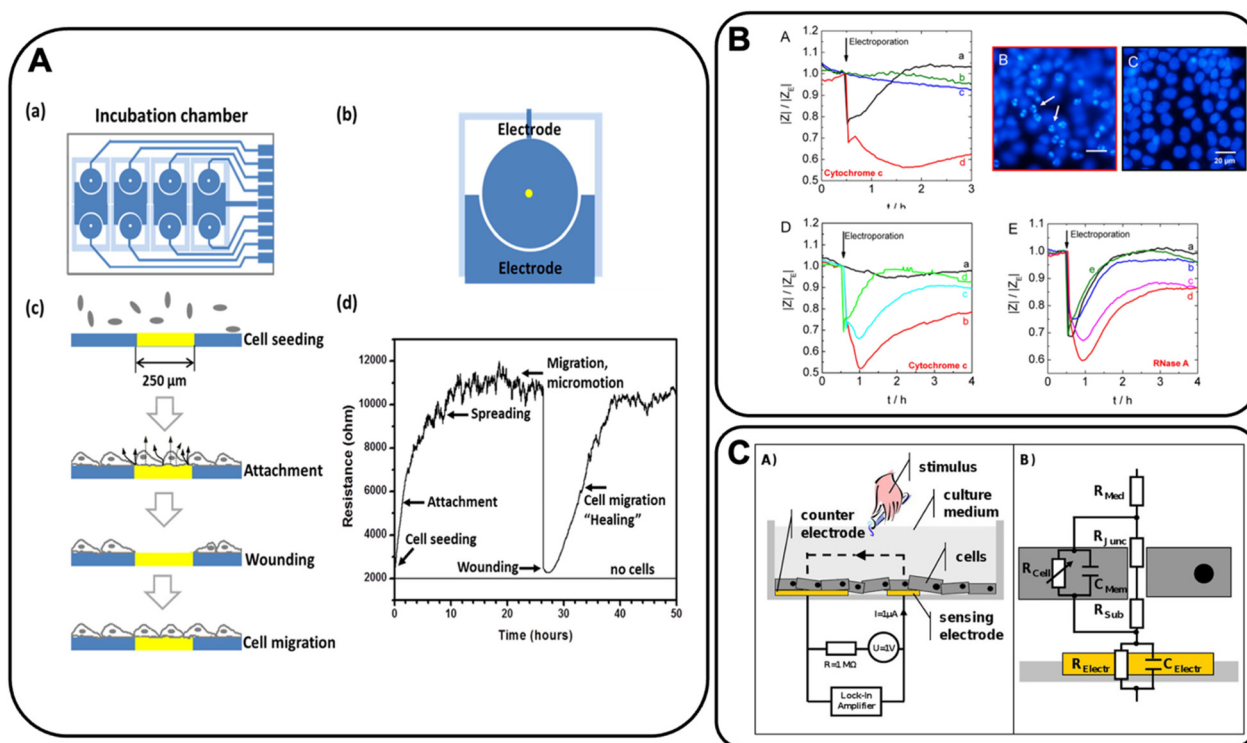


Fig. 4 ECIS for adherent cell layer analysis: (A) impedance signal corresponding to cell seeding, attachment, wounding and migration.¹⁵⁵ Reprinted from ref. 155, Copyright 2023, with permission from Elsevier, (B) impedance signal caused by the cellular response after electroporation-mediated loading of normal rat kidney cells with bioactive molecules,¹⁵⁶ and (C) electrical model of adherent cell layers in the ECIS technique and the cellular response to a stimulus.¹³⁶

3.3. Electrochemical impedance biosensor applications

The world has been challenged by a number of virus outbreaks worldwide including the influenza virus, dengue virus (DENV), human immunodeficiency virus (HIV), Ebola virus (EBV), and the most recent one, the acute respiratory-related coronavirus (COVID-19), which poses a serious threat to human health. As of February 2022, the COVID-19 pandemic has affected more than 170 countries worldwide, with over 430 million confirmed cases including over 5.92 million deaths.¹⁴¹ Besides the vaccine development for human protection against diseases, there is an urgent need for accurate, early, and rapid disease diagnostics. The traditional polymerase chain reaction (PCR) technique has its limitations, such as requiring expensive and low-flexibility machines, trained personnel, and a time-consuming process that can take several hours. This review will discuss the use of impedance electrochemical biosensors as a promising approach for virus detection, offering advantages such as fast response, low LoD, and real-time tracking. In electrochemical impedance biosensors, target viruses are captured by various bioreceptors modified on sensing electrodes, resulting in changes in electrode interfacial impedance due to specific binding.

A recent study demonstrated an aptasensor that used an aptamer and artificial nucleic acids in combination with a microfluidic platform for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).¹⁴² The electrode

array, coated with a specific aptamer in a microfluidic device, enabled the capture of Trace N-protein, a biomarker from SARS-CoV-2, in the sensing region (Fig. 5A). The biosensor could detect at the picofarad level in a range of capacitance changes, resulting in ultralow LoDs of nanograms per milliliter within just 15 seconds in both PBS and practical samples. Using similar concepts, a specific electrochemical aptasensor embedded in the microfluidic platform was proposed to detect norovirus in one-step.¹⁴³ The microfluidic platform consisted of a filter zone to remove solid or larger particles from the sample before the selective capture of norovirus. The electrodes were modified with the viral capsid-specific aptamer. The proposed chip could distinguish between the target virus and other molecules, and had a detection range from 100 pM to 3.5 nM. Regarding influenza virus H1N1 detection, Singh *et al.* proposed an immunosensor coupled with the microfluidic platform.¹⁴⁴ The working electrode was modified using reduced graphene oxide and specific monoclonal antibodies to enhance biomolecule immobilization. The results showed better selectivity with an improved LoD of 0.5 PFU mL⁻¹.

Recently, a microfluidic chip integrated differential impedance measurement was proposed for high-sensitivity antibody detection.¹⁴⁵ The gold-interdigitated active and reference electrodes were functionalized with a copolymer layer that contained a synthetic peptide probe. Fig. 5B shows the concepts, electrode modification, immunosensor, and the corresponding

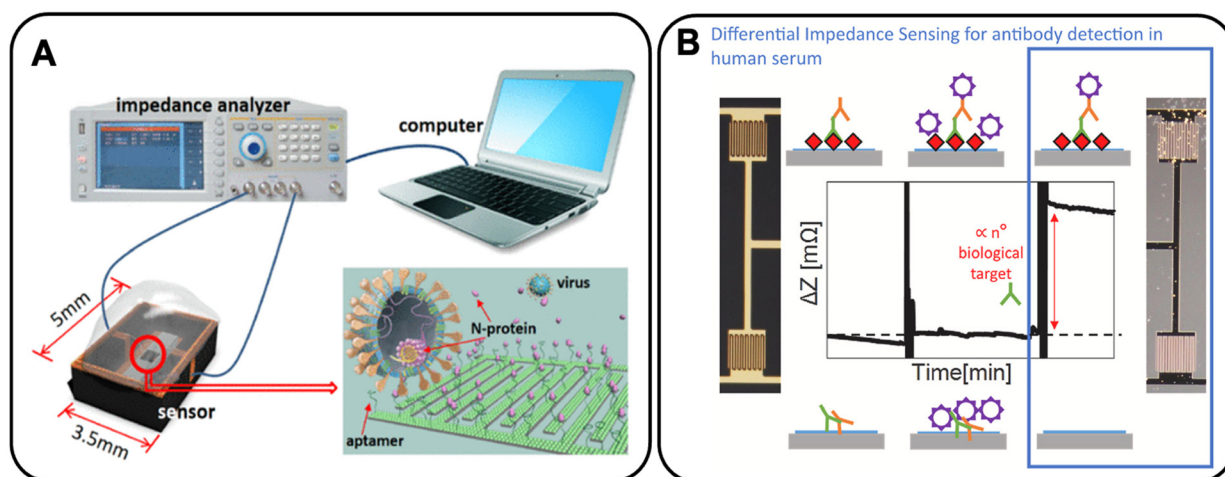


Fig. 5 Electrochemical impedance biosensor for virus detection: (A) SARS-CoV-2 nucleocapsid protein (N-protein) detection using an aptasensor combined with a microfluidic chip: concepts of the proposed sensor, impedance measurement and analysis systems and N-protein, a reliable biomarker for early SARS-CoV-2 infection capturing and detection.¹⁴² Reprinted with permission from ref. 142. Copyright 2023 American Chemical Society; (B) anti-dengue virus antibody detection for dengue virus diagnosis based on the differential impedance sensing platform: active electrode modification with a copolymer layer containing a synthetic peptide probe, corresponding to electrical impedance signal measurements.¹⁴⁵ Reprinted from ref. 145, Copyright 2023, with permission from Elsevier.

electrical impedance signals. The biosensor, which had a LoD of below 100 pg mL^{-1} for IgG antibody detection, was initially tested with anti-dengue virus antibodies and was found to have superior sensitivity compared to a commercial ELISA kit. Regarding SARS-CoV-2, Zeng *et al.* presented an impedimetric biosensor. The biosensor comprised gold micro-interdigitated electrodes and viral Spike-S proteins from the virus that were coated between the electrode digits to trap anti-S antibodies in the samples.¹⁴⁶ The work introduced two approaches to improve the sensitivity of the impedimetric sensor, including the use of secondary antibody-gold nanoparticles (AuNPs) and dielectrophoresis force to concentrate the target antibody. The AuNP approach reached a LoD of 200 ng mL^{-1} , while the DEP method had a LoD of $2 \text{ } \mu\text{g mL}^{-1}$ but had a faster testing time of only 30 minutes.

Besides applications in virus and antibody detection, a microfluidic platform combined with electrochemical impedance spectroscopy has been used to study the expression of multiple glycans on a cell surface.¹⁴⁷ The electrodes were modified with specific lectins to capture the corresponding carbohydrate epitopes on the cell surface. The electrical impedance signals resulting from the immobilization of cells in the electrode array enabled the assessment of surface glycan expression. This process was further confirmed by impedance spectroscopy and an optical microscope simultaneously. Thus, it enhances the sensitivity and accuracy of the proposed device.

4. Machine learning applications in impedance sensing devices

In recent years, machine learning has made significant contributions in various fields and is being widely used for accurate

decision making and predictions based on historical data. This technology is particularly useful for data analysis and image recognition. The application of machine learning in biological data analysis is an emerging area that offers great potential. Machine learning algorithms can extract cell signals hidden in the measured signal of bioimpedance data,¹⁴⁸ predict cell parameters,^{117,149} classify cell types,^{80,117,125,150} and analyze cell fates.^{117,124,151} Specifically, supervised machine learning, a subset of machine learning, has shown promising results in evaluating the viability of tumor cells after drug treatment using high-dimensional impedance data.¹²⁴ The combination of IFC and supervised machine learning has enabled cell viability assessment without the use of traditional staining techniques, allowing for the completion of subsequent analysis. A support vector machine (SVM) classifier with a Gaussian kernel is used for better accuracy. In this work, the authors used a support vector machine (SVM) classifier with a Gaussian kernel for better accuracy.¹²⁴ The SVM classifiers used amplitude change and phase changes in the frequency range of 300 kHz to 30 MHz as features and achieved an accuracy of 95.9%, with a true positive of 95%, and a true negative of 97% when compared to the Trypan blue test as ground truth. This system has potential as a PoC diagnostic tool for the patient response to drug treatment because of its portability, affordability, and label-free nature and rapid response. In a similar study, supervised learning-based classification and unsupervised learning-based clustering were used to distinguish early, late apoptotic, and necrotic tumor cell subpopulations from unknown datasets, as shown in Fig. 6.¹⁵¹ Besides, single-cell intrinsic parameters (size, dielectric properties) which are normally obtained from complex and time-consuming model-fitting methods are extracted by using a neural network approach. The design of microfluidic devices

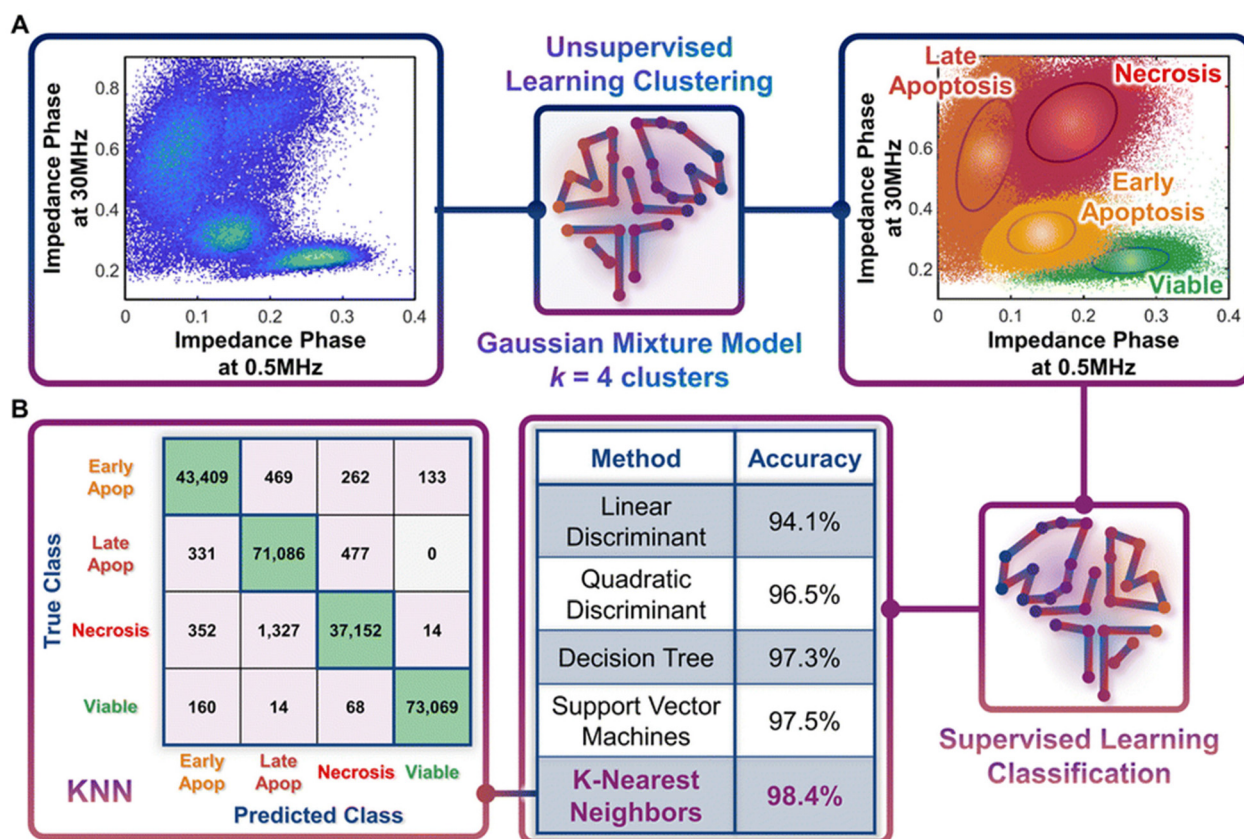


Fig. 6 Machine learning algorithms including unsupervised learning and supervised learning for classification and assessment of pancreatic cancer cell subpopulation states under cytotoxicity effects: (A) unsupervised learning clustering for the determination of different cell subpopulations based on impedance data at 30 MHz and 0.5 MHz; (B) supervised learning techniques for classification of viable, early apoptotic, late apoptotic, and necrotic cells based on clustered data.¹⁵¹

integrates coplanar electrodes and a cell-equivalent circuit model for the neural network approach. The fully connected neural network includes five layers, with each layer consisting of multiple neurons and the input layer with four-frequency impedance peaks.¹¹⁷ The neural network was trained using a stochastic gradient descent (SGD) optimizer and using backward propagation of loss values. This approach was successful in determining the cell parameters with a calculation speed of 0.3 ms per cell, a 10 000-fold enhancement compared to the conventional method in real-time and online. The root mean square errors (RMSEs) of the predicted values with reference to the true values were $0.28\ \mu\text{m}$, $0.02\ \text{S m}^{-1}$, and $0.78\ \text{mF m}^{-2}$ for cell radius, cytoplasm conductivity, and membrane capacitance, respectively. Besides single-cell characterization, the neural network approach proved to be an effective technique for cell type classification. The predicted parameters were used as inputs for a neural network classifier between tumor cells and lymphocytes for the assessment of the cell status. The classification accuracy was up to 91.5%, with classification precision values of 98.5%, 83.6%, and 89.8% obtained for living, fixed, and heat-shocked cells, respectively. Another study has used neural networks to classify different tumor cells and differentiate tumor cells before and after epithelial-mesenchy-

mal transitions based on measuring the specific membrane capacitance and cytoplasm conductivity in a constriction channel.⁸⁰ The two-layer neural network was able to discriminate between H1299 and HeLa cell lines with a success rate of 90.9% and differentiate A549 cells before and after epithelial-mesenchymal transitions with a classification rate of 76.5%. Various inputs, such as diameter, opacity, phase shift, specific membrane capacitance, and cytoplasm conductivity, can be introduced to the neural network model for cell discrimination.^{80,125}

Although machine learning has not been widely applied in impedance analysis yet, recent results achieved from its application have proven to be effective in assisting biological analysis based on impedance data. These initial results show the significant advantages of machine learning such as rapid calculation time and high accuracy, which need to be improved before being transferred to clinical settings.

5. Conclusions and perspectives

Cellular biophysics has become increasingly essential in the biomedical field, particularly in developing diagnostics and

therapeutics. The biophysical characterization of cells, especially at the single-cell level, has provided accurate markers for human diseases. In recent years, microfluidic platforms integrated with impedance sensing techniques have emerged as a label-free, non-invasive, and robust tool for the biophysical characterization of both cell populations and single cells. This review presents the basic principles and single-cell modeling of impedance sensing techniques, and summarises the two primary impedance sensing methods: EIS and IFC. EIS involves the electrical measurement of trapped cells over a broad range of frequencies, while IFC performs impedance analysis at one or several frequencies of flowing cells. To enhance the sensitivity of the whole system, recent reports have optimized the microchannel geometry, and electrode configuration, and developed cell-focusing techniques to enhance the sensitivity of the entire system.

This review highlights recent advancements in impedance sensing techniques in biomedical applications, including suspended cell analysis (such as cell quantification, cell phenotypic analysis, classification, and status assessment), adherent cell analysis, and electrochemical impedance biosensor applications. Recent efforts in impedance sensing have been made to develop a compact, portable, fully integrated, and user-friendly platform for PoC testing. The geometry of impedance sensing devices and cell models has been optimized to measure more cell biophysical characteristics, including electrical and mechanical properties. Moreover, machine learning algorithms and innovative technologies have been widely used to extract electrical signal features and achieve accurate cell type classification and assessment.

Despite the promising results, impedance sensing techniques still have apparent drawbacks that need to be addressed before they can be transferred to clinical settings. One challenge is the complex process of patterning electrodes on a microchannel. Another is the lack of clinical validations for impedance sensing devices. However, 3D printing techniques can simplify the fabrication process for microfluidic channels with integrated electrodes and streamline the data processing of the collected electrical signals. This will increase the potential for device commercialization and widespread use. Ideally, further research and developments can be carried out to establish compact multifunctional platforms that integrate sample preparation and pre-processing, cell sorting and enrichment, and optical imaging analysis. Additionally, the entire system should be optimized and made user-friendly, portable, low-cost, and disposable, while also enhancing the sensitivity and accuracy of the device.

Author contributions

Thu Hang Nguyen, Hung Anh Nguyen, and Loc Do Quang carried out the literature review and analyzed the data. Thu Hang Nguyen and Y-Van Tran Thi presented the data and wrote the manuscript. Hung Anh Nguyen, Donna Hoang Tran, and Hung Cao reviewed and edited the manuscript. Trinh Chu

Duc and Tung Thanh Bui reviewed, edited the manuscript, and supervised the project.

Conflicts of interest

There are no conflicts to declare.

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Tuesday - Microfluidics Platform Technologies

T3P.041 A DEEP-LEARNING-BASED APPROACH FOR IN VITRO MATURATION MONITORING UTILIZING HEXAGONAL MICROPILLAR TRAP MICROFLUIDIC PLATFORM

Hang Nguyen Thu¹, Anh Nguyen Thi Ngoc¹, Trung Nguyen Hoang¹, Junhan Xiao², Tung Le Thanh¹, Hang Nguyen Thi¹, Hanh Nguyen Van³, Hoang Anh Phan¹, Thien Nguyen Duy², Tung Thanh Bui¹, William C. Tang², Hung Cao², Trinh Chu Duc¹, and Loc Do Quang¹

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T3P.042 ACOUSTIC LENS INTEGRATED WITH A MICROFLUIDIC DEVICE TOWARD HIGH-POWER AND HIGH-THROUGHPUT CELL MANIPULATION

Souta Kurihara and Takeshi Hayakawa

Chuo University, JAPAN

T3P.043 DETECTION OF MICROPLASTIC WASTE BY USING A NOVEL MICROFLUIDIC SYSTEM WITH AN INTEGRATED OBJECT TRACKING ALGORITHM

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T3P.044 EFFICIENT HYDROPHILIC ARRAY GENERATION USING ULTRASONIC ATOMIZATION AND OFF-THE-SHELF PERFORATED MASKS

Xiaochen Lai, Xicheng Wang, Yanfei Sun, and Yong Zhu

Nanjing University of Information Science & Technology, CHINA

T3P.045 3D-PRINTED TRIAXIAL ELECTROSPRAY MICROFLUIDICS FOR UNIFORM, CORE-SHELL-SHELL MICROPARTICLE GENERATION

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T3P.046 HYBRID DIW-SLA TISSUE PRINTING SYSTEM WITH A MULTI-MATERIAL MICROFLUIDIC NOZZLE PRINTHEAD

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T3P.047 LCD 3D PRINTING OF A NORMALLY CLOSED FLUIDIC TRANSISTOR VIA ADDITIVE ASSEMBLY

A. Muhyamin Chowdhury, Thaddaeus Stine, Kalp B. Upadhayay, Carolyn G. Catan, Adithya Kidambi, Althea Marielle G. Eclarin, Catherine W. Lim, Michelle Liu, and Ryan D. Sochol

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T3P.048 OOCYTE BIOMECHANICS USING A MICRODEVICE TO CORRELATE TRANSIENTS OF MICRO-FLOW IMPEDANCE AND ELECTRICAL IMPEDANCE

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T3P.049 STUDY OF RECTANGULAR MEMBRANE PMUT-BASED ACOUSTIC STREAMING MICROPUMP

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A DEEP-LEARNING-BASED APPROACH FOR IN VITRO MATURATION MONITORING UTILIZING HEXAGONAL MICROPILLAR TRAP MICROFLUIDIC PLATFORM

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ABSTRACT

Oocyte maturation is a critical determinant of outcomes in assisted reproductive technologies (ART). Precise control and real-time monitoring of oocyte maturation at the single-cell level are essential for optimizing *in vitro* maturation (IVM) protocols and enhancing treatment efficacy. Recent advancements in microfluidics have facilitated the creation of controlled microscale culture environments on-chip, enabling tunable nutrient exchange, physiologically relevant shear stress, and cellular interactions. This study proposes a novel microfluidic platform incorporating a hexagonal micropillar trap (HMT) structure to enable single oocyte trapping and controlled maturation. Integrating a deep-learning-based approach, the system analyzes 24-hour timelapse images to assess cumulus cell area expansion, a key indicator of oocyte maturation. In comparison to conventional culture methods, this platform enables automated oocyte handling, reduces variability, and supports objective and intelligent assessment of the maturation process. The combination of microfluidics and artificial intelligence establishes a robust framework for refining IVM protocols, improving oocyte maturation, and enhancing ART outcomes.

KEYWORDS

In vitro maturation, single oocyte trapping, microfluidic, deep learning

INTRODUCTION

Assisted reproductive technology (ART) plays a critical role in addressing a wide range of reproductive challenges, from managing infertility and mitigating the effects of population aging to expanding family-building options for same-sex couples and single individuals [1], [2]. Additionally, ART enables genetic screening and selection, contributing to improved reproductive health outcomes [3]. IVM has emerged as a promising approach within ART, offering a safer and more cost-effective alternative to conventional ovarian stimulation. This technique is especially beneficial for patients at risk of ovarian hyperstimulation syndrome (OHSS), such as individuals with polycystic ovary syndrome (PCOS) [4]. Traditional IVM methods typically utilize bulk static culture systems that lack dynamic regulation of the cellular microenvironment. These setups fail to replicate the

complex *in vivo* conditions, both biochemical and mechanical, required for optimal oocyte development, often resulting in inconsistent and heterogeneous maturation outcomes. Additionally, the highly manual nature of conventional IVM workflows contributes to procedural variability and limits scalability. To date, much of the progress in ART has focused on optimizing culture media composition, while the development of physical tools for handling and manipulating oocytes and embryos has received comparatively less attention.

Microfluidic systems have been developed to address these constraints by establishing regulated, physiologically relevant culturing conditions [5], [6]. These technologies provide the individual trapping of oocytes, modulate biochemical and mechanical signals. However, existing microfluidic IVM platforms lack optimal trapping geometries for single-oocyte and integrated real-time automated monitoring systems, limiting their application in high-throughput and reproducible analyses.

This study presents a novel microfluidic platform incorporating hexagonal micropillars arranged in staggered trapping chambers to trap single oocytes, enhance nutrient exchange and waste removal while minimizing mechanical stress on the oocyte. By integrating deep-learning techniques, the system enables automated, high-precision analysis of single oocyte maturation, addressing key limitations of conventional approaches. This framework represents a step forward in IVM technology, offering improved experimental control and standardized maturation assessment, while also establishing a foundation for future advances in dynamic culture and intelligent monitoring systems.

MATERIALS AND METHODS

Device design and working principle

The HMT microfluidic chip features a single inlet for oocyte and medium loading, an outlet for waste removal, and trapping units composed of five hexagonally shaped micropillars arranged in a staggered pattern to optimize hydrodynamic trapping efficiency (Figure 1). Hydrodynamic single oocyte trapping leverages staggered micropillar arrays to create localized low-velocity zones and streamline deflections, enabling passive and efficient capture without external forces. The geometric parameters of the chip are detailed in Table 1. The unit trap sizes progressively decrease from 350 μm to 80 μm . The final

trapping array consists of uniformly distributed micropillars spaced 80 μm apart to screen out oocytes lacking cumulus cell layers. The decrease in unit trap dimensions is intended to accommodate oocytes of varying sizes while enabling the natural exclusion of smaller, potentially lower-quality oocytes, thereby enhancing the overall selection process [7].

Table 1: Geometrical parameters of microfluidic chip

Name	Value
Chip length	20000 μm
Chip width	6000 μm
Radius of micropillars	150 μm
Distance between two trap units in a row	400 μm
Distance between two rows of traps	400 μm
Unit trap size	80-350 μm

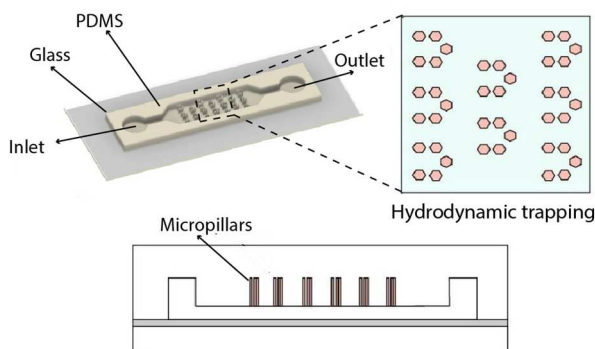


Figure 1: Design of the proposed HMT microfluidic chip for single oocyte trapping

Materials and fabrication

The HMT was fabricated using standard polydimethylsiloxane (PDMS) soft lithography techniques. Initially, a silicon wafer was spin-coated with SU-8 50 negative photoresist (MicroChem Corp., Newton, MA, USA) to form a single-layer mold. The mold pattern was then defined through UV photolithography, establishing the structural features of the chip. The thickness of the SU-8 features was measured to be approximately 220 μm using a surface profilometer (Dektak ST System Profilometer). Following mold fabrication, a PDMS prepolymer was prepared by mixing the Sylgard-184 silicone elastomer base with its crosslinker (Dow Corning, Midland, MI, USA) at a 10:1 (w/w) ratio. This mixture was poured over the SU-8 mold and cured at 65°C for two hours. After curing, the PDMS replica was carefully peeled from the mold. Inlet and outlet ports were then created by punching holes at designated locations to facilitate fluidic connections. Finally, the PDMS layer underwent oxygen plasma treatment to enhance bonding and was irreversibly sealed to a glass slide, completing the fabrication of the chip.

Oocyte preparation and culture

Ovaries from prepubertal crossbred pigs were obtained at a local slaughterhouse and transported to the laboratory

in Dulbecco's phosphate-buffered saline (DPBS; Gibco, USA) supplemented with 1% Penicillin-Streptomycin (Thermo, USA), maintained at 35°C during transit. Oocyte-cumulus complexes were retrieved by excising 3–6 mm follicles with a No. 21 scalpel, allowing the follicular fluid to settle in a 15-ml tube at 38.5°C, and selecting only highest quality oocytes (with round, uniform cytoplasm, numerous, well-distributed cumulus cells), free of any signs of damage or defects. The culture medium was prepared containing ((POM) (Research Institute for the Functional Peptides, Higashine, Japan) supplemented with 1 mM dibutyryl cAMP (dbcAMP; Sigma), 10 ng/ml epidermal growth factor (EGF), 10 IU/ml eCG (Serotropin; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan), and 10 IU/ml hCG (500 units; Puberogen, Novartis Animal Health, Tokyo, Japan). The oocytes were placed into the culture medium within a cleaned chip and incubated at 38.5°C with 5% CO₂. The culture process was continuously monitored for 48 hours to ensure the oocytes' development and maturation.

Experimental setup

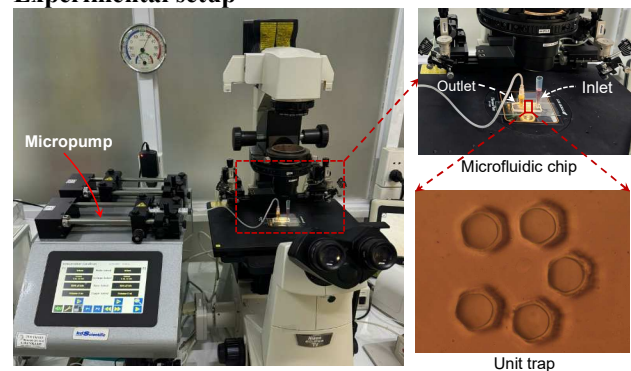


Figure 2: Experimental setup for single oocyte trapping

The microfluidic chip was placed under a Nikon Eclipse Ti microscope equipped with a high-resolution camera to facilitate observation and evaluation during experimental procedures (Figure 2). The inlet of the microfluidic system was fitted with a 1000 μl pipette tip to load oocytes suspended in culture medium, while the outlet was connected to a syringe driven by a micropump. The micropump plays a crucial role in controlling the continuous flow of the culture medium through the channel. The micropump system operated with high stability, helping to maintain a consistent microenvironment and adjust the flow rate to ensure adequate nutrient and oxygen supply to the cells throughout the experiment.

RESULTS AND DISCUSSION

The microfluidic chip incorporating HMT units was successfully manufactured using soft lithography technique. As depicted in Figure 3, the completed HMT chip was bonded to a glass slide, highlighting its compact dimensions relative to a 5-cent coin. The micropillar configuration and fabrication quality were further assessed through scanning electron microscopy (SEM) (Figure 3). The trapping efficiency of the HMT demonstrates the effectiveness of the microfluidic chip in trapping oocytes

based on hydrodynamic principles.

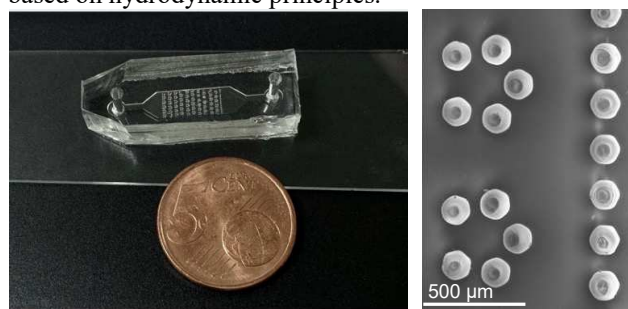


Figure 3: Fabricated microfluidic chip (left) and Scanning electron microscope (SEM) images of the micropillars (right)

In Figure 4, a single trapping unit is shown at higher magnification, where an oocyte has been successfully captured and cultured within the micropillar structure. The trapping rate, defined as the ratio of oocytes successfully captured within the chip to the total number introduced, was found to average 88.37% with a standard error of approximately 1.5% in the experimental results. The distribution of captured oocytes confirms the hydrodynamic trapping mechanism, wherein once a trapping unit is occupied, subsequent oocytes are directed toward unoccupied traps. This observation validates the efficiency of the staggered micropillar arrangement in facilitating size-dependent trapping while preventing overlap within individual trap units.

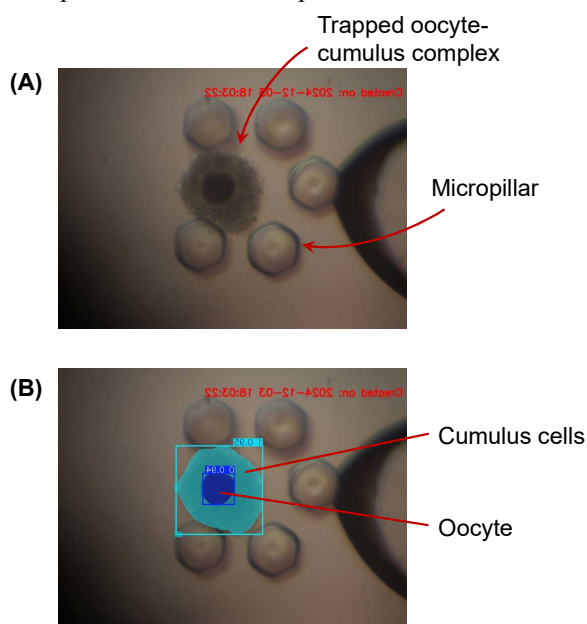


Figure 4: (A) Example of timelapse images of trapped oocyte on microfluidic chip in IVM procedure, (B) AI-assisted image segmentation. Class 0 corresponds to oocyte, class 1 represents cumulus cells.

Static culture experiments were performed on the microfluidic HMT chip, where oocytes remained securely confined within the micropillar traps. Figure 4 illustrates the trapping of an oocyte within an HMT unit and the corresponding AI-assisted segmentation distinguishing the oocyte (class 0) from surrounding cumulus cells (class 1).

This approach enables the automated measurement of cumulus cell area. It provides valuable insights into oocyte maturation and contributes to advancements in IVM optimization [8]. Deep learning-based segmentation of cumulus cells and oocytes improves assessment accuracy and objectivity. By minimizing the subjective bias of manual evaluation, this approach offers a robust framework for real-time automated tracking of oocyte maturation.

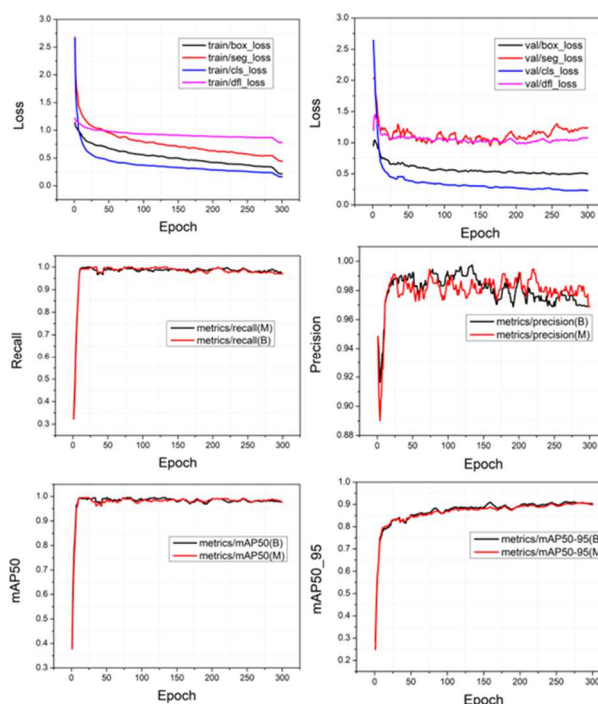


Figure 5: Efficiency of the deep learning segmentation model.

In this work, YOLOv8-segmentation model has been used to assess cumulus cell expansion. After 300 training epochs, the results indicate that the model performs exceptionally well on the segmentation task (Figure 5). Specifically, the loss functions for both the training and validation sets exhibit a strong convergence trend, with a relatively fast convergence rate as loss values decrease sharply within the first 50 epochs. However, a potential issue observed is the risk of overfitting, as the dfs_loss (distribution focal loss) and seg_loss (segmentation loss) for the validation set show a slight increasing trend in the later epochs and remain higher than their corresponding loss values in the training set.

In terms of precision and recall, the model achieves consistently high average values for both bounding boxes and masks, exceeding 95%. Additionally, the mAP50 (mean average precision at IoU = 0.5) remains close to 100%, while the mAP50-95 is above 90%. These results indicate a high degree of accuracy in the predicted masks compared to the ground truth, demonstrating the model's capability to precisely segment the boundaries of individual oocytes.

CONCLUSIONS

In this study, we developed an integrated microfluidic system combined with deep learning to enable intelligent and automated single oocyte trapping, culture, and evaluation. The microfluidic chip was designed based on hydrodynamic principles using HMT structures, with tailored trap dimensions to achieve high-efficiency single oocyte capture. Oocyte-cumulus complexes were successfully trapped and cultured on-chip under regulated nutrient and gas conditions, which supported their sustained viability and developmental progression. A machine learning model was also established to segment the oocyte and cumulus cell regions, enabling accurate and efficient assessment of cumulus cell expansion - an important indicator of oocyte maturation. These results highlight the potential of the proposed platform to advance IVM protocols and contribute more broadly to assisted reproduction field.

ACKNOWLEDGEMENTS

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Technical Program

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Monday - Microfluidics Platform Technologies

M4P.041 A BIOSENSOR-INTEGRATED FILTRATION DEVICE FOR INTACT HIV ISOLATION AND LABEL-FREE DETECTION IN POINT-OF-CARE ENVIRONMENTS

Leyang Liu¹, Takhmina Ayupova¹, Saurabh Umrao¹, Lucas D. Akin¹, Han-Keun Lee¹, Joseph Tibbs¹, Xing Wang¹, Utkan Demirci², and Brian T. Cunningham¹

¹University of Illinois, Urbana-Champaign, USA and ²Stanford University, USA

M4P.042 **A MINIATURIZED PLATFORM FOR INVESTIGATING THE STRENGTH OF THROMBUS INDUCED BY BLOOD SHEAR RATES

Dong-Hwi Ham¹, Seong-hyeon Lee², Jae-Hyun Choi¹, Ji-Seob Choi¹, Woo-jun Jung², Yong-ha Hwang², and Woo-Tae Park¹

¹Seoul National University of Science and Technology, KOREA and ²Korea University, KOREA

M4P.043 CONTINUOUS AND SHEATHFLOW-LESS MICRO-/NANOPARTICLE SEPARATION COMBINING THERMOPHORESIS AND THERMOELECTRICITY WITH A 3D SPIRAL MICROCHANNEL

Junho Kim, Hwisu Jeon, and Taesung Kim

Ulsan National Institute of Science and Technology, KOREA

M4P.044 EFFECT OF NANOPARTICLES ON THE SUPPRESSION OF REAGENT EVAPORATION IN SINGLE-PLATE EWOD SYSTEM

Xiaoping Li, Duo Fu, Yifu Xu, Mingrui He, Caiqin Zhao, and Dachao Li

Tianjin University, CHINA

M4P.045 EXPERIMENTAL EVALUATION OF MULTI-QUADRILATERAL-PILLAR ARRAY MICROFLUIDIC PLATFORM FOR OOCYTE DENUDEATION UTILIZING DEEP-LEARNING APPROACH

Hang Nguyen Thu¹, Trung Nguyen Hoang¹, Anh Nguyen Thi Ngoc¹, Mobina Malekifar², Tung Le Thanh¹, Mai Anh Tran¹, Hanh Nguyen Van³, Ha Tran Thi Thuy⁴, Tung Thanh Bui¹, Hoang Anh Phan¹, Hung Cao², Trinh Chu Duc¹, and Loc Do Quang¹

¹Vietnam National University (VNU), VIETNAM, ²University of California, Irvine, USA,

³Vietnam Academy of Science and Technology, VIETNAM, and

⁴Posts and Telecommunications Institute of Technology, VIETNAM

M4P.046 **HOMOGENIZATION OF SOLUTE CONCENTRATION IN VERTICALLY COALESCENT DROPLETS USING ACOUSTIC WAVE

Yoshinori Miyata, Shinji Bono, and Satoshi Konishi

Ritsumeikan University, JAPAN

M4P.047 INTERACTION OF AN ACCELERATED LIQUID INTERFACE WITH A MESH FOR SATELLITE FREE DROPLET EJECTION.

Imnatoshi Jamir, Bheema S R. Bapuram, and Allu S. Reddy, and Prosenjit Sen

Indian Institute of Science, INDIA

M4P.048 MICROFLUIDIC SENSOR FOR MICROPLASTIC DETECTION IN SALINE FRESHWATER: ENHANCEMENTS BY WHEATSTONE BRIDGE AND MXENE-COATED ELECTRODES

Haider Warraich¹, Alireza Zabihhesari^{1,2}, Shooka Karimpour¹, and Pouya Rezai¹

¹York University, CANADA and ²Dalhousie University, CANADA

M4P.049 SELF-PRESSURIZING MICROFLUIDIC CHIP FOR BUBBLE SUPPRESSION

Zizhen Wang^{1,2}, Meiyan Qin^{1,2}, Rui Jiang^{1,2}, Yang Zhao¹, Haiyang Mao¹, Lingqian Zhang¹, Chengjun Huang^{1,2}, and Mingxiao Li

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EXPERIMENTAL EVALUATION OF MULTI-QUADRILATERAL-PILLAR ARRAY MICROFLUIDIC PLATFORM FOR OOCYTE DENUDATION UTILIZING A DEEP-LEARNING APPROACH

Hang Nguyen Thu¹, Trung Nguyen Hoang¹, Anh Nguyen Thi Ngoc², Mobina Malekifar³, Tung Le Thanh¹, Mai Anh Tran², Hanh Nguyen Van⁴, Ha Tran Thi Thuy⁵, Tung Thanh Bui¹, Hoang Anh Phan¹, Hung Cao³, Trinh Chu Duc¹, and Loc Do Quang^{2*}

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ABSTRACT

Oocyte denudation is the procedure of removing the cumulus cells encasing an oocyte to expose the oocyte itself. During oocyte maturation, cumulus cells play a crucial role in providing nutritional factors. However, it is necessary to isolate cumulus cells from the cumulus-oocyte complex (COC) to assess the oocyte maturity and enable procedures like intracytoplasmic sperm injection (ICSI). Traditional oocyte denudation procedures are often labor intensive and operator dependent, leading to variability in denudation efficacy and potential mechanical damage to the oocyte during manual pipetting. In this work, we designed and experimented with a novel microfluidic system for oocyte denudation using a multi-quadrilateral-pillar array structure. The micropillar array was configured to generate sharp shear angles that mechanically disrupted the connections between the oocyte and surrounding cumulus cells as the COC passed through while also creating a zigzag flow trajectory that facilitates thorough denudation across different regions of the oocyte surface. Image processing and computer vision techniques were applied to evaluate denudation efficiency, confirming that the chip performed effectively in removing cumulus cells from porcine COCs. The proposed denudation on-chip shows strong potential for integration into automated and streamlined workflows in assisted reproductive technologies (ART). Its modular micropillar array can be expanded to increase COC throughput, enabling rapid and efficient large-scale oocyte denudation.

KEYWORDS

Microfluidic chip, oocyte denudation, micropillar array, segmentation

INTRODUCTION

According to WHO, infertility is emerging as a global health issue, with estimates indicating that approximately 186 million women of reproductive age in developing countries “wish to give birth to a child” [1]. This highlights the urgent need to advance assisted reproductive technologies (ART), including in vitro fertilization (IVF), intrauterine insemination (IUI), intrafallopian transfer (IFT), intracytoplasmic sperm injection (ICSI), and especially in vitro maturation (IVM) [2]. *In vitro* maturation (IVM) has been used widely as the procedure only requires immature oocytes, which is suitable for patients with polycystic ovaries, patients who are unresponsive to ovarian stimulation, or cancer [3]. IVM is

a sophisticated, multi-phase process used for the cultivation of oocytes. Oocyte denudation is the post-culture process where cumulus cells are separated from the COC [4]. Cumulus cells contain FSH and LH receptors that manage to absorb nutrients through the maturation process. However, in certain ART such as ICSI, it is necessary to remove the granulosa cell mass, using only oocyte covered by the zona pellucida. Manual oocyte denudation is a time-consuming process and labor-intensive procedure with inconsistent outcomes among various operators. To mitigate these limitations, several automated oocyte denudation devices have been developed using robotic and microfluidic technologies. Zhai *et al.* developed a robotic system combining machine vision and automated alignment to locate oocytes and cumulus cells precisely and enhance denudation efficiency, achieving a $97.0 \pm 2.8\%$ success rate [5]. However, the method remains technically complex, requiring specialized fabrication and advanced control systems. Meanwhile, Wang *et al.* developed a microfluidic system with a jagged multi-channel configuration to mechanically separate cumulus cells, yet challenges such as channel clogging, limited throughput, and processing delays persist [6].

In response to these challenges, we introduce a novel microfluidic-based oocyte denudation featuring quadrilateral micropillar arrays arranged in an alternating pattern within a microchannel. This design generates mechanical shear forces to effectively separate cumulus cells from oocytes while maintaining structural integrity. The micropillars are strategically engineered with pointed edges and a zigzag alignment to maximize COC interaction, enhancing denudation efficiency when combined with enzymatic pretreatment. High denudation efficiency was achieved through the use of image processing and computer vision to evaluate oocyte output and denudation performance. Our device has significant prospects in assisted reproduction due to its simple operation, compact system, high throughput, scalable, and high efficiency.

PROPOSED DESIGN

Device configuration

Oocyte denudation via conventional techniques involves initially treating COCs with the enzyme hyaluronidase to loosen the cumulus extracellular matrix. Subsequently, mechanical force is employed to entirely remove the cumulus cell [7]. A microfluidic system with multi-row quadrilateral pillars was designed, inspired by

this theoretical mechanism, as depicted in Figure 1. The proposed microfluidic device is designed as a U-shaped structure, measuring 21.5 mm in length and 1.42 mm in width for each side.

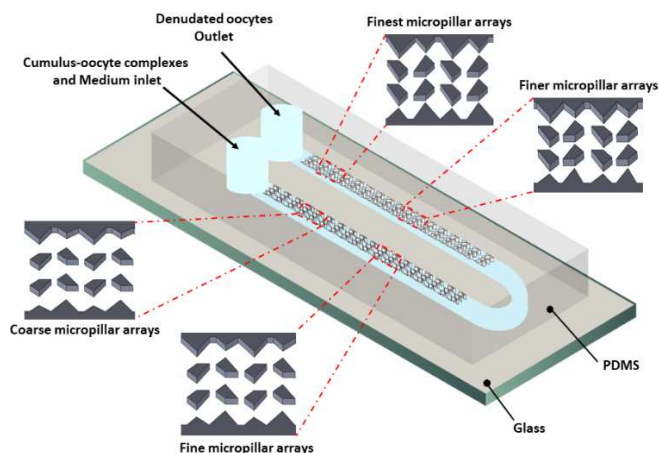


Figure 1: Design of the proposed microfluidic chip for oocyte denudation.

The device comprises four sequential sections of micropillar arrays, arranged from coarse to finest structures. The horizontal spacing between adjacent pillars decreases progressively across these sections as illustrated in Figure 1, starting from 240 μm in the coarse section, 200 μm in the fine section, 160 μm in the finer section, and finally, 120 μm in the finest section. Each section consists of 24 rows of pillars, with each row including 4 pillars: 2 central pillars and 2 lateral pillars. The center pillar is a quadrilateral prism, constructed by aligning an isosceles triangle at the top with a shown right isosceles triangle. Pillar width varies across sections to regulate inter-pillar spacing according to each section's design. The pillars are organized into multi-micropillar arrays featuring small channels and sharp edges, enabling the extraction of cumulus cells. Furthermore, the inverse alignment of adjacent micropillar rows guides oocytes along a zigzag path, increasing contact area and enhancing denudation efficiency.

MATERIALS AND METHOD

Fabrication process

The microfluidic device was fabricated using soft lithography. A silicon wafer was chemically cleaned, coated with SU-8 photoresist, and patterned via UV exposure through a photomask to form microstructures with a height of around 220 μm . PDMS was then cast onto the SU-8 mold, cured, and peeled off to form the channel layer. The PDMS structure was bonded to a glass slide using oxygen plasma treatment, and inlet/outlet ports were created to complete the device.

Porcine oocyte preparation and enzyme hyaluronidase treatment

IVM was performed according to a previous protocol [8] using a porcine oocyte medium (POM; Functional Peptides Institute, Japan) supplemented with 10 ng/mL EGF, 10 IU/mL eCG (Serotropin; ASKA, Japan), and 10 IU/mL hCG (Puberogen; Novartis, Japan). Groups of 40–50 COCs were cultured in 500- μL droplets in 4-well plates (Nunc; Thermo Fisher, USA) without oil, at 38.5°C under

5% CO_2 , 5% O_2 , and 90% N_2 . For the first 22 h, 1 mM dbcAMP was added, then removed for an additional 22–24 h of culture. Prior to microfluidic denudation, COCs were briefly treated with 0.1% (w/v) hyaluronidase.

Experimental setup

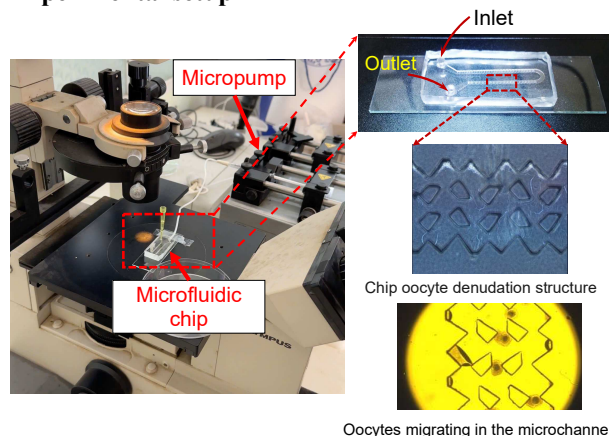


Figure 2: Experimental setup

On-chip denudation could be performed using two approaches: either automatically with a micropump, as illustrated in Figure 2, or manually using a pipette. To ensure a sterile environment, the manual method was carried out inside a biosafety cabinet. Subsequent to denudation, the oocytes were collected in a culture dish and analyzed using an epifluorescence microscope (Nikon Eclipse Ti). The microscope, fitted with a camera and a Thermal Plate heater (Thermo Plate TPi – Series), preserved the oocyte's physiological parameters, reduced environmental impacts, and delivered clear, high-resolution images for further research.

Deep learning model for evaluation of denudation efficiency

Figure 3 illustrates the morphology of oocytes before and after the denudation procedure. Cumulus cells from partially denuded oocytes can promote embryo quality and blastocyst development, as previous studies have highlighted their crucial role in enhancing the fertilization potential and developmental competence of the oocyte cytoplasm [9], [10]. Therefore, to evaluate denudation efficiency, this study considered denudation successful when the area of residual cumulus cells surrounding the oocyte was less than a threshold 20% of the oocyte area.

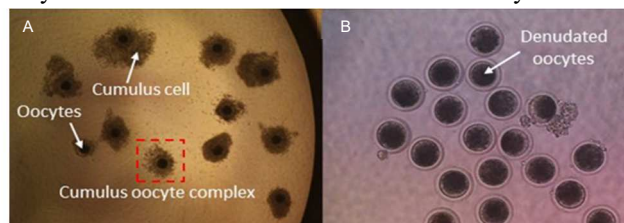


Figure 3: Oocyte morphology before (A) and after (B) the on-chip denudation procedure

This work investigated the application of deep learning techniques for precise segmentation of oocytes and cumulus cells, aiming to evaluate the denudation efficiency of the proposed method. The dataset used for model training consist of images capturing oocyte populations with a sample size of more than 100 individuals after the

denudation process. These images were annotated using Roboflow, defining two distinct classes: oocytes and cumulus cells. The annotated dataset was then employed to train a segmentation model based on YOLOv8. Although YOLOv8 did not reach the accuracy levels of more advanced architectures, such as YOLOv9 or YOLOv11, it offered a well-balanced trade-off between computational efficiency and segmentation precision. This made it a practical choice for the automated assessment of denudation efficiency in this study.

After segmentation, a key challenge was matching each cumulus cell cluster to its corresponding oocyte. This required determining the centroid of each mask. For oocytes, which are nearly circular, centroids were estimated using the Least Squares circle fitting method. The general equation of a circle is:

$$(x - a)^2 + (y - b)^2 = r^2 \quad (1)$$

where (a, b) was the center and r was the radius. Expanding the equation and rewriting it in a linear form, we obtained:

$$x^2 + y^2 = Dx + Ey + F \quad (2)$$

where $D = -2a, E = -2b, F = r^2 - a^2 - b^2$. With n pairs of (x, y) , we got the matrices:

$$\begin{bmatrix} x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \\ \vdots & \vdots & \vdots \\ x_n & y_n & 1 \end{bmatrix} \begin{bmatrix} D \\ E \\ F \end{bmatrix} = \begin{bmatrix} x_1^2 + y_1^2 \\ x_2^2 + y_2^2 \\ \vdots \\ x_n^2 + y_n^2 \end{bmatrix} \quad (3)$$

Solving the least squares system would get optimal result for a, b and r .

In contrast, for cumulus cell masks, the centroid coordinates would be computed using the standard centroid formula for polygons. The centroid of a polygon with n vertices of (x, y) was given

$$C_x = \frac{1}{6A} \sum_{i=1}^n (x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \quad (4)$$

$$C_y = \frac{1}{6A} \sum_{i=1}^n (y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \quad (5)$$

where (C_x, C_y) was the coordinate of the cumulus cell mask's centroid, and A was the area of the polygon calculated by Shoelace formula:

$$A = \frac{1}{2} \left| \sum_{i=1}^n (x_i y_{i+1} - x_{i+1} y_i) \right| \quad (6)$$

(x_i, y_i) represents the coordinates of the i -th vertex of the polygon, and n is the total number of vertices. Once the centroids are determined, the Euclidean distance between the centroid of the oocyte and the centroid of a cumulus cell cluster is calculated as follows:

$$d = \sqrt{(x_c^{oocyte} - x_c^{cell})^2 + (y_c^{oocyte} - y_c^{cell})^2} \quad (7)$$

where d denoted the Euclidean distance between the centroids of the oocyte and the cumulus cell mask. If $d \leq d_{threshold}$, the cumulus cell region was identified as the one associated with the oocyte under consideration.

After identifying the cumulus cell region associated with the oocyte, the areas of both the cumulus cell region and the oocyte were computed using the Shoelace formula, then calculating the area proportion, as given below:

$$P = \frac{A_{cumulus\ cell}}{A_{oocyte}} \quad (8)$$

The area proportion between cumulus cell ($A_{cumulus\ cell}$) and oocyte (A_{oocyte}) was denoted as P , and if the denudation factor was less than or equal to the denudation threshold - a predefined standard value - then the oocyte was considered to have undergone a successful denudation process.

RESULTS AND DISCUSSION

Deep learning model's performance

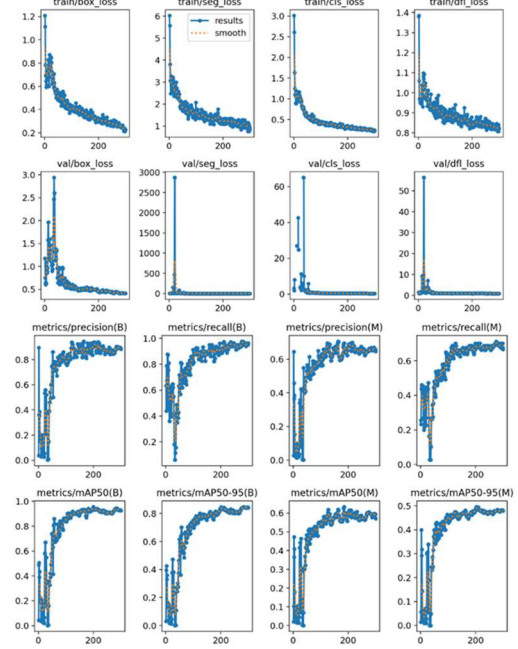


Figure 4: Model performance for box and mask prediction after 250 training epochs (batch size = 12, close mosaic = 4).

The training results indicated that the loss converges rapidly for the training set, indicating that the model was still learning effectively and has the potential for further development (Figure 4). However, the model exhibited signs of overfitting due to the limited amount of training data relative to the number of parameters required. The average recall and precision values for the bounding box remained high, exceeding 90%, demonstrating that the model can localize objects relatively well. Nevertheless, its ability to predict accurate segmentation masks remained relatively low, with the average recall, precision, and the mAPs value for masks reaching only around 60%.

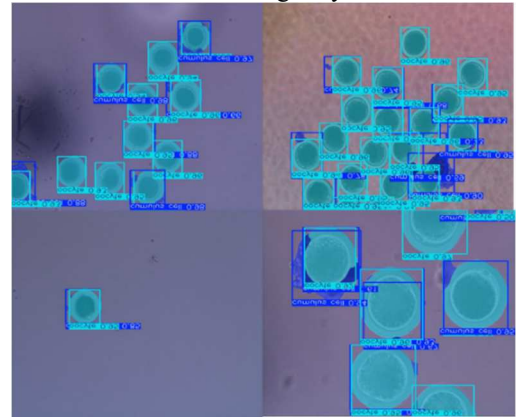


Figure 5: Example prediction of model for 4 input images

Although the model's performance is not yet optimal, it is important to acknowledge that its relatively accurate bounding box localization allows for a reasonably effective segmentation of objects, as illustrated in Figure 5. However, it is also evident that the segmentation masks, particularly for cumulus cells, have not yet achieved the desired level of accuracy for certain instances. Nonetheless, this remains a promising approach for further development toward the automation of denudation efficiency assessment.

Recovery rate and denudation efficiency

The recovery rate and denudation efficiency of oocytes were evaluated to quantitatively measure the efficacy of the microfluidic device depicted in Figure 6. Recovery rate is defined as the proportion of collected oocytes at the outlet to those introduced at the inlet. Denudation efficiency is calculated as the number of successfully denuded oocytes divided by the total number of collected oocytes. Damage rate refers to the proportion of oocytes damaged during denudation relative to the total number collected at the outlet.

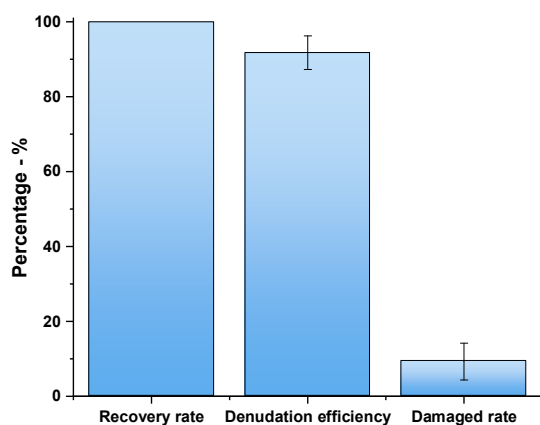


Figure 6: Performance of the microfluidic chip

A total of four independent experiments were performed, involving 151 oocytes. As depicted in Figure 6, the recovery rate remains at 100%. The denudation rate reaches $91.8 \pm 4.49\%$, indicating the effective removal of surrounding cells. Meanwhile, the damage rate remains at a satisfactory level of $9.31 \pm 4.91\%$. This proportion depends on the quality of cultured oocytes as well as the experimenters' experience.

CONCLUSION

This study proposed a microfluidic device for oocyte denudation, offering key advantages over traditional manual methods through automation and high-throughput capability. Utilizing a novel quadrilateral micropillar array design, the system leverages mechanical shear forces to achieve a high recovery rate and denudation efficiency of approximately 90% while preserving oocyte integrity. With the application of deep learning-based evaluation, we provide promising prospects for automation in oocyte and cumulus cell segmentation and evaluation. These findings underscore the potential of microfluidic technology to standardize and optimize ART procedures, thereby advancing automation, minimizing variability, and improving outcomes across laboratories.

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CIRCULATING TUMOR CELL (CTC) SEPARATION FROM BLOOD CONSTITUENTS USING A DIELECTROPHORESIS-BASED MICRODEVICE

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Abstract— Numerous studies have focused on circulating tumor cell separation, given its pivotal role in cancer diagnosis, prognosis, and treatment. This study presents an effective microfluidic system that integrates a specialized dielectrophoresis structure, namely the facing-electrode configuration dielectrophoresis (FEC-DEP) structure, to isolate circulating tumor cells from various blood constituents, including red blood cells, white blood cells, and platelets. The FEC-DEP structure comprises a bottom slanted electrode array positioned parallel to a basic rectangular top electrode. These parallel electrodes generate a dielectrophoresis-induced non-homogeneous electric field. Therefore, when the FEC-DEP is integrated into a flow, it can guide different objects in the flow along distinct trajectories. Consequently, objects with similar characteristics can move together within the same stream. This design has the potential to reduce reliance on particle position within the microchannel and streamline the microfabrication process. The finite element method was employed for numerical analysis of the separation process, and device parameters were adjusted to optimize performance. The results demonstrate the ability of the microfluidic device to efficiently enrich tumor cells, achieving a high efficiency rate of approximately 80%, in a label-free and cost-effective manner.

Keywords—dielectrophoresis, microfluidic chip, cell separation, cancer cell analysis

INTRODUCTION

Separating blood cells in a sample is an important testing process because each blood component has its own function, especially for cancer patients. In the pre-metastatic stage, information about circulating cancer cells moving in the circulatory and lymphatic systems provides clinical analytical value and the ability to diagnose the medical condition to provide appropriate treatment regimens for patients.

Dielectrophoresis (DEP) is a modern, non-invasive technology used for determining the electrical properties of biological objects, which can then be analyzed and utilized to manipulate and separate target cells from the sample. DEP is a method whereby a force is applied on a dielectric particle when it encounters a non-homogeneous electric field, regardless of whether the particles possess an inherent charge. Varmazyari et al. proposed a DEP-based microfluidic system with parallel sidewall electrodes that showed promising results with over 90% separation efficiency of circulating tumor cells (CTCs) [1]. However, the fabrication process is complex for setting up electrodes at the sidewall. Additionally, Wu et al. introduced a biochip with coplanar electrodes at only bottom of the microchannel to trap yeast cells and PS microbeads suspended in diverse aqueous solutions, each boasting unique conductivities [2]. Results were promising, with single yeast cell occupancy of over 72% achievable. Nevertheless, this technique requires a strong

concentration of the electric field around the electrode area and faces a particle position dependence problem.

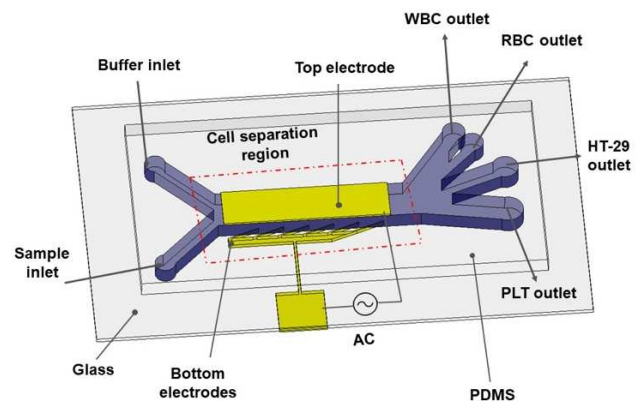


Fig. 1. The proposed microfluidic chip with integrated facing-electrode configuration dielectrophoresis (FEC-DEP) structure for separating of CTCs from blood constituents.

Our work aims to apply dielectrophoresis methods to isolate CTCs from cancer patients' blood cells. The proposed microfluidic system is illustrated in Fig. 1. It is a microfluidic system with two inlets and four outlets and an integrated dielectrophoresis structure, namely the facing-electrode configuration dielectrophoresis (FEC-DEP) structure. The FEC-DEP structure comprises a bottom slanted electrode array positioned parallel to a basic rectangular top electrode. These parallel electrodes generate a dielectrophoresis-induced non-homogeneous electric field.

In our previous study, we proposed a microfluidic device to enrich CTCs from RBCs and WBCs with three output configurations [3]. In this study, the system has been further optimized to separate CTCs from cells in blood samples, including red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). Initially, the blood sample is preprocessed to preserve four different cell types and introduced into the inlet. After that, all cells experience a non-uniform electric field. Because of the distinguishable electrical properties and sizes of blood cells and tumor cells, under the different effects of DEP force, cell types move along different trajectories inside the microchannel and concentrate at distinctive exits of the channel.

The presented microfluidic system is expected to provide a potentially effective, small-size, easily integrated, low-cost solution for CTC separation.

THEORETICAL ANALYSIS

In microfluidic systems, laminar flow occurs when the Reynolds number (Re) is small, typically $Re < 1$, indicating

that inertial forces are negligible compared to viscous forces. In this regime, described by the Navier-Stokes equation, viscous forces dominate, and inertial forces can be disregarded. Torsional flow, also known as Stokes flow, is prevalent in systems with high viscosity or small geometric length scales. Fluids in microfluidic systems are incompressible, and exhibit Newtonian behavior. In the analysis of such flows, momentum balance and mass conservation equations are simplified due to the dominance of viscous forces, characteristic of Stokes flow [4].

Hydrodynamic force is governed by Navier Stokes's law in a microfluidic channel:

$$\vec{F}_{HD} = 6\pi\eta R_p(\vec{v}_m - \vec{v}_p) \quad (1)$$

Here, R_p is the radius of the biological objects, η is the dynamic viscosity coefficient of the medium and $\vec{v}_m$ is the velocity of the flow.

The motion of particles induced by DEP commences when a non-uniform electric field is applied, creating a polarization gradient between the cells and the surrounding medium. DEP relies on the size and electrical properties of particles, as well as the binding characteristics of proteins and particle surfaces, to produce specific particle movements. When particles encounter this non-homogeneous electric field, two distinct forces are exerted between them and the surrounding medium, resulting in a net force. The direction of particle movement can be influenced by either positive DEP (p-DEP) or negative DEP (n-DEP) effects, determined by the relative polarizability of the particle and the suspension medium. p-DEP happens as particles migrate towards regions of high electric field gradients, whereas n-DEP occurs when particles move towards areas of low electric field gradients. Both of these effects are influenced by the Clausius-Mossotti factor. The DEP force driving the particle is calculated by the equation [5]:

$$F_{DEP} = 2\pi R_p^3 \epsilon_0 \text{Re} \left\{ \frac{\epsilon_c^* - 2\epsilon_f^*}{\epsilon_c^* + \epsilon_f^*} \right\} \nabla |\vec{E}|^2 \quad (2)$$

where, R_p is the cell radius, $\epsilon_0 = 8,854181817 \times 10^{-12} \text{ F/m}$ is the permittivity of vacuum. The Clausius-Mossotti (CM) factor associated with the dielectric characteristics of both particle (or cells in this study (c)) and the fluid (f) is applied to the bracket, where the complex permittivity is defined as:

$$\epsilon^* = \epsilon - j \frac{\sigma}{\omega} \quad (3)$$

The polarizability of the particles is measured by the CM factor which depending on the real part of permittivity ϵ and the electrical conductivity σ ; $\omega = 2\pi f$ is the angular frequency of driving AC voltage, and j is the imaginary part defined as $j^2 = -1$. The $\text{Re}\{\text{CM}\}$ can be adjusted within the range of -0.5 to 1 by altering the frequency of the voltage, leading to negative DEP (nDEP) or positive DEP (pDEP). In the circumstance of $\text{Re}\{\text{CM}\}$ equals 0, the frequency is termed the crossover frequency. The magnitude of the $(\nabla |\vec{E}|^2)$ is another important factor influencing the interaction with a particle which is dependent on the driving voltage. The design of the fluidic chip integrated electrode design is required to increase the $\nabla |\vec{E}|^2$ and regulate other electrokinetic forces [6].

The corresponding relative complex dielectric constant of a cell, encompassing the cell's shell and interior, is substituted for the complex dielectric constant of the particle when

calculating the DEP force (Figure 2). The single-shell model describes the complicated permittivity of the cell ϵ_c^* as follow [7]:

$$\epsilon_c^* = \epsilon_{mem}^* \left(\frac{R}{R - d_{mem}} \right)^3 + 2 \frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*} \left(\frac{R}{R - d_{mem}} \right)^3 - \frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*} \quad (4)$$

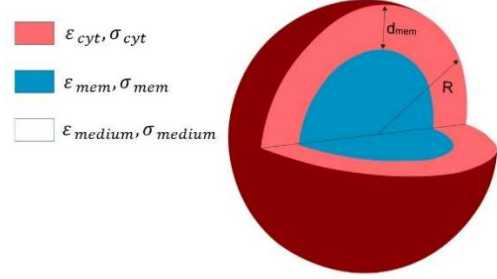


Fig. 2. Single cell model.

Biological cells in the microchannel undergo the DEP force as they follow the streamlines of fluid flow. The DEP force ($\vec{F}_{DEP}$), which is directed perpendicular to the flow, and the hydrodynamic force ($\vec{F}_{HD}$) are the primary factors influencing cell movement. Minor affected forces such as gravitation and Brownian forces are generally ignored as the cells' radius is greater than $1 \mu\text{m}$ as indicated in Table 1. Newton's second law, which accounts for the combined vector forces of DEP and hydrodynamics, determines the velocity and trajectory of the cell:

$$\vec{F}_{HD} + \vec{F}_{DEP} = m_p \frac{d\vec{v}_p}{dt} \quad (5)$$

where v_p represent the velocity and m_p stand for the mass of the cell, respectively.

Based on DEP principle, a microfluidic system has been proposed for cells manipulation and cancer cells separation.

DESIGN OF MICROFLUIDIC DEVICE AND SIMULATION MODEL

The utilization of FEC-DEP with a herringbone-patterned electrode array integrated into the bottom and a parallel electrode on top of a microfluidic system as depicted in Figure 3 has been proposed to achieve the DEP effect and isolate CTCs from blood cells. The biochip platform features two inlets: one for the blood sample containing PLTs, RBCs, WBCs, and HT-29 tumor cells, and the other for the buffer solution. The electrode area is positioned in the middle of the microchannel. The chip has four different outputs for concentrating various cell types. The electrode design comprises a ground electrode located at the top of the microchannel and a herringbone-patterned electrode array with positive polarity at the bottom.

During operation, the blood sample, comprising RBCs, WBCs, PLTs, and CTCs, is pumped through the microchannel at a velocity of $200 \mu\text{m/s}$, while the buffer solution flows at $900 \mu\text{m/s}$. Phosphate-buffered saline (PBS), characterized by a relative permittivity of 80 and a conductivity of 55 mS/m , serves as buffer solution. Due to variations in size and electrical properties among different cell types, distinct DEP forces are applied to HT-29 cells, WBCs, RBCs, and PLTs, directing each cell type along a

specific trajectory. Notably, as the angle of the electrodes increases, the DEP effects exerted on cells along the y-axis become more pronounced. This strategy enables effective separation and isolation of CTCs from other blood cells, offering promising prospects for cancer diagnostics and research.

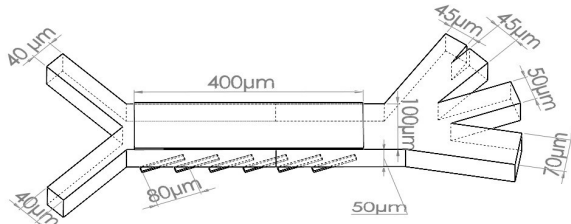


Fig. 3. Illustration of the proposed microfluidic system with dimensions of fluidic channel and FEC-DEP structure.

TABLE I. ELECTRICAL PROPERTIES OF EACH CELL TYPE [8]

Parameter	HT-29	RBC	WBC	PLT
Density (kg/m^3)	1077	1110	1080	1064.5
Diameter (μm)	11	6	14	2.5
Relative permittivity	120	59	150	50
Electrical conductivity (S/m)	0.72	1.31	0.76	0.25
Shell thickness (mm)	4	8	7	7
Shell relative permittivity	11.1	4.44	6.01	6
Shell electrical conductivity (S/m)	34×10^{-6}	1×10^{-6}	24×10^{-6}	1×10^{-6}

When subjected to an uneven electric field, all particles undergo DEP force, causing them to migrate upward away from the electrode region. However, due to pressure from the buffer flow, HT-29 cells and PLTs tend to deviate towards the wall near the electrodes' region. Notably, the amplitude of the DEP force is closely linked to the third-order cell size, resulting in WBCs experiencing a stronger DEP force and being directed to outlet 1. Conversely, red blood cells (RBCs) and HT-29 cells are collected at outlets 2 and 3, respectively. PLTs, with the smallest diameter, are gathered at outlet 4 and experience the least DEP force. The ratio of blood sample to buffer flow rate is 1:4.5, while the applied voltage is 6 V and the AC voltage frequency is 1 kHz.

The separation mechanism and the performance of the microfluidic chip were investigated in a 3D model using COMSOL's Electric Currents, Creeping Flow, and Particle Tracing for Fluid Flow modules, which allows assessing factors such as velocity distribution, electric field within the microchannel, DEP influence on cells, and particle trajectories under external forces. The channel walls were configured with a "no-slip" and "freeze" condition, ensuring negligible fluid velocity near the walls and maintaining particle position and velocity upon collision with the wall. All cells were randomly introduced into the sample inlet. The entire mesh comprised 93,356 domain elements, 14,238 boundary elements, and 1,354 edge elements. The numerical calculation converged with the applied time step and mesh resolution. Table 1 provides the electrical characteristics of

single-shell cells used in simulations. These findings elucidate the mechanism underlying the separation and isolation of specific cell types using DEP in microfluidic systems, offering valuable insights for biomedical applications.

RESULTS AND DISCUSSION

The electric field distribution within the microfluidic device is depicted in Figure 4. Within the electrode region, the electric potential increases as the distance to the electrode decreases, leading to a higher electric field intensity. The configuration of positively slanted electrodes located at the base of the channel, combined with ground electrodes positioned atop the channel, generated an uneven electric field across the separation area. Consequently, this uneven electric field induced DEP forces acting on cells in both horizontal and vertical orientations.

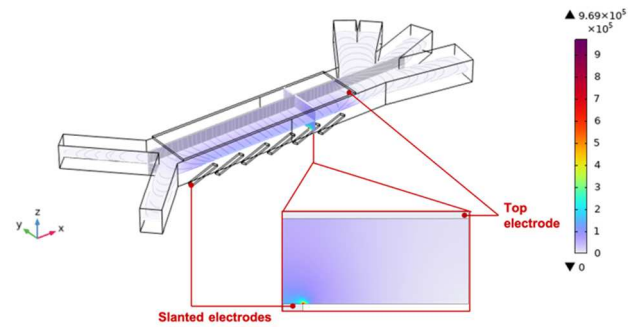


Fig. 4. Distribution of the electric field norm within the microchannel.

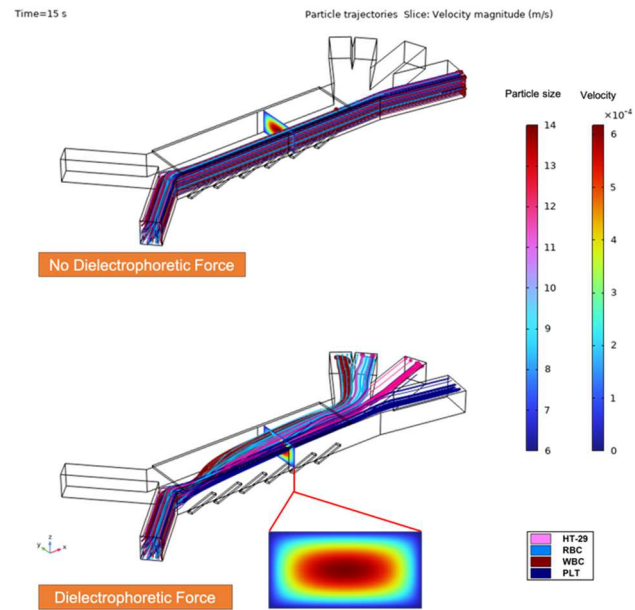


Fig. 5. Separation of CTCs from RBCs, WBCs, and PLTs in the microfluidic device in case of with or without DEP force applied. Inset shows the fluidic velocity distribution.

Figure 5 depicts the trajectories and velocity distribution of cells. In the absence of DEP force acting on particles, all cells would migrate along the same path, pushed against the channel wall solely by pressure from the buffer flow. Consequently, the outlet would contain cells randomly arranged according to the inlet. As the velocity progresses towards the channel's side, it gradually decreases from its

maximum value at the channel's center. In this simulation, a velocity ratio of 1:4.5 was maintained between the sample and buffer flows.

Numerical computations were conducted using an excitation AC voltage of 6 V at 1 kHz. Initially, the buffer flow injected into inlet 1 directed four different cell types towards the electrode region after they were introduced into inlet 2. The DEP force applied on all cells results in repulsion in both horizontal and vertical directions away from the electrode region. Subsequently, based on their distinctive responses to the DEP force during separation, WBCs, RBCs, HT-29 cancer cells, and PLTs concentrate at specified outlets (outlets 1, 2, 3, and 4 respectively), reflecting the distinguishing characteristics of the four simulated cell types.

Two criteria including separation efficiency and purity for each cell type, were used to assess the performance of the DEP-based device:

$$SE(A) = \frac{\text{Total type A cells found at the outlet of A}}{\text{Total type A cells included at the inlet}} \quad (6)$$

$$SP(A) = \frac{\text{Total type A cells found at the outlet of A}}{\text{Total all cell types found at the outlet of A}} \quad (7)$$

For HT-29 cells, RBCs, WBCs, and PLTs, the SE was 80%, 74%, 100%, and 86%, respectively, at the ideal circumstances suggested in this study.

The moving trajectories of cells were extensively studied through parametric research to investigate the effects of various operating and design factors. Figure 6 illustrates the separation efficiency and cell trajectories at driving voltages between 5 and 7 V. The electric voltage frequency and particle-to-buffer ratio were set at 1 kHz and 1:4.5, respectively. The electric field gradient in the DEP equation is proportional to the voltage magnitude, theoretically leading to increased particle deflection with higher voltage. However, results indicate a significantly higher DEP impact at 7 V applied voltage, with particles tending to gravitate towards the WBC outlet. Approximately 38% of RBCs were collected there, and a notable portion of HT-29 cells were redirected towards the RBC outlet, suggesting comparable scenarios. Similar occurrences were observed with PLTs at the HT-29 exit.

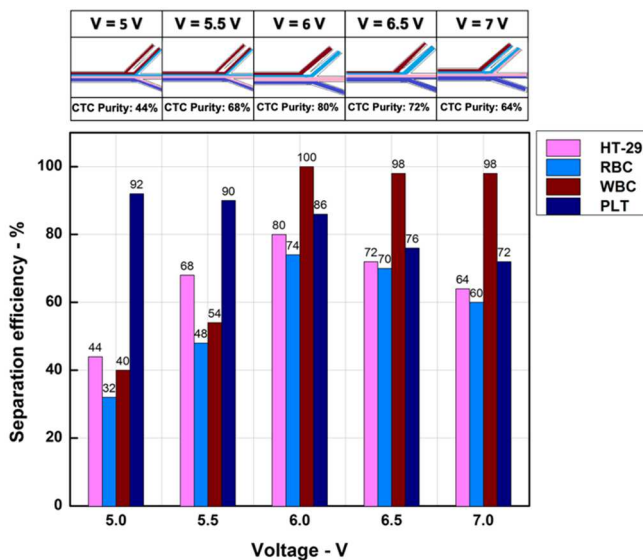


Fig. 6. Impacts of the magnitude of the applied voltage on separation performance.

Conversely, at an applied voltage of 5 V, HT-29 cells, WBCs, RBCs, and PLTs followed a slightly deflected course as the DEP force was insufficient to produce significant deflection. At the optimal voltage of 6 V (as depicted in Figure 6), cells were deflected and followed the predicted trajectory, resulting in maximum efficiency for all cell types.

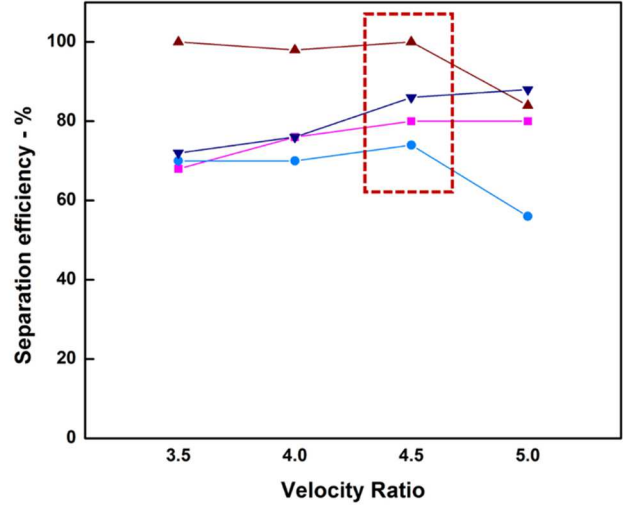


Fig. 7. Effects of the velocity ratio between the buffer inlet and the sample inlet on separation efficacy.

The impact of buffer medium flow rate on cell trajectories is depicted in Figure 7. The buffer solution plays a crucial role in guiding particle movement along a specific path before entering the separation region, emphasizing the importance of design control. The study focused on input velocity, defined as the ratio of buffer velocity to cell velocity, with a constant driving voltage of 6 V and an electric voltage frequency of 1 kHz. Cells' velocity from inlet 2 was fixed at 200 $\mu\text{m/s}$. High buffer velocity increases the drag force acting on particles, pressing them against the wall and deflecting them downwards, while lower buffer velocity prompts migration towards upper outlets. Successful separation of cancer cells and blood cells occurred at a buffer velocity of 900 $\mu\text{m/s}$ or a blood sample to buffer speed ratio of 1:4.5, as particles migrated towards the bottom electrode area, avoiding contact and entrapment along the channel's top wall. HT-29 separation efficiency obtained 80%, with a sample purity level of nearly 80%.

Cell separation was facilitated by the non-uniform electric field generated by the electrode array, with the field's strength depending on electrode quantity and arrangement. Alterations in the electric field affected cell separation efficiency and purity by modifying the force acting on cells.

CONCLUSIONS

This work introduces an effective DEP-based biochip device aimed at distinguishing HT-29 cancer cells from RBCs, WBCs, and PLTs, subsequently validated through numerical evaluation to confirm biochip design performance. DEP force is applied to particles via a FEC-DEP structure, facilitating reduced cell position dependence in the channel and simplified fabrication complexity. Geometric and operational characteristics' effects were investigated, resulting in high

separation efficiency for WBCs, RBCs, PLTs, and HT-29 under ideal conditions, with HT-29 tumor cells efficiently and accurately collected at the designated outlet. Particle trajectories were significantly influenced by applied voltage and the ratio between blood sample and buffer flow velocity. These promising results serve as a foundation for further theoretical and applied research. The proposed system offers numerous advantages, including small size, low applied voltage, optimal efficiency, non-invasive, and label-free, holding potential for cancer identification, evaluation, and analysis.

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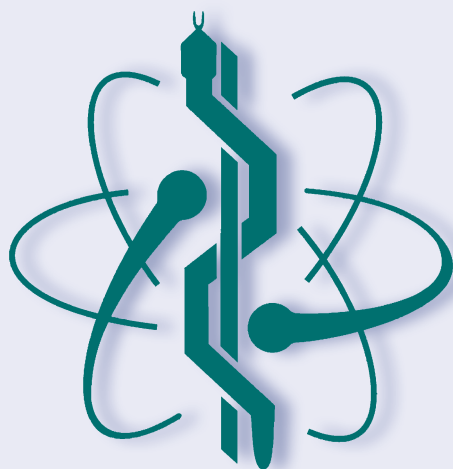
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Phan Thiet, Vietnam: Healthcare Evolution
Towards 5P Medicine in Low- and
Middle-Income Countries Ecosystem - Volume
1: Medical Devices, Biomedical Data Science
and Models, and Entrepreneurship and
Management in Healthcare

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Development of a Micropillar-Based Concept for Hydrodynamic Trapping and Long-Term Culture of Single Oocytes

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Abstract. Oocyte growth and analysis are critical in assisted reproductive procedures. Traditional microdrop culture might encounter several disadvantages, including time consumption, manual handling, and lack of automation. These methods may struggle to replicate the dynamic follicular environment, hence reducing oocyte competence. This study introduces a biochip designed for the hydrodynamic trapping and long-term culture of single oocytes based on the micropillar concept that includes interleaved trap units. Each unit consists of a three-micropillar-based structure that not only captures single oocyte automatically but also enhances its exposure to the culture medium. This design enhances the oocytes' exposure to the culture medium by increasing surface area and facilitating dynamic culture with a continuous supply of nutrients, hormones, and waste removal. The hydrodynamic trapping mechanism utilizing a micropillar structure was verified using the finite element method. The simulation results verified the trapping mechanism and the interaction between oocytes and medium using the proposed pillar-based microfluidic structure. Initial fabrication results have also been reported in this work. The obtained results validated the ability of the proposed microfluidic chip device for automatic trapping and long-term culture of single oocytes, establishing a solid foundation for optimizing and further exploring the proposed biochip. This biochip can be integrated with other on-chip functions to create a multifunction lab-on-a-chip platform for assisted reproduction procedures.

Keywords: microfluidic chip · micropillar-based concept · single-oocyte trapping

1 Introduction

Assisted reproductive technology (ART) is vital for addressing various challenges, including population aging, infertility, expanding family options for same-sex couples and single individuals, and enabling genetic screening and selection [1]. Despite significant progress in ART, only 5% of aspirated oocytes have the potential to develop into a child [2]. To date, most advancements in this area have concentrated on altering media components, while the physical instruments for handling and manipulating oocytes and embryos in ART laboratories have received comparatively less attention. This gap highlights the urgent need for new and improved physical tools in ART to enhance procedural outcomes.

A key component of ART is *in vitro* maturation (IVM), where immature cumulus-oocyte complexes from antral follicles mature *in vitro* [3]. The main goal of IVM is to minimize or eliminate the risk of ovarian hyperstimulation syndrome (OHSS) in PCOS patients, reduce medication costs, and alleviate patient stress [4]. This technique is also recommended for normo-ovulatory women, fertility preservation, and rare cases such as oocytes failing to mature or consistently poor-quality embryos.

Traditional IVM uses microdrop oocyte culture, a static method widely utilized for years. Although simple and cost-effective, this method can limit nutrient exchange and waste removal, potentially affecting oocyte development. IVM technique is challenging to mimic dynamic *in vivo* oocyte maturation, which might reduce oocyte competence [5]. On the other hand, microfluidic technology, which is gaining increasing attention, permits dynamic culture systems [6]. These systems provide a continuous flow of culture medium around tissues, enhancing the exchange of nutrients and metabolites between the medium and the tissue. This dynamic environment more accurately replicates natural conditions for oocyte development [7, 8].

Additionally, microfluidic systems conserve resources by using microscale volumes of medium and allow for continuous modulation of different concentrations according to the chip design. This precise control of culture conditions supports optimal oocyte development. Besides the medium aspect, microfluidic chips can also be used for single oocyte trapping, a promising technique in assisted reproduction. Hydrodynamic trapping simplifies oocyte handling, allowing for quick and easy medium changes and automated monitoring and analysis of individual oocyte development [9, 10].

In our study, we developed a microfluidic chip specifically designed for trapping and long-term dynamic culture of single oocytes. Finite element method (FEM) simulations with COMSOL Multiphysics software have been used to model single oocyte trapping based on a hydrodynamic concept in the proposed microfluidic chip. These simulations revealed a dynamic and low-velocity field around the trapped oocyte, which is beneficial for its long-term culture. To enhance oocyte development, a micropillar-based design has been integrated to increase the oocyte surface area exposed to the medium. Using advanced lithography techniques, we successfully fabricated the proposed device. The simulation and fabrication results laid a strong foundation for future experimental investigations. Additional on-chip functions could be integrated into the proposed microfluidic chip to create a multifunctional lab-on-a-chip platform tailored for assisted reproduction procedures.

2 Design and Mechanism of Proposed Microfluidic Device

2.1 Design

Figure 1 illustrates the structural configuration of the microfluidic chip designed for the trapping and nurturing of individual oocytes. The proposed structure consists of two inlets and one outlet. These inlet ports are positioned diagonally on both sides to facilitate the introduction of oocytes, mediums, and additional hormones into the microchannel. For single oocyte trapping purposes, trap units are arranged in a staggered configuration within the microchannel. This configuration is based on the hydrodynamic principle underlying the single-cell trapping mechanism. When an oocyte becomes trapped within a trap unit, the velocity of fluid flow around the trapped cell decreases due to obstruction. Consequently, the other single oocytes passing through this region tend to navigate to avoid the trapped cell with high resistance to access other trap units. The configuration of trap units in interleaved rows enhances trapping efficiency. Each trap unit comprises three micropillars. The spacing between these micropillars has been meticulously calculated and arranged for each trap cell to accommodate a single oocyte. In addition, the micropillar structure helps enhance the interface area between the oocyte and the culture medium in comparison to other structures [11, 12]. In this study, a single oocyte was used to evaluate the functioning mechanism of the proposed biochip, utilizing the simulation method. The reference mechanical parameters considered in the simulations encompassed cell size, Young's modulus, and Poisson's ratio, as shown in Table 1 [13, 14].

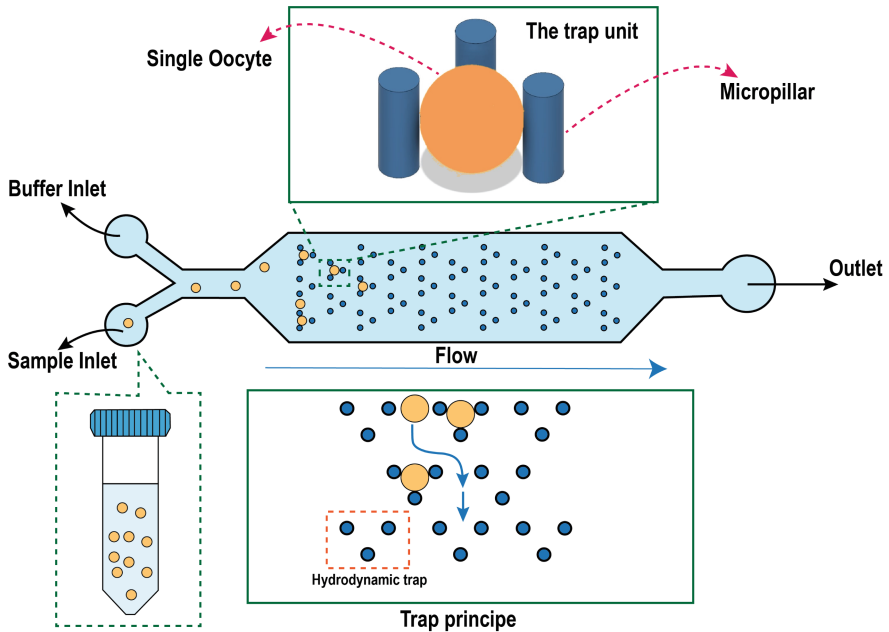


Fig. 1. Schematic of the proposed microfluidic chip design.

2.2 Mechanism and Simulation Model

Figure 2 illustrates the simulation model of the proposed microfluidic chip. The simulations were conducted using COMSOL Multiphysics software version 6.0. To simplify the analysis, the simulation employed a 2D model of the chip’s main channel, where the solution and oocytes were introduced and allowed to interact over a period of time. When culturing immature oocytes, the oocytes are always co-cultured with surrounding cumulus cells, forming oocyte-cumulus complexes. Therefore, the oocyte size used in this simulation is 320 μm .

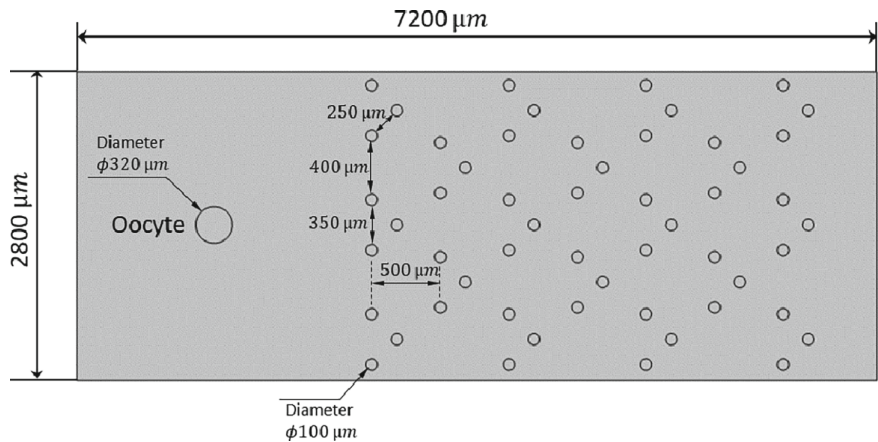


Fig. 2. Simulation model and geometry parameters

Table 1. Physical parameters

Name	Parameter
Fluid density	10^3 kg/m^3
Fluid viscosity	$10^{-3} \text{ Pa}\cdot\text{s}$
Oocyte density	1150 kg/m^3
Oocyte Young’s modulus	300 MPa
Oocyte Poisson’s ratio	0.3
Polydimethylsiloxane (PDMS) density	970 kg/m^3
PDMS Young’s modulus	$3 \times 10^8 \text{ Pa}$
PDMS Poisson’s ratio	0.49

Fluid flow was modeled as creeping flow using the Navier-Stokes equations, while cell motion followed linear elastic dynamics and Newton’s laws. This study explored fluid-solid interaction (FSI), where hydrodynamic forces deform the structure, thereby altering the flow field. The Arbitrary Lagrangian-Eulerian (ALE) method was employed

to handle the dynamic mesh and moving boundaries, ensuring numerical stability and accuracy throughout the computational process. Reynold number is extremely small, then the flow is considered Stokes flow. The equations are given as follows [15]:

$$\nabla \cdot [-p\mathbf{I} + \mathbf{K}] + \mathbf{F} = 0 \quad (1)$$

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}_f) = 0 \quad (2)$$

where p represents the pressure, $\mathbf{K}$ denotes the viscous stress tensor, $\mathbf{F}$ is the volume force vector, ρ signifies the fluid density, and $\mathbf{u}_f$ indicates the fluid velocity. For an incompressible fluid, the density is assumed to be constant. Modeling cells as solid structures allows for accurate simulation of their mechanical properties using FEM in FSI models, providing an understanding of their behavior in different fluid mediums. This approach integrates biological insights with solid mechanics, thereby advancing computational models of cellular dynamics. The displacement and deformation of solids conform to the governing equations [16]:

$$\varepsilon_s = \frac{1}{2}[(\nabla \mathbf{u}_s)^T + \nabla \mathbf{u}_s + (\nabla \mathbf{u}_s)^T (\nabla \mathbf{u}_s)], \quad (3)$$

$$\nabla \sigma_s + \mathbf{F}_s = \rho_s \frac{\partial^2 \mathbf{u}_s}{\partial t^2}, \quad (4)$$

Equation (3) represents the strain-displacement equation, where ε_s is the infinitesimal strain tensor and $\mathbf{u}_s = (u_s, v_s, w_s)$ denotes the solid displacement field (m, m, m). Equation (4) corresponds to Newton's equation of motion, where σ_s the Cauchy stress tensor, $\mathbf{F}_s$ is the boundary force per unit area in 2D (N/m^2), and ρ_s represents the solid density (kg/m^3).

FSI involves the interplay between fluid dynamics and structural mechanics. A structure deforms under fluid forces, which in turn affects the fluid flow by changing its velocity [16]. The interaction equations are determined by:

$$\mathbf{F}_A = [-\rho \mathbf{I} + \mathbf{K}] \cdot \mathbf{n} \quad (5)$$

$$\mathbf{u}_{tr} = \frac{\partial \mathbf{u}_s}{\partial t} \quad (6)$$

in which $\mathbf{F}_A$ is the combined force between fluid pressure and viscosity on the solid boundary, while $\mathbf{n}$ denotes the outward normal to the boundary. According to Eq. (6), $\mathbf{u}_{tr}$ is the displacement rate of the solid, which equals the fluid velocity $\mathbf{u}_f$. Thus, a condition of no-slip boundary exists at the fluid-solid interface.

The simulation applies specific boundary conditions to ensure physical accuracy and numerical stability. At the inlet, a parabolic velocity profile is defined as $\vec{u}_f = U_0 \vec{n}$, where $U_0 = u_0 \frac{6(W-Y)Y^1}{W^2}$, with u_0 as the characteristic velocity and W as the channel width. For the outlet, a zero-gauge pressure condition is applied to ensure a smooth exit flow and match experimental setups. Along solid walls, no-slip conditions are imposed. On specified walls, a zero-velocity condition is enforced: $\vec{u}_f = 0$. These boundary conditions effectively model the flow and interaction with domain boundaries.

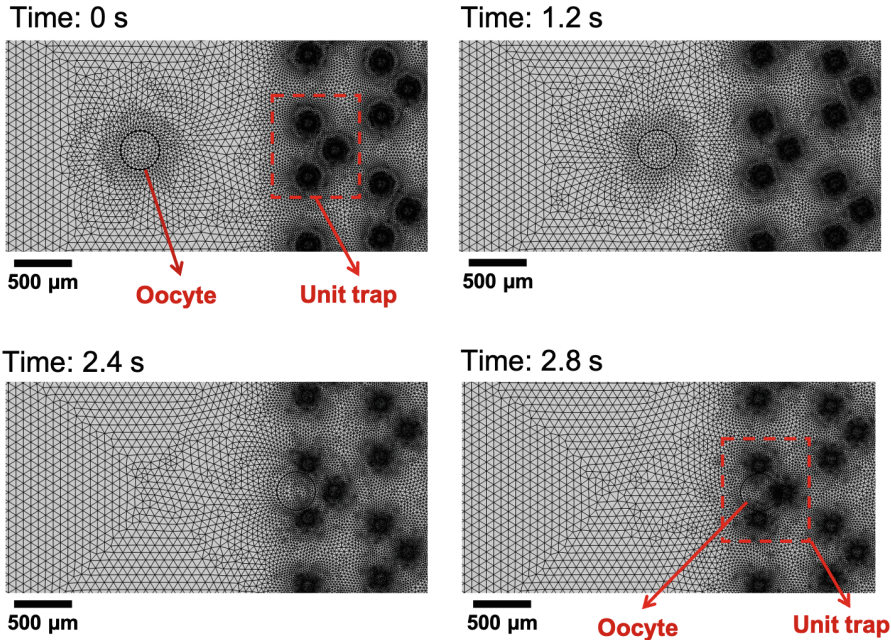


Fig. 3. Moving mesh around the oocyte as it moves to the trap

Employing a moving mesh in FSI simulations ensures an accurate representation of evolving geometries, precisely tracks deformations and movements, and enhances computational stability and efficiency [17]. This is achieved by continuously adapting the mesh to correspond with the dynamic shapes of both structures and fluids. Figure 3 depicts the mesh movement in response to the simulated cell displacement. The mesh is nonuniform, featuring more compact and smaller elements at the fluid-solid interfaces and less dense, larger elements within the fluid domain.

2.3 Microfluidic Fabrication Process

Microfluidic chip devices were fabricated using standard soft lithography techniques. These devices are composed of polydimethylsiloxane (PDMS) (Sylgard-184 Silicone Elastomer Kit, Dow Corning, Midland, MI, USA), a material extensively utilized in microfluidics due to its high biocompatibility. A negative photoresist SU-8 (SU-8 50, MicroChem Corp, Newton, MA, USA) was patterned on a silicon wafer via photolithography, employing a mask with a pre-designed device structure to create an SU-8 master mold for microfluidic channel fabrication. The mold was spin-coated at 500 rpm for 10 s and 1000 rpm for 30 s, following the material's data sheet to achieve the desired channel height. Subsequently, PDMS prepolymer mixed with curing agent Sylgard 184 (Sylgard-184 Silicone Elastomer Kit, Dow Corning, Midland, MI, USA) at a 1:10 ratio was poured onto the mold and degassed in a vacuum chamber. The polymer was then partially cured in an oven at 60 $^{\circ}\text{C}$ for 45 min. This cure time was determined to be optimal as shorter durations led to structural collapse, while longer durations hindered

the release of PDMS from the mold. After partial curing, the PDMS was peeled from the mold, and fluid inlets and outlets were punched through using a suitably sized chisel tip. The PDMS layer was then permanently bonded to a standard glass slide via oxygen plasma treatment. The primary SU-8 mold can be reused multiple times, thereby reducing costs and fabrication time.

3 Results and Discussions

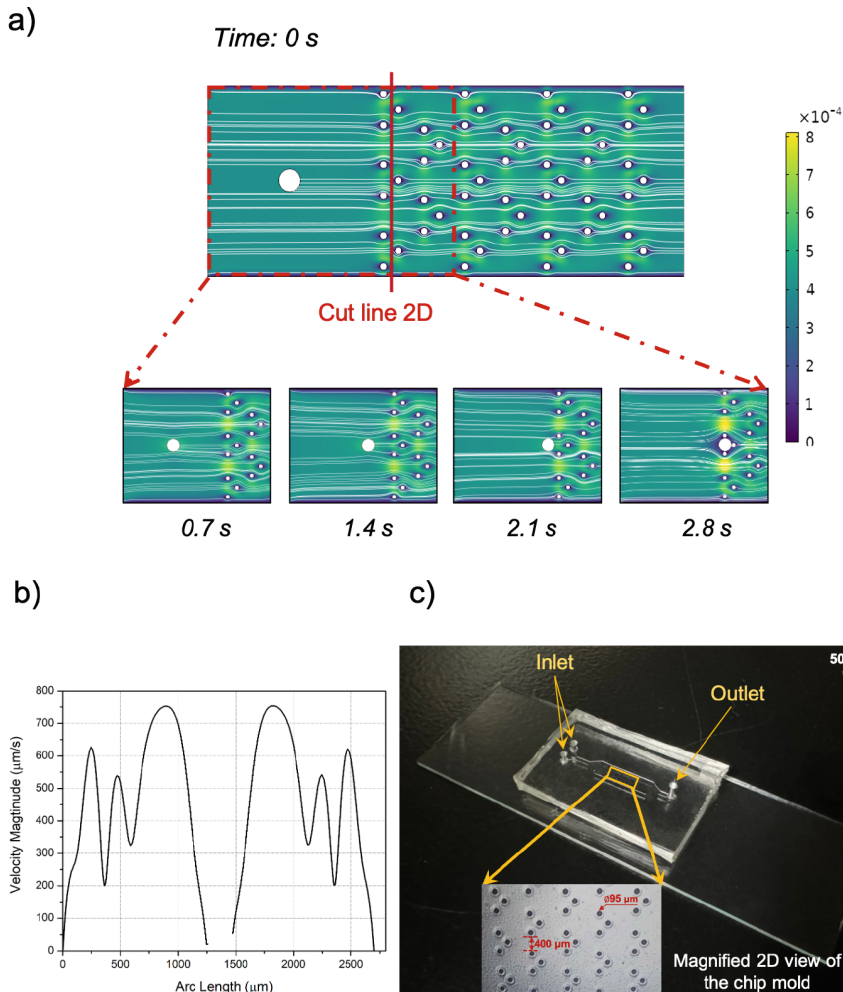


Fig. 4. a) Velocity magnitude distribution inside the device during cell trapping at different times, b) Velocity magnitude along the cutline at $t = 2.8$ s when the oocyte was trapped within the unit, c) Demonstrates the fabricated microfluidic chip.

To analyze the flow dynamics critical for oocyte trapping and cultivation in a microfluidic device, Fig. 4a and Fig. 4b illustrate the simulation results of the velocity magnitude within the channel and the velocity profile across the 2D cut line at $t = 2.8$ s, corresponding to the instant the oocyte is trapped in the trap unit. The detailed velocity distribution provides insights into the flow dynamics around the trapped oocyte, highlighting areas of high and low velocity within the channel. The inlet velocity in the simulation is set to $400 \mu\text{m/s}$. Velocity analysis within the channel is conducted throughout the cell's migration until it is captured at the trapping site. Results are plotted at 0 s, 0.7 s, 1.4 s, 2.1 s, and 2.8 s. The data indicate that the flow velocity in the channel varies between 0 and $800 \mu\text{m/s}$. Initially, the oocyte is located far away from the trapping unit, and the velocity within the trap units exceeds $600 \mu\text{m/s}$, significantly higher than outside the traps, guiding the oocytes to the empty trap units. During the intervals from 0.7 s to 2.1 s, the velocity field remains constant, with the velocity around the target trap unit being higher than that of adjacent traps. At 2.8 s, when the cell is fully trapped, the velocity around and inside the trap unit significantly decreases. The velocity magnitude graph drawn on a 2D cut line analysis reveals that the minimum velocity around the trapped cell is approximately $15\text{--}25 \mu\text{m/s}$, which is beneficial for dynamic long-term culture. The flow field velocity within this trap unit is notably lower than the velocity within the channel. This lower velocity mitigates shear stress, thereby decreasing the likelihood of cell damage or deformation due to fluid flow influences. However, this velocity is still enough to allow the oocyte to continue receiving nutrients from the culture medium and facilitates the automatic expulsion of waste. Furthermore, the microfluidic chip designed for capturing and cultivating single cells has been successfully fabricated. Figure 4c demonstrates the fabricated microfluidic chip. The microfluidic chip has two inlets and one outlet. The device has micropillar structures that are well-defined and relatively uniform with a diameter of approximately $95 \mu\text{m}$, and the gap between unit traps is $400 \mu\text{m}$. The chip height is $220 \mu\text{m}$ suitable for oocyte manipulation. The results of the chip fabrication process indicate that the fabricated structures exhibit a shape and size that closely align with the original design specifications. Notably, the dimensions of the pillars, particularly the top sections, are reduced by approximately 5%. This minor reduction is attributed to challenges encountered during the fabrication process, which prevented the complete removal of excess SU-8 material from the mold. Nevertheless, this dimensional reduction might be advantageous in subsequent applications, particularly in oocyte culture. The slightly reduced pillar dimensions enhance the contact area between the oocyte and the culture solution, potentially promoting more efficient interaction and improved outcomes in the oocyte culture. Figure 5 analyzes the movement of an oocyte when another oocyte is confined within a trapping unit. The results demonstrate that the free oocyte tends to bypass the trapping unit containing the trapped oocyte because the velocity on the bypass direction is much higher than the filled trap unit. This observation aligns with the principles of hydrodynamic trapping [18], wherein the cell follows the path of least resistance. The staggered arrangement of the trapping units ensures that the oocyte is subsequently captured by alternative trapping units. The successful fabrication of the chip according to the proposed design, along with the successful simulation of the chip model, lays a strong foundation for ongoing

research. These results promise to create a potential platform for future applications in biomedical engineering.

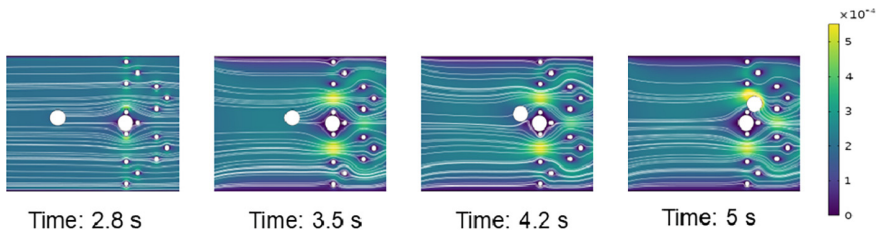


Fig. 5. The oocyte movement bypass a filled trap unit

4 Conclusions

This research introduced a microfluidic chip on a micropillar platform designed for the hydrodynamic trapping and cultivation of single oocytes. Simulation results confirmed the ability of the proposed microfluidic chip for automatic trapping and the potential long-term culture of individual oocytes, laying a robust groundwork for future optimization and experimental validation. The chip was successfully fabricated using soft lithography techniques. This biochip can be integrated with additional on-chip functionalities to develop a multifunctional lab-on-a-chip platform for assisted reproduction procedures. The micropillar structure supports dynamic cell cultivation, offers high-throughput capabilities, and shows potential for applications in organ-on-a-chip systems.

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