

VIETNAM NATIONAL UNIVERSITY, HANOI
UNIVERSITY OF ENGINEERING AND TECHNOLOGY



NGUYEN THU HANG

**RESEARCH AND DEVELOPMENT OF AN
ORGAN-ON-A-CHIP PLATFORM FOR
ASSISTED REPRODUCTIVE TECHNOLOGIES
BASED ON MICROSYSTEMS**

(Nghiên cứu phát triển cấu trúc organ-on-a-chip ứng
dụng trong kỹ thuật hỗ trợ sinh sản dựa trên nền tảng
vi hệ thống)

PhD DISSERTATION IN ELECTRONIC ENGINEERING

HANOI – 2026

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Abstract

Infertility has become a major global health concern, affecting approximately one in six individuals of reproductive age worldwide, with Vietnam ranking among the countries with the highest infertility rates. Assisted reproductive technologies (ART), particularly *in vitro* maturation (IVM) have emerged as essential procedures in modern fertility treatment. However, current workflows still rely heavily on manual handling, subjective assessment, and static culture conditions that inadequately replicate the dynamic ovarian microenvironment. These limitations highlight the need for advanced and automated platforms capable of providing controlled culture conditions, continuous monitoring, and objective evaluation of oocyte development to improve the efficiency, consistency, and reliability of reproductive treatments.

This thesis presents the design, fabrication, simulation, and experimental validation of a modular microfluidic system for streamlining multiple stages of the oocyte-processing workflow in assisted reproductive technologies. The proposed system integrates OvaOoC for single-oocyte trapping, dynamic co-culture of oocytes and cumulus cells, and continuous maturation monitoring combined with time-lapse imaging and AI-assisted analysis. In addition, high-throughput automated on-chip denudation and DEP-based oocyte selection were developed to support downstream fertilization procedures through more objective and standardized oocyte handling. Porcine oocytes were employed as a biologically relevant model for proof-of-concept validation of the developed platform.

The proposed system consists of three microfluidic modules for oocyte trapping and culture, on-chip denudation, and DEP-based oocyte selection. In the OvaOoC module, hydrodynamic trapping units with progressively decreasing dimensions enable size-dependent immobilization of individual oocytes, while dynamic perfusion and co-culture with cumulus cells provide a more physiologically relevant microenvironment. Continuous time-lapse imaging combined with deep learning-based analysis further enables automated assessment of cumulus expansion and morphological changes during oocyte maturation. Following maturation, cumulus–oocyte complexes are transferred into the denudation chip, which incorporates a multi-row quadrilateral micropillar array for automated stripping of cumulus cells. Finally, denuded oocytes are introduced into a DEP-integrated microfluidic chip, where diagonally arranged electrodes generate a non-uniform electric field for label-free separation of oocytes according to their dielectric

properties and developmental competence.

The simulation and experimental results confirmed the effectiveness of the proposed microfluidic platform. Hydrodynamic simulations demonstrated favorable flow and shear stress conditions for oocyte culture, while experiments achieved a high trapping efficiency of 88.37% and improved maturation performance under dynamic on-chip culture compared with conventional microdrop culture. The integrated time-lapse imaging and deep learning framework enabled automated and objective evaluation of cumulus expansion. The developed on-chip denudation system achieved a high denudation efficiency of 95.94% (± 0.94) with superior throughput compared with existing systems, while maintaining IVF outcomes comparable to conventional manual procedures. In addition, the DEP-integrated chip successfully distinguished and separated normal and abnormal oocytes based on dielectric properties, with good agreement between simulation and experimental results.

This thesis contributes to the field of assisted reproductive technologies by presenting a modular microfluidic platform toward integrated ART-workflow for oocyte handling, culture, monitoring, and selection within a unified workflow. The integration of emerging microfluidic, organ-on-chip, artificial intelligence, and dielectrophoretic technologies provides a promising approach toward more efficient, standardized, and objective ART systems. Future work will focus on improving biomimetic microenvironmental control, enhancing analytical capabilities, and extending the platform toward fully integrated systems for fertilization and embryo development applications.

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Dissertation Introduction

Background and context of the research

Infertility has become a major global health concern, with millions of couples worldwide experiencing reproductive failure and posing significant challenges to reproductive healthcare systems. *In vitro* maturation (IVM) has attracted considerable attention due to its ability to reduce ovarian stimulation, minimize patient burden, and lower the risk of ovarian hyperstimulation syndrome. However, current workflows still rely heavily on manual handling, subjective evaluation, and static culture systems that inadequately mimic the physiological ovarian microenvironment. Recent advances in microfluidic, organ-on-chip, artificial intelligence, and dielectrophoretic technologies provide promising opportunities for developing biomimetic and automated platforms for oocyte culture, monitoring, and selection in next-generation ART applications.

Objective and significance of the research

The primary objective of this research is to develop and optimize an integrated microfluidic platform that provides a controlled and biomimetic microenvironment for key oocyte-related procedures in assisted reproductive technologies. To overcome the limitations of current IVM workflows, the proposed system incorporates dynamic co-culture, deep learning-assisted monitoring, automated on-chip denudation, and dielectrophoretic selection within a microfluidic framework. This approach has the potential to establish a more objective, physiologically relevant, and automation-compatible platform for improving the efficiency, consistency, and standardization of ART.

Main contributions of the dissertation

This dissertation presents the development of an integrated microfluidic platform for assisted reproductive technologies, with contributions spanning microfluidic culture, automated oocyte manipulation, and label-free oocyte selection. The main contributions of this study are summarized as follows:

- Development of the OvaOoC platform for hydrodynamic single-oocyte trapping and dynamic co-culture of oocytes and cumulus cells under controlled microenvironmental conditions. The proposed organ-on-chip system enables continuous perfusion, stable single-cell immobilization, and improved nutrient exchange for oocyte maturation.
- Integration of time-lapse imaging and deep learning-assisted image analysis for automated and objective monitoring of oocyte maturation. The developed framework enables segmentation and quantitative assessment of cumulus cell expansion and morphological changes during in vitro development.
- Design and experimental validation of a high-throughput on-chip denudation system based on a quadrilateral micropillar array. The proposed hydrodynamic denudation mechanism enables efficient removal of cumulus cells while reducing operator dependency and improving process standardization compared with conventional manual methods.
- Development of a DEP-integrated microfluidic chip for label-free oocyte selection based on dielectric properties. The proposed electrode configuration and microchannel structure enable the discrimination and separation of normal and abnormal oocytes in a non-invasive and automation-compatible manner.

Scientific and practical significance

Scientifically, this dissertation contributes to the understanding of microfluidic hydrodynamics, fluid–cell interactions, and dielectrophoretic manipulation in assisted reproductive technologies. The study also advances the integration of organ-on-chip systems, deep learning-assisted analysis, and DEP-based cell manipulation within a microfluidic framework for automated and objective oocyte assessment. Practically, the developed platform provides a promising foundation for more standardized, scalable, and automation-compatible ART workflows, helping bridge the gap between microfluidic engineering technologies and real-world reproduction applications.

Methods and scope of the study

This study employs a combination of numerical simulation, microfabrication, and experimental validation to develop a microfluidic platform for assisted reproductive technologies. The scope of the research includes the design and fabrication of microfluidic chips, characterization of fluid flow, shear stress, and DEP-induced cell behavior, as well

as experimental evaluation of single-oocyte trapping, dynamic co-culture, automated denudation, and DEP-based oocyte selection using porcine oocytes as biological samples.

Overview of the dissertation structure

- **Chapter 1** presents the research background, challenges in assisted reproductive technologies, and recent advances in microfluidic and organ-on-chip systems for oocyte applications.
- **Chapter 2** introduces the theoretical foundations, system design, and FEM-based simulations for microfluidic flow, hydrodynamic trapping, fluid–cell interactions, and DEP-based manipulation.
- **Chapter 3** describes the microfabrication processes, experimental setups, biological sample preparation, and oocyte-related experimental procedures.
- **Chapter 4** presents and discusses the simulation, fabrication, and experimental results of the proposed microfluidic platforms for oocyte trapping, culture, denudation, assessment, and selection.
- **Conclusions** chapter presents the key findings of the thesis and discusses potential directions for future research.

Chapter 1

Introduction

1.1 Background significance

Reproductive and endocrine failure are increasingly recognized as major global health concerns [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. According to the WHO report Infertility Prevalence Estimates, 1990–2021, approximately one in six people of reproductive age worldwide experience infertility in their lifetime, showing an urgent need to do in-depth research on the reproductive systems and provide access to high-quality, reasonably priced fertility care. Vietnam is among the countries with the highest infertility rates worldwide. Notably, approximately 50% of infertility cases occur in individuals under 30 years of age, highlighting an increasingly younger trend in disease onset. It is estimated that around one million couples in Vietnam experience infertility each year, with about 7.7% of reproductive-age couples affected [2]. Therefore, the development and refinement of infertility treatment techniques are of critical importance to public health.

Assisted reproductive technology (ART) has been established as a group of medical techniques aimed at addressing this global challenge, including all infertility treatments that involve the handling of oocytes or embryos. ART procedures typically include the surgical retrieval of oocytes from the ovaries, fertilization with sperm in the laboratory, and subsequent transfer of the resulting embryos either to the patient or to a gestational carrier [3]. Since its early beginnings with ovarian graft transplantation in 1895 [4], ART has undergone more than a century of advancement and refinement, enabling the successful treatment of increasingly complex reproductive cases [5]. Milestones in this progression include improvements in reproductive tissue and organ transplantation and *in vitro* culture techniques, such as the first human uterus transplantation in 1931 [5], [6], the first human birth from *in vitro* fertilization (IVF) in 1978 [7], and ability to give birth from a frozen embryo in 1984 [8]. More recent technological innovations,

driven by the integration of reproductive biology, materials science, bioengineering, and advanced manufacturing, have further expanded the capabilities of ART [5]. Notable achievements include the first human birth from transplanted ovarian tissue in 2005 [9], live animal birth from follicles grown *in vitro* within a biomaterial scaffold in 2006 [10], and in 2017 a miniature handheld female reproductive tract, namely EVATAR, were introduced that could eventually change the future of research and treatment of diseases in women's reproductive organs [11].

IVF is regarded as the most common assisted reproductive technology currently available for infertility treatment. The procedure involves fertilizing oocytes *ex vivo*, culturing embryos in the laboratory, and subsequently transferring the embryos into the uterus. The birth of Louise Brown, the first child conceived via IVF in the United Kingdom, marked a major milestone in modern medicine and offered new hope for infertile couples [12]. Vietnam welcomed the first three IVF infants in 1998. As part of the IVF process, patients typically undergo ovarian stimulation and continuous clinical monitoring for several consecutive weeks. The combination of these steps can make the overall treatment feel even more overwhelming for patients. Patients usually receive ovarian stimulation hormone injections over a period of approximately two weeks, with a total of around 30 injections, to induce the simultaneous development of multiple follicles. They also undergo frequent ultrasound examinations and blood tests to monitor follicular growth. Once the follicles reach the appropriate size, oocyte retrieval is performed, followed by in-laboratory oocyte culture and fertilization to generate embryos, which are subsequently cryopreserved. After the woman's menstrual cycle resumes, she returns to the clinic for a two-week regimen of hormonal therapy to prepare the endometrium, after which embryo thawing and transfer are carried out. Overall, the process is not only time-consuming and costly but also places considerable physical and psychological demands on patients, particularly due to medication-related side effects and the risk of ovarian hyperstimulation syndrome (OHSS).

To address these limitations, *in vitro* maturation (IVM) has been developed. With IVM, patients can undergo IVF with no or only minimal ovarian stimulation, thereby substantially reducing the side effects and risks associated with hormonal treatment. IVM involves the collection of immature oocytes from small antral follicles in the ovary, which are typically at a stage before entering dominant growth, followed by culture in a specialized laboratory environment that supports oocyte maturation [13]. Once maturation is achieved, the oocytes are fertilized with sperm to form embryos, which are subsequently transferred following procedures similar to conventional IVF. The first report of IVM in humans appeared in 1965; however, successful pregnancies and live births were not documented until 1989, marking an important milestone in the field of assisted reproduction [14].

In IVM, immature oocytes are retrieved instead of mature oocytes as in conventional

IVF. A key advantage of *in vitro* oocyte maturation is that it is less invasive and reduces patient burden. A comparison of the IVM and IVF workflows is presented in Figure 1.1. Because IVM does not require ovarian stimulation with hormones, it minimizes treatment-related complications and eliminates the risk of ovarian hyperstimulation syndrome, making it particularly suitable for women with polycystic ovary syndrome (PCOS), who are at elevated risk when exposed to stimulation protocols [15]. In addition, IVM shortens the overall treatment duration, decreases the frequency of monitoring and laboratory testing, and consequently reduces financial costs and psychological burden for patients. The method is also considered safer for specific patient groups, such as women seeking fertility preservation prior to cancer therapy. IVM represents one of the most suitable options for patients who cannot delay cancer treatment long enough to obtain fully matured oocytes through conventional stimulation protocols. By enabling the retrieval of immature oocytes for later use, IVM helps preserve a woman's potential for future pregnancy and childbirth. Moreover, for individuals with estrogen-sensitive malignancies, IVM offers an important advantage by avoiding the elevated estrogen levels associated with standard ovarian stimulation approaches. Owing to these advantages, IVM is being increasingly adopted and offers renewed hope for many couples experiencing infertility.

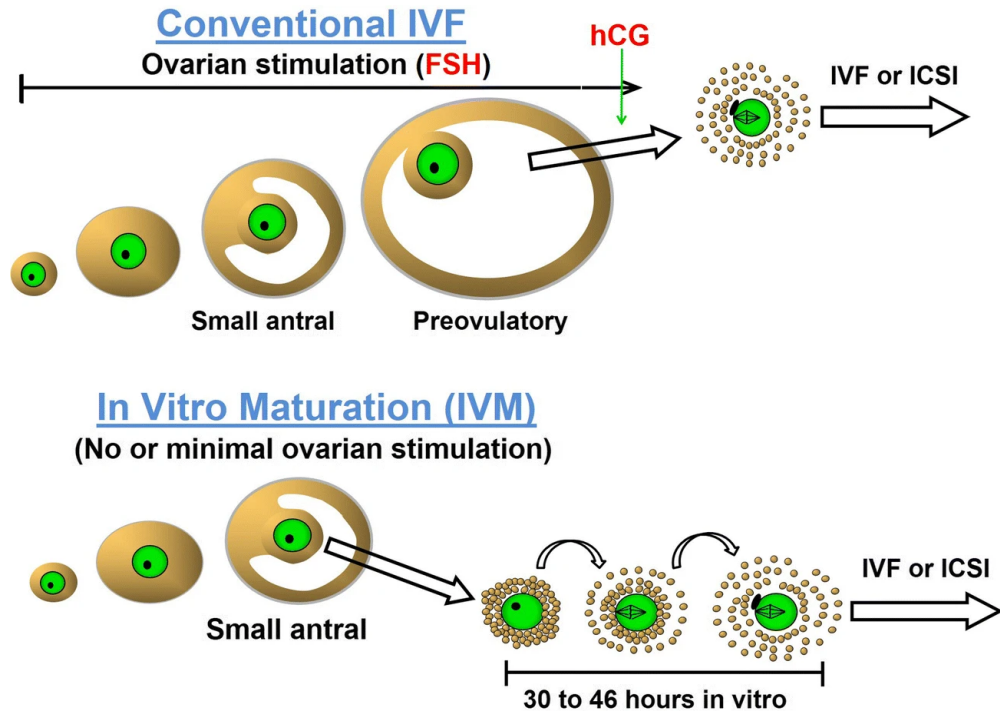


Figure 1.1: Overview of the In vitro Maturation (IVM) technique in comparison with conventional IVF [16]

Although IVM offers several significant advantages, particularly in reducing the need for ovarian stimulation and lowering the risk of ovarian hyperstimulation syndrome, it still presents several limitations. Foremost among these is the lack of automation

in key steps of handling, culturing, monitoring, and assessing oocyte quality. At present, most IVM procedures remain highly dependent on manual operations performed by embryologists, from the retrieval of immature oocytes and their placement into appropriate culture environments to the monitoring of maturation and the selection of the competent oocytes for fertilization. This reliance on manual practice not only prolongs the overall workflow but also increases the likelihood of procedural errors and elevates the risk of oocyte damage resulting from abrupt environmental changes or inconsistent handling. Manual manipulation may also introduce variability in culture conditions, which can adversely affect oocyte maturation outcomes. Furthermore, many laboratories still lack standardized and harmonized protocols for oocyte handling, contributing to substantial variability in IVM results across clinical centers.

In addition, current culture systems are not optimized for continuous monitoring and assessment of oocyte development, an essential requirement for determining oocyte quality and maturation potential. Culturing and evaluating development at the single-cell level remain challenging, resulting in the loss of important biological information during critical stages of oocyte maturation. Moreover, oocyte quality assessment still relies largely on the expertise and direct visual inspection of embryologists under a microscope. This approach is time-consuming and inherently subjective, which increases the likelihood of error or the omission of oocytes with developmental potential [17]. The absence of standardized, automated systems in IVM further limits efforts to optimize the procedure and improve consistency across treatment cases.

Another challenge lies in the difficulty of controlling microenvironmental factors such as hormone concentrations and growth factors, all of which play critical roles in supporting optimal oocyte development. Accurately replicating the natural physiological environment of the ovary, where oocytes grow and mature within the female body, remains a major challenge for conventional IVM protocols. *In vivo*, oocyte maturation occurs within a highly dynamic and complex microenvironment that depends on multiple interacting parameters, many of which traditional static culture systems cannot adequately reproduce. Static culture conditions often lead to the accumulation of metabolic waste products and fail to provide the natural circulatory flow present *in* the ovarian *milieu*. Insufficient control over these factors can result in heterogeneous oocyte development and reduced fertilization and embryonic growth potential. Furthermore, the inability to mimic the physiological microenvironment of the maternal ovary with precision may contribute to suboptimal or unpredictable outcomes in infertility treatment.

In this context, microfluidic technology has emerged as a promising approach for enhancing the efficiency and precision of the IVM process. Culturing cumulus–oocyte complexes (COCs) within microfluidic platforms not only helps preserve their three-dimensional architecture and the natural biological interactions between oocytes and

cumulus cells, but also enables precise control of microenvironmental conditions through microscale fluid flow, integrated sensing, and accurate chemical gradient generation. Organ-on-a-chip (OoC) technology, which relies on integrated microfluidic systems, is capable of recapitulating key structural and functional characteristics of human organs within a compact chip-based platform. These systems can co-culture multiple cell types and physiological parameters to reproduce tissue-level functions in a controlled environment. When applied to IVM, such systems can create an environment that more closely resembles the native ovarian niche and facilitate deeper investigation into the physiology of oocyte maturation, thereby supporting the optimization of culture conditions and improving fertilization outcomes [18]. Microfluidic chips also support the manipulation, positioning, and real-time monitoring of individual oocytes through integrated imaging modules, allowing dynamic biological data to be collected without disruptive manual handling. This capability contributes to improved oocyte quality and enhances the likelihood of successful fertilization. Furthermore, microfluidic systems help minimize errors associated with manual procedures and offer more precise control over critical environmental parameters such as temperature, pH, and gas composition.

1.2 Microfluidic technology for female reproduction research

1.2.1 Definition and core operating principles

In recent years, the term “*microfluidic technology*” has gained significant global attention due to its wide range of potential applications [19]. Microfluidics focuses on the design, fabrication, and utilization of microscale devices and systems for controlling and manipulating the behavior of fluids and suspended particles at the microscale level. These systems typically incorporate components such as channels, chambers, pumps, valves, and sensors, many of which have dimensions on the order of micrometers or even smaller.

The use of this technology to manipulate and regulate very small volumes of fluids, typically at the microscale, has enabled a wide range of important applications in research and healthcare (Figure 1.2). Microfluidic technology is transforming cellular biology research by enabling precise control and the integration of multiple biological functions within a compact platform. One of its most prominent applications is single-cell analysis, which allows the isolation and detailed examination of individual cells at the genomic, transcriptomic, proteomic, and metabolic levels. This capability facilitates the detection and characterization of cellular heterogeneity within populations, including rare cell types such as stem cells or circulating tumor cells [20]. In addition, microfluidics plays a critical role in drug screening by enabling the creation of hundreds of microscale

compartments in which multiple drug candidates can be tested on single cells using minimal sample volumes. This approach accelerates preclinical research and significantly reduces associated costs.

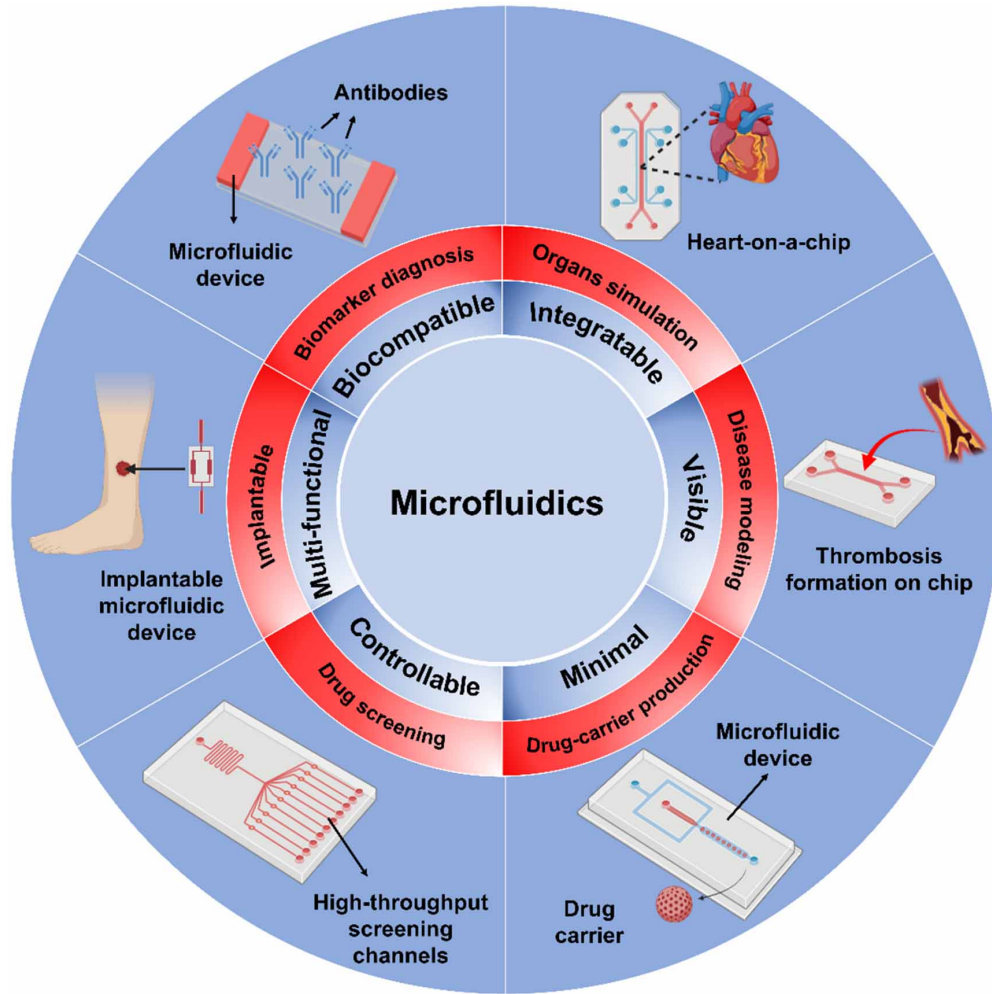


Figure 1.2: Microfluidics applications [21]

The small dimensions of microfluidic systems allow precise control over physical parameters such as flow rate, pressure, solute concentration, and interactions among biological components [22]. This capability enables the simulation of complex physiological microenvironments, thereby supporting the development of more sophisticated and accurate cell culture models. In cell culture applications, microfluidic technology facilitates the construction of microscale systems that mimic living tissues, allowing the recapitulation of chemical gradients, mechanical forces such as shear stress, and three-dimensional structures required for cells to function as *in vivo*. Microfluidic chips can also be designed to provide continuous perfusion while integrating sensors for monitoring pH, oxygen levels, and metabolite concentrations throughout the culture process. These features collectively ensure that the culture environment is maintained under optimal conditions for cell growth and differentiation.

1.2.2 Applications in female reproduction research

a. Organ-on-a-chip

The emergence of OoC technology in the early 2000s was driven by advances in biomimetic microfluidic devices designed to simulate diverse biological functions and components. This development has led to a new class of *in vitro* models capable of recapitulating essential features of human physiology, including the intricate human reproductive system [23], [24]. Leveraging its capacity to co-culture multiple cell types and to emulate inter-organ communication through complex endocrine pathways and signaling mechanisms, OoC technology has garnered substantial interest among researchers in ART. After years of refinement, OoC systems have demonstrated the ability to model various stages of sexual reproduction (Figure 1.3). These biomimetic platforms facilitate deeper insights into the mechanisms underlying *in vivo* physiological processes and disease states. Furthermore, microfluidics-based physiological models provide a powerful framework for drug screening and toxicological evaluation. Recent advances in biosensors and bioelectronics integrated within microfluidic platforms have enabled real-time monitoring and precise regulation of these biomimetic systems.

Organ-on-chip for hormone recapitulation

Microfluidic chips, owing to their ability to precisely manipulate multiple fluidic channels at the microscale, provide a powerful platform for replicating hormone stimulation and delivering other regulatory factors such as nutrients, drugs, and toxicants. Female reproductive processes are mostly stimulated by hormonal cues: primordial follicles in the ovaries are activated at puberty by follicle-stimulating hormone (FSH) released from the anterior pituitary gland to become primary follicles; follicular fluid supplies essential hormones for follicle growth; ovulation is triggered by a surge in luteinizing hormone; and fetal development depends on coordinated hormonal signals from the mother, fetus, and placenta. These *in vivo* hormone-regulated pathways can be recreated on-chip through precise control of fluid flow within microfluidic devices. Several approaches have been employed to establish dynamic culture environments, including gravity-driven flow, syringe-pump-mediated perfusion, and piezo-Braille pin actuation. When designing dynamic culture systems, parameters such as flow rate, flow profile, channel volume, and fluid recirculation must be carefully optimized to support embryo development. Notably, shear stress exceeding 1.2 dyn/cm^2 has been shown to damage blastomeres and contribute to embryo degeneration [25].

Ferraz *et al.* developed an oviduct-on-a-chip platform designed to reproduce key physiological features of the peri-ovulatory oviduct under dynamic culture conditions, thereby supporting bovine oviduct epithelial cell growth and differentiation more effectively than conventional static IVF systems. The device contains two independently perfusable compartments separated by a porous membrane lined with bovine oviduct

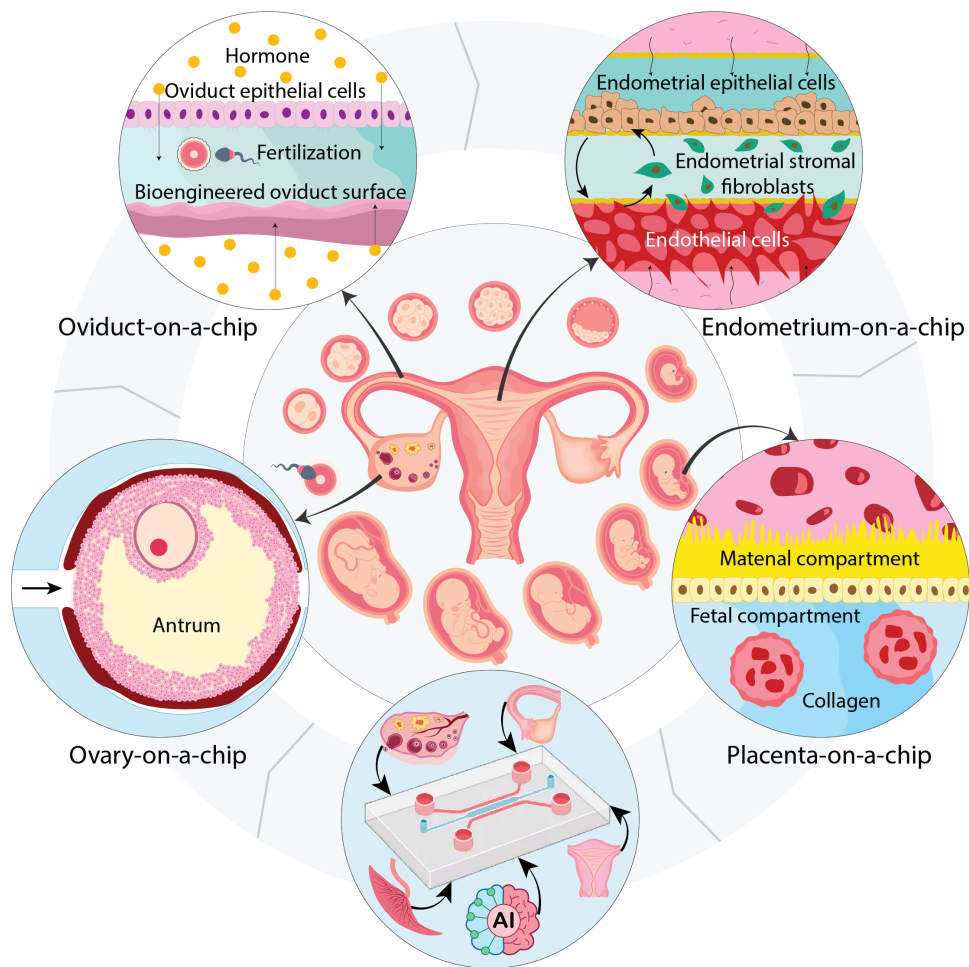


Figure 1.3: The footage of female reproductive organ-on-a-chip technology on the growth from oocyte to fetus and their integration to build up female reproductive system-on-a-chip in ART

epithelial cells, while the apical side includes micro-pillars for retaining oocytes and embryos during continuous perfusion. Under hormonally regulated dynamic flow, the epithelial cells displayed *in vivo*-like gene expression, morphology, and structural differentiation, and the resulting zygotes exhibited DNA methylation and transcriptomic profiles closer to those of *in vivo*-derived embryos [26]. In subsequent work, the same group extended this microfluidic strategy to a canine oviduct-on-a-chip model for on-chip CRISPR-Cas9 gene editing and disease modeling. Using this system, they reproduced key pathological features associated with serous tubal intraepithelial carcinoma, demonstrating that the platform can serve not only reproductive studies but also investigations of early disease development in the oviduct [27].

A recently engineered system, known as EVATAR, integrates dynamic and three-dimensional culture to recapitulate the 28-day human menstrual hormone cycle [11]. This microscale fluidic platform incorporates five interconnected organ modules: the ovary, fallopian tube, uterus, cervix, and liver, and is designed to investigate ovarian hormonal regulation of downstream reproductive tissues and peripheral organs. The system operates through electromagnetically controlled micropumps that independently actuate 60 valves, enabling precise modulation of fluid flow without requiring separate air lines for each channel. To approximate *in vivo* physiological conditions, ovarian follicles were cultured within the microfluidic environment in the presence of follicle-stimulating hormone, which maintained follicular architecture and spatial organization. The successful induction of ovulation on-chip demonstrated that the platform supports not only follicular growth but also maturation, ovulation, and granulosa cell luteinization.

Decidualization, a critical endometrial transformation that prepares the uterus for embryo implantation, has been successfully modeled in microfluidic systems that recreate hormonally regulated interactions between stromal and endothelial cells [28], [29]. These platforms support *in vivo*-like cellular and morphological changes, providing useful tools for studying normal endometrial physiology. In addition, endometrium-on-a-chip models have been used to reproduce menstrual cycle phases, examine hormone-dependent vascular responses, and evaluate drug effects such as levonorgestrel [30]. Microfluidic approaches have also been applied to disease modeling, particularly endometriosis, where studies of interactions between endometrial stromal cells and peritoneal mesothelial cells suggest that disease progression may depend not only on abnormalities in endometrial cells but also on changes in the surrounding peritoneal environment [31].

Organ-on-chip for mimicking cell-cell interaction

Microfluidic chips not only generate precise and physiologically relevant fluid flows but also recreate the three-dimensional organization of multiple cell types by enabling co-culture within compartments separated by thin, porous membranes. Such configurations facilitate cell–cell communication and endocrine signaling, both of which are essential features of *in vivo* microenvironments. For example, Virant-Klun *et al.* demon-

strated that immature human oocytes co-cultured with cumulus cells from mature oocytes exhibited higher maturation rates and enhanced expression of genes related to transcriptional regulation, embryogenesis, epigenetics, and development compared with oocytes matured without accompanying cumulus cells [32]. Numerous microfluidic platforms have adopted similar co-culture strategies to study interactions among various reproductive cell types, including the oocyte–cumulus–granulosa complex during folliculogenesis, human endometrial stromal and endothelial cells during decidualization [33], embryos and fallopian tube epithelial cells, blastocysts and endometrial stromal cells during early embryonic development, and fetal extravillous trophoblasts and maternal uterine cells during implantation [34].

Promising outcomes have been achieved using these co-culture systems. The bio-engineered model developed by Gnecco *et al.* successfully recapitulated stromal cell differentiation during decidualization [33]. In subsequent work, the same group co-cultured human endometrial stromal cells with uterine microvascular endothelial cells in an OoC platform to investigate how stromal differentiation depends on endometrial vasculature [35]. Hemodynamic forces generated within the microfluidic system activated endothelial cells, which in turn markedly enhanced stromal decidualization. In the context of embryo development, the combination of co-culture and dynamic perfusion improved blastocyst formation rates from 21.40% under static off-chip conditions to 37.50% on-chip [36]. An implantation-on-a-chip model has also been developed to reproduce the critical process of embryo implantation [34]. The device includes three parallel compartments that simulate the directed migration of extravillous trophoblasts toward maternal arteries and their interactions with endothelial cells – interactions essential for vascular remodeling. Using this co-culture platform, the study revealed previously uncharacterized effects of maternal immune cells and decidualized stromal cells on trophoblast migration and invasion.

Positive outcomes have been demonstrated using co-culture platforms that model the maternal–fetal interface by integrating trophoblasts (maternal side) and endothelial cells (fetal side) to form an artificial placental barrier [37]. In this system, a Matrigel-filled microchannel positioned between two parallel cell channels successfully recapitulated the structural morphology of the native barrier. The presence of continuous VE-cadherin junctions, an essential endothelial adherens junction protein, indicated full confluence and proper barrier formation in both trophoblast and endothelial layers. When TiO₂ nanoparticles were introduced into the maternal channel, disruption of barrier permeability resulted in multiple placental dysfunctions. Although low concentrations of TiO₂ produced minimal effects on oxidative stress and cell motility, compromised barrier integrity and reduced immune cell populations were observed, suggesting potential risks of nanoparticle exposure during pregnancy. A similar approach was used to model the multistep inflammatory responses of the placental barrier following *E. coli*

exposure [38]. In this design, trophoblasts and endothelial cells were cultured in two compartments separated by a porous membrane, with maternal and fetal “bloodstreams” perfused through their respective channels. Introduction of *E. coli* into the maternal channel induced substantial cell death in both cell types, triggered the release of inflammatory cytokines, and activated maternal macrophages. The model revealed active communication across the barrier, as evidenced by cytokine secretion detected on the fetal side. Building on the concept of trophoblast–endothelial co-culture within a three-dimensional microfluidic environment, standardized and higher-throughput placental barrier-on-a-chip systems have been developed to capture essential features of the dynamic *in vivo* maternal–fetal interface. These platforms are proving valuable for studying placental pathologies, particularly preeclampsia, and hold significant promise for advancing therapeutic development and deepening our understanding of placental disease mechanisms [39].

Organ-on-chip for modelling biophysical cues

In addition to co-culture and dynamic culture approaches, researchers have also endeavored to replicate *in vivo* biophysical cues. Mechanical contractions in the reproductive tract play essential roles in ART [40]. During embryo transport from the oviduct to the uterus, both smooth muscle contractions and ciliary activity contribute significantly to this process [41]. To examine the impact of muscle-like contractions on embryonic development, Kim *et al.* developed a microfluidic system featuring a partially constricted channel designed to mimic peristaltic movements of the oviduct [42]. Each device contained two side reservoirs, an inlet, and an outlet. Three microchannel configurations were tested to determine which best supported embryo development: a straight channel and two constricted channels with widths of 150 μm and 160 μm , respectively. Twenty fertilized embryos were introduced into each channel, placed on a tilting platform, and cultured for three days. The proportion of embryos reaching the 8-cell stage was 23.9% in the straight channel and 56.7% in the 160- μm constricted channel. These findings suggest that early bovine embryo development is enhanced by mechanical stimulation, underscoring the physiological importance of muscular contractions.

b. Microfluidic systems for oocyte handling and culture

Oocytes represent a fundamental determinant in the reproductive process of animals and human. They not only carry the genetic material required for embryo formation but also exert a direct influence on subsequent embryonic development and viability. The maturation of an oocyte from its early growth stages to its acquisition of fertilization competence is a highly intricate process that encompasses multiple biological and biochemical transitions. In recent years, researchers have successfully applied microfluidic chips across multiple aspects of oocyte handling and assisted reproduction, from *in vitro* maturation to cryopreservation and even microscale manipulations. Microfluidic

chips have been employed for oocyte maturation using diverse oocyte sources, including isolated denuded oocytes, COCs, ovarian tissue fragments, and early-stage follicles [43].

Choi *et al.* demonstrated that recreating ovarian mechanical heterogeneity using alginate (stiff cortex) and collagen (soft medulla) within a microfluidic 3D culture system markedly improved follicle development to the antral stage [44]. Remarkably, their platform also induced ovulation of COCs without the addition of luteinizing hormone (LH) and epidermal growth factor (EGF). These results show that biomechanical gradients within the ovary play a central role in regulating follicle maturation and ovulatory function. A recent work reported improved oocyte maturation efficiency by using a microfluidic device for dynamic culture, compared to the traditional static culture in Petri dishes. A microfluidic device is designed with a microchamber array that facilitates the trapping and maturation of individual oocytes within a controlled microenvironment. The results showed that dynamic culture conditions (both passive and active) significantly reduced lipid peroxidation (MDA concentration) and oocyte apoptosis rates compared to static culture. These findings indicate that on-chip dynamic culture enhances oocyte viability and maturation efficiency in *in vitro* conditions [45]. Han *et al.* developed a microwell-based microfluidic device that integrates single-oocyte trapping, fertilization, and subsequent embryo culture [46]. The microwell array was designed to retain individual oocytes during sperm and oocyte loading, medium replacement, and debris removal. Most recently, Franko and Ferraz (2025) introduced *OoTrap*, a microfluidic device designed for field-based ART applications that enables the efficient capture, isolation, and culture of oocytes outside of laboratory [47]. The device operates in both static and perfusion modes, with perfusion offering optimal conditions for oocyte maturation. Their study demonstrated that *OoTrap* achieved higher IVM rates, reduced chromosomal abnormalities, and preserved spindle morphology integrity, especially under perfusion conditions. This research underscores the potential of *OoTrap* to improve ART outcomes by minimizing manual handling, providing a controlled microenvironment, and making ART accessible in resource-limited settings. Despite these promising advances, most existing platforms rely on microwell- or microchamber-based structures for single-oocyte confinement. Such closed or partially confined geometries may hinder the effective removal of cellular debris, thereby compromising the culture environment and obstructing clear visualization of oocytes during maturation. In addition, simple chamber configurations may generate stagnant flow regions, nonuniform shear-stress distributions, and localized nutrient depletion. A further limitation arises from the characteristics of the biological samples used in IVM. Oocytes are commonly cultured together with surrounding cumulus cells as COCs, which exhibit considerable variation in size. Consequently, uniformly sized trapping chambers may not accommodate the full range of COC dimensions, resulting in inconsistent or reduced trapping efficiency. Continuous monitoring of individual oocytes

is also important for evaluating maturation dynamics. However, current microfluidic systems remain limited in their ability to provide real-time, automated, and continuous evaluation of individual oocytes throughout the maturation process.

c. Microfluidic systems for fertilization and culture, evaluation of embryos

Microfluidic platforms have emerged as powerful tools for real-time, non-invasive, multiparametric embryo evaluation, offering precise control over imaging conditions, fluid dynamics, and single-embryo handling. For examples, microfluidic culture devices can culture embryos in stable microenvironments for continuous morphological imaging of developing embryos, as demonstrated by an eggbox chip that enabled extended observation of preimplantation development [48]. These systems also permit the application and monitoring of mechanical stimuli at the microscale. Bae *et al.* showed that controlled hydrodynamic pressure pulses can influence embryo development, demonstrating that both the magnitude and the duration of compression are critical determinants of embryonic response. Their system revealed that excessively strong or prolonged mechanical stimuli induce detectable morphological changes, indicating compromised developmental potential [49]. Mohagheghian *et al.* used a microfluidic force-mapping chip to quantify blastocyst traction forces, showing that oscillatory tensile and compressive stresses dominate over shear within the confined microenvironment [50]. Furthermore, the integration of viability and metabolic sensing modules into microfluidic chips allows embryos to be evaluated through distinct biophysical readouts. For example, apoptotic status can be resolved through fluorescence-based miniaturized assays, whereas metabolic competence becomes accessible via micro-NMR (nuclear magnetic resonance) measurements that reveal molecule-level signatures relevant for embryo selection [51]. A microfluidic electrochemical platform was designed to measure oxygen usage in individual mammalian embryos with high precision, using an array of microscale electrodes to stabilize and monitor a single embryo throughout the assay. By capturing stage-specific respiratory activity from two-cell to blastocyst, the device demonstrated that microfluidic confinement and integrated sensing enable reliable discrimination of metabolic progression, thereby supporting viability assessment *in vitro* [52]. Another study employed a microfluidic single-embryo analysis system to quantify oxygen consumption in individual human embryos, demonstrating that microscale confinement enables highly sensitive detection of metabolic differences associated with developmental competence [53]. In addition, lab-on-a-chip immunoassays allow non-invasive monitoring of embryo-secreted biochemical markers. By embedding the glucose assay workflow into a mechanically actuated microfluidic platform, the microfluidic device enabled precise sampling and fluorescence-based quantification of glucose depletion in nanoliter volumes, thereby allowing sensitive, real-time assessment of embryo metabolism without compromising viability [54]. A recent study presented a droplet-based microfluidic immunoassay that enables ultrasensitive detection of trace

amounts of hCG β secreted by embryos in spent culture medium, with a detection limit as low as 0.1 pg/mL. The authors demonstrated that hCG β levels measured in microfluidic droplets were significantly higher in spent media from top-quality blastocysts than from non-blastocyst or lower-quality embryos, highlighting the potential of micro-scale secretome analysis for non-invasive embryo selection [55]. In 2020, Lee *et al.* implemented an on-chip cytokine assay (interleukin-1 β and TNF- α) to differentiate embryos by developmental competence based on their secreted factor profiles [56]. Taken together, these studies demonstrate that microfluidic platforms substantially improve the precision and depth of embryo evaluation by enabling stable imaging conditions, controlled microscale environments, and integrated analyses of key developmental indicators. Such capabilities allow embryos to be assessed non-invasively and in real time, offering a more comprehensive and physiologically relevant basis for determining developmental competence.

d. Toxicology and drug screening

Microfluidic technologies have emerged as powerful platforms for drug screening and toxicity testing in female reproductive systems due to their ability to recapitulate key physiological, biochemical, and biomechanical cues. Compared with animal models, microfluidic reproductive systems offer improved human relevance, reduced ethical concerns, and higher throughput, while enabling real-time monitoring of cellular responses under dynamic conditions. A recent study presents a physiologically relevant, vascularized three-dimensional endometrium-on-a-chip that recapitulates the multilayered human endometrial microenvironment for in vitro drug testing [30]. Using emergency contraception levonorgestrel as a model compound, the system enables quantitative, dose-dependent assessment of drug-induced changes in endometrial permeability and vascular regression. Another uterus-on-a-chip study applies microfluidic perfusion to model obesity-associated hyperinsulinaemia as a clinically relevant metabolic exposure rather than a static culture condition [57]. By continuously delivering insulin under controlled flow, this platform enables mechanistic evaluation of insulin-induced dysregulation of endometrial epithelial function.

Microfluidic models of the placenta–decidual interface have been extensively employed to dissect complex biological processes governing maternal–fetal exchange, including active and passive drug transport, barrier integrity, cellular uptake mechanisms, and endocrine or environmental toxicity [39]. These platforms enable controlled comparison between cell-free and cell-containing systems, static versus dynamic perfusion, and parent drugs versus metabolites, allowing researchers to isolate the functional contributions of placental tissues to drug disposition. Importantly, placenta-on-a-chip systems have revealed the role of efflux transporters, trophoblast signaling pathways, and vascular dynamics in regulating fetal drug exposure, while also provided quantitative readouts such as permeability coefficients, drug enrichment ratios, and cellular

uptake efficiency. In addition to pharmacological compounds, these models have been applied to evaluate nanomaterial toxicity, endocrine disruptors, and plastic-derived contaminants, demonstrating their versatility for safety screening across diverse exposure scenarios relevant to pregnancy. Among representative studies, Abostait *et al.* demonstrated that dynamic microfluidic perfusion enhances liposomal uptake at the placenta–decidual interface [58]. This effect was significantly greater than under static conditions. The study indicates that flow-induced transport mechanisms play a critical role in nanoparticle–tissue interactions. It highlights the importance of incorporating physiologically relevant hemodynamic forces. These forces should be considered when evaluating drug and nanocarrier delivery during pregnancy. In another example, Pemathilaka *et al.* investigated maternal–fetal transport of naltrexone and its active metabolite using a placenta-on-a-chip platform and showed that placental cells significantly limit drug accumulation relative to acellular controls. These findings provide direct evidence that placental metabolism and barrier function must be considered in preclinical drug screening [59]. Besides, ovary-on-a-chip systems offer a complementary platform to study drug-induced reproductive toxicity, particularly in the context of ovarian health. As demonstrated by Aziz *et al.*, the ovary-on-a-chip model can replicate the dynamic environment of ovarian follicles, enabling the evaluation of chemotherapeutic drug effects, such as those induced by doxorubicin [60]. This model revealed that exposure to doxorubicin led to reduced follicular growth, impaired hormone secretion, and increased apoptosis, demonstrating the system’s potential in assessing the impact of drugs on ovarian function. By integrating such platforms, researchers can simultaneously evaluate the effects of pharmaceutical compounds on various organs within the female reproductive system, offering a more comprehensive understanding of maternal–fetal interactions and reproductive toxicity. The ability to model these critical biological processes *in vitro* not only reduces reliance on animal testing but also opens new avenues for drug safety screening, especially for therapies that may impact fertility, pregnancy outcomes, and overall reproductive health.

1.2.3 Fabrication of microfluidic chips

a. Polydimethylsiloxane (PDMS) material in microfluidic chip fabrication

Polydimethylsiloxane (PDMS) is a silicone polymer characterized by its flexible and stable structure, widely used in various technological applications, particularly in the fields of microfluidics and lab-on-a-chip devices. The chemical formula of PDMS is $[(\text{CH}_3)_2\text{SiO}]_n$, where n represents the number of monomer units, allowing the production of polymers with varying chain lengths. This material is renowned for its notable properties, such as elasticity, optical transparency, chemical resistance, and ease of fabrication. PDMS was first synthesized in the 1940s and rapidly became one of

the preferred materials in the fields of biotechnology and biomedical engineering. The widespread adoption of PDMS in the fabrication of microfluidic chips is primarily due to its ability to replicate biological structures with high precision while maintaining the necessary flexibility for micro-scale applications.

One of the key advantages of PDMS is its flexibility and ease of fabrication. With its ability to bend and shape, PDMS allows researchers to create microfluidic channels with custom sizes and shapes, simplifying the design and production of microfluidic chips. The fabrication process of these chips typically employs photolithography combined with molding techniques, enabling the creation of microstructures with high precision. Furthermore, the optical transparency of PDMS allows scientists to monitor processes occurring within the microfluidic chip without interfering with the measurement process. This capability is particularly valuable for observing biological reactions or cell movements in real time. The versatility of PDMS extends to its ability to integrate with other components within a microfluidic system. This material can be combined with various polymers or other materials to create microfluidic chips with enhanced functionalities, such as chemical or biological detection through the integration of sensors. This opens up new opportunities for the development of innovative applications in biomedical diagnostics and therapeutic treatments.

Additionally, PDMS exhibits high biocompatibility, making it suitable for safe use in applications involving cells and tissues. This material is non-toxic to cells, preserving the viability and biological activity of cell samples cultured within the microfluidic chip. The biocompatibility of PDMS is attributed to its chemical structure. PDMS possesses stable chemical properties and is non-reactive with biological components (chemical resistance), meaning that when used in biological systems, it does not generate harmful by-products or negatively impact the cells. Furthermore, PDMS can be easily sterilized and cleaned without damaging its microstructure, ensuring a safe and sterile environment for cell culture. The use of PDMS in microfluidic chips also allows for surface modification to enhance interactions with cells or biomolecules. Techniques such as surface coating can be employed to improve the adsorption of cells or molecules of interest, optimizing the efficiency of experiments and research in microfluidic environments.

Therefore, PDMS-based microfluidic chips are used to control cell culture environments and study biological responses, aiding in therapy development and disease understanding. They are also utilized in drug screening, optimizing testing and drug delivery, and are being researched for personalized diagnostics and treatments.

b. Photolithography technique for microfluidic channel fabrication

Photolithography is a widely used method for fabricating PDMS with microfluidic channel cavities, offering microfluidic pattern resolution that can range from nanometer to micrometer precision, depending on the master mold's resolution. This method involves the use of light to create detailed patterns on the surface of materials such as

silicon or glass, facilitated by a photosensitive coating (resist). As shown in Figure 1.4, photolithography is commonly applied in the fabrication of microcircuits, MEMS, and other micro-systems. The principle of photolithography is a sophisticated process based on the photochemical reaction of photosensitive materials (photoresist) when exposed to ultraviolet (UV) light, enabling the formation of detailed patterns on the surface of microchips or microsystems [61]. An essential component in creating these patterns is the optical mask. The mask is an optical film containing the desired image patterns and functions as a template. Typically, the mask is made from transparent materials such as glass, quartz, or plastic, with the desired structures and shapes etched onto it. The opaque regions on the mask block light, allowing only the exposed areas to permit light transmission, thereby transferring the pattern onto the photoresist layer.

When ultraviolet (UV) light with a short wavelength and high energy passes through a mask, the light only transmits through the unshielded areas of the mask and contacts the photoresist layer. This light acts as a catalyst, triggering chemical reactions in the photoresist that alter its chemical properties. Depending on the type of photoresist used, these reactions will result in different changes [62]. In the case of positive photoresist, the areas exposed to light undergo molecular degradation, making them more soluble in the developing solvent. This allows the illuminated portions to be washed away, creating empty regions that follow the mask pattern. In contrast, with negative photoresist, when exposed to UV light, the molecules in the photoresist layer bond more tightly, causing those areas to harden and become less soluble in the solvent, thereby protecting the illuminated regions.

After light passes through and the mask successfully transfers the pattern onto the photoresist layer, the layer undergoes a process known as development. During development, a special solvent is used to dissolve and remove the areas of the photoresist that no longer retain their original properties. The lift-off technique (photoresist-based photolithography) is a process in which a positive photoresist is applied to the substrate surface and then exposed to light through a mask. The areas of the photoresist that are not covered by the mask (exposed to light) will undergo a change in their properties and dissolve in the developing solution, while the areas covered by the mask (unexposed) will remain adhered to the substrate surface. Subsequently, a layer of material is deposited onto the substrate and photoresist through methods such as evaporation or sputtering. The material that adheres to the photoresist layer is then removed by immersing the sample in an acetone solution and using ultrasonic agitation. This process will wash away the photoresist and the overlying material, leaving only the material securely adhered to the substrate.

The etching technique (negative photoresist photolithography) is the reverse process of positive photolithography. In this process, the unexposed regions dissolve in the developing solution, while the exposed photoresist areas remain intact. Then, the

material adhering to the non-photoresist regions is etched away using an electron beam, and the remaining photoresist layer is removed with alcohol, leaving the desired material on the substrate. Etching the material on the substrate is more complex than washing away the solidified photoresist, as it requires precise control of the electron beam. If this process is not well-controlled, the electron beam can penetrate the substrate. In the lift-off technique, besides using positive photoresist with a positive mask, the negative properties of negative photoresist (photoresist revert) can also be utilized with a negative mask. The result of this process is an accurate pattern etched onto the material surface, faithfully replicating the original design of the mask.

Photolithography requires high precision, as the light must be evenly exposed, and the mask must be properly aligned with the substrate surface to ensure the pattern is accurately reproduced. Each step, from exposure to development and final etching, must be tightly controlled to achieve high resolution, meeting the stringent requirements of the semiconductor, microelectromechanical systems (MEMS), and microelectronic industries. As a foundational technology in integrated circuit and micro-system fabrication, photolithography enables the creation of intricate details with high precision at the nanoscale. Therefore, the fabrication of microfluidic channel structures is typically achieved through photolithography to create molds for PDMS structures. The molds used for PDMS microchannel casting can be made from various materials, and different types of photoresists are used in the photolithography process.

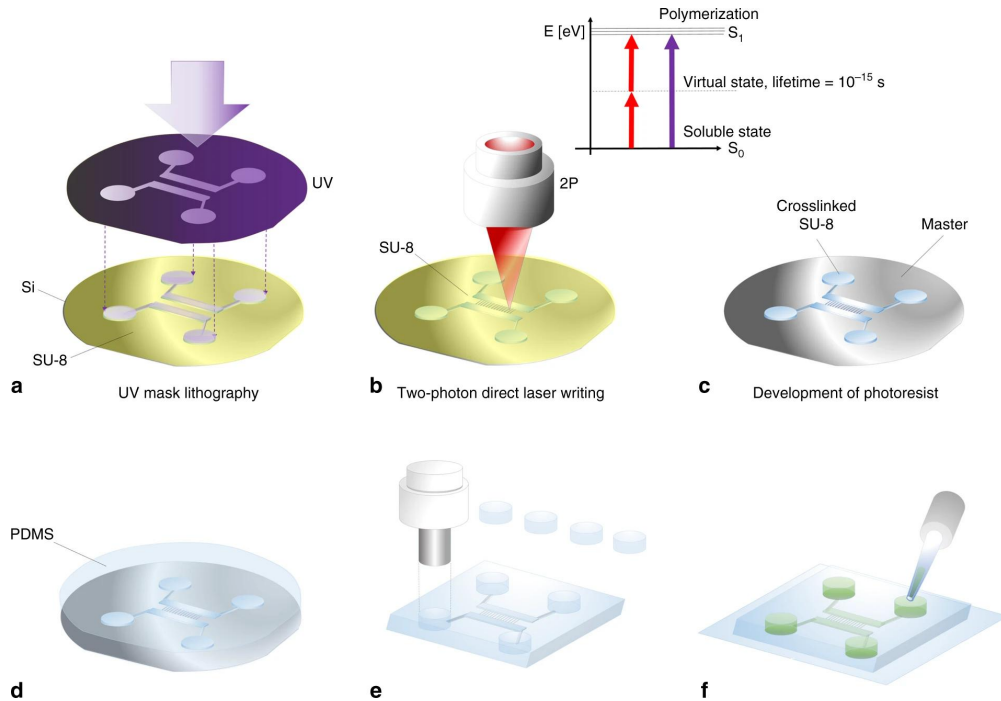


Figure 1.4: An illustration of the process for fabricating PDMS microchannels using photolithography [63]

Typically, for mold fabrication of channels, negative photoresist is used. Dry film

photoresist and SU-8 are two popular materials for mold creation in microsystem and microelectronic fabrication, particularly in lab-on-a-chip applications. Dry film photoresist is a thin film applied to the surface of the material through a thermal bonding process, followed by UV exposure through a mask to create patterns. The dry film exposed to light hardens, while the unexposed areas can be easily removed with a solvent. Due to its simplicity and low cost, dry film is often chosen for rapid processes that do not require thick mold layers, making it ideal for experimental setups or printed circuit boards. However, dry film has limitations in mold thickness (typically no more than a few tens of microns) and poorer adhesion, making it less suitable for applications requiring long-term durability.

In contrast, SU-8 is a liquid epoxy-based photoresist that allows for the creation of thick layers, up to hundreds of microns, while maintaining high strength and adhesion. As a negative photoresist, SU-8 hardens upon UV exposure, enabling the creation of highly accurate microstructures that can withstand harsh chemical etching processes. Due to these properties, SU-8 is often used to create durable micro-molds in applications requiring thicker layers, such as microchannels in lab-on-a-chip devices or MEMS structures. However, SU-8 has the drawback of being difficult to remove once hardened, with a more complex processing procedure and longer processing times compared to dry film, in addition to being more expensive. With their distinct characteristics, dry film and SU-8 fulfill different requirements in micro-system fabrication: dry film is suited for quick, simple designs, while SU-8 is ideal for applications needing high durability and precision.

In this thesis, SU-8 photoresist was chosen, with various formulations suitable for producing different thicknesses. SU-8 is the most commonly used photoresist for creating molds for microfluidic structures based on PDMS. Typically, when using SU-8 with the simple spin-coating process, the maximum mold thickness is limited to around 250 μm . To fabricate channels using PDMS material, microfabrication processes based on photolithography were performed on a silicon wafer, using the epoxy-based negative photoresist SU-8.

1.3 Advanced technologies integrated with microfluidic systems in ART

1.3.1 Dielectrophoresis

In recent years, biomedical engineering has experienced significant progress through the integration of microelectronics and MEMS, particularly with the application of dielectrophoresis (DEP) for the manipulation and separation of biological cells and biomarkers. The development of microfluidic devices capable of precisely handling

biological entities has created new opportunities for research and clinical applications in reproductive medicine. In ARTs, these platforms enable the selective isolation and assessment of reproductive cells based on their intrinsic electrical properties. For example, dielectrophoretic approaches can be applied to separate and enrich high-quality sperm cells from heterogeneous semen samples, providing valuable information for sperm selection and supporting improved outcomes in procedures such as IVF and ICSI [64].

Dielectrophoresis (DEP) is a phenomenon in which the motion of polarizable particles is influenced by a non-uniform electric field due to differences in dielectric properties between the particles and the surrounding medium [65]. When exposed to such a field, biological particles become polarized and experience a net force that causes them to move toward regions of higher or lower electric field intensity depending on their electrical characteristics. This principle enables the manipulation, trapping, and separation of biological particles such as cells, microorganisms, and biomolecules. DEP is widely regarded as a rapid, label-free, and cost-effective analytical technique, as it relies on the intrinsic electrical properties of particles rather than chemical labeling. Because of these advantages, DEP has been extensively applied in a variety of fields including biosensing, environmental monitoring, polymer and materials research, as well as biomedical and clinical diagnostics. The ability of DEP-based systems to selectively manipulate biological entities with high precision makes them particularly valuable for applications involving cell characterization and separation in lab-on-a-chip platforms.

Motion in DEP arises from differences in polarizability between a particle and the surrounding medium when exposed to a non-uniform electric field (Figure 1.5). If the particle moves toward the electrode edges, where the electric field gradient is strongest, the phenomenon is referred to as positive dielectrophoresis (pDEP). Conversely, when the particle is repelled from these high-field regions and moves toward areas of lower electric field intensity, it is termed negative dielectrophoresis (nDEP).

Significant progress in exploiting the principles of DEP has been observed over the past decades, particularly during the period from 2010 to 2020. The phenomenon itself was first described in the 1920s by Hatschek and Thorne, who reported characteristic behaviors of particles in non-uniform electric fields. Around the same period, H. S. Hatfield filed a patent for a dielectric separation technique and established a pilot plant to separate cassiterite from quartz [67]. In 1951, the term “dielectrophoresis” was formally introduced by H. A. Pohl in his seminal work entitled “The Motion and Precipitation of Suspensoids in Divergent Electric Fields.” Figure 1.6 illustrates the rapid growth in the number of patents and scientific publications related to DEP from 2010 to May 2020, based on records from PubMed and the United States Patent and Trademark Office. Prior to 2010, most studies on DEP focused primarily on exploratory investigations and proof-of-concept demonstrations. In contrast, the past decade has witnessed substantial progress toward practical and applied implementations of DEP

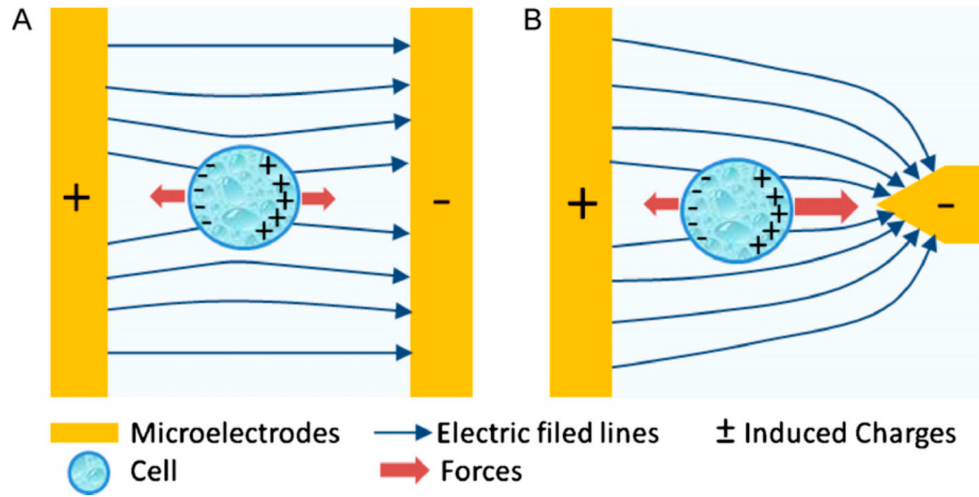


Figure 1.5: Illustration of the principle of positive dielectrophoresis (pDEP): (A) In a uniform electric field, the particle is symmetrically polarized, resulting in a net dielectrophoretic force (FDEP) of zero; (B) In a non-uniform electric field, the particle becomes asymmetrically polarized, generating a net dielectrophoretic force that drives the particle toward regions of maximum electric field intensity [66]

technologies. Between 2000 and 2010, the number of publications increased steadily, while from 2010 to 2020 the overall publication output remained relatively stable, with two notable surges in 2015 and 2019, corresponding to increases of approximately 21% and 37% above the decade average. A search for the keyword “dielectrophoresis” on PubMed reveals approximately 112 publications from 1951–2000, 613 publications from 2000–2010, and 981 publications from 2010–2020, highlighting the growing interest and expanding applications of DEP in the era of modern biotechnology [68].

In ART, where maintaining cell viability and developmental competence is essential, DEP has attracted growing interest as a tool for gamete and embryo manipulation [64], [69]. Applications of DEP in reproductive biology include the selection of high-quality oocytes, the sorting and preparation of sperm cells, the characterization of embryos, and the integration of these processes within microfluidic platforms designed to automate aspects of IVF.

The use of DEP for oocyte selection has received particular attention because oocyte quality is one of the most critical determinants of successful fertilization and embryo development. Traditional oocyte selection in IVF laboratories relies largely on morphological criteria such as cytoplasmic appearance, zona pellucida characteristics, and the presence of the first polar body. However, the evaluation of oocyte morphology is often subjective and depends heavily on the experience and personal judgment of embryologists, resulting in considerable variability between laboratories and the absence of universally standardized selection criteria [71]. DEP provides a potential evaluation method because the electrical properties of cells reflect their membrane composition, cytoplasmic conductivity, and intracellular organization [72]. These properties influence

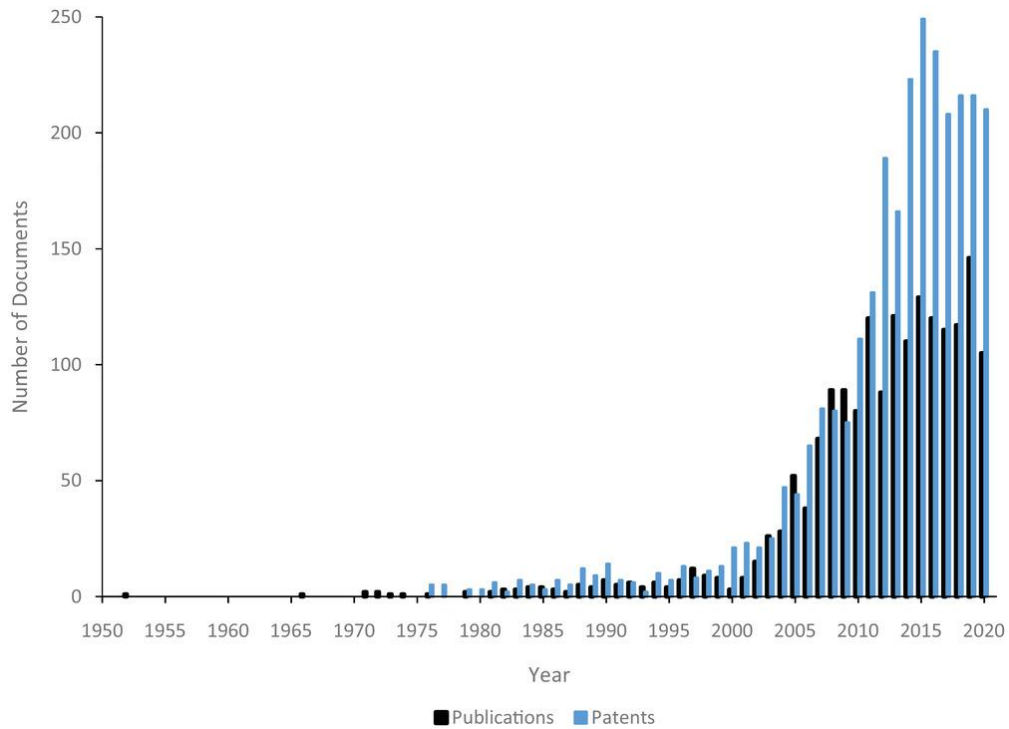


Figure 1.6: Growth in patents and scientific publications on DEP over time, from Pohl’s pioneering work in 1952 to March 2020 [70]

the Clausius–Mossotti factor, which determines the magnitude and direction of the DEP force acting on a cell in an alternating electric field.

One of the earliest studies demonstrating the potential of DEP for evaluating oocyte quality was reported by Dessie *et al.* (2007) [71]. In this study, the dielectrophoretic behavior of bovine oocytes and zygotes produced in vitro was investigated to determine whether their electrical response could be associated with developmental competence. Individual oocytes were exposed to a non-uniform alternating electric field, and their movement toward the electrodes was monitored and categorized according to their migration speed. The authors observed that oocytes exhibiting faster dielectrophoretic movement showed significantly higher rates of embryo development compared with those displaying slower responses. Moreover, differences in gene expression patterns were detected between the groups, suggesting that the DEP response reflects underlying cellular and molecular characteristics related to developmental potential. These findings indicate that dielectrophoretic behavior may serve as a noninvasive biophysical indicator for assessing the quality of oocytes and zygotes and could potentially complement conventional morphology-based selection methods in assisted reproductive technologies. Another study further explored the application of DEP in reproductive technologies by developing a microfluidic chip for oocyte selection in IVF [73]. In this study, a castellated electrode array was integrated into a microfluidic device to evaluate the dielectrophoretic velocity of individual oocytes under an alternating electric field. The

results showed that oocytes exhibiting movement in the DEP field displayed significantly better developmental competence compared with those that remained stationary, as evidenced by higher blastocyst formation rates and lower incidences of polyspermic fertilization. Importantly, the developmental outcomes of oocytes selected by the DEP device were comparable to those selected through conventional manual assessment by experienced embryologists. Moreover, the integration of DEP within a microchip platform highlights the potential for developing automated and standardized systems for gamete selection in assisted reproductive technologies. During oocyte maturation, structural rearrangements occur within the plasma membrane and cytoplasm, including redistribution of organelles, changes in ion transport activity, and modifications to membrane lipid composition [74]. These changes influence the polarizability of the cell and therefore its response to non-uniform electric fields. Because dielectrophoresis (DEP) can detect such electrical variations without requiring chemical labeling or physical contact, it provides a non-invasive approach for evaluating the physiological status and developmental competence of oocytes while preserving their viability. Although differences in gene expression have been observed between oocyte groups exhibiting distinct DEP responses, this association does not demonstrate that DEP can directly detect genetic or chromosomal abnormalities. Nevertheless, exploring whether such abnormalities produce detectable dielectric signatures represents a novel and potentially valuable direction for future research.

In addition to oocyte selection, DEP has also been explored for sperm manipulation and selection, which represents another critical step in assisted reproduction. Sperm cells exhibit unique dielectric properties due to their highly polarized structure, consisting of a head containing condensed chromatin, a midpiece rich in mitochondria, and a flagellum responsible for motility. These structural differences influence the distribution of electrical charges within the cell and thus its response to electric fields [75]. Early experimental work demonstrated that DEP could selectively manipulate spermatozoa based on their electrical polarization behavior and morphological characteristics. For example, E. Rosales-Cruzaley *et al.* (2013) applied insulator-based dielectrophoresis (iDEP) in a microfluidic device containing cylindrical insulating structures to enrich and separate sperm populations under DC electric potentials ranging from 200 to 1500 V. Their results showed that differences in cell shape and structure between mature sperm cells and spermatogenic cells produced distinct polarization responses in the electric field, enabling selective concentration and separation of these cell types. Importantly, viability assessments indicated that a substantial proportion of sperm cells remained viable after the dielectrophoretic treatment. These findings highlight the potential of DEP as a promising tool for sperm enrichment and selection in infertility studies and assisted reproductive technologies. Further evidence of the potential of DEP for sperm manipulation was reported in a recent study investigating the use of dielectrophoresis to

separate X- and Y-chromosome-bearing sperm cells [76]. By exploiting small differences in DNA content and dielectric properties between these sperm populations, DEP-based microfluidic systems can induce distinct movement behaviors in a non-uniform electric field, enabling partial enrichment of specific sperm fractions. It has been suggested that the effectiveness of DEP-based sperm sorting arises from differences in dielectrophoretic properties between X- and Y-sperm, which may be associated with variations in cell membrane proteins and the zeta potential of the sperm surface. These biophysical differences influence the polarization of sperm cells under electric fields and contribute to their differential responses during DEP manipulation.

Another promising application of DEP in reproductive biology is the characterization and manipulation of embryos. Recent studies have demonstrated that DEP can be employed as a non-contact tool for precise embryo handling [77]. For instance, a robotic dielectrophoresis tweezers system using quadrupole electrodes was developed to manipulate mouse embryos on a Petri dish without physical contact. By generating controlled DEP forces and integrating vision-based feedback algorithms, the system was able to move embryos to specified locations and orient them accurately for subsequent processing. This approach eliminates the need for precise mechanical alignment required in conventional pick-and-place techniques, thereby reducing the risk of mechanical damage to embryos while improving manipulation efficiency. Such DEP-based systems highlight the potential of electric-field-driven tools for automated, gentle, and flexible embryo handling in assisted reproductive technologies.

DEP has also been incorporated into microfluidic biochip systems designed to mimic the physiological environment of the mammalian oviduct. In such systems, positive DEP forces are applied within microfluidic channels to simultaneously manipulate and position both oocytes and sperm cells, thereby increasing their interaction probability [78]. Under appropriate electrical conditions, both gamete types exhibit positive DEP responses that guide them toward regions of higher electric field intensity, resulting in localized sperm accumulation around the oocyte. Experimental results from these microfluidic platforms demonstrated that this DEP-assisted positioning significantly enhanced the likelihood of natural fertilization within the channel. Subsequent embryo culture showed normal early development, with embryos reaching the two-cell stage after 24 hours and the four-cell stage after 48 hours. These findings highlight the potential of DEP-integrated microfluidic systems to facilitate controlled gamete interaction and fertilization, offering a promising approach for developing automated and physiologically relevant platforms for assisted reproductive technologies.

Since DEP manipulation is performed directly on living cells, maintaining cell viability and developmental competence during electric-field exposure is a critical technical consideration. In addition to the intended DEP force, the applied electric field may induce Joule heating, electrothermal flow, electrode polarization, and electrochemical

reactions. Joule heating is particularly important because electrical current passing through the conductive medium dissipates energy as heat. The resulting local temperature gradients may alter the electrical conductivity and permittivity of the medium and potentially affect cellular viability and function. The magnitude of Joule heating depends not only on the applied voltage and frequency, but also on the conductivity of the suspending medium, electrode spacing and geometry, local electric-field distribution, channel dimensions, substrate thermal properties, fluid flow, and exposure duration. To minimize these effects, DEP systems are typically operated under low-conductivity media, moderate voltage amplitudes, and optimized frequencies, while electrode geometry and microchannel design are carefully engineered to reduce power dissipation and facilitate heat removal.

1.3.2 Time-lapse and artificial intelligence

Morphological analysis of oocytes and embryos represents an important step in the IVM and IVF processes. Morphological assessment not only assists scientists and clinicians in selecting the highest-quality embryos for uterine transfer, but also contributes to improving the likelihood of successful pregnancy. Numerous studies have been conducted in this field to investigate the relationship between oocyte and embryo morphology and their implantation potential as well as subsequent developmental competence. Furthermore, with the continuous advancement of imaging technologies, researchers now have access to increasingly powerful tools for observing and analyzing the structural characteristics of oocytes and embryos across different stages of development.

Previous studies have extensively focused on the morphological evaluation of oocytes. Researchers have investigated parameters such as oocyte size, shape, zona pellucida appearance, as well as the number and distribution of nuclear structures within the oocyte. A mature oocyte is generally characterized by a specific size range, a regular spherical shape, and an intact zona pellucida without visible abnormalities or fractures. In addition, the ooplasm is expected to appear homogeneous and free of unusual inclusions or granules. The nucleus also plays an important role, as it is typically expected to be centrally located and exhibit a regular round shape, which is often considered indicative of good oocyte quality. These morphological features are closely associated with the implantation potential and developmental competence of the resulting embryo, thereby influencing the overall success of the IVF process.

For embryos, morphological assessment is equally important. Previous studies have focused on parameters such as cell number, cell size, embryonic symmetry, the presence of cellular debris, and signs of fragmentation. High-quality embryos are generally characterized by a progressive increase in cell number over time and by blastomeres of relatively uniform size, reflecting favorable cleavage dynamics. Symmetry is also

considered an important indicator, as embryos with a more symmetrical appearance are often associated with better developmental potential. In addition, the absence of significant cellular debris, fragmentation, or large intercellular spaces is commonly regarded as a hallmark of good embryo quality. These features are valuable not only for embryo selection but also for providing important insights into embryonic health and developmental competence under *in vitro* culture conditions.

Morphological analysis extends beyond the selection of high-quality embryos for IVF and has potential applications in several other areas. For example, it may be used to evaluate the effectiveness of emerging infertility treatments, such as ICSI, by examining their association with improvements in embryonic morphology. Researchers may also employ morphological assessment to investigate the underlying mechanisms of reproductive disorders, including polycystic ovary syndrome (PCOS) and oocyte dysfunction, thereby contributing to the development of more effective therapeutic strategies. In addition, morphological analysis can be applied in the development of animal models for studying embryonic development in other species, which may further broaden our understanding of developmental biology and human reproduction.

Time-lapse imaging is a technique for acquiring visual data by capturing sequential still images at fixed time intervals and subsequently compiling them into a video that represents dynamic processes over time. In biology, this technology is widely used to monitor the development and transformation of cells, tissues, and organs in living systems or under *in vitro* culture conditions. It provides a direct and continuous view of biological events that conventional observation methods often fail to capture, particularly when such processes occur too slowly to be readily appreciated in real time. In biological research, time-lapse imaging has become a valuable tool for tracking cellular development, cell division, cell-cell interactions, and oocyte maturation during *in vitro* culture experiments.

Time-lapse imaging has been widely applied in studies of cellular development. Owing to its ability to continuously record images over extended periods, this technique enables researchers to observe key biological events such as cell division, including both mitosis and meiosis, cellular differentiation, and structural changes in cells under different conditions. It also facilitates the observation of programmed cell death (apoptosis) and helps reveal how cells respond dynamically to different therapeutic agents. For example, Figure 1.7 illustrates the time-lapse photography of the embryo's division process and the expulsion of cell debris from within the zona pellucida, with arrows pointing to the cell fragments [79]. Time-lapse imaging also has important implications for infertility research and treatment. In particular, this technology enables continuous monitoring of oocyte maturation during procedures such as IVF. It can capture the developmental progression from immature to mature oocytes and facilitate the assessment of key cellular events, including the division and spatial arrangement of structures such as

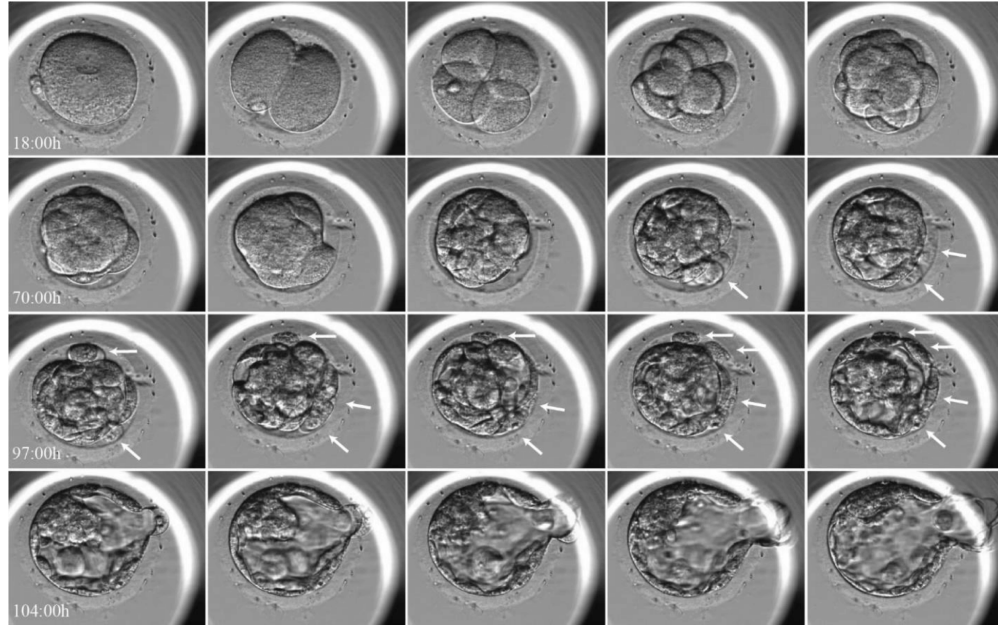


Figure 1.7: Time-lapse photography using the EmbryoScope™ shows the embryo expelling cell debris and fragments within the zona pellucida during its division process. Arrows indicate the expelled cell debris [79].

the polar body, which may provide useful indicators of oocyte fertilization potential. In IVF, oocytes or embryos are cultured in specialized media following fertilization, during which their development can be monitored using time-lapse imaging. This approach allows observation of critical developmental stages, including cleavage events, blastocyst formation, and hatching. Based on these image sequences, embryologists can evaluate embryo quality more comprehensively and select embryos with more favorable developmental characteristics for transfer, thereby potentially improving the success rate of IVF.

The application of artificial intelligence (AI) in reproductive research not only contributes to optimizing embryo culture workflows but also opens new avenues for investigation. AI-based systems can support the automated regulation of culture conditions and enable precise monitoring of embryonic development, thereby facilitating the development of more advanced therapeutic strategies and potentially improving success rates. The integration of AI into reproductive research has the potential to drive significant progress in the field by providing novel approaches to enhance both the efficiency and safety of assisted reproductive treatments.

Advances in AI and computer vision have made the automated assessment of oocyte and embryo maturity increasingly accurate, rapid, and practical. This progress may not only contribute to improving the success rates of assisted reproductive treatments, but also enhance the overall patient experience and support continued research in reproductive science. AI is expected to remain an important supportive tool in this field, contributing to the sustainable advancement of assisted reproduction and helping

more families achieve parenthood. Within ART, AI has increasingly been applied to support clinicians in the evaluation and selection of high-quality gametes and embryos, with the aim of improving treatment efficiency and reproductive outcomes.

Among the earliest applications of computational methods in reproductive medicine were automated image-processing systems developed for semen analysis. Conventional platforms such as FERTECH [80] and the Integrated Visual Optical System [81] have provided relatively reliable and standardized assessments of sperm characteristics based on World Health Organization (WHO) criteria. These systems have contributed to reducing subjectivity in routine analysis and have facilitated more consistent evaluation across laboratories. However, their performance is still largely constrained by the predefined WHO criteria on which they are based, and in many cases they depend on staining procedures that may alter sample appearance and potentially influence expert interpretation. As a result, although these conventional tools have been clinically useful, they remain limited in their ability to capture more subtle or complex sperm characteristics associated with fertilization potential.

Meanwhile, machine learning techniques have shown considerable potential for sperm quality assessment by analyzing parameters such as morphology, motility, DNA integrity, and other relevant characteristics [82]. A range of conventional algorithms, including K-Nearest Neighbor (KNN), Support Vector Machine (SVM), Decision Trees, and Naive Bayes, have been applied to distinguish normal from abnormal sperm based on sperm head images. In these approaches, images were first processed to extract handcrafted shape descriptors, including Hu moments, Zernike moments, and Fourier descriptors, which were then used to train the models. More recently, with advances in computational power, deep learning has emerged as a preferred approach for complex image analysis tasks. Unlike traditional machine learning methods, deep learning models can automatically extract and learn relevant features directly from input images, often resulting in improved performance. For example, Javadi *et al.* developed a model to detect morphological abnormalities in the sperm head, acrosome, and vacuole, achieving $F_{0.5}$ scores of 83.86%, 84.74%, and 94.65%, respectively, while maintaining good performance even on non-stained and low-quality images [187]. In addition, recent smartphone-based semen analysis platforms have combined image acquisition, microfluidic sample preprocessing, and convolutional neural network (CNN)-based analysis to enable low-cost and portable at-home testing. One such system predicted the proportion of normal sperm with a sensitivity of 80% and a specificity of 92%, highlighting the potential of AI-assisted point-of-care fertility assessment [83].

Beyond morphology, AI has also been applied to predict sperm DNA integrity and motility. Machine learning-based methods have enabled DNA integrity assessment at the single-sperm level, which remains challenging with conventional techniques. For instance, McCallum *et al.* developed a CNN that predicted sperm DNA quality from brightfield

images in less than 10 ms [84]. Similarly, sperm motility, a key factor associated with fertility outcomes, has been analyzed using AI models trained on features derived from CASA systems or sperm tail dynamics. Goodson *et al.* classified five sperm motility patterns using an SVM-based decision tree and achieved an accuracy of 89.9% [85]. Collectively, these studies suggest that AI could support a more comprehensive assessment of sperm quality by integrating multiple indicators, including morphology, DNA integrity, and motility.

Embryo selection for transfer is a critical determinant of success in both IVF and ICSI. In current clinical practice, embryo selection is still largely based on manual visual assessment of morphological appearance and developmental kinetics. Although this approach is widely used, it may produce results that are uncertain, variable, and inherently subjective, as evaluations can be influenced by the experience of individual embryologists and by inter-observer differences. To address these limitations, AI models have increasingly been developed to provide rapid, automated, and more standardized assessment of embryo quality across multiple developmental stages, including the pronuclear, cleavage, and blastocyst stages. At the earliest stages of development, AI has been applied to assist in the confirmation of normal fertilization. For example, Dimitriadis *et al.* developed a CNN to distinguish normally fertilized embryos exhibiting two pronuclei (2PN) from abnormally fertilized embryos (non-2PN) at approximately 18 hours after insemination [86]. Using embryo images as input, the proposed model achieved a sensitivity exceeding 90%, indicating its potential as a supportive tool for early embryo assessment. Such applications are important because accurate identification of normal fertilization is a prerequisite for subsequent embryo selection and may help reduce the burden of manual inspection in busy IVF laboratories. With the adoption of time-lapse imaging systems in assisted reproduction, AI-based embryo assessment has advanced further by incorporating both morphological and morphokinetic information [87]. Time-lapse imaging enables continuous, non-invasive monitoring of embryo development and provides a much richer dataset than conventional static observations. This has created favorable conditions for machine learning and deep learning algorithms, which can extract subtle spatial and temporal patterns that may not be readily recognized by the human eye. Among the notable examples, STORK, a deep learning framework built on Google's InceptionNet architecture, was developed to rank blastocyst quality using approximately 50,000 time-lapse images [88]. When compared with embryologists, STORK demonstrated superior performance relative to individual evaluators in embryo quality assessment. In another study, a CNN combined with a genetic algorithm was used to predict whether embryos at the cleavage stage would successfully develop into blastocysts [89]. The results suggested that this AI-based approach outperformed highly trained embryologists in identifying embryos with the greatest developmental potential.

Beyond morphological grading and developmental prediction, AI has also shown promise in non-invasive chromosomal screening. This is particularly significant because the current standard approach, preimplantation genetic testing for aneuploidy, although clinically valuable, is associated with several limitations, including invasiveness, cost, and the potential complications arising from embryonic mosaicism. AI-based image analysis has therefore been investigated as an alternative or complementary strategy for predicting ploidy status without embryo biopsy. For instance, ERICA (Embryo Ranking Intelligent Classification Algorithm), a deep learning-based system, was developed to predict euploidy using 1,231 blastocyst images [90]. ERICA achieved an accuracy of 70% and a positive predictive value of 0.79, while requiring less than 25 seconds to rank four embryos, and it outperformed two senior embryologists in this task. Similarly, STORK-A, an extension of the STORK framework for aneuploidy screening, was trained and validated on a large dataset of 10,378 embryos imaged at 11 hours after ICSI and showed encouraging predictive performance [91]. More recent studies have also reported that AI models can detect abnormalities associated with chromosomes 21 and 16 from embryo morphology with accuracies exceeding 70%, further highlighting the potential of AI-assisted image analysis as a non-invasive alternative to traditional PGT-A [92].

AI has also shown considerable potential in oocyte assessment and selection within assisted reproductive technologies. Various AI approaches, including neural networks, support vector machine (SVM) classifiers, and machine learning pipelines combined with image segmentation, have been applied to evaluate oocyte quality using different types of biological and imaging features. In the context of IVM, neural network-based models have been used to analyze time-lapse microscopic images of live oocytes and to extract dynamic cytoplasmic movement characteristics throughout the culture period. Such non-invasive analysis enabled the discrimination between developmentally competent and incompetent oocytes with an accuracy of 91.03% [93]. In addition to dynamic imaging features, machine learning has also been employed to assess the mechanical properties of oocytes and zygotes shortly after fertilization. One study demonstrated that viscoelastic properties measured within hours after fertilization could be used to predict blastocyst formation with high precision, specificity, and sensitivity, suggesting that AI-assisted biomechanical analysis may provide useful indicators of subsequent embryonic development [94]. Furthermore, AI has been applied to phenotypic oocyte classification through the segmentation of the oocyte and zona pellucida, followed by the extraction of multiple morphological features, including zona pellucida characteristics and cytoplasmic particle size. Using these features, machine learning models were able to anticipate oocyte maturation with an accuracy ranging from 80% to 90% [95]. Collectively, these findings indicate that AI can support a more objective, rapid, and non-invasive evaluation of oocyte quality, thereby offering promising tools for improving oocyte selection in reproductive medicine [93].

Despite these encouraging findings, several important limitations remain. Many AI systems still suffer from the so-called “black box” problem, in which their decision-making processes are not easily interpretable to clinicians [96]. In addition, model performance can be strongly influenced by the quality, size, and diversity of the training datasets, raising concerns about bias, limited generalizability, and potential reductions in accuracy when models are applied across different clinics, patient populations, or imaging systems. These challenges suggest that, while AI-based embryo assessment is promising, further validation, standardization, and multicenter testing are still required before widespread clinical adoption can be fully justified.

1.4 Direction of the Research

In this study, an integrated microfluidic platform is established to streamline key oocyte-processing steps throughout the IVM workflow in preparation for fertilization, including single-oocyte trapping, dynamic culture, maturation monitoring, denudation, quality-based selection, as illustrated in Figure 1.8. At the core of this platform is **OvaOoC**, an oocyte-centered organ-on-chip module designed for single-oocyte trapping, dynamic co-culture, and continuous on-chip assessment during *in vitro* development. Precise single-cell trapping and manipulation enable individual oocytes to be immobilized with high accuracy, thereby facilitating the investigation of their morphological characteristics, biological behavior, and responses to different culture conditions. In parallel, the chip supports dynamic culture and co-culture of oocytes with cumulus cells, thereby allowing certain aspects of the ovarian microenvironment to be more closely recapitulated than in conventional static systems. Continuous medium perfusion, preserved oocyte–cumulus cell interactions, and localized microenvironmental control help reproduce physiologically relevant conditions for nutrient exchange, paracrine signaling, and waste removal, all of which are important for oocyte maturation and developmental competence. The chip was specifically designed to increase the contact area between the oocyte and the culture medium, thereby promoting more efficient mass transfer and potentially improving maturation outcomes. The OvaOoC also allows for the automated exclusion of low-quality oocytes prior to downstream fertilization procedures. By combining microfluidic single-oocyte trapping and culture with time-lapse imaging and deep learning-based segmentation, automated and objective quantification of cumulus cell expansion and oocyte morphological changes is enabled. This platform supports continuous monitoring and systematic assessment of single-oocyte maturation directly on-chip. Collectively, the integration of microfluidic culture control in OvaOoC with AI-assisted image analysis may provide a useful foundation for improving IVM monitoring, supporting more consistent assessment of maturation, and advancing automation in assisted reproductive technologies.

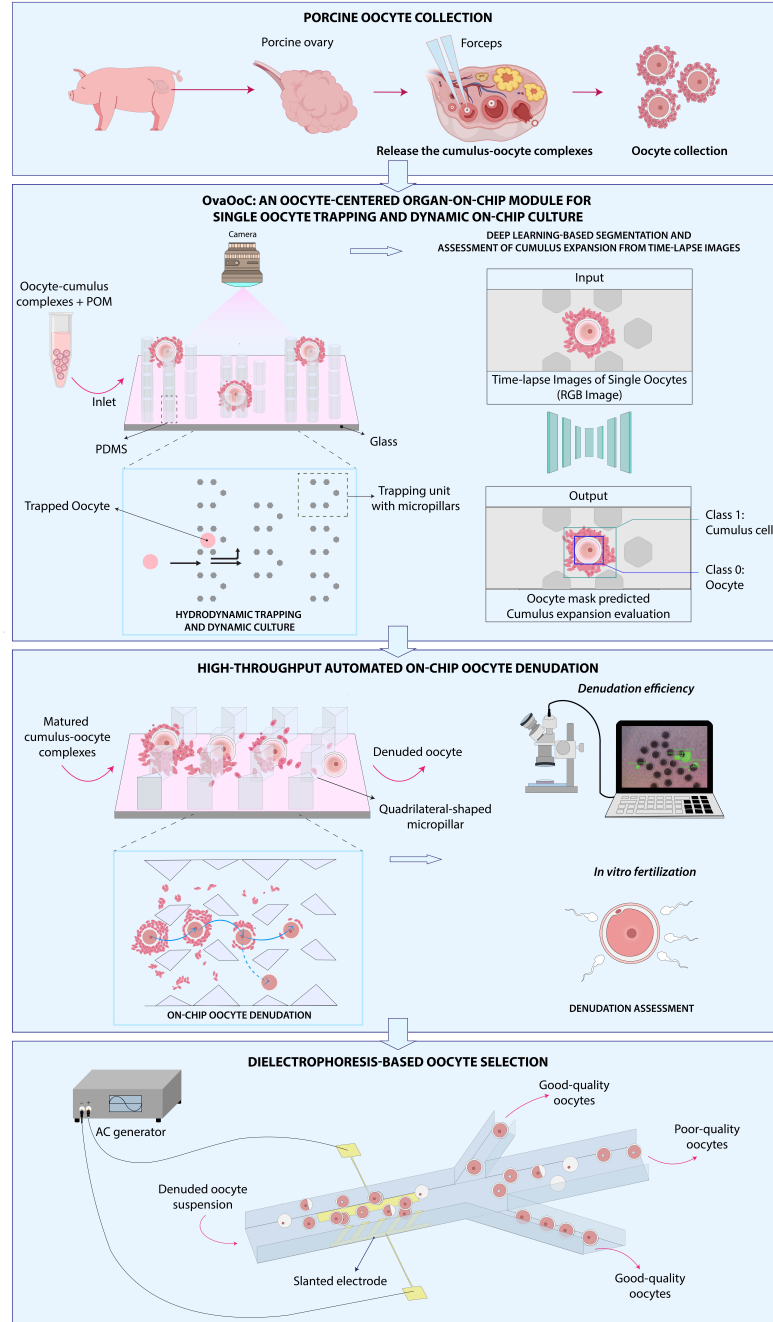


Figure 1.8: Schematic overview of the proposed microfluidic-assisted reproductive platform. Oocytes are first collected from animal ovaries and introduced into the OvaOoC module for single-oocyte trapping, dynamic co-culture with cumulus cells, and continuous on-chip monitoring during in vitro maturation. After maturation, cumulus cells were mechanically removed through automated on-chip denudation. Finally, the denuded oocytes were subjected to DEP-based selection according to their dielectric properties for downstream fertilization applications

Following *in vitro* culture, denudation is an essential step prior to oocyte assessment and ICSI/IVF. During oocyte maturation, cumulus cells maintain close communication with the oocyte through gap junctions and play important roles in the transfer of growth-related signals, energy substrates, and regulatory molecules that support oocyte competence and early embryonic development. Once maturation has been achieved, however, these surrounding cumulus cells must be removed so that the denuded oocyte, enclosed only by the zona pellucida, can be properly evaluated and used for fertilization. In conventional practice, oocyte denudation is typically performed through enzymatic treatment followed by repeated mechanical pipetting. Although this approach is widely used, it remains labor-intensive, operator-dependent, and difficult to standardize, particularly when large numbers of oocytes are processed. These limitations highlight the urgent need for a more controlled and automated strategy for cumulus cell removal.

To address this challenge, on-chip oocyte denudation was designed, fabricated, and experimentally validated for oocyte denudation through the mechanical removal of cumulus cells from cumulus–oocyte complexes. The chip incorporates a specifically designed quadrilateral micropillar array that facilitates controlled stripping of cumulus cells as the complexes pass through the microstructure. Numerical simulations and experimental validation were conducted to evaluate the hydrodynamic behavior and denudation performance of the system. Key outcomes, including denudation efficiency, processing time, throughput, and embryo developmental potential after IVF, were compared with those obtained using standard manual protocols. This microfluidic platform provides a scalable and cost-effective alternative to manual denudation procedures and may also be adapted for other high-throughput cell separation applications involving heterogeneous biological samples.

After denuded oocytes had been obtained for fertilization, the present work was further directed toward the development of a microfluidic system integrated with DEP for oocyte selection based on dielectric properties. DEP-based manipulation and selection offer several advantages for assisted reproductive applications. Because the method relies on the intrinsic electrical properties of cells, selection can be performed without biological labeling, while remaining non-invasive and minimizing mechanical damage to the oocyte. In addition, DEP can be readily integrated into microfluidic platforms, thereby providing a favorable basis for process automation and standardization. The ability to classify oocytes according to dielectric characteristics also offers a more objective alternative to conventional morphology-based assessment, which is often influenced by subjective interpretation. Accordingly, this study aimed to propose a microfluidic DEP structure integrated with an electrode system capable of generating a non-uniform electric field for the separation of high-quality and abnormal oocytes based on their dielectric properties. Through this design, a more objective, non-invasive, and automation-compatible platform for oocyte selection is expected to be established,

thereby contributing to improved efficiency and consistency in ART workflows.

For the initial biological validation of the microfluidic system, porcine oocytes were chosen due to their relevance in reproductive biology and similarities to human oocytes, including comparable size, maturation timelines, and proteomic expression. Using porcine oocytes also addressed ethical and practical constraints, as human oocytes are limited, while providing a biologically relevant model for proof-of-concept studies in reproductive research.

This thesis is organized into five chapters.

Chapter 1, *Introduction* introduces the research background, motivation, objectives, and scope of the study, together with an overview of recent advances in microfluidic technologies for assisted reproductive applications.

Chapter 2, *Theory and Simulation*, presents the fundamental physical theories underlying the proposed microfluidic platform, including fluid dynamics in microfluidics, fluid–cell interaction, and DEP for cell manipulation. Based on these theoretical foundations, microfluidic chip structures are proposed and optimized for single-oocyte trapping, dynamic culture, on-chip denudation, and DEP-based oocyte selection. This chapter also describes the finite element method (FEM)-based simulation approach, including the governing equations, material models, and boundary conditions used to analyze fluid flow, shear stress distribution, trapping performance, and DEP-induced particle behavior.

Chapter 3, *Experimental Setup*, describes the fabrication procedures of the microfluidic chips, together with the experimental equipment and setups used throughout the study. This chapter also presents the protocols for oocyte collection and preparation, culture media and incubation conditions, as well as the experimental procedures for single-oocyte trapping, dynamic culture, denudation, fertilization and DEP-based manipulation.

Chapter 4, *Results and Discussion*, presents and discusses the simulation, fabrication, and experimental results obtained from the proposed microfluidic systems. The chapter focuses on the evaluation of single-oocyte trapping performance, dynamic culture in the OvaOoC platform, on-chip denudation efficiency, maturation assessment, and DEP-based oocyte selection. Detailed analyses and discussions are provided to validate the effectiveness and applicability of the developed platform for assisted reproductive technologies.

Finally, ***Conclusions*** summarizes the main findings and contributions of this thesis and outlines future research directions for further improving the microfluidic platform and extending its applications in reproductive engineering and assisted reproduction technologies.

1.5 Conclusion

This chapter has presented an overview of assisted reproductive technologies (ART), with particular emphasis on *in vitro* maturation (IVM), including its advantages, current limitations, and existing research gaps. Recent advances in biotechnology for reproductive applications were also reviewed, highlighting the potential of microfluidic systems to provide controlled and biomimetic microenvironments for oocyte manipulation and culture. In addition, emerging integrated technologies, including dielectrophoresis (DEP), time-lapse imaging, and artificial intelligence (AI)-based analysis, were discussed for their potential to improve automation, monitoring, and objective assessment in ART workflows. Based on these scientific and technological backgrounds, this thesis proposes a modular microfluidic-assisted platform to streamline multiple stages of the oocyte culture and preparation workflow prior to fertilization. The proposed research is expected to contribute toward the development of more efficient, automated, and physiologically relevant systems for next-generation assisted reproductive technologies.

Chapter 2

Theory and Simulation

2.1 Theory and Numerical model

2.1.1 Fluid mechanics

Fluid mechanics in microchannel

The Navier–Stokes equations constitute a system of nonlinear partial differential equations describing the conservation of momentum in fluid motion and form the theoretical foundation of classical fluid mechanics [97]. These equations govern the behavior of both gases and liquids by accounting for the balance between inertial forces, pressure gradients, viscous stresses, and external body forces within a fluid domain. Together with the continuity equation, they provide a comprehensive mathematical framework for modeling fluid flow phenomena in both natural and engineered systems. Because of their generality and physical rigor, the Navier–Stokes equations are widely used in theoretical analysis, experimental interpretation, and computational fluid dynamics (CFD) simulations across a broad range of applications, including aerodynamics, chemical engineering, and microscale transport processes.

Physically, the Navier–Stokes equations describe how momentum is transported through a fluid due to convection and viscous diffusion. The equations capture the interaction between velocity, pressure, density, and viscosity fields, allowing researchers to predict how a fluid evolves over time under given boundary and initial conditions. This capability enables the construction of mathematical models that accurately represent the behavior of fluid flows in real experimental systems. Consequently, the Navier–Stokes framework serves as the basis for many numerical simulation techniques used in modern fluid dynamics research and engineering design.

To translate a physical fluid system into a computational model, the relevant fluid properties and governing parameters must be explicitly defined and quantified. These parameters typically include fluid density, dynamic viscosity, pressure distribution, velocity field, and any external body forces acting on the fluid. For an incompressible

Newtonian fluid, the Navier–Stokes equation [98] can be written as:

$$\rho \left(\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right) = -\nabla p + \mu \nabla^2 \vec{u} + \vec{f} \quad (2.1)$$

where p and μ denote the pressure and dynamic viscosity of the fluid, respectively, ρ represents the fluid density, and $\vec{u}$ is the velocity vector of the flow field. Here f represents body force densities.

In microfluidic systems, fluid motion typically occurs in the laminar flow regime, which is characterized by very small Reynolds numbers. The Reynolds number is a dimensionless parameter defined as the ratio between inertial forces and viscous forces acting within a fluid:

$$Re = \frac{\rho U L}{\mu} \quad (2.2)$$

where ρ is the fluid density, U is the characteristic velocity, L is the characteristic length scale, and μ is the dynamic viscosity. This parameter plays a crucial role in determining the nature of fluid motion and is widely used to distinguish between laminar and turbulent flow regimes. In microfluidic channels, the characteristic length scale is typically on the order of micrometers, while flow velocities are relatively low. As a result, the Reynolds number becomes extremely small, indicating that viscous forces dominate the flow dynamics [99]. The unique hydrodynamic characteristics of low-Reynolds-number flows have been extensively studied in the microfluidics literature. For example, Squires and Quake (2005) provided a comprehensive review of fluid physics at the nanoliter scale, highlighting the importance of viscous-dominated flows in microfluidic systems. Similarly, Stone *et al.* (2004) discussed the engineering principles of microfluidic flow control and emphasized the role of laminar flow in microscale transport phenomena [99]. These studies demonstrate that understanding the Navier–Stokes framework under low-Reynolds-number conditions is essential for accurately modeling and designing microfluidic systems.

Single cell hydrodynamic trapping

Single-cell trapping is a fundamental technique in cellular biology and microfluidics, enabling the isolation, immobilization, and precise manipulation of individual cells within controlled microscale environments. This capability is essential for studying cellular heterogeneity, cell–cell interactions, and dynamic biological processes at the single-cell level. Various trapping strategies have been developed, among which the most commonly used include hydrodynamic, magnetic, optical, acoustic, and electric-field-based trapping methods.

Hydrodynamic trapping relies on fluid flow to generate forces that immobilize cells at predefined locations within microfluidic channels. As cells are transported by the flow, specially designed microstructures, such as constrictions, weirs, or trapping chambers,

create localized pressure gradients and flow resistance that capture individual cells. This method offers the advantage of being label-free and does not require external fields, thereby preserving the biological integrity and viability of the cells. However, precise control over cell positioning can be challenging, particularly for small cells or in highly viscous environments where flow conditions become less predictable.

Magnetic trapping, on the other hand, utilizes magnetic fields to manipulate cells that are either inherently magnetic or labeled with magnetic nanoparticles. When exposed to an external magnetic field, these particles generate forces that allow cells to be captured and positioned with high precision. This technique provides excellent controllability and is widely used in cell separation and enrichment applications. Nevertheless, it requires the attachment of magnetic labels to the cells, which may alter their biological properties or interfere with downstream analyses, especially in sensitive biological systems [100].

Electric-field-based trapping, including DEP, exploits the interaction between cells and applied electric fields to control cell motion and positioning. In DEP-based trapping, cells are polarized in a non-uniform electric field and experience forces that drive them toward regions of high or low electric field intensity depending on their dielectric properties. This approach allows rapid, precise, and label-free manipulation of cells and has been widely applied in microfluidic systems for cell sorting and single-cell analysis. However, exposure to strong electric fields may induce cellular stress, heating effects, or membrane damage, potentially affecting cell viability if not carefully controlled [101].

In this study, hydrodynamic trapping was selected for oocyte immobilization and culture due to its biocompatibility, and ability to preserve cellular integrity. Unlike electric or magnetic trapping methods, hydrodynamic trapping does not require external fields or labeling agents, thereby minimizing the risk of cellular stress, membrane damage, or alteration of physiological properties. This is particularly important for oocytes, which are highly sensitive to environmental conditions and mechanical perturbations [102]. In addition, hydrodynamic trapping allows gentle and stable confinement of oocytes within microfluidic structures while maintaining continuous nutrient exchange through controlled fluid flow, creating a favorable microenvironment for *in vitro* culture. These advantages make hydrodynamic trapping a suitable and reliable approach for handling and maintaining the developmental potential of oocytes in assisted reproductive applications.

2.1.2 Fluid-cell interaction

Solid mechanics is one of the fundamental branches of physical science that deals with the deformation, stress, and motion of solid materials subjected to external loads and environmental influences. These loads may include mechanical forces, prescribed displacements, inertial effects arising from acceleration, thermal variations, chemical

interactions, and electromagnetic forces acting on the material body. Within the framework of continuum mechanics, solids are treated as continuous media in which the internal stress distribution and deformation can be described using field variables such as displacement, strain, and stress tensors. The theoretical foundation of solid mechanics enables researchers to predict how materials deform, store elastic energy, and eventually fail when subjected to external loading conditions [103]. Such formulations are essential in engineering mechanics, materials science, and increasingly in biomechanics, where biological structures are analyzed using the same continuum mechanics principles.

Applying the principles of solid mechanics to cellular modeling allows the simulation framework to capture important mechanical responses such as strain distribution, stress concentration, and deformation patterns within the cell structure. These models provide insight into how cells interact with their surrounding microenvironment, particularly in microfluidic systems where cells are subjected to hydrodynamic forces and mechanical constraints imposed by channel geometries. In such systems, the deformation of cells can significantly influence their motion, orientation, and interactions with other particles or boundaries. Accurate mechanical modeling is therefore essential for predicting cellular behavior in applications such as cell sorting, mechanophenotyping, and biomedical diagnostics.

For small deformations and relatively low external loads, many solid materials can be accurately approximated using the theory of linear elasticity, which assumes that the relationship between stress and strain is linear and reversible. Under these conditions, the deformation of a material is sufficiently small such that geometric nonlinearities can be neglected. If the material properties are also assumed to be identical in all directions, the material is considered isotropic, meaning that its mechanical response does not depend on orientation. The stress–strain relationship for isotropic linear elastic materials is commonly described by Hooke’s law, which relates stress to strain through elastic constants such as Young’s modulus and Poisson’s ratio [104]. This simplified constitutive model is widely used in computational mechanics because it provides an accurate representation of the mechanical behavior of many materials under moderate loading conditions while remaining computationally efficient.

To numerically analyze these mechanical behaviors, the finite element method (FEM) is widely employed as a powerful computational technique for solving the governing equations of solid mechanics. FEM discretizes the solid domain into small elements and approximates the displacement field within each element, enabling the numerical solution of complex deformation problems that cannot be solved analytically. Fluid–solid interaction (FSI) refers to multiphysics problems in which fluid flow and structural deformation influence each other simultaneously. When integrated into FSI models, FEM allows simultaneous simulation of both the fluid dynamics and the mechanical response of deformable solid structures such as biological cells. This coupled modeling

framework makes it possible to investigate how hydrodynamic forces generated by the surrounding fluid interact with cellular mechanical properties, producing deformation and motion within microfluidic environments. By combining biological insight with classical solid mechanics and advanced computational methods, this modeling strategy enables a realistic representation of cellular dynamics in fluidic systems. The use of FEM-based FSI simulations allows researchers to capture complex interactions between fluid flow and deformable biological structures, providing deeper understanding of cell mechanics and improving the predictive capability of computational models used in biomedical and microfluidic research [105].

The displacement and deformation of the solid satisfy the governing equations of linear elastodynamics [106]:

$$\varepsilon_s = \frac{1}{2} [(\nabla \mathbf{u}_s)^T + \nabla \mathbf{u}_s + (\nabla \mathbf{u}_s)^T (\nabla \mathbf{u}_s)] \quad (2.3)$$

$$\nabla_{\sigma_s} + \mathbf{F}_s = \rho_s \frac{\partial^2 \mathbf{u}_s}{\partial t^2} \quad (2.4)$$

$$\sigma_s = \mathbf{C} \varepsilon_s. \quad (2.5)$$

Here, Equation (2.3) is the strain-displacement (compatibility) equation, with ε_s denoting the infinitesimal strain tensor and $\mathbf{u}_s = (\mathbf{u}_s, v_s, w_s)$ denoting the solid displacement field (m, m, m). Equation (2.4) is Newton's equation of motion, with σ_s the Cauchy stress tensor, $\mathbf{F}_s$ 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 (2.5) is the linear elastic stress-strain law with $\mathbf{C} = \mathbf{C}(E, \nu)$ as the stiffness matrix given by

$$\mathbf{C} = \frac{E}{(1+\nu)(1-2\nu)} \begin{bmatrix} 1-\nu & \nu & \nu & 0 & 0 & 0 \\ \nu & 1-\nu & \nu & 0 & 0 & 0 \\ \nu & \nu & 1-\nu & 0 & 0 & 0 \\ 0 & 0 & 0 & 1-2\nu & 0 & 0 \\ 0 & 0 & 0 & 0 & 1-2\nu & 0 \\ 0 & 0 & 0 & 0 & 0 & 1-2\nu \end{bmatrix} \quad (2.6)$$

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

In FSI systems, a structure immersed in a fluid is subjected to hydrodynamic forces, including pressure and viscous stresses generated by the fluid motion. These forces act on the fluid-structure interface and may cause the structure to deform or move depending on its mechanical properties. Conversely, the deformation of the structure

alters the geometry of the surrounding fluid domain, thereby modifying the velocity field and pressure distribution of the flow. As a result, the fluid and solid domains are strongly coupled and must be solved together through the governing equations of fluid dynamics and structural mechanics. The interaction equations are determined through:

$$\mathbf{F}_A = [-\rho\mathbf{I} + K] \cdot \mathbf{n} \quad (2.7)$$

$$\mathbf{u}_{tr} = \frac{\partial \mathbf{u}_s}{\partial t} \quad (2.8)$$

In these equations, $\mathbf{F}_A$ presents the total force (caused by the fluid pressure and viscous force) exerted on the solid boundary, K is shear stress tensor and $\mathbf{n}$ is the outward normal to the boundary. From Equation (2.8), $\mathbf{u}_{tr}$ is the rate of change for the displacement of the solid. In this case, $\mathbf{u}_{tr}$ equal to the fluid velocity $\mathbf{u}_f$. In other words, at the liquid-solid interface, a non-slip boundary condition is formed for the liquid domain.

The fluid is assumed Newtonian with the corresponding constitutive equation for the viscous stress tensor K is written by:

$$K = \mu \left(\nabla_{\mathbf{u}_f} + (\nabla_{\mathbf{u}_f})^T \right) \quad (2.9)$$

where T indicates the transposition, μ is the fluid dynamic viscosity (Pa.s).

2.1.3 Dielectrophoresis for cell manipulation

DEP refers to the manipulation of polarizable particles in a non-uniform electric field. When a non-uniform electric field is applied, it generates differences in polarization among cells due to variations in their electrical properties. For example, oocytes at different physiological states or developmental qualities may exhibit distinct dielectric responses because their membrane structure, cytoplasmic composition, and intracellular organization differ. As a result, cells experience motion within the electric field gradient. The magnitude and direction of this motion depend on several factors, including particle size, electrical properties such as membrane capacitance and cytoplasmic conductivity, and interactions between the particle surface and the surrounding medium. Larger cells or particles with higher polarizability generally experience stronger dielectrophoretic forces. Similarly, differences in the electrical characteristics of the cell membrane or intracellular components can significantly influence the DEP response.

The DEP force can generally be classified into two main types depending on the direction of particle movement in a non-uniform electric field as shown in Figure 2.1. Positive dielectrophoresis (pDEP) occurs when a particle is attracted toward regions of high electric field intensity, typically near the edges or tips of electrodes where the electric

field gradient is strongest. This situation arises when the particle is more polarizable than the surrounding medium. In contrast, negative dielectrophoresis (nDEP) takes place when a particle is repelled from regions of high electric field intensity and moves toward areas of lower electric field strength [107]. This occurs when the surrounding medium is more polarizable than the particle itself. The distinction between these two behaviors forms the basis for many DEP-based separation and manipulation techniques used in microfluidic systems.

The DEP force affecting the cell is described by [108]:

$$\overrightarrow{F_{DEP}} = 2\pi R_p^3 \varepsilon_0 \text{Re} \left\{ \frac{\varepsilon_c^* - \varepsilon_f^*}{\varepsilon_c^* + 2\varepsilon_f^*} \right\} \nabla |\vec{E}|^2 \quad (2.10)$$

while R_p is the particle radius, $\varepsilon_0 = 8.854181817 \times 10^{-12} \text{ F/m}$ is the permittivity of vacuum, ε_c^* and ε_f^* represent the complex permittivities of the particle and the surrounding fluid medium, respectively. The term inside the brackets corresponds to the Clausius–Mossotti (CM) factor, whose real part determines both the magnitude and the direction of the dielectrophoretic force. The CM factor measures the polarizability of the particles, which depends on the real part of permittivity and the electrical conductivity, $\omega = 2\pi f$ 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 nDEP that pushes the particles away from the highest electric field gradient and 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. Another important parameter influencing the interaction between the electric field and a particle is the magnitude of the gradient of the squared electric field ($\nabla |\vec{E}|^2$). This term is strongly dependent on the applied voltage and determines the strength of the dielectrophoretic force acting on the particle. In practical DEP systems, the distribution and intensity of ($\nabla |\vec{E}|^2$) are largely governed by the design of the electrode geometry and the configuration of the microfluidic channel. Proper electrode arrangement can generate strong and well-controlled electric field gradients, which enhance the effectiveness of particle manipulation. Therefore, careful design of the fluidic flow and electrode structures is essential not only to maximize the electric field gradient but also to regulate other electrokinetic effects that may influence particle motion within the system.

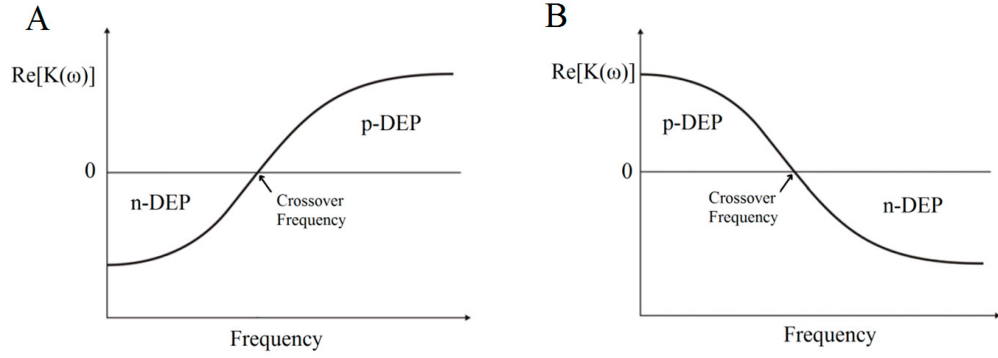


Figure 2.1: Dielectrophoretic (DEP) spectrum of a polarized particle (A): $\sigma_p < \sigma_m$ and $\varepsilon_p > \varepsilon_m$; (B): $\sigma_p > \sigma_m$ and $\varepsilon_p < \varepsilon_m$ [109]

2.2 Design

2.2.1 Single oocyte trapping and on-chip maturation

The objective of this study is to propose, develop, and optimize a microfluidic chip that partially mimics the *in vivo* physiological environment. The system is designed to precisely trap individual oocytes while simultaneously providing a stable and controlled microenvironment that supports efficient oocyte maturation and assessment. In particular, the implementation of single-cell trapping facilitates monitoring of individual oocytes, which is essential for streamlining the culture process and reducing operator-dependent variability.

Many previously reported microfluidic platforms for oocyte trapping and culture employ fixed microwell or microchamber structures for single-oocyte confinement [45], [46], [47]. Although these structures can provide stable retention, their confined and uniformly sized geometries may limit accommodation of COCs with different dimensions, restrict debris removal and optical observation, and provide limited capability for continuous automated monitoring. Simple chamber structure may cause flow stagnation, non-uniform shear stress, and nutrient depletion. To address these limitations, the proposed platform uses an open micropillar-based trapping structure with variable gaps and continuous flow paths around the trapped COCs.

In this thesis, **OvaOoC**, an oocyte-centered organ-on-chip module was designed for single-oocyte trapping, dynamic co-culture, and continuous on-chip assessment during *in vitro* development. The microfluidic chip structure consists of hexagonal micropillar trapping (HMT) units to effectively trap and culture single COCs of varying sizes based on hydrodynamic principles, making it highly suitable for IVM (Figure 2.2). The design of the chip is aimed at enhancing the interaction between oocytes and the culture medium by increasing the contact area, which improves nutrient exchange and optimizes maturation outcomes. Incorporating single-cell trapping within a microfluidic environment, the system facilitates the simultaneous culture of oocytes and cumulus cells,

ensuring that the complex interactions between these two cell types are maintained in a controlled and dynamic setting. The addition of deep learning-driven image segmentation enables real-time, automated, and objective analysis of cumulus cell expansion and the morphological changes of the oocytes during maturation. This AI-assisted approach provides a highly efficient means of tracking the progression of single oocyte maturation on-chip.

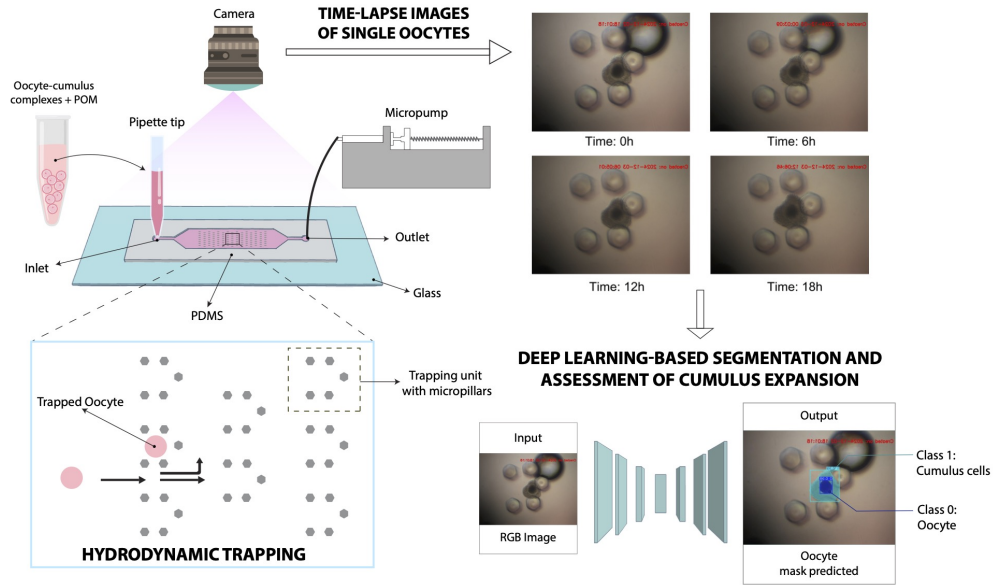


Figure 2.2: Schematic and functionality of the OvaOoC system

The proposed system is specifically designed to trap single oocytes and maintain them in culture over extended periods. Unlike conventional single-cell trapping platforms, this system incorporates a size-dependent trapping mechanism that ensures the efficient capture of oocytes, regardless of their size. The trapping mechanism operates through hydrodynamic flow control. The progressively narrowing size of the trapping units ensures that larger oocytes are retained in the larger upstream traps, while smaller oocytes continue migrating downstream until they encounter a unit that matches their size, optimizing the capture of oocytes with different dimensions.

Hydrodynamic trapping operates on a micro-scale flow control mechanism. As the fluid passes through narrow channels and micropillar structures, localized changes in velocity and pressure occur. These changes create low-pressure regions where cells of appropriate sizes, such as oocytes, are retained. When a trap unit is occupied, approaching cells are guided to bypass the unit by choosing the path of least resistance. This prevents cells from stacking in filled traps and ensures that each trap captures a single cell. The staggered arrangement of the traps enhances this process, directing cells toward unoccupied units without overlap. The microfluidic design and fluid flow control guide the cells efficiently into separate traps, minimizing mechanical interaction and reducing the risk of damage. This selective trapping mechanism ensures high

capture efficiency and allows for continuous, non-invasive cell processing in applications like single-cell analysis and cell culture. Unlike mechanical trapping methods, which often rely on direct contact and may induce physical stress on the cells, hydrodynamic trapping utilizes the fluid pressure to position cells, minimizing physical damage. The micro-adjustment of the flow plays a critical role in keeping the cell in place during manipulation and culture, reducing the risk of damage while providing a stable environment.

The choice of a hexagonal geometry for the micropillars was based on established microfluidic principles and further refined through preliminary experiments. Traditional U-shaped or chamber-based traps may cause flow stagnation, restricting continuous nutrient exchange [110], [111]. These designs can also result in non-uniform shear stress and reduced cell-medium contact [112], while square, inverted trapezoidal, and triangular pillar geometries tend to generate high localized shear stress at sharp corners [112], [113]. Although circular pillars offer smooth flow profiles, our initial experiments highlighted a major drawback in retaining highly deformable single cells [114]. The curvature of circular pillars only partially confined the oocytes at the tangential point, making them susceptible to slipping when flow rates increased. In contrast, the hexagonal design, with its flat, sloped surfaces, offers a longer restriction zone, which enhances the structural holding capacity and prevents the oocyte from escaping under varying flow conditions, all while maintaining low-shear streamlines. The 5-pillar array was specifically sized to create a spacious pocket, allowing gentle shifts of the oocyte under continuous culture flow. This design is critical for our platform, as it enables seamless, real-time tracking and automated morphological evaluation of cumulus expansion using the YOLOv8 deep learning module.

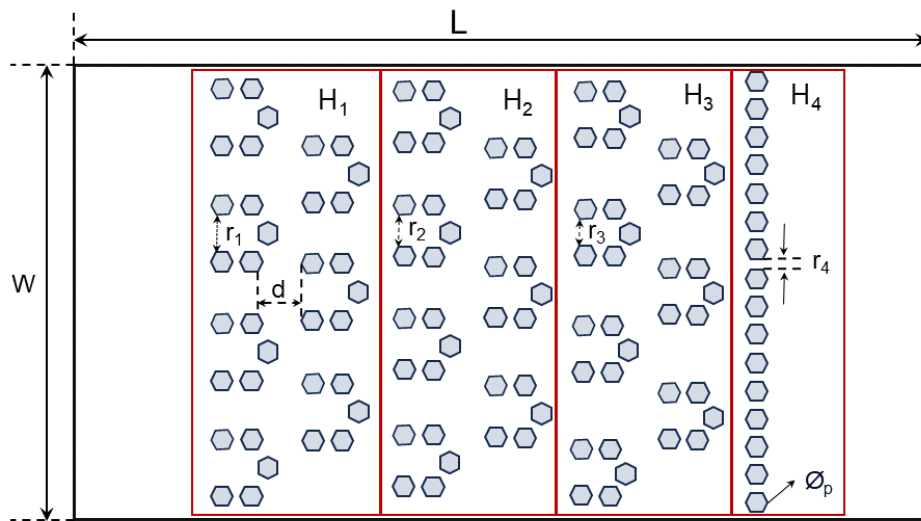


Figure 2.3: Refinement of the trapping region design in the microfluidic chip

The microfluidic chip is designed with an overall length of 28 mm (from inlet to

Table 2.1: 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
r₁	Width of the trap unit in the H ₁ region	250 μm
r₂	Width of the trap unit in the H ₂ region	200 μm
r₃	Width of the trap unit in the H ₃ region	150 μm
r₄	Width of the trap unit in the H ₄ region	80 μm
$\varnothing_p$	Diameter of the micropillar	200 μm

outlet) and a width of 5 mm. The trap region spans 12,000 μm in length and is methodically divided into four distinct zones, each featuring uniformly sized trap units that progressively decrease in size. This design aims to enhance trapping efficiency for cells of varying sizes. As shown in Table 2.1, each zone's trap unit size gradually narrows, allowing for optimal capture of cells ranging from large aggregates to smaller single cells. A simplified schematic of the trapping architecture is provided in Figure 2.3, which offers an overview of the structural hierarchy. However, the actual layout is denser than depicted, ensuring a higher trapping density and efficient use of the available microfluidic space.

Based on the experimentally observed size range of the COCs, which varied from approximately 100 to 350 μm , the trapping structures were dimensioned to ensure reliable capture, positioning, and immobilization of COCs of different sizes. To maintain consistent fluid distribution and stable trapping performance across all zones, the spacing between consecutive trap rows is set at 400 μm , and each micropillar has a diameter of 200 μm . In region H₁, the trap units are the largest, with a width of 250 μm . This design allows for the initial capture of large cells or multicellular aggregates, facilitating coarse filtration and reducing flow resistance. As the flow progresses to region H₂, the trap width is reduced to 200 μm , providing a more confined space suitable for capturing medium-to-large single cells. Further narrowing occurs in region H₃, where the trap width decreases to 150 μm . This size is particularly important, as it closely aligns with the dominant cell size range of 150–210 μm , which is typically observed in experimental settings. Region H₃, designed with the highest density of trap units, plays a critical role in efficiently capturing cells within this size range. The width of the traps in H₃ was initially designed to be 150 μm , but fabrication variations can result in a width of up to 180 μm . Finally, region H₄ contains the smallest traps, measuring 80 μm in width, which serve as a fine filter for trapping smaller cells or residual particles that have passed through the previous zones.

This progressive size variation across the four trap regions creates a gradient-based

trapping mechanism that allows for selective, size-dependent cell capture, ensuring that only cells of the desired size are retained. This feature also acts as a passive quality filter, as oocytes smaller than $100\text{ }\mu\text{m}$, which are often developmentally compromised or damaged, are excluded [115]. The systematic approach to size selection not only facilitates the entrapment of oocytes of varying sizes but also serves as a highly effective tool in oocyte screening for ART. Specifically, this microfluidic platform can significantly improve processes such as IVM and IVF. Since oocyte size is a critical determinant of developmental competence, the platform enables the automated and reproducible selection of oocytes, reducing dependence on manual microscopic evaluation. Additionally, the transparent microchannel design allows for real-time observation of the oocytes, enabling studies on their positioning, morphological dynamics, and response to different culture conditions.

2.2.2 Deep learning-based evaluation of cumulus cell expansion

The evaluation of oocyte maturity is a critical aspect of ART, including IVF and ICSI, as it directly influences fertilization outcomes and subsequent embryo development [116]. Traditional methods of assessment, which primarily rely on manual inspection by embryologists, are inherently subjective, time-consuming, and prone to variability. These methods depend on the clinical experience of the embryologist, leading to potential inconsistencies in evaluation and treatment decisions. Furthermore, the manual nature of these techniques limits their efficiency, as they are labor-intensive and hinder continuous monitoring of oocyte maturation. The inherent limitations of these traditional approaches include the risk of misidentifying oocytes as immature, which can result in the unnecessary exclusion of viable oocytes. Additionally, the time and effort required from embryologists restrict the number of patients they can manage, leading to higher operational costs.

To overcome these challenges, there has been a growing emphasis on the integration of automation in ART. Recent advancements in AI have focused on automating the assessment of oocyte and embryo maturity, offering significant benefits such as improved accuracy, faster diagnostic processes, and optimized research workflows. AI-based systems enable more precise, objective, and efficient evaluations of oocyte maturity, reducing the subjective nature of manual assessments. This not only alleviates the workload on embryologists but also enhances the overall success rates of fertility treatments, improving patient experience and offering greater prospects for couples seeking to conceive.

This study investigates the use of deep learning techniques for automating oocyte image segmentation, with a particular focus on analyzing the expansion of cumulus cells, a critical marker for assessing oocyte maturation. The expansion of cumulus cells is

often indicative of the oocyte's maturation stage, with an increase in the area of cumulus cells correlating with improved oocyte quality and a greater potential for successful fertilization [117]. Recent studies have highlighted that abnormalities in cumulus cell expansion can lead to poor oocyte quality, adversely affecting embryo development and implantation success [118]. By analyzing the temporal changes in cumulus cell area, researchers can gain valuable insights into the maturation process, which aids in optimizing IVM procedures and improving the chances of successful fertilization.

To monitor the maturation process of oocytes, the changes in the area of the surrounding cumulus cell region are relied upon. This requires accurate segmentation of the cumulus cell area. Conventional image processing algorithms, such as thresholding, color clustering, and edge detection, are not able to fulfill this requirement due to the unstable nature of the environment, particularly in terms of color segmentation. Furthermore, microscopic images of cells often present overlapping objects, such as oocytes and cumulus cells, or cumulus cells and the trapping structure. These factors cause the boundaries between regions to become blurred, making it difficult to definitively delineate them using linear algorithms or fixed-threshold methods. Therefore, an AI-based approach using deep learning was employed to segment the two target objects: “oocytes” and “cumulus cells”. Specifically, the YOLOv8-nano segmentation model was selected because of its lightweight architecture, high inference speed, and suitability for the complexity of the target structures [119]. As part of the Ultralytics YOLOv8 framework, this model enables object detection and instance segmentation within a unified architecture. This means that the model not only detects the location of objects through bounding boxes but also generates precise segmentation masks for each individual object. Unlike pure segmentation models like U-Net [120], which focus primarily on semantic segmentation (global segmentation), YOLOv8-nano provides more detailed and localized segmentation. YOLOv8-nano is built on an anchor-free principle, where each pixel predicts bounding box coordinates directly, eliminating the need for fixed anchor boxes. It uses the compact CSPDarknet backbone to reduce parameters, saving memory and improving processing speed. The model also features dynamic label assignment during training to enhance spatial learning and reduce overfitting. Its segmentation head generates higher resolution masks with more detail compared to previous YOLO versions. Additionally, the Ultralytics framework allows for easy training monitoring and real-time evaluation of metrics like mAP, IoU, Precision, and Recall.

To evaluate this, a time-lapse image dataset of individual on-chip oocytes was collected over a 24-hour period. These images are then uploaded to Roboflow for annotation, where two classes are defined: oocytes and cumulus cells [121]. After the annotation process, the dataset is used to train a segmentation model based on YOLOv8. After training, the model is deployed to automatically segment oocyte and

cumulus cells from input images as shown in Figure 2.2. 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.

The dataset was randomly split in an 80:20 ratio for training and validation purposes. The training process was conducted over 300 epochs, with a batch size set to 16. Mosaic augmentation was applied, with the close-mosaic parameter set to 4, in order to provide the model with a broader view of the data. The Adam optimizer [122] was chosen to ensure fast convergence, while a dropout rate of 0.1 was employed to mitigate overfitting. Other parameters were kept at their default values in the YOLOv8 model or were automatically adjusted during training by the Adam optimizer or the internal optimization mechanisms of the Ultralytics framework.

After image segmentation, the model returns a binary mask corresponding to the identified objects; in this case, the oocyte and cumulus cell region. These masks can be converted into polygonal shapes by extracting the closed contours, which enables the calculation of the area of each segmented region. To perform this accurately and efficiently, the shoelace formula, an established geometric method is applied to calculate the area of the polygon based on the coordinates of its vertices [123]. In this approach, each mask after segmentation is converted into a series of coordinate points representing the outer boundary of the object's region. The area calculation using the shoelace formula does not require the model to understand the complex internal shape of the object but focuses solely on the boundary.

$$A = \frac{1}{2} \left| \sum_{i=1}^n (x_i y_{i+1} - x_{i+1} y_i) \right| \quad (2.11)$$

in which (x_i, y_i) represents the coordinates of the i -th vertex of the polygon, and n is the total number of vertices.

The area of the object region is calculated based on the initial segmentation mask, represented in pixel units, reflecting the number of pixels (N) that cover the object region (either the oocyte or cumulus cells). However, to make the results meaningful in biological experiments, this area needs to be converted into real-world units, specifically square micrometers (μm^2).

In this study, the conversion is performed through a system calibration step using a reference sample with known physical dimensions. This process allows for the determination of a scaling factor, denoted as r , which represents the real-world length corresponding to each pixel in the image plane. The actual area of the object region is then calculated using the formula:

$$A_{real} = N \cdot r^2 \quad (2.12)$$

where A_{real} is the actual area in μm^2 , N is the number of pixels forming the

segmentation mask, and r is the scaling factor, representing the real-world length corresponding to one pixel.

By combining accurate segmentation results from the model with this area calculation method, the system not only identifies the regions associated with oocytes and cumulus cells but also quantifies the expansion of cumulus cells over time. This step is crucial for the quantitative evaluation of oocyte development, providing a more detailed understanding of maturation dynamics.

2.2.3 High-throughput on-chip oocyte denudation

ARTs encompass various fertilization techniques such as IVF and intracytoplasmic sperm injection (ICSI). One crucial procedure in the pre-fertilization process is oocyte denudation, where cumulus cells are carefully removed from the oocyte-cumulus complexes (COCs). Matured oocytes are covered by cumulus cell mass, commonly referred to as the COC. These cumulus cells are essential for oocyte maturation as they possess receptors for follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which are involved in nutrient absorption and the development of the oocyte. Despite the crucial role of cumulus cells in oocyte maturation, they need to be removed during ART procedures to facilitate proper evaluation and fertilization [124]. The removal of cumulus cells ensures that only the oocyte, which is protected by the zona pellucida, is used for fertilization. The process of oocyte denudation not only facilitates the subsequent fertilization procedure but also helps ensure that only viable oocytes are selected, optimizing the chances of successful fertilization and embryo development.

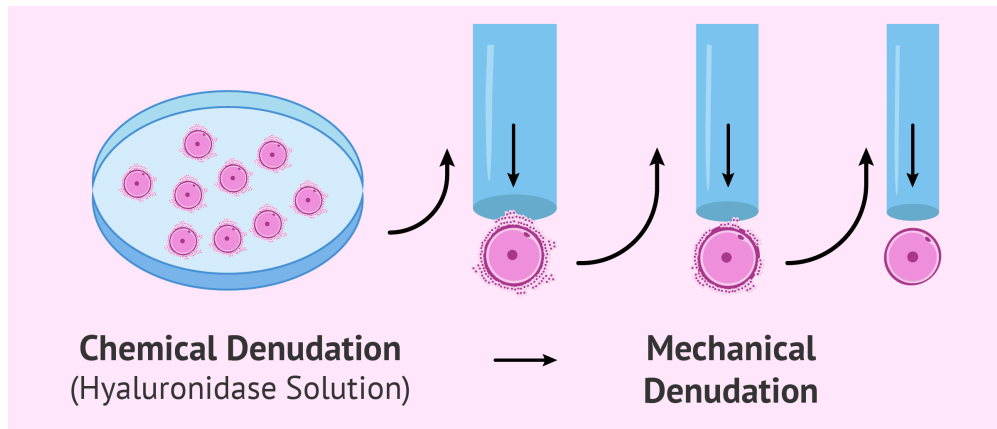


Figure 2.4: Traditional oocyte denudation process [125]

Traditionally, to perform oocyte denudation using traditional techniques, COCs are first treated with the enzyme hyaluronidase to break down the cumulus extracellular matrix (Figure 2.4). Hyaluronidase is an endoglycosidase enzyme that degrades hyaluronic acid into monosaccharides by cleaving its glycosidic bonds. Hyaluronic acid, a glycosaminoglycan found in connective tissues, skin, and joint fluids, is particularly

abundant in the cumulus cells surrounding the oocyte. After enzymatic treatment, mechanical force is applied to fully remove the cumulus cells. The primary mechanism for denudation involves the shear force generated at the tip of the pipette during aspiration, which plays a key role in separating the cumulus cells from the oocyte in the complex [126].

However, the use of hyaluronidase carries the risk of toxicity to the oocytes, making precise control over enzyme degradation timing critical. This is particularly challenging in manual procedures. Additionally, when working with a large number of oocytes, accurately identifying and manipulating both the oocytes and their surrounding cumulus cells during pipetting and washing becomes difficult. Furthermore, manual oocyte denudation is a time-consuming and labor-intensive process, often yielding inconsistent results between different operators, which leads to reduced efficiency in the separation process.

Inspired by the traditional theoretical mechanism, the proposed chip incorporating a multi-row quadrilateral micropillar array was designed, as illustrated in Figure 2.5. The device adopts a U-shaped microfluidic configuration with an overall length of 21.5 mm, while each channel has a width of 1.42 mm, enabling controlled fluid transport and stable manipulation of COCs. Prior to on-chip processing, COCs were briefly exposed to a collection medium containing hyaluronidase to partially digest the extracellular matrix surrounding the cumulus cells. The treated COCs were then immediately introduced into the microfluidic inlet using a pipette and driven toward the denudation region under an imposed pressure gradient. Subsequently, they were pushed through the inlet to the denudation region, where the spacing between the micropillars gradually decreased. The chip incorporates a chemical mechanism, similar to conventional denudation protocols, where enzymes like hyaluronidase are used to digest the extracellular matrix and facilitate the removal of cumulus cells. In addition to the chemical contribution of hyaluronidase, which weakens cell-matrix adhesion, the denudation process is governed by two complementary mechanical mechanisms. First, fluid flow through the microchannel generates localized hydrodynamic shear stress along the surface of the COCs, particularly within the narrow gaps between micropillars. This shear force helps dislodge and pull away cumulus cells from the oocyte, essentially “washing” cells off as the COC moves through the channel. Second, the sharp-edged quadrilateral micropillars provide direct mechanical interaction, physically scraping and shearing off remaining adherent cells upon contact. The staggered arrangement of these micropillars induces continuous rotational motion of the COCs, ensuring uniform exposure of the oocyte surface to both shear forces and mechanical contact. Through this synergistic combination of enzymatic treatment, hydrodynamic shear, and mechanical scraping, cumulus cells are efficiently removed. Finally, driven by the pressure difference between the inlet and outlet, fully denuded oocytes are transported to the outlet and collected

using a pipette for subsequent analysis.

Considering that COCs typically range from approximately 100 to 350 μm in size, whereas denuded oocytes are generally about 100–120 μm , the micropillar spacing was designed to gradually dissociate the surrounding cumulus cells while allowing the denuded oocytes to pass safely through the device. The device is composed of four sequential sections, in which the horizontal spacing between adjacent micropillars (W) progressively decreases, as illustrated in Figure 2.5. Specifically:

- **Primary microchannel (Section 1):** The spacing between micropillar rows ranges from 200 to 240 μm , and the spacing between micropillars within each row ranges from 150 to 180 μm , enabling the removal of loosely attached cumulus cells.
- **Secondary microchannel (Section 2):** The spacing between rows ranges from 160 to 200 μm , and between micropillars within each row from 120 to 150 μm , facilitating the detachment of adherent cell layers surrounding the oocyte.
- **Conditioning microchannel (Section 3):** The spacing between rows ranges from 120 to 160 μm , and between micropillars from 100 to 120 μm , targeting the removal of cells closely associated with the oocyte membrane.
- **Purification microchannel (Section 4):** A uniform spacing of approximately 120 μm between rows and 100 μm between micropillars within each row ensures complete removal of residual cells.

This gradual reduction in spacing enables stepwise removal of cumulus cells, from the outermost to the innermost layers surrounding the oocyte. Each section consists of 24 rows of micropillars, with each row containing four pillars, including two central pillars and two lateral pillars. The pillar size increases with the section number to enhance mechanical interaction in regions of narrower spacing. The odd rows of micropillars refer to those positioned at odd-numbered row in the microchannel, starting from the inlet, while the even rows are positioned at even-numbered locations. This staggered configuration induces a zigzag trajectory of the COCs, thereby increasing contact frequency and surface interaction with the micropillars, which contributes to improved denudation efficiency. More specifically, the middle pillar is a quadrilateral prism, formed by matching an isosceles triangle at the top and a right isosceles triangle illustrated in Figure 2.5. The width of the pillar varies according to the order of the section. The length of the pillar is fixed at 350 μm , and the distance between the two rows is 100 μm . The pillars are arranged to form multi-micropillar arrays with narrow channels and sharp edges, facilitating the removal of cumulus cells.

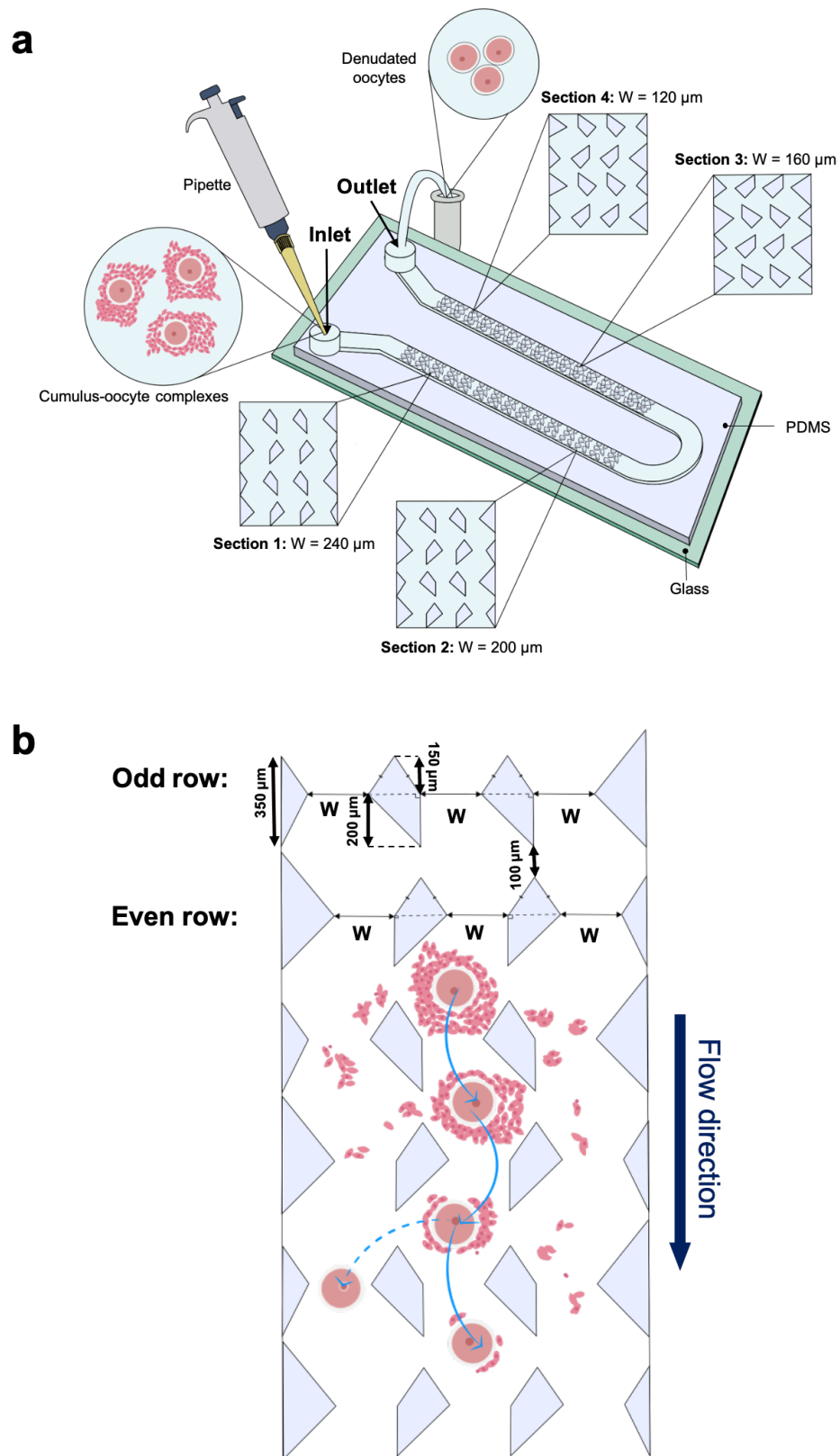


Figure 2.5: (a) Design and working principle of the proposed chip, (b) Schematic representation of the alternating odd–even micropillar configuration, illustrating the passage of a COC through successive pillar rows

2.2.4 Dielectrophoresis-based microfluidic chip for oocyte manipulation

Following oocyte denudation, the oocytes are introduced into a microfluidic platform that employs DEP to manipulate and automatically separate normal and abnormal oocytes, using an embedded electrode array arranged in a herringbone configuration, as illustrated in Figure 2.6. The proposed device consists of an inlet for introducing the oocyte suspension following denudation and buffer solution. The microfluidic design was optimized to discriminate oocytes based on their dielectric properties, which are associated with cellular integrity and developmental competence. Different oocyte conditions exhibit distinct “electrical signatures”, leading to varied responses under DEP forces.

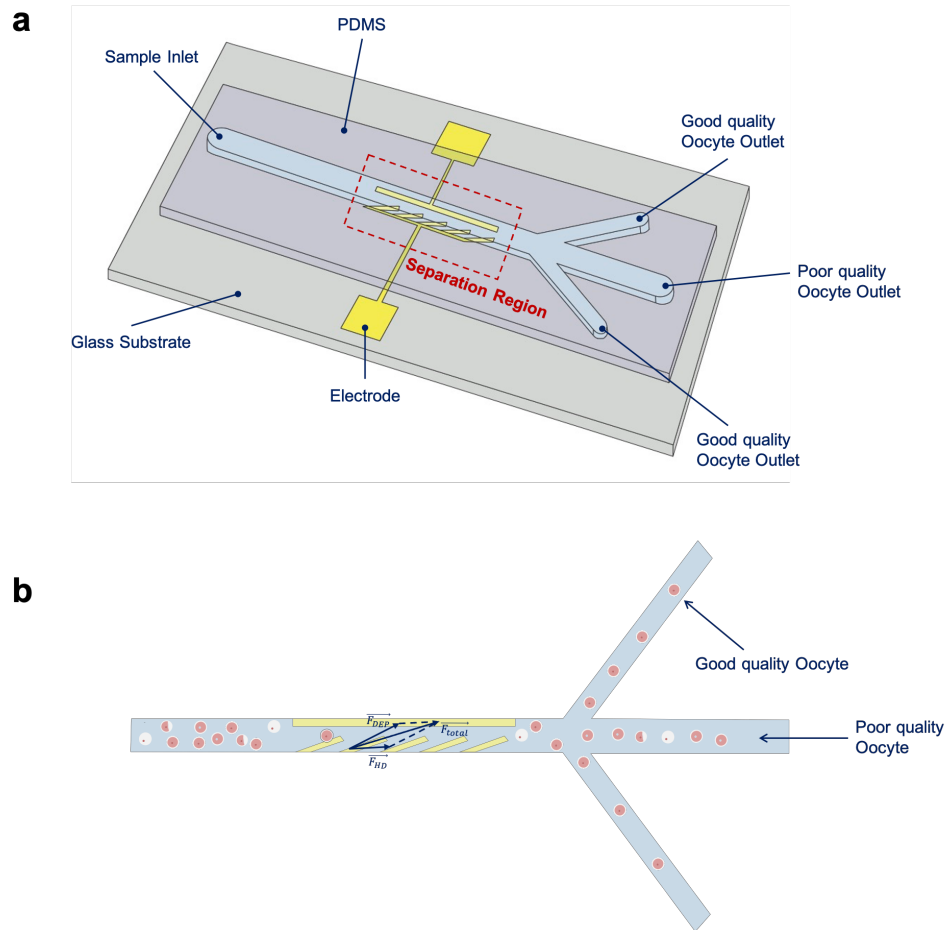


Figure 2.6: Design of the proposed biochip: (a) Illustration of the DEP-based microfluidic chip for oocyte separation, (b) Mechanism of the device

The central region of the microchannel serves as the active separation zone, where an array of electrodes generates a non-uniform electric field. The electrode system comprises five identical positive electrodes patterned diagonally in a herringbone arrangement,

along with a coplanar ground electrode located on the same substrate above the electrode array, as shown in Figure 2.6. This configuration establishes a three-dimensional non-uniform electric field. The outlets are designed to collect separated oocyte populations. The electrode length of $1,800\ \mu\text{m}$ inclined at an angle of 20° was determined based on a combination of factors including manufacturability, the scale of the biological samples, and the electrical field requirements for effective dielectrophoretic manipulation of oocytes. The width of each slanted electrode is $250\ \mu\text{m}$, while the spacing between adjacent branches along the main electrode is $800\ \mu\text{m}$. The rectangular electrode is designed as a straight conductive strip with a total length of $7.75\ \text{mm}$ ($7,750\ \mu\text{m}$) and a uniform width of $400\ \mu\text{m}$. The outlets are inclined at angles of 30° to the horizontal axis for the normal and abnormal oocytes, respectively. The tilted angles and geometrical parameters of the outlets are optimized based on the movement trajectories of each cell type.

For the experimental setup, the oocyte suspension containing a mixture of normal and abnormal oocytes is introduced into the microfluidic channel simultaneously with a buffer solution. The buffer, consisting of phosphate-buffered saline (PBS) with controlled conductivity and permittivity, is injected to hydrodynamically focus the oocytes at the entrance of the microchannel. Within the separation region, the inclined electrode array generates a non-uniform electric field, producing DEP forces with both vertical and lateral components. Due to differences in electrical properties and structural integrity, normal and abnormal oocytes exhibit distinct DEP responses. Normal or high-quality oocytes experience stronger attractive pDEP forces toward regions of high electric field intensity and are consequently drawn toward and retained at the electrode surfaces within the microchannel. As a result, these oocytes are preferentially directed into and collected through the two lateral branch outlets. In contrast, abnormal oocytes experience nDEP under the selected operating conditions, causing them to be repelled from high electric field regions near the electrodes and guided along the main flow path toward the central outlet for collection.

2.3 Modelling and simulations

2.3.1 Finite element method

The Finite Element Method (FEM) is a general and effective approach for solving various engineering problems. It is widely used in analyzing stress and strain states in mechanical structures, such as automotive parts, aircraft, ships, high-rise buildings, bridge beams, and more. Additionally, it is applied to field theory problems, including heat transfer theory, fluid mechanics, hydroelasticity, elasticity of gases, and electromagnetism. With the support of information technology and CAD systems, complex structures can now be

easily computed and precisely designed. FEM is an approximate method used to solve mechanical problems, with the solution derived from solving the equilibrium equations of the entire computational domain based on the principle of limit equilibrium. The FEM computation process typically involves solving a system of linear algebraic equations, expressed in matrix form, which can be automated using computers. There are several well-known FEM software tools available, such as ANSYS, COMSOL Multiphysics, ABAQUS, and SAP, among others.

- **Geometric Nodes:** A geometric node refers to a set of n points in the domain V that define the geometry of the FEM. The domain V is divided based on these nodes, and the domain is replaced with a set of elements $\mathbf{v}^e$ that have simpler shapes. Each element $\mathbf{v}^e$ must be chosen such that it is analytically defined by the coordinates of its geometric nodes, meaning the coordinates must lie within or on the boundary of the element.
- **Mesh Division Rules:** The division of the domain V into elements $\mathbf{v}^e$ must satisfy two rules:
 - Two different elements can only share points on their boundary. This eliminates the possibility of overlapping elements. The boundaries between elements may consist of points, lines, or surfaces.
 - The collection of all elements $\mathbf{v}^e$ must closely approximate the original domain V , ensuring that no gaps or holes are created between the elements.
- **Types of Finite Elements:** There are several types of finite elements: one-dimensional, two-dimensional, and three-dimensional elements. Within each type, the quantities being analyzed may vary in first order (known as first-order elements), second order, third order, and so on.
- **Steps for Implementation**
 - Create a mesh by discretizing the system into a finite number of finite elements connected at nodes.
 - Formulate the weak form of the differential equation for the representative elements.
 - Establish the characteristic matrices for the representative elements.
 - Transform the characteristic matrices of all elements into a common coordinate system and perform the assembly process.
 - Apply the boundary conditions of the problem, resulting in a system of non-degenerate linear algebraic equations.

- Solve the system of equations to determine the unknowns at the nodes in the overall coordinate system.
- Perform the necessary calculations and output the results through graphical post-processing. Adjust parameters and recalculate if necessary.

2.3.2 Simulation environment: COMSOL Multiphysics software

COMSOL, Inc. (USA), founded in 1986, specializes in developing mathematical modeling software aimed at enabling new advances in physics and engineering. Multiphysics simulation refers to the analysis of systems involving multiple interacting physical phenomena, such as the coupling of chemical kinetics with fluid dynamics or the integration of finite element methods with molecular dynamics. These problems typically require solving coupled systems of partial differential equations (PDEs).

COMSOL Multiphysics is a cross-platform finite element analysis (FEA) software designed for modeling, simulation, and post-processing. It allows users to build models using physics-based interfaces and to solve coupled PDE systems in a unified environment. The software provides an integrated development environment (IDE) and a consistent workflow for applications in electromagnetics, structural mechanics, fluid dynamics, and chemical engineering.

The software features an intuitive graphical user interface that enables users to construct and customize models, define boundary conditions, and visualize simulation results efficiently. In addition, COMSOL Multiphysics supports integration with external programming languages such as MATLAB and Python, enhancing flexibility and extending computational capabilities.

2.3.3 Simulation model and boundary conditions

2.3.3.1. Single oocyte trapping and on-chip maturation

To investigate the fundamental mechanisms of hydrodynamic trapping and the pillar-based design concept, the simulation model is focused specifically on the trapping region of the microfluidic device. The model consists of three main components: an inlet, a trapping zone, and an outlet, representing the essential sections required to capture the flow behavior within the system. Simulated cells are introduced into the microfluidic channel to analyze the operating principles of the device and to evaluate how hydrodynamic forces influence cell motion and trapping efficiency.

In the governing equations for solid mechanics and fluid–structure interaction, only the cells are treated as deformable solid entities, while the trapping structures are assumed to be rigid. These structures act as fixed boundaries that enforce the no-

slip condition on the fluid, ensuring that the fluid velocity at the solid surfaces is zero. The physical properties of the system, such as mechanical behavior and fluid characteristics, are incorporated into the model through appropriate mathematical formulations assigned to the corresponding elements, as detailed in Table 2.2. This modeling approach simplifies the geometry and reduces computational cost while preserving the essential hydrodynamic characteristics of the system.

The fluid flow is driven by pressure gradients, with the flow direction defined from regions of higher pressure to lower pressure. A boundary condition is applied at the outlet by setting the pressure to zero, thereby establishing a reference for the pressure field. The simulation is initiated by introducing one or more cells into the channel at a given time point. As time progresses, the cells are transported by the fluid flow and move toward the trapping region, where their trajectories and interactions with the micropillar array can be analyzed to evaluate trapping behavior and efficiency.

Table 2.2: Physical properties [127], [128].

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

CFD simulations constitute a powerful approach for analyzing and visualizing the effects of fluid-induced forces and stresses on cells, while also enabling the prediction and optimization of key parameters that influence cellular behavior. In this study, the inlet boundary is defined under fully developed laminar flow conditions, characterized by a parabolic velocity profile with an average velocity of U_0 (m/s). At the outlet, a pressure-based boundary condition is applied, where viscous stress is neglected and a Dirichlet condition is imposed on the pressure to ensure a stable flow field. All solid boundaries within the simulation domain, including channel sidewalls and fixed microstructures, are assigned no-slip boundary conditions, ensuring that the fluid velocity at these surfaces is zero. Additionally, the mesh displacement at these boundaries is set to zero, reflecting their rigid and stationary nature throughout the simulation. These boundary conditions collectively establish a realistic representation of microfluidic flow behavior and are summarized in detail in Figure 2.7.

In FSI simulations, maintaining mesh compatibility between the fluid and solid

domains is essential to ensure numerical accuracy and stability. These simulations are typically formulated using the Finite Element Method (FEM) in combination with the Arbitrary Lagrangian–Eulerian (ALE) framework. The ALE method enables the mesh to move independently of both the material (Lagrangian description) and the spatial frame (Eulerian description), making it particularly suitable for capturing the interaction between moving or deformable boundaries and the surrounding fluid. In COMSOL Multiphysics, the ALE solver is specifically designed for FSI applications. It operates by iteratively updating the computational mesh and solving the coupled fluid–solid governing equations to accurately represent the dynamic interaction between the two domains. A segregated solver scheme is employed, in which the fluid and solid problems are solved separately at each time step, with information such as forces and displacements exchanged between them to account for coupling effects.

Additionally, mesh smoothing techniques such as Winslow smoothing and hyperelastic smoothing are commonly employed to improve mesh quality and effectively handle deformation in FSI simulations [129]. Winslow smoothing is a well-established approach for enhancing mesh regularity, reducing element distortion, and maintaining numerical stability, particularly in regions experiencing low to moderate deformation. In contrast, hyperelastic smoothing is more suitable for scenarios involving large mesh deformation, as it treats the mesh as a pseudo-elastic material, allowing it to accommodate significant stretching without loss of quality. This capability is especially important in simulations involving solid-like particles such as cells, where complex interactions with the surrounding fluid may induce substantial local deformation of the mesh. By preserving mesh integrity under such conditions, hyperelastic smoothing enables more accurate representation of particle dynamics and fluid–structure interactions. Therefore, selecting an appropriate mesh smoothing strategy based on the deformation characteristics of the system is critical for achieving reliable simulation results and computational efficiency. In this study, hyperelastic smoothing was used due to its superior performance in handling large deformations and maintaining mesh quality throughout the simulation.

Figure 2.7 illustrates the evolution of mesh deformation corresponding to the displacement of the simulated cell at different time points ($t = 0$ s, 0.3 s, 0.6 s). The mesh is intentionally non-uniform, with finer and denser elements concentrated near the fluid–solid interface to accurately capture interaction effects, while coarser elements are used in regions farther from the cell to reduce computational cost. Notably, a relatively uniform mesh distribution is maintained around the cell to ensure accurate resolution of its motion and interaction with the surrounding fluid. A refined mesh resolution is adopted in this study to achieve convergence and improve numerical accuracy.

However, in the ALE framework, significant mesh deformation may occur as the simulated cell approaches the trapping region. Under such conditions, mesh quality

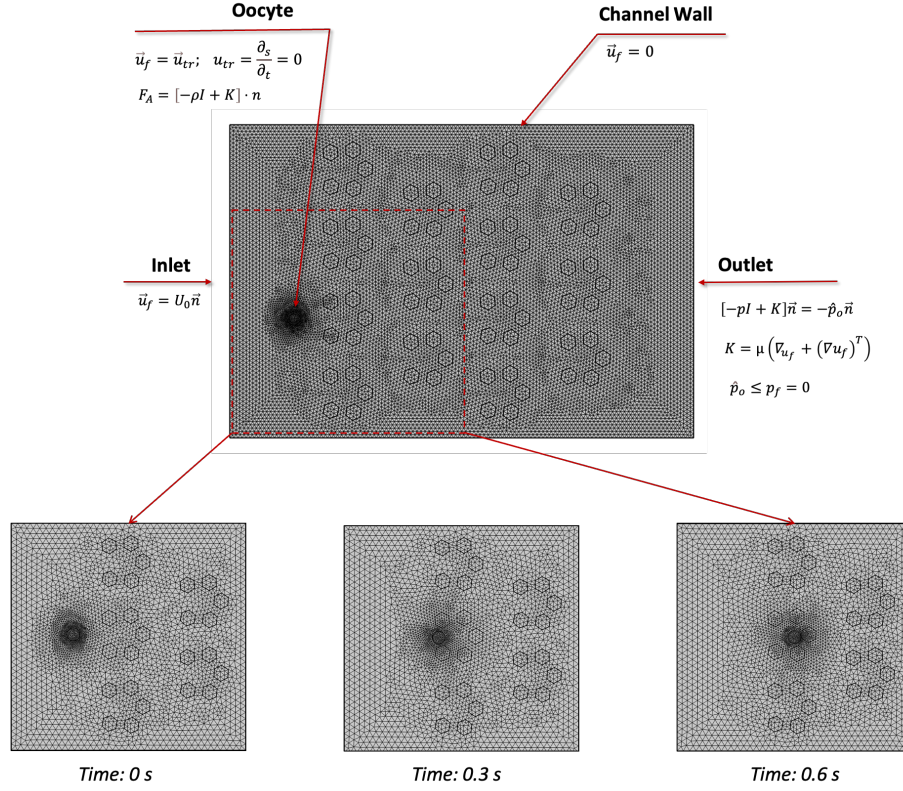


Figure 2.7: Meshing model (boundary conditions and moving mesh method)

can deteriorate despite the application of smoothing techniques, potentially leading to distorted or inverted elements. While the presence of inverted elements does not necessarily cause immediate simulation failure, it renders the affected elements unreliable for subsequent computations. If these elements are located outside critical regions, their impact on overall accuracy may be limited; however, excessive mesh distortion can reduce solution accuracy and hinder convergence.

To address this issue, a remeshing strategy is implemented. When the mesh quality falls below a predefined threshold (typically less than 0.2 on a normalized scale from 0 to 1), the solver is configured to pause and revert to a previous time step. A new mesh is then generated in the deformed region, after which the simulation resumes using the updated mesh configuration. This adaptive remeshing approach ensures numerical stability and maintains solution accuracy, even under conditions of significant mesh deformation.

2.3.3.2. Oocyte denudation on-chip

A three-dimensional computational model was developed to evaluate the flow distribution and shear stress within the proposed microfluidic chip, enabling a detailed and accurate characterization of the fluid dynamics. Shear stress analysis within the denudation chip was conducted using COMSOL Multiphysics 6.1. The fluid behavior in the microchannel was described using the Stokes flow regime, which is appropriate for low Reynolds number conditions typically observed in microfluidic systems. The

Creeping Flow module was used to simulate the velocity field and pressure variations within the microchannel, making it ideal for low-Reynolds-number fluid dynamics applications such as microfluidics, where viscous forces dominate. This module solves the Stokes equations for momentum conservation and the continuity equation for mass conservation, accommodating both compressible and incompressible fluids.

The simulation domain includes an inlet for introducing COCs, a denudation region containing micropillar structures, and an outlet for collecting oocytes after separation from surrounding cumulus cells. To ensure high numerical accuracy, a fine mesh with multiple element refinement was employed, resulting in a total of 1,623,706 domain elements. The simulation workflow consisted of several sequential steps, including geometry construction, material selection and parameter definition, physical setup with appropriate boundary conditions, mesh generation, numerical simulation, and post-processing under various operating conditions. A schematic representation of the simulation setup and analysis is presented in Figure 2.8. Parametric simulations were performed using inlet flow rates of 0.25, 0.5, 0.75, and 1.0 mL/min to investigate their effects on shear stress distribution within the channel. Fluid motion is driven by the pressure gradient between the inlet and outlet, resulting in flow from regions of higher pressure to lower pressure. This approach enables efficient evaluation of the hydrodynamic conditions required for effective cumulus cell removal while minimizing potential mechanical damage to the oocytes.

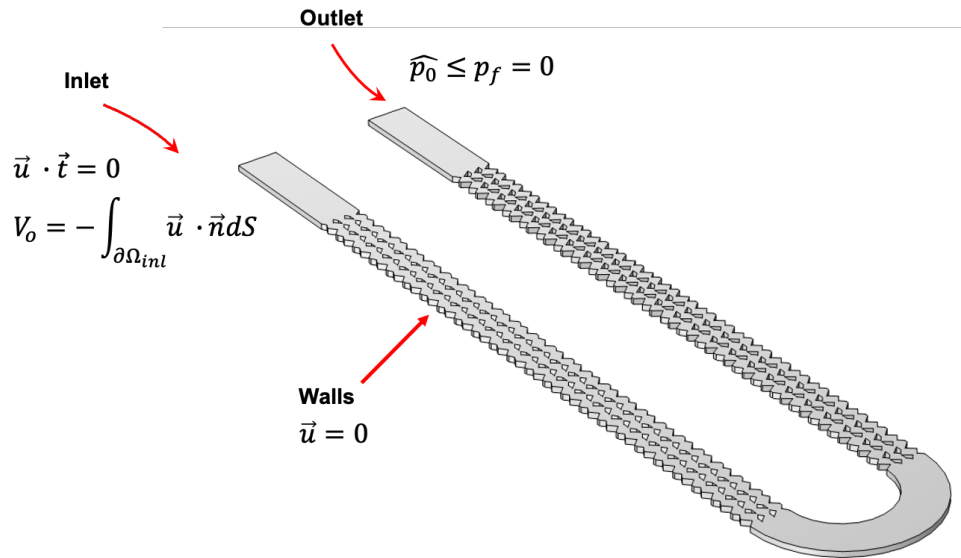


Figure 2.8: Simulation model and boundary conditions of the denudation chip

2.3.3.3. DEP-based chip for oocyte separation

A three-dimensional simulation model was constructed based on the previously de-

scribed microfluidic chip design to evaluate the coupled electrokinetic and hydrodynamic behavior within the system (Figure 2.9). The model retains the essential structural features of the device, including the inlet, the electrode-based separation region, and multiple outlets for cell collection. The central region of the microchannel functions as the active separation zone, where an array of electrodes is integrated to generate DEP forces. Specifically, five identical positive electrodes are arranged diagonally in a herringbone configuration on the bottom surface of the channel, while a common ground electrode is positioned on the top surface. This configuration produces a non-uniform electric field with both vertical and lateral components, enabling controlled manipulation and lateral displacement of cells within the flow.

The working fluid is phosphate-buffered saline (PBS), characterized by a conductivity of 55 mS/m and a relative permittivity of 80, ensuring biocompatibility and stable electrical conditions. At the inlet, a relatively high buffer flow velocity is imposed to achieve hydrodynamic focusing of cells at the entrance of the microchannel, thereby improving separation efficiency. The system operates under an applied AC voltage of 2.5 V at a frequency of 1 MHz, with a controlled flow rate of 270 $\mu\text{m/s}$. Under these conditions, the combined effects of fluid flow and DEP forces enable the selective manipulation and separation of different cell types, which are subsequently directed toward distinct outlet channels for collection.

Within a microchannel, oocytes align with the fluid streamlines and are subjected to dielectrophoretic forces. These cells primarily experience hydrodynamic forces ($\overrightarrow{F_{HD}}$) and DEP ($\overrightarrow{F_{DEP}}$) forces which act in perpendicular directions. Other forces, such as gravitational forces and Brownian motion, are generally insignificant for cells larger than 1 μm in diameter. The resultant force acting on the cells can be described by Newton's second law:

$$\overrightarrow{F_{HD}} + \overrightarrow{F_{DEP}} = m_p \frac{d\overrightarrow{v_p}}{dt} \quad (2.13)$$

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

The operational principles and separation efficiency of the proposed microfluidic system were analyzed using a three-dimensional numerical model developed in COMSOL Multiphysics 6.1, which incorporated several key physics modules to accurately simulate the system's behavior. The **Electric Currents** module was employed to model the electric field distribution generated by the interdigitated electrodes. This module calculates electric field distributions, current densities, and potential gradients in conductive media, assuming inductive effects are negligible when the skin depth is much larger than the device dimensions. It supports both time-domain and frequency-domain simulations, incorporating capacitive effects in both transient and steady-state analyses. The **Creeping Flow** module simulates velocity and pressure fields in microchannels,

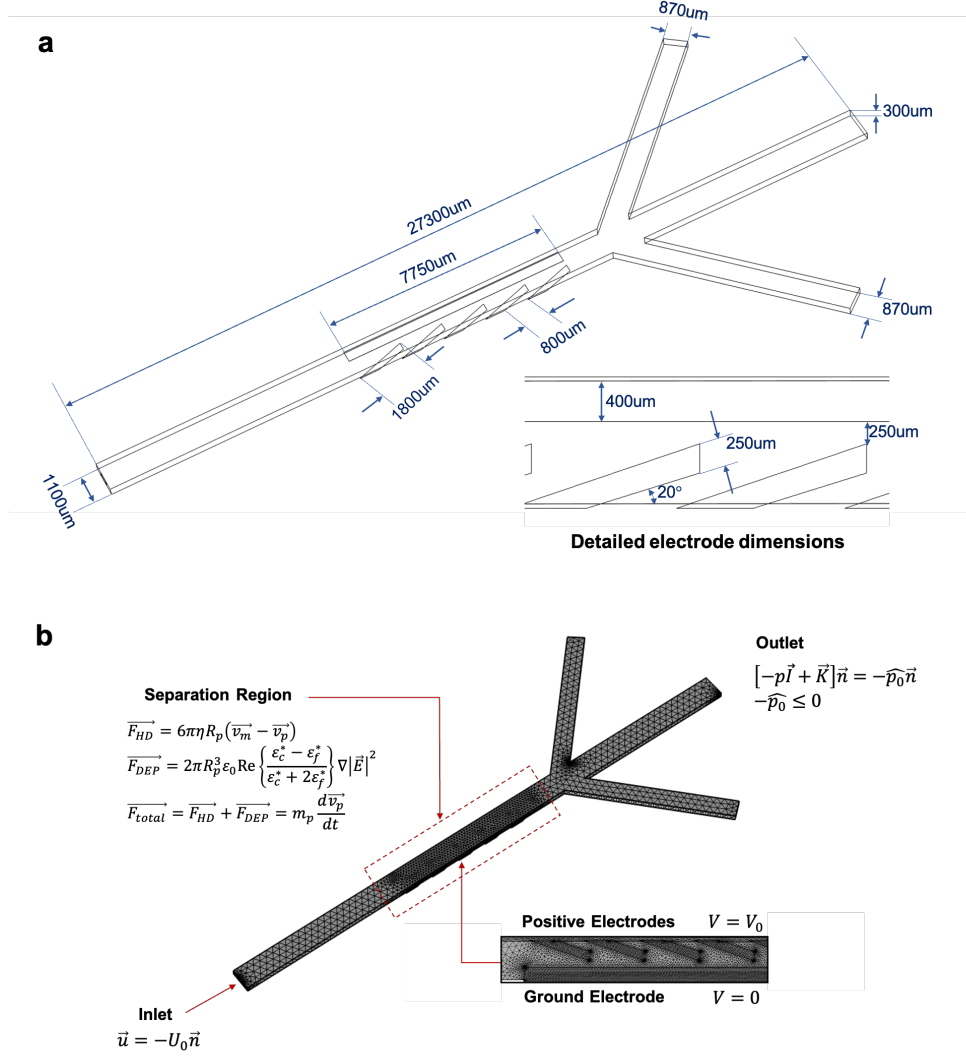


Figure 2.9: (a) Simulation model and detailed dimensions of DEP-based chip for oocyte manipulation, (b) Meshing model and boundary conditions

solving the Stokes and continuity equations for momentum and mass conservation, making it ideal for low-Reynolds-number fluid dynamics in microfluidics. Additionally, the **Particle Tracing for Fluid Flow** module was employed to track the movement of normal and abnormal oocytes under the combined influence of DEP forces and hydrodynamic flow. This module simulates the motion of individual particles under external forces, including DEP, hydrodynamic, and electrostatic interactions, and supports both one-way and two-way coupling, allowing particles to interact with surrounding fields and boundaries.

Together, these modules provided a comprehensive understanding of the microfluidic device's flow dynamics, the influence of electric fields on cell behavior, and the interactions between cells and fluid flow, thus enabling a detailed analysis of the DEP-based separation mechanism. The simulation results were used to evaluate the system's efficiency in selectively manipulating oocytes within the microfluidic environment, contributing to advancements in biomedical applications.

The model utilized a refined computational mesh consisting of 21429 mesh vertices and 99033 tetrahedra elements, ensuring high-resolution numerical accuracy. The microchannel walls were assigned no-slip boundary conditions to accurately reflect real-world flow dynamics, and a freeze condition was applied at the outlet to prevent artificial particle reflections. To replicate realistic biological conditions, cells were introduced into the system at randomized positions. The simulation results provided valuable insights into the DEP-based separation mechanism within the microfluidic environment, significantly contributing to advancements in biomedical applications.

In this simulation, oocytes are modeled as protoplasts [73], as shown in Figure 2.10. Protoplasts are delicate, balloon-like cellular structures that lack a rigid cell wall, making them highly vulnerable to mechanical and osmotic stress. From a structural perspective, they are modeled as a conductive cytoplasmic interior enclosed by a thin, capacitive membrane, which functions as a dielectric barrier regulating ion exchange and electrical interactions [130]. Mammalian cells, including oocytes, share structural similarities with protoplasts, particularly in the absence of a cell wall. Like protoplasts, mammalian cells consist of a conductive cytoplasmic core surrounded by a thin, selectively permeable plasma membrane that acts as a capacitive barrier, regulating ion exchange and cellular interactions. This structural configuration significantly influences their electromechanical properties, making them highly responsive to external forces such as DEP, electroporation, and shear stress. The analytical formulation of the dielectric model provides a critical tool for characterizing the electrical properties of protoplasts.

On average, oocytes have a diameter of $120\ \mu\text{m}$, with a membrane capacitance of $1.25\ \mu\text{F}/\text{cm}^2$ and a conductance of $400\ \Omega \cdot \text{cm}$. The dielectric constant and conductivity of the cytoplasm are assumed to be 70 and $5\ \text{mS}/\text{cm}$, respectively [73], [131]. Because the dielectric properties of abnormal oocytes have not been directly characterized, they

are assumed to be comparable to those of normal oocytes, except for the electrical conductivity associated with membrane integrity. Cell death and severe degeneration may compromise the membrane and increase ionic permeability. Therefore, in the present model, the conductivity of abnormal oocytes was assumed to increase by approximately four orders of magnitude relative to that of normal oocytes, following the range reported for damaged or permeabilized cells [132]. This assumption was introduced to represent severe membrane deterioration and to investigate its influence on the DEP response. During operation, the sample fluid containing oocytes is introduced through the central inlet at a velocity of $600 \mu\text{m/s}$, while phosphate-buffered saline (PBS) is injected through the lateral inlets at a velocity of $475 \mu\text{m/s}$. This controlled flow strategy ensures that the sample stream remains aligned along the central axis of the microchannel, maximizing exposure to the applied DEP field. Differences in dielectric properties create varying DEP forces on normal and abnormal oocytes, directing them along distinct paths within the microfluidic channel.

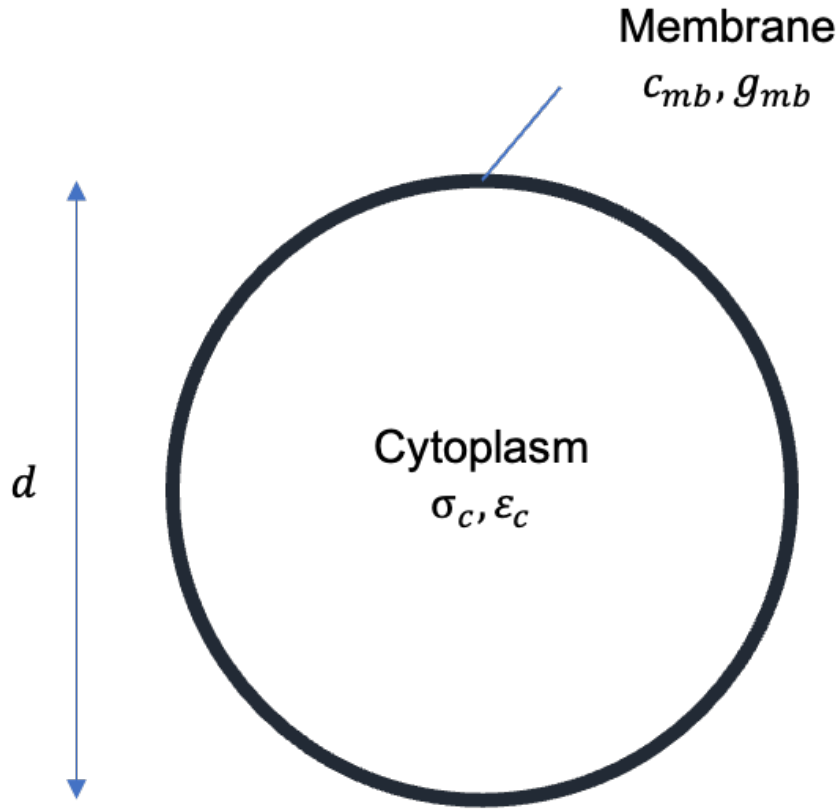


Figure 2.10: Dielectric model of the protoplast

The microchannel is equipped with an interdigitated electrode array located at its base, consisting of two alternating electrode sets. The first set operates at an alternating current (AC) voltage of 2.5 V with a positive polarity, while the second set is grounded at 0 V. The driving oscillating voltage frequency is set to 1 MHz. This alternating electrode configuration generates localized non-uniform electric field gradients, enabling

precise cellular manipulation through DEP. Consequently, the cells undergo directed migration based on their dielectric properties, ensuring efficient sorting while minimizing thermal effects and maintaining system stability.

2.4 Conclusion

In summary, this chapter has presented the theoretical foundations and simulation methodologies underlying the proposed microfluidic platform. Fundamental principles of fluid flow in microfluidic systems, including the Navier–Stokes equations and low-Reynolds-number flow behavior, were introduced together with the hydrodynamic mechanisms governing single-cell trapping. In addition, the principles of dielectrophoresis (DEP), DEP-induced forces, and other forces affecting the trajectory and manipulation of oocytes within microchannels were discussed. The interactions between fluid flow and biological cells were also described to provide a better understanding of the mechanical microenvironment experienced by oocytes during on-chip manipulation and culture.

This chapter further presented the design concepts and operating principles of the proposed microfluidic chip for oocyte trapping, culture, denudation, and DEP-based selection. Moreover, the finite element method (FEM)-based simulation framework was described in detail, including the simulation modules, meshing strategies, cell models, material properties, governing equations, and boundary conditions employed throughout the numerical analyses. These theoretical and computational foundations provide the basis for the experimental implementation and performance evaluation presented in the following chapters.

Chapter 3

Experimental Setup

3.1 Materials and fabrications

3.1.1 Equipment utilized in the fabrication process

- *WS-650MZ-8NPPB Spin Coater*

The WS-650MZ-8NPPB spin coater is a specialized device designed for the thin-film coating process using the centrifugal spinning method. It is commonly used in microelectronics technology and materials research. This device is capable of producing a uniform coating on the surface of samples, making it suitable for high-precision applications such as microchip manufacturing, sensor production, conductive films, and various applications in the field of microbiology.

The WS-650MZ-8NPPB spin coater utilizes a vacuum system to securely hold the sample onto the substrate, facilitating the creation of uniform and high-quality coatings. The device consists of three main components: the coating chamber, which houses a vertical rotating shaft with a small hole to generate vacuum force between the substrate and the sample, ensuring it remains fixed during rotation; the vacuum pump, which generates the necessary suction to keep the sample in place at high speeds, ensuring a continuous and precise coating process; and the control panel, which allows for easy adjustment of parameters such as rotation speed, time, and vacuum force, enabling precise control and optimization of the coating process based on specific requirements.

- *Photolithography System*

The Midas MDA-400M Mask Aligner is a manual alignment and exposure system designed for research and development in microelectronics and MEMS. It can process samples up to 4 inches in diameter, ensuring high precision for prototyping and experimental production. The system includes key components that contribute to the accuracy and efficiency of the alignment and exposure processes. The Substrate Holder

secures wafers or samples during the process, accommodating sizes up to 4 inches, providing flexibility for various sample types. The Mask Holder holds the mask firmly during alignment and exposure, supporting masks up to 5 inches by 5 inches, suitable for complex microelectronics processes. The UV Light Source provides the necessary UV light for exposure, with a 350W lamp and wavelengths ranging from 260 nm to 350 nm, ensuring uniform illumination with an intensity of approximately 10 mW/cm² at 365 nm.

Additionally, the dual CCD microscope system assists with precise alignment between the sample and mask. With magnification capabilities ranging from 5X to 20X and connection to a 19-inch LCD screen, the system enables users to observe and align the sample with high accuracy, down to less than 1 micrometer. The UV beam control ensures even light distribution across the entire sample surface, maintaining uniformity within $\pm 3\%$ over an area of 4.25 inches by 4.25 inches. Moreover, the Contact Control system provides flexibility by allowing different contact modes, such as soft, hard, vacuum, and non-contact, enabling adaptability for various processes.

- *Dektak 150 Thickness Measurement System*

The Dektak 150 thickness measurement system consists of several key components: the stylus probe, sample stage, electronic control unit, and analysis software connected to the computer. The stylus probe is the most critical component, directly contacting the sample surface and recording height changes as it scans across it. The sample stage allows precise movement of the sample along different axes, ensuring high scanning accuracy. The electronic control unit adjusts the probe's parameters and scanning speed while transmitting data to the analysis software. The software displays and processes the data, enabling detailed analysis of the measurement results. The Dektak 150 operates on the Alpha Step method using stylus profilometry. During measurement, the stylus probe gently contacts the sample surface and moves along predefined scan paths. As the stylus moves, it records changes in surface height, creating a high-resolution topography map, with precision down to the nanometer scale. The data is then processed by the analysis software to generate charts and images of thickness, roughness, and other surface characteristics. This measurement process allows for the detection and recording of fine surface details, supporting high-precision applications in material research and development.

- *Vacuum chamber*

The LabTech vacuum chamber is an essential laboratory device used to dry samples under vacuum conditions, protecting them from oxidation and high temperatures. This device allows for precise control of both temperature and vacuum pressure, optimizing the drying process for different types of samples. A pressure gauge located on the

front of the chamber enables accurate monitoring and adjustment of the vacuum level. Additionally, the heat-resistant glass door allows users to observe the samples during the drying process without opening the chamber, thereby maintaining the stability of the vacuum environment. The integrated digital control system allows for setting and monitoring parameters such as temperature, time, and pressure, ensuring a stable and precise drying process.

- *Sample Processing Chamber*

During the photolithography process, cleaning, drying, and rinsing the samples are crucial steps to ensure the accuracy and quality of the photoresist coating. All of these procedures are carried out in the sample processing chamber, which is designed to house the necessary equipment and chemicals for safe and efficient sample preparation. The chamber includes key components such as a hotplate, a drying gun, and cleaning chemicals like alcohol, acetone, deionized water (DI water), and developer solutions, all of which support the surface treatment of the sample before photolithography.

- *Hot plate*

The hotplate is a key device in the drying process, ensuring the complete removal of any residual water molecules from the sample surface after cleaning. It offers flexible temperature control, enabling the drying of samples at various temperatures depending on the material type or photolithography process requirements. Typically, the hotplate is set at temperatures ranging from 60°C to 150°C, or even higher, to ensure the sample is thoroughly dried and unaffected by moisture during the photoresist coating process.

In addition to its drying function, the hotplate is also equipped with a stirring function, which is useful in nanoparticle synthesis. However, in photolithography, the stirring function is seldom used, and the focus is mainly on the heating capability. The hotplate allows for the adjustment of several parameters, including the target temperature, heating rate (the rate at which temperature increases), and stirring speed, if needed.

For samples coated with photoresist, the most critical requirement is maintaining a stable surface temperature, with deviations allowed only within $\pm 1^\circ\text{C}$. This temperature stability ensures the uniformity and quality of the photoresist layer, preventing issues such as peeling or deformation caused by temperature fluctuations. Without precise temperature control, the photoresist layer may be damaged or exhibit defects, compromising the accuracy of the photolithography process. Additionally, it is essential that the surface of the hotplate is flat to avoid discrepancies in film thickness.

3.1.2 Fabrication process of PDMS microchannel

3.1.2.1. Development and optimization of microfluidic structure fabrication

The microfluidic chip fabrication process was optimized through iterative cycles of structural design, SU-8 mold fabrication, PDMS replication, dimensional characterization, and experimental evaluation. The primary objective was to obtain mechanically stable and dimensionally uniform micropillar arrays suitable for oocyte trapping, separation, and recovery.

The initial structure consisted of micropillars arranged in triangular trapping units. However, the low pillar height and relatively large spacing between adjacent structures allowed oocytes to pass through the device without sufficient interaction. The photomask was therefore modified by reducing the pillar spacing and introducing trap openings of 170, 200, 250, 300, and 350 μm to accommodate the broad size distribution of oocytes and cumulus–oocyte complexes.

In a subsequent fabrication attempt, the SU-8 mold containing 170- μm trap openings and micropillars with a diameter of 100 μm exhibited relatively well-defined features. Nevertheless, after PDMS casting and demolding, the micropillar height varied between approximately 40 and 70 μm . Several pillars were also deformed, fractured, or absent. Although this represented an improvement over the initial fabrication, the structures remained too short and nonuniform for reliable oocyte processing.

These defects were associated with variations in both SU-8 mold fabrication and PDMS replication. Nonuniform spin coating and residual SU-8 at the substrate edges produced local differences in photoresist thickness, which were subsequently transferred to the PDMS structures. The spin-coating speed and duration were therefore adjusted according to the SU-8 viscosity and target mold thickness, while excess photoresist was removed to improve surface flatness.

The soft-baking conditions were optimized to remove residual solvent while minimizing shrinkage, cracking, and internal stress in the thick SU-8 layer. A controlled heating and cooling procedure was applied to improve the stability and uniformity of the photoresist before ultraviolet exposure.

The ultraviolet exposure dose and post-exposure baking conditions were also adjusted to achieve sufficient crosslinking throughout the SU-8 layer. Underexposure produced weak or incomplete structures, whereas excessive exposure altered pillar dimensions and reduced the designed gaps. The development time and rinsing conditions were subsequently optimized to remove uncrosslinked SU-8 without eroding or damaging the high-aspect-ratio micropillars.

The PDMS replication procedure was improved in parallel. The PDMS mixture was thoroughly degassed before casting to prevent air bubbles from becoming trapped within the closely spaced microstructures. The curing conditions were adjusted to provide adequate mechanical strength while retaining sufficient elasticity for demolding. The PDMS layer was then removed gradually from the mold to minimize lateral forces on the micropillars. Cutting was performed using a sharp blade on a fixed support to

prevent pillar fracture and channel deformation.

The microfluidic structure was further refined based on experimental observations. A preliminary design incorporated trap openings decreasing from 350 to 80 μm along the flow direction. However, the 300–350 μm regions exhibited very limited oocyte retention, whereas effective interactions occurred mainly at openings of 250 μm or smaller. The trap-opening range was therefore revised to decrease from 250 to 80 μm , and the micropillar diameter was increased to 200 μm . These changes increased the contact area with the oocytes, improved structural strength, and reduced the likelihood of oocytes passing directly through the pillar array.

Different micropillar geometries and spatial arrangements were subsequently fabricated and evaluated. Among the investigated configurations, staggered hexagonal micropillars provided the most favorable combination of fabrication fidelity, structural stability, and oocyte retention. The staggered arrangement created multiple sequential contact points with the oocytes while maintaining interconnected pathways for fluid, smaller cells, and suspended impurities.

Following optimization, the micropillar height reached approximately 150–220 μm , while the microchannel height ranged from 180 to 220 μm , as measured using a Dektak 150 surface profilometer. The optimized micropillars occupied a substantial proportion of the channel height, enabling effective interaction with oocytes and cumulus–oocyte complexes while maintaining sufficient clearance for fluid and smaller components to pass through the device.

3.1.2.2. Optimized fabrication process

The microfluidic chip fabrication process is carried out in the following steps:

Step 1: Prepare the silicon wafer and cut it into appropriately sized pieces. Then, perform a cleaning procedure by immersing the silicon wafer in methanol for 10 minutes, followed by acetone for 10 minutes. After rinsing with deionized water, dry the wafer with nitrogen gas (N_2) and bake it at a temperature ranging from 90 to 120°C for 10 minutes. Alternatively, the standard cleaning procedure can be carried out by immersing the wafer in Piranha solution, a mixture of H_2SO_4 and H_2O_2 in a 3:1 ratio, at 120°C for 2 hours.

Step 2: Apply a layer of SU-8 onto the wafer surface, using approximately 1 ml per inch, ensuring the SU-8 spreads evenly without forming air bubbles. Perform the spin-coating process by adjusting the speed and duration to achieve the desired coating thickness. For a target thickness range of 160 μm to 225 μm (the maximum achievable with the material), the spin-coating process is divided into two steps: Step 1 for 10 seconds at 500 rpm, and Step 2 for 30 seconds at 1000 rpm.

Step 3: Prebake (Softbake): Bake the silicon wafer coated with SU-8 (2050, MicroChem Corp., Newton, MA, USA) by heating from room temperature to 95°C. The temperature is gradually increased by 5°C every 2 minutes. The wafer is maintained at

65°C for 7 minutes, then the temperature is increased from 90°C to 95°C and held for 40-45 minutes. Afterward, allow the wafer to cool back to room temperature.

Step 4: Expose the silicon wafer, which has cooled to room temperature, under UV light. The silicon wafer is aligned with the mask containing the defined structures placed above it. The exposure energy is set according to the photoresist supplier's specifications, with the energy set to a range of 260-350 mJ/cm² in this case.

Step 5: Postexposure Bake: The exposed wafer is heated from room temperature to 90°C. The temperature is increased by 5°C every 2 minutes. Once the temperature reaches 65°C, it is held for 7 minutes, then returned to room temperature. After that, the temperature is increased to 90°C and maintained for 15 minutes. Finally, the silicon wafer is cooled back to room temperature.

Step 6: Develop the SU-8 photoresist using SU-8 Developer solution to wash away the unwanted photoresist areas. The remaining SU-8 photoresist will serve as a mold for the microfluidic channels. After rinsing with the developer solution, the wafer is washed with IPA for 10 seconds until no white streaks appear. Then, the wafer is dried with N₂ and allowed to return to room temperature.

Step 7: Perform the hard bake to stabilize and strengthen the microchannel structures. This step is carried out at temperatures ranging from 120°C to 150°C for 15-20 minutes, followed by gradual cooling to room temperature.

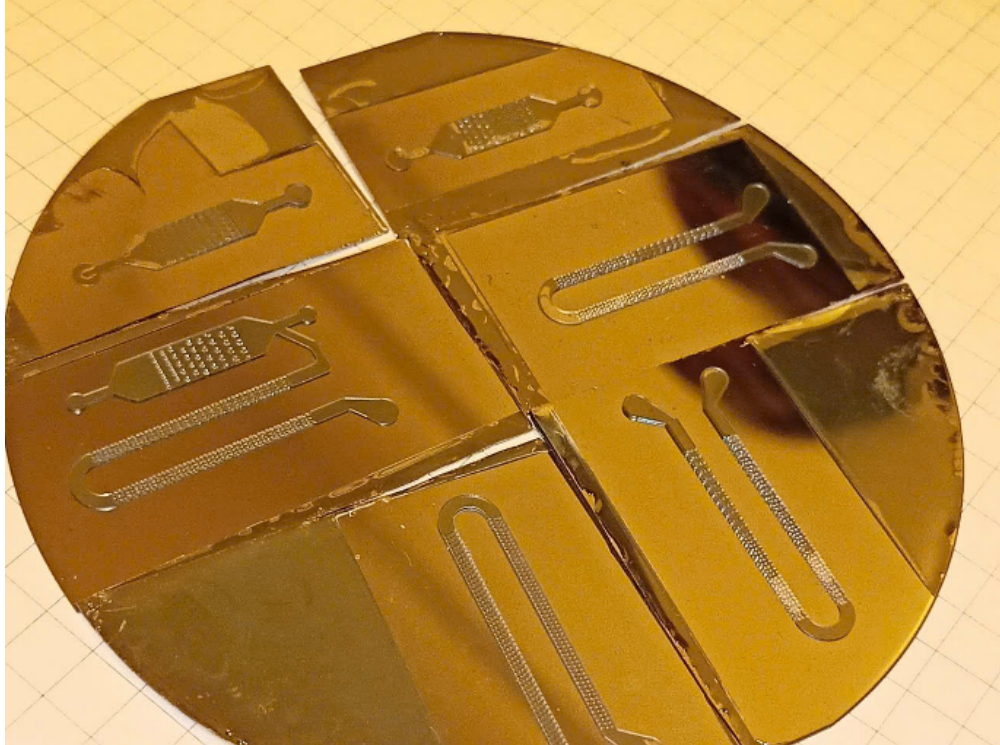


Figure 3.1: The completed SU-8 mold

Step 8: After completing the microfluidic mold from the SU-8 photolithographic material (Figure 3.1), the preparation of the PDMS polymer mixture begins by mixing

two components of PDMS, Part A and Part B from the Sylgard-184 Silicone Elastomer Kit (Dow Corning, Midland, MI, USA), in a 10:1 weight ratio. The mixture is thoroughly stirred to ensure homogeneity before being poured into the prepared SU-8 mold. To eliminate all air bubbles inside and on the surface of the mixture, the PDMS mixture is then placed into a vacuum chamber to minimize any defects that may affect the transparency and mechanical properties of the microchannels. During this stage, the PDMS mixture remains in a liquid state, ensuring flexibility in uniformly distributing the mixture into the fine details of the mold.

The next step involves heating the SU-8 mold with the PDMS mixture at a stable temperature of 65°C for approximately 2 hours. This heating process initiates the polymerization, causing the PDMS to harden and form a durable layer that replicates the microstructure of the SU-8 mold. After the heating process is complete, the mold and PDMS layer are gradually cooled to room temperature to prevent thermal stresses that could distort the microchannel structure. Finally, the solidified PDMS layer is carefully separated from the SU-8 mold, completing the microchannel mold fabrication process. The inlet and outlet holes of the microchannels are then manually created using a punching technique, ensuring accurate connection to the microfluidic system.

Step 9: After completion, the PDMS base containing the microchannel structure retains hydrophobic properties and cannot naturally bond with other materials. To reduce its hydrophobicity and enable bonding with other materials, surface treatment methods are applied, with oxygen plasma exposure being the most common. For assembling the PDMS microchannel structure onto a glass wafer containing metal microelectrodes, both the PDMS and glass wafer surfaces are treated with oxygen plasma to clean and activate the surfaces, using a plasma chamber (Model PDC-32G, Harrick Plasma Corp., Ithaca, NY, USA).

The bonding process requires high precision between the microchannel and the electrode structures on the glass wafer. Therefore, a monitoring camera is used to adjust the position of the PDMS base to align perfectly with the electrode structures on the glass before the two wafers are permanently bonded. This process is summarized in Figure 3.2. Additionally, during the alignment, a small amount of methanol may be used as a temporary separating agent, preventing the two surfaces from sticking together for a short time and facilitating precise adjustment before completing the bonding process.

High-quality master molds with intact channel features and dimensions matching the design were reused for PDMS replication. Only fabricated chips with dimensional deviations below 5% from the designed values were selected for subsequent experiments.

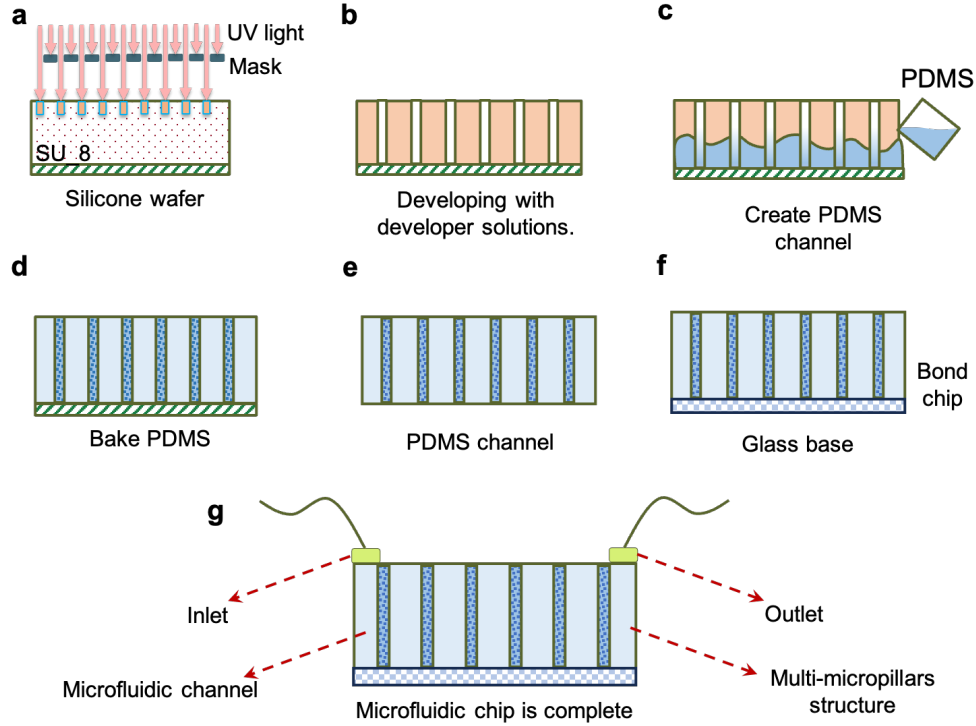


Figure 3.2: Steps for fabricating a PDMS microfluidic chip for oocyte maturation applications

3.1.3 Fabrication process of microfluidic electrode integration

The microelectrode fabrication process involves several sequential steps, as shown in Figure 3.3:

Step 1: The AZ1505 photoresist layer (Merck Performance Materials, Germany) is coated onto a glass substrate that has been sputter-coated with an ITO layer and surface-treated. The coating process is performed in two stages: first, spinning at 700 rpm for 10 s, then increasing to 1700 rpm for 20 s to create a 2 μm thick photoresist layer. Next, the glass substrate is heated at 90°C for 1 min 30 s to fix the coating.

Step 2: The freshly coated photoresist layer is exposed to UV light at an exposure energy range of 260-350 mJ/cm^2 for 8 s. After exposure, the glass plate is heated at 90°C for 1 min 30 s to fix the photoresist layer.

Step 3: Prepare the developer solution by diluting 25% TMAH with DI water at a ratio of 1:6. The photoresist activated by UV light in the exposure step is developed by immersing the wafer in the developer solution for about 7 s. Then, rinse the wafer again with DI water and dry it with N_2 gas. The wafer is baked at 90°C for 2 min to completely remove any remaining moisture on the surface.

Step 4: Etch the ITO layer not protected by photoresist by immersing the wafer in an $\text{HCl} + \text{FeCl}_3$ solution maintained at 60°C for about 35 s. Then, rinse the wafer again with DI water and dry it with N_2 gas.

Step 5: Remove the remaining photoresist layer by rinsing the wafer with Acetone solution. Then, rinse the wafer again with DI water and dry it with N₂ gas.

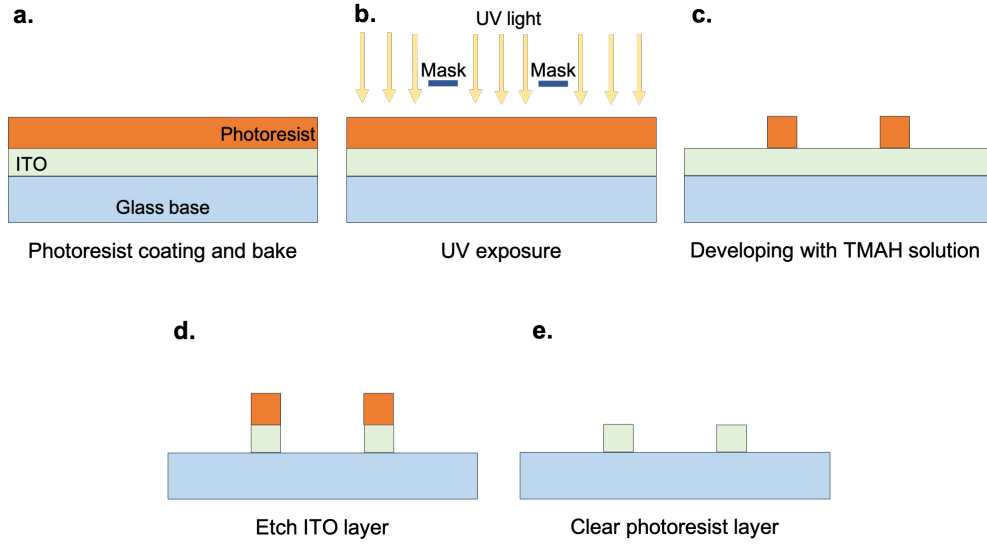


Figure 3.3: Steps for fabricating microelectrodes for oocyte separation applications

3.2 Sample preparation

3.2.1 Porcine oocyte in vitro maturation

For the initial biological validation of the microfluidic system, porcine oocytes were chosen because of their recognized 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 [133], [134], [135].

Materials:

- Tubes, Petri dishes, pipettes, conical tips, scalpels
- Mineral oil
- Glass pipettes, capillary pipettes

Equipment and machinery:

- CO₂ Incubator 6%, 38°C
- Stereomicroscope and inverted microscope

- Hot plate
- Biosafety cabinet

Preparation of culture media:

- Oocyte Collection Medium

Prepare the oocyte collection medium (Medium 199 (with Hank's salts; Sigma-Aldrich Corp., St. Louis, MO)) supplemented with 5% fetal bovine serum (Gibco; Invitrogen Corp., Carlsbad, CA), 20 mM HEPES (Dojindo Laboratories, Kumamoto, Japan), and antibiotics [100 units/ml penicillin G potassium (Sigma-Aldrich Corp.) and 0.1 mg/ml streptomycin sulfate (Sigma-Aldrich)] in 60 mm Petri dishes (Falcon 351007, Thomas Scientific, NJ).

- Oocyte Culture Medium

Prepare the oocyte maturation medium (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).

IVM Procedure

The IVM procedure for animal oocytes (porcine) is carried out at the Institute of Biotechnology, Vietnam Academy of Science and Technology. The specific process is as follows [136]:

Follicular fluid collection and immature oocyte isolation: Immature oocytes are selectively chosen from pigs of good breeding, aged 8-9 months, with a body weight of approximately 19-25 kg. The ovaries are excised and transported to the laboratory, immersed in saline for 30 minutes at 35-37°C (Figure 3.4). Afterward, the ovaries are washed three times in Dulbecco's phosphate-buffered saline (PBS) (Sigma-Aldrich Corp., St. Louis, MO, USA) supplemented with antibiotics (100 units/ml penicillin G potassium; Sigma-Aldrich Corp. and 0.1 mg/ml streptomycin sulfate; Sigma-Aldrich) at 37°C.

Follicular fluid containing oocytes is collected by puncturing ovarian follicles using a scalpel. The collected fluid is then allowed to settle in a tube at 38°C for 15 minutes. After sedimentation, the supernatant is carefully removed, and the remaining fraction containing oocytes and debris is retained. This sediment is subsequently transferred into a collection medium in a 90-mm Petri dish for microscopic observation and oocyte retrieval. COCs are then selected based on morphological criteria, including uniform cytoplasm and the presence of at least one intact layer of cumulus cells (Figure 3.5), to be used for subsequent IVM procedures.

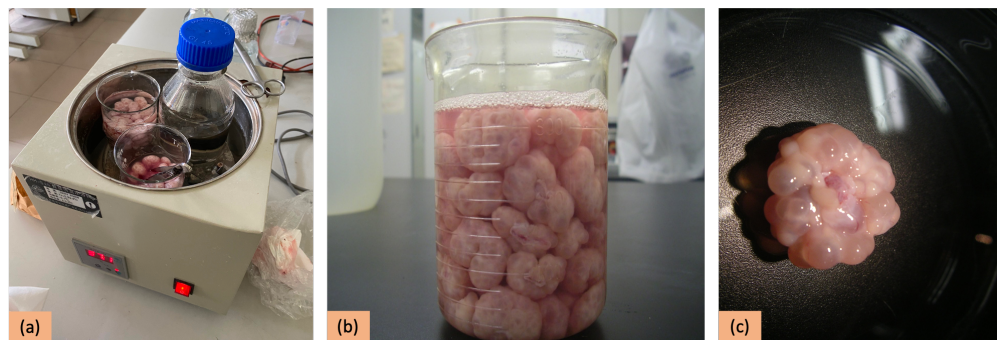


Figure 3.4: The porcine ovaries are stored in a sterile incubator before being processed for oocyte aspiration

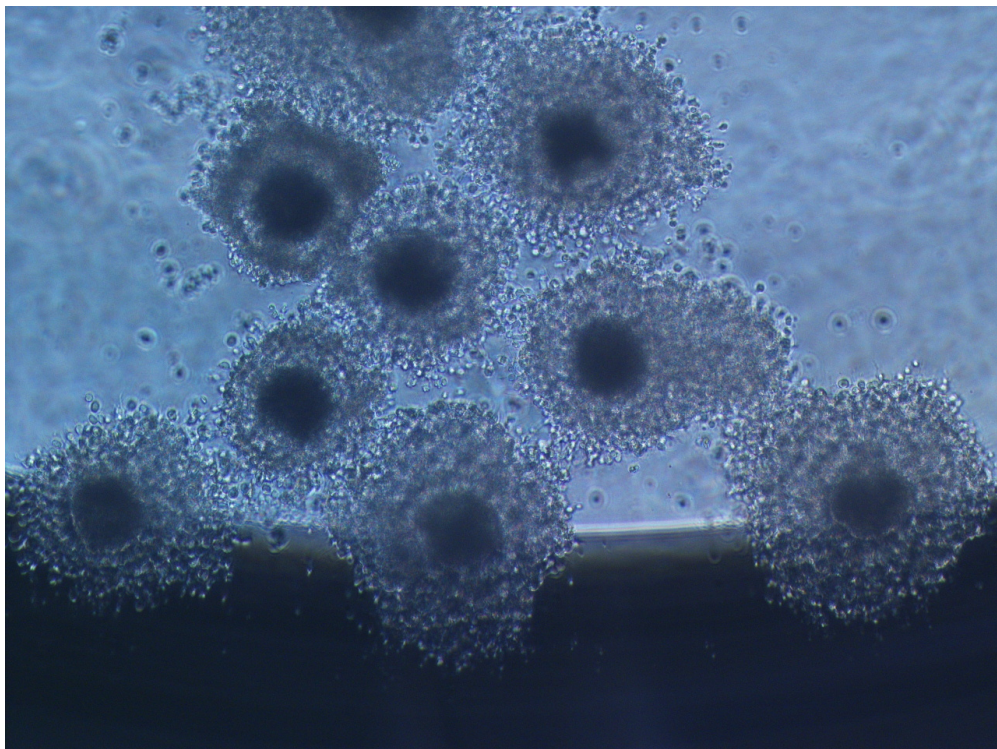


Figure 3.5: Image of the COCs under a microscope

Preparation of IVM culture dish: The IVM culture dish is prepared by dispensing droplets of POM culture medium (10–20 μL per oocyte) into the wells. These droplets are then covered with mineral oil to prevent evaporation. The dish is subsequently placed in a CO_2 incubator for 2 hours to allow equilibration before initiating the culture process. COCs are individually transferred into droplet of culture medium within the wells using a glass pipette.

Oocyte *in vitro* culture: The culture dish is placed in a CO_2 incubator at 6% CO_2 , 38°C , and $\geq 95\%$ humidity. After 24 hours, the medium is replaced with a hormone- and dbcAMP-free medium. The oocytes are then cultured for an additional 24 hours, resulting in a total culture duration of 48 hours.

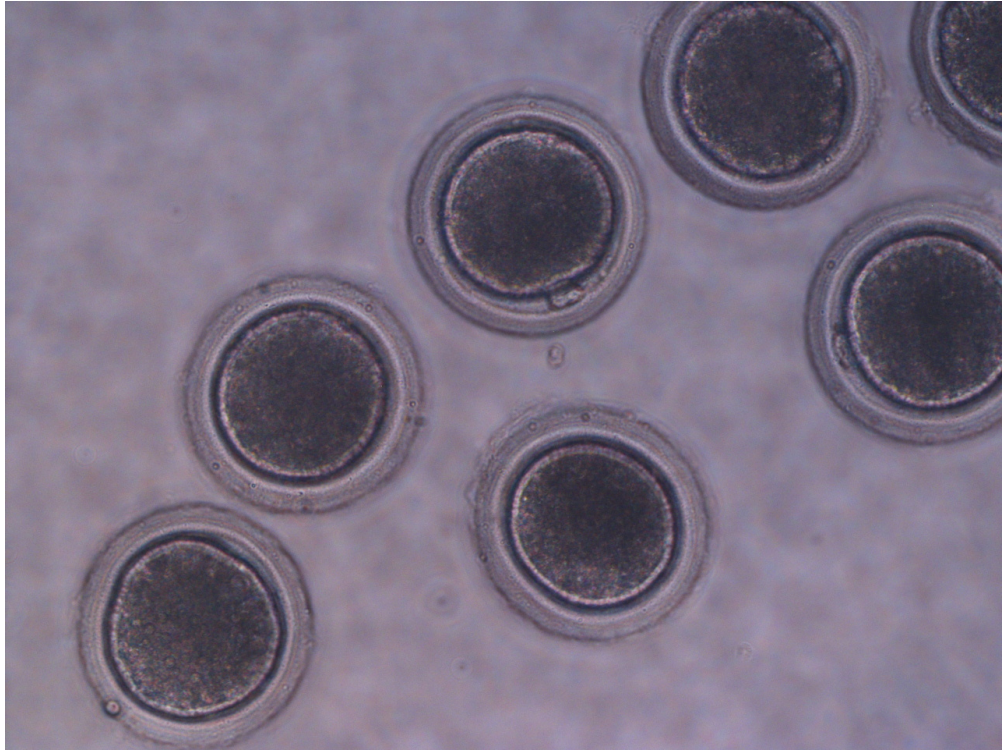


Figure 3.6: After 48 hours of culture, the oocytes undergo denudation to remove the surrounding cumulus cells for evaluation of the polar body

After 48 hours, the oocytes are observed under a microscope for the presence of the first polar body (PB1) as shown in Figure 3.6, which indicates successful maturation. Oocytes that meet the required criteria are then transferred to the denudation process for subsequent ICSI or IVF procedures.

3.2.2 Porcine oocyte denudation

Following IVM, the COCs were processed using the microfluidic chip for denudation. Oocytes presenting the PB1 were identified as mature. After 48 hours of culture, the matured oocytes were subjected to cumulus cell removal prior to subsequent fertilization

procedures.

To evaluate the denudation efficiency, the COCs were divided into two groups: one group was processed using the proposed microfluidic chip-based system, while the other was treated using the conventional manual method performed by a trained embryologist. In the traditional approach, COCs were incubated in a collection medium containing 0.1% (w/v) hyaluronidase for 2 minutes, followed by mechanical denudation through gentle pipetting using a fine glass pipette [137].

In contrast, the proposed method significantly modified both the enzymatic and mechanical conditions. The COCs were briefly exposed to a collection medium containing 0.01% (w/v) hyaluronidase, corresponding to a tenfold reduction in enzyme concentration compared to the conventional protocol. Immediately afterward, the COCs were introduced into the DENO microfluidic chip, where denudation was performed directly under continuous flow conditions for 1 minute 30 seconds, without requiring a separate pre-incubation step.

This on-chip denudation strategy not only simplifies the workflow but also substantially reduces enzyme usage and processing time, while maintaining effective removal of cumulus cells. Such improvements highlight the potential of the microfluidic approach for enhancing reproducibility, minimizing operator dependency, and reducing chemical exposure during oocyte preparation.

3.2.3 In vitro fertilization

IVF was carried out following the standard protocol established in our previous study [138]. After denudation, the mature oocytes obtained from both experimental groups were carefully transferred into a porcine-specific fertilization medium. This medium was formulated to maintain ionic homeostasis, support sperm capacitation and activation, and optimize the conditions required for effective sperm–oocyte interaction during fertilization. Prior to insemination, the oocytes were washed three times in fresh IVF medium to remove residual handling solution and to equilibrate them under fertilization conditions. Subsequently, the oocytes were placed into 90 μL droplets of IVF medium, with approximately 10–20 oocytes allocated to each droplet. Each droplet was overlaid with sterile paraffin oil (Paraffin Liquid; Nacalai Tesque) to prevent evaporation, maintain osmotic stability, and protect the culture environment from fluctuations in pH and temperature.

For sperm preparation, frozen–thawed semen was diluted in 7 mL of sperm washing medium consisting of Medium 199 with Earle’s salts (Gibco), with the pH adjusted to 7.8. The sperm suspension was then centrifuged at $580 \times g$ for 2 min to remove cryoprotectant residues and other contaminants that might compromise fertilization efficiency. After centrifugation, the supernatant was discarded, and the sperm pellet

was gently resuspended in 50 μL of sperm washing medium. Sperm motility was then evaluated subjectively under a stereomicroscope, and only sperm samples showing at least 30% motility were selected for subsequent IVF procedures. This quality control step was necessary to ensure the use of sperm populations with sufficient fertilizing potential.

Following assessment, 100 μL of the prepared sperm suspension was transferred into a 30 mm Petri dish, covered with paraffin oil, and incubated at 37°C for 15 min. This short pre-incubation period was intended to allow the sperm to acclimate to the fertilization environment and to enhance their readiness for interaction with the oocytes. Sperm were then introduced into the fertilization droplets at final concentrations of 10^5 and 10^6 sperm/mL, enabling evaluation of fertilization performance under different insemination densities. The IVF medium was supplemented with 5 mmol/L caffeine, which is known to stimulate sperm motility and promote hyperactivation, and 5 mg/mL bovine serum albumin (BSA), which serves as a protein source, stabilizes the plasma membrane, and supports physiological sperm function during fertilization.

Fertilization was performed under controlled atmospheric conditions at 38.5°C in an incubator containing 5% CO_2 and 90% N_2 for 3–6 h. These conditions were selected to mimic the physiological environment and to maximize the likelihood of successful sperm penetration and gamete fusion. After the fertilization period, presumptive zygotes were collected, gently washed to remove loosely attached sperm, and transferred to conventional embryo culture dishes for subsequent in vitro culture. Embryo development was then monitored in an incubator maintained at 38.5°C and 5% CO_2 for 7 days. The developmental competence of the fertilized oocytes was evaluated by assessing key embryological outcomes, including cleavage rate and blastocyst formation.

3.3 Experimental Setup

3.3.1 On-chip single oocyte trapping

The single-cell trapping process for oocytes is carried out in a specialized microfluidic system, where modern equipment and controlled environmental conditions are synchronized, as shown in Figure 3.7. The objective of this process is to position each oocyte accurately within the microfluidic trap structure on the chip without compromising the cell's integrity, while creating optimal conditions for subsequent monitoring and analysis of the cell's morphology and behavior.

Before use in single-cell trapping experiments, the microfluidic chip must be sterilized to eliminate any risk of contamination. The chip is placed in an autoclave at 120°C for 30 minutes following the standard wet autoclaving method, and then allowed to cool naturally in a sterile environment for 15 - 30 minutes to avoid exposure to external air.

This cooling stage is crucial to ensure that the chip does not deform due to temperature differences and to prevent condensation that could damage the internal microstructures.

The microfluidic chips are designed with microchannels sized in the range of several hundred micrometers, ensuring stable laminar flow during cell transport. The handling and culture medium used for experiment is POM (Porcine Oocyte Medium), a specialized medium that supports *in vitro* oocyte maturation. To enhance the stability of the medium and reduce unwanted adhesion within the microfluidic channels, BSA (Bovine Serum Albumin) is added at an appropriate concentration. The medium is sterile-filtered and maintained at 38.5°C before use to prevent thermal shock to the cells.

After selection through the collection and classification process, immature oocytes are introduced into the chip via the microchannel inlet. This is achieved using a micro-pump that controls the flow, delivering the cell-containing solution into the channel without creating sudden pressure. The continuous addition of medium during the process is essential to prevent the microfluidic channels from drying out or air bubbles from forming. Both of these issues could harm the cells or interfere with the flow rate within the system.

One of the core components of the system is the syringe pump (GEMINI 88 Plus, KD Scientific, USA), which enables precise control of the fluid flow rate, typically in the $\mu\text{L}/\text{min}$ range. In this study, the pump, equipped with a stepper motor, was used to drive two syringes containing the liquid through the inlet and outlet ports of the microfluidic device. The flow rate was carefully adjusted according to the viscosity of the medium, cell concentration, and the geometry of the microchannel. Specifically, the selected flow rate was low enough to prevent the cells from being swept away too rapidly, while still sufficiently high to transport them efficiently toward the desired trapping positions. To ensure stable fluid delivery, the syringes and connecting tubes were attached to the microchannel ports using L-shaped connectors compatible with the 1 mm punched holes. This configuration provided a secure connection and minimized the risk of liquid leakage during solution injection into the microchannel.

Oocytes are gently aspirated from droplets or containers using a pipette or micro-pump system, ensuring that all cells are introduced into the chip. If a cell passes the trap, the system can reverse the flow slightly to return the cell to the desired position, or adjust the flow rate in the branches to provide more accurate guidance.

The entire trapping and positioning process was monitored using an optical microscope integrated with a high-speed digital camera system. To ensure suitable physiological conditions for the cells during observation, the microscope was placed on a heated stage maintained at 38.5°C. Real-time images displayed on the digital screen enabled continuous observation of the cells, allowing their position, movement, and condition within the microchannel to be assessed throughout the experiment. In particular, a high-speed camera (Phantom VEO-710 L-72G-C, USA) was employed to

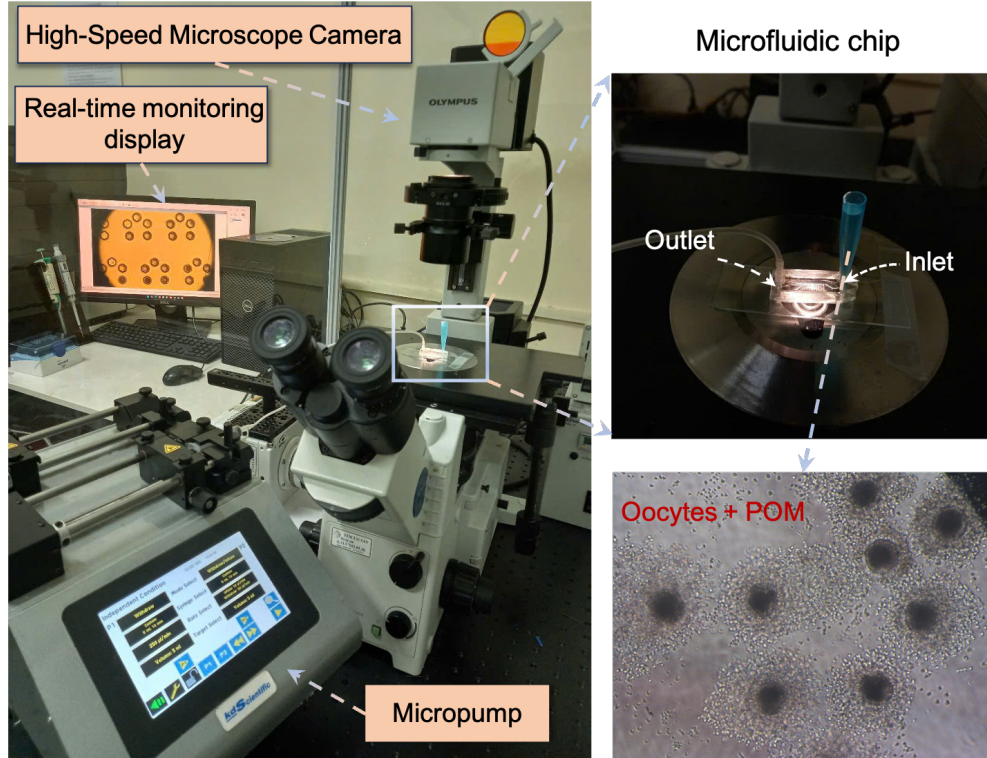


Figure 3.7: Experimental setup for on-chip single oocyte trapping

capture cell movements inside the microchannel at high frame rates, thereby facilitating detailed analysis of the effects of the electric field within the microfluidic environment. This camera is capable of recording at up to 7400 fps with an image resolution of 1280×800 pixels, making it well suited for acquiring detailed videos of fast dynamic events and microscopic objects of interest. The recorded images and videos were exported using the *Phantom Camera Control* software, with the output files saved at a setting of 100 fps in MP4 format for subsequent analysis and presentation. In parallel, high-magnification optical observation was performed using a microscope with $400\times$ magnification and high resolution, which significantly enhanced the ability to visualize and analyze COCs in the microfluidic environment. The imaging system was also capable of capturing high-resolution images and recording videos when required, providing a valuable dataset for evaluating trapping efficiency, assessing cell viability, and supporting subsequent image analysis, including the development of machine vision-based processing algorithms.

After the cells are positioned in the traps, POM medium is continuously added to maintain moisture and nutrients within the microfluidic channel. Additionally, it is essential to closely monitor any abnormalities, such as air bubble formation, changes in cell shape, or unstable flow, to intervene promptly. The entire single-cell trapping process is carried out under strict sterile conditions, with all procedures performed on a sterile workbench in a laboratory environment. Ensuring a clean working environment is essential to maintaining the viability and development potential of the oocytes throughout the cultivation and analysis process.

3.3.2 Dynamic on-chip single oocyte culture

Unlike traditional methods of culture in petri dishes or static droplets, the microfluidic chip system enables the creation of a dynamic microenvironment that closely mimics the *in vivo* biological conditions, including gentle fluid flow, precise control of temperature, pH, and gas concentrations. This helps optimize the developmental conditions for oocytes, increasing maturation rates and improving the quality of the cells after cultivation. Once the oocytes are successfully trapped at designated positions on the microfluidic chip, the chip is placed into an automated system and cultured for 48 hours. As shown in Figure 3.8, the system is also connected to a micro-pump for medium replacement after 24 hours of culture. Continuous microfluidic perfusion at a flow rate of 20 nL/min was implemented to regulate mass transport, improving the delivery of nutrients and gases while assisting in the removal of metabolic byproducts surrounding the oocytes. The flow is designed to be laminar to minimize shear forces acting on the COCs, while ensuring even exposure of the culture medium to each cell. The microchannels are appropriately sized to accommodate the oocyte, ensuring no obstruction or excessive mechanical pressure. Simultaneously, traditional static microdrop culture on Petri dishes was used as a control condition. After 2 days of on-chip culture, the COCs were collected and denuded to evaluate the maturation rate. During the 48-hour culture period, key environmental parameters such as temperature, pH, and CO₂ concentration are continuously monitored through integrated sensors or a controlled gas chamber. The pH is maintained between 7.2 and 7.4 using a bicarbonate buffering system and 5% CO₂ to ensure stability in the medium's alkalinity. Additionally, the heating system consistently maintains the temperature at 38.5°C.

Throughout the culture period, the development and maturation of the oocytes are monitored using an optical microscope integrated with a heated stage. The monitoring primarily focuses on the morphological changes of the COC complexes, such as the expansion of the cumulus cells, the clarity of the cytoplasm, and the overall shape of the oocyte. One of the key indicators of maturation is the appearance of the first polar body (PB1), signaling that the oocyte has completed the first stage of meiosis and is ready for fertilization. Additionally, more advanced systems allow the use of time-lapse techniques to continuously record the cell's transformation without direct intervention, minimizing the risk of contamination and environmental disturbances.

To objectively assess the quality of the oocytes after culture, image processing software can be integrated with the microscope system to semi-automatically analyze parameters such as diameter, roundness, cumulus cell spread, and cytoplasmic density. This approach ensures that the evaluation is not solely based on visual observation but is also supported by reliable quantitative data.

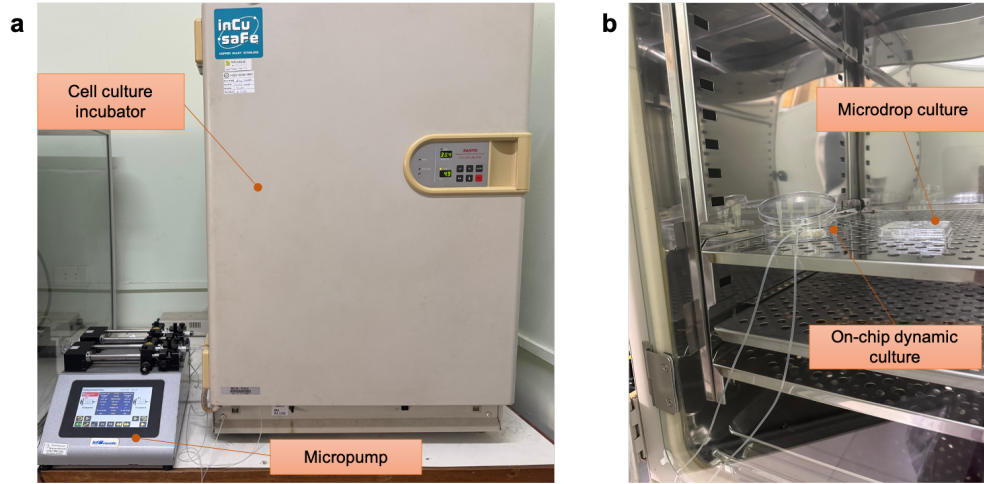


Figure 3.8: (a) Experimental setup for dynamic on-chip oocyte culture, (b) Oocyte culture inside the incubator, either on-chip or in microdrops within a commercial culture dish (control)

3.3.3 On-chip oocyte denudation

Figure 3.9 illustrates the experimental setup for the denudation process of cumulus cells surrounding the oocyte using a microfluidic device. Initially, a micro pump is used to pump the COCs into the microfluidic device. However, to ensure sterility, the entire denudation process on the chip is performed manually in a biosafety cabinet, where a micropipette is used to introduce the oocyte sample into the device.

The magnified image clearly shows the microfluidic device structure with two main points: the inlet and the outlet, which help control the flow of the solution containing the COCs through the microchannels in the chip. The central part of the device is where the hexagonal micropillars with sharp angles are arranged. A high-speed camera is used to record the movement and collisions of the COCs within the microchannel. The data captured from the video shows that the mechanical forces generated at the points of collision between the COCs and the micropillars effectively remove most of the surrounding cumulus cells. Specifically, after each collision, the oocyte tends to rotate, increasing its surface area in contact with the sharp edges of the micropillars during subsequent movements. This enhances the efficiency of cumulus cell removal and maintains a continuous denudation process along the flow direction within the microfluidic device.

After denudation, the oocytes were collected into a culture dish and examined under an epifluorescence microscope (Nikon Eclipse Ti). The microscope, fitted with a camera and a Thermal Plate heater (Thermo Plate TPi – Series), ensured the maintenance of the oocytes' physiological conditions, reduced environmental influences, and provided clear, high-resolution images for further analysis.

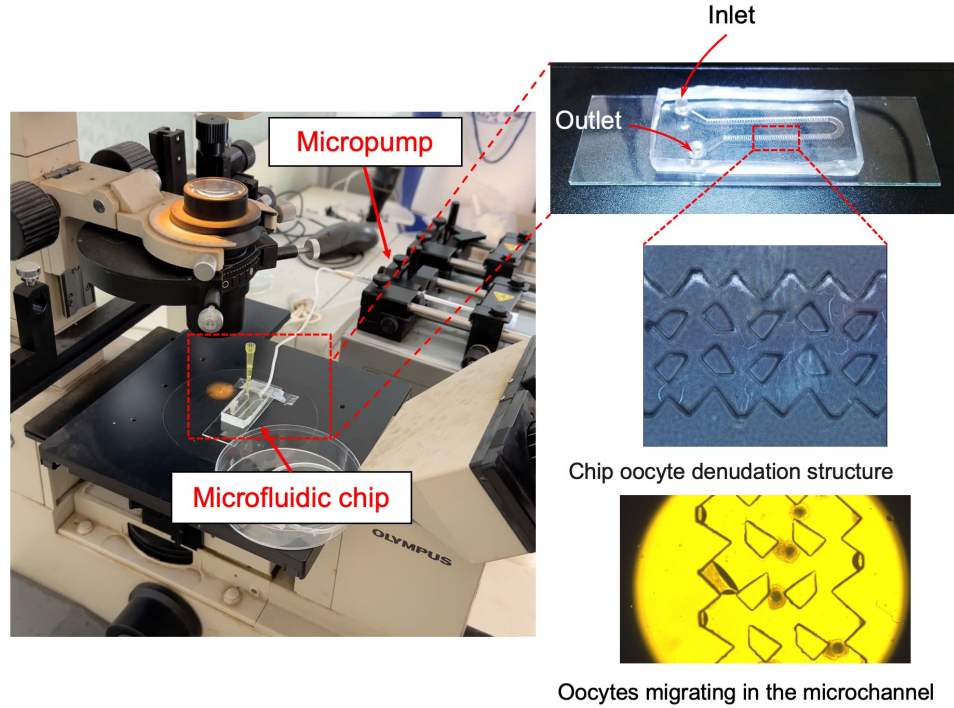


Figure 3.9: Experimental setup for on-chip oocyte denudation

3.3.4 DEP-based oocyte separation

In this study, oocyte-based micromanipulation was performed using a non-uniform electric field generated by microelectrodes supplied with an alternating current (AC) voltage. The applied AC signal ranged from 1 to 20 V over a frequency range of 1 kHz to 1 GHz. This voltage was delivered to the exciting electrode and the reference electrode to generate the DEP force acting on the cells. The electrical stimulation was maintained for approximately 5 min during each experiment. A function generator (PeakTech P 4046, 2-Channel Arbitrary Signal Waveform Generator) was used to provide the required AC signal. In addition, the electrical setup was integrated with the syringe pump system, high-speed camera, and optical microscope, enabling simultaneous microfluidic manipulation and real-time observation of the DEP-induced behavior of the oocytes. The experimental setup for DEP-based oocyte manipulation is illustrated in Figure 3.10.

3.4 Conclusion

In summary, this chapter has presented the experimental methodologies and procedures employed throughout this study. The chapter described the microfabrication processes of the proposed microfluidic chip, including the fabrication of micropillar structures and the integration of microelectrodes for DEP-based manipulation. Detailed information

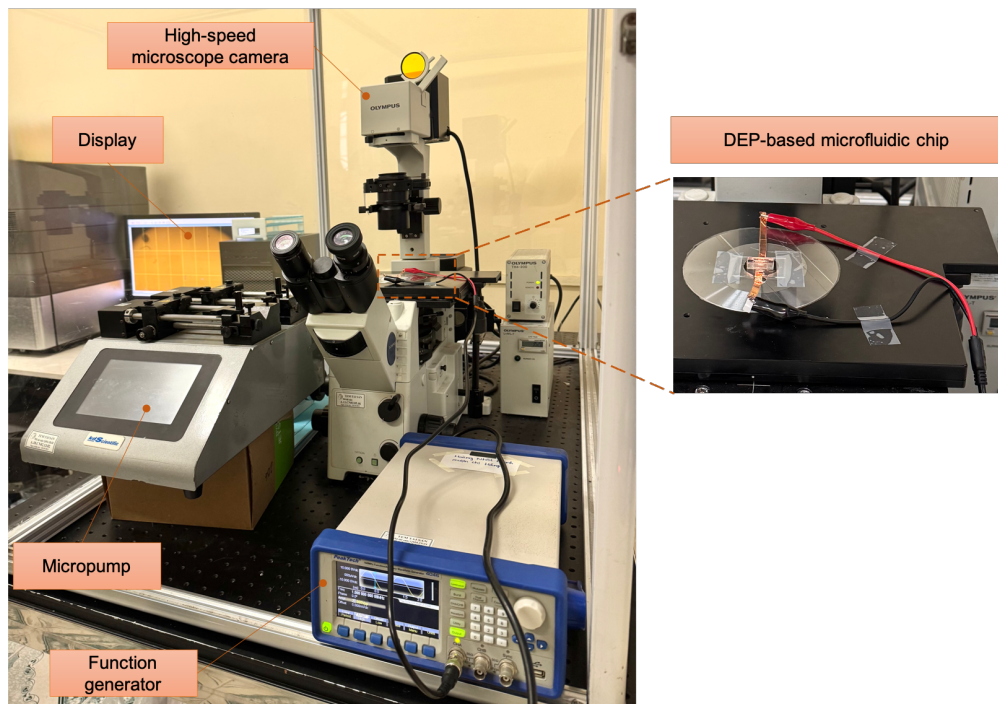


Figure 3.10: Experimental setup for DEP-based oocyte manipulation and separation

regarding the fabrication materials, equipment, and step-by-step procedures was also provided to ensure the reproducibility of the developed microfluidic platform.

In addition, this chapter introduced the protocols and materials used for porcine oocyte collection, handling, *in vitro* maturation, and fertilization-related procedures. The experimental setups for single-oocyte trapping, dynamic culture, on-chip denudation, and DEP-based manipulation were also described, including the associated instruments, fluidic and electrical connections, and imaging systems. Finally, the evaluation methods and analytical approaches used to assess device performance and biological outcomes were presented, providing the experimental foundation for the results and discussions in the following chapter.

Chapter 4

Results and Discussion

4.1 Fabrication of the proposed system

4.1.1 Fabrication of the microfluidic chip for single oocyte trapping and maturation

The microfluidic chip was successfully fabricated using soft lithography techniques. As shown in Figure 4.1(a), the PDMS chip is bonded to a glass slide, demonstrating its compact size, which is comparable to a 5-cent coin. To assess the micropillar arrangement and the overall quality of the fabrication, scanning electron microscopy (SEM) was employed, as illustrated in Figure 4.1(b). At higher magnification, the micropillars show uniform spacing and smooth surfaces.

Height measurements of the channels were taken using a Dektak ST System Profilometer (Veeco Instruments Inc., Plainview, NY, USA). The recorded values ranged from approximately 220 μm to 230 μm , aligning closely with the proposed design specifications, thus confirming the accuracy and reproducibility of the soft lithography process. However, both optical and SEM characterizations revealed some geometric deviations between the fabricated PDMS structures and the idealized 3D CAD models, particularly in the rounding of the hexagonal corners. These discrepancies are inherent in the standard SU-8 photolithography and PDMS bonding processes. Despite the rounding of the corners, the main bodies of the pillars retain enough angularity to maintain the necessary flow resistance and ensure the proper function of the trap units.

The rounding of sharp corners may result in smoother flow streamlines when compared to the idealized geometry, which may lead to a reduction in shear stress. Additionally, the fabrication and bonding process induced slight height variations between the micropillars and the channel, preventing the complete confinement of the oocyte along the vertical walls. This slight variation allows for lateral and vertical medium circulation, which could promote long-term cultivation of the oocytes. However, this fabrication imperfection may slightly reduce the oocyte confinement, increasing

the likelihood of the oocytes slipping at higher flow rates, potentially impacting the trapping performance.

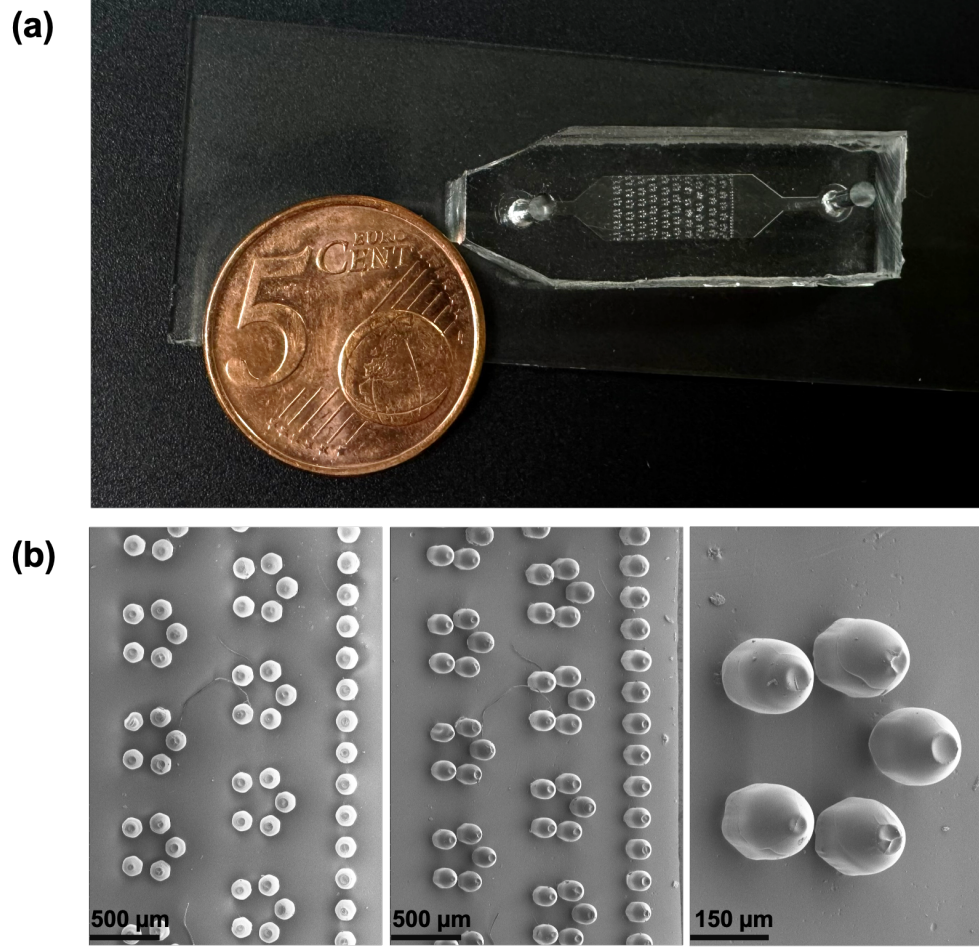


Figure 4.1: (a) Fabricated microfluidic chip, (b) SEM characterization of the chip

4.1.2 Fabrication of the microfluidic chip for oocyte denudation

Figure 4.2 presents SEM images of the fabricated microfluidic device, offering detailed insights into the morphology and surface characteristics of the micropillars. Figure 4.2(a) provides an overview of the micropillar structure, showcasing the accuracy and precision of the fabrication process. Figure 4.2(b) and Figure 4.2(c) display the dimensions of the micropillars, with images captured from both an overview and vertical viewing angle. The height of the micropillars is measured to be approximately $200\ \mu\text{m}$, while the spacing between the micropillars at the fourth section is around $120\ \mu\text{m}$. These measurements confirm that the fabricated device aligns with the proposed design specifications, validating the accuracy of the fabrication process.

Figure 4.2(d) offers a closer inspection of a single micropillar, highlighting the surface texture and any potential defects or irregularities. This high magnification view reveals the fine details of the micropillar's structure, providing crucial information about the

uniformity of the pillar surfaces and any subtle variations that may have occurred during the fabrication. This level of scrutiny ensures that any imperfections in the micropillar geometry are identified, which is essential for understanding the overall performance and reliability of the microfluidic device.

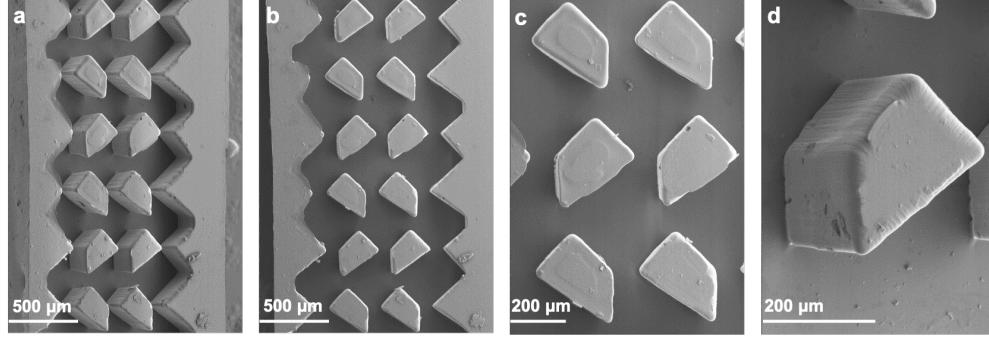


Figure 4.2: Scanning electron microscope (SEM) evaluation of denudation chip

4.1.3 Fabrication of the microfluidic chip for oocyte separation

The microfluidic chip integrated with electrodes, as observed under a microscope, is shown in Figure 4.3. First, to evaluate the influence and effects of DEP forces on cells, a simplified microfluidic chip was designed consisting solely of a microwell with a radius of $1000\ \mu\text{m}$. The microwell is positioned surrounding the electrode region to establish an optimal electric field distribution for effective interaction with the cells. Following the photolithography and material processing steps, the electrodes were patterned directly onto the ITO surface, ensuring sufficient electrical conductivity while maintaining the optical transparency necessary for microscopic observation. As shown in Figure 4.3, stereomicroscope images confirm that the microelectrodes were fabricated with good structural uniformity and alignment with the intended design. More specifically, the electrode configuration consists of a combination of rectangular electrode and diagonally oriented (inclined) electrode arrays arranged in an interdigitated manner. This arrangement generates localized regions with strong electric field gradients, particularly at the electrode edges. As a result, the structure is capable of inducing distinct DEP forces in different regions of the channel, thereby facilitating directional manipulation and separation of oocytes based on their dielectric properties.

4.2 Simulation results

Figure 4.4 presents a comprehensive simulation analysis of flow dynamics and mechanical stress distribution during oocyte trapping within the microfluidic channel. The inlet flow velocity was set at $400\ \mu\text{m/s}$. In Figure 4.4(a), the simulation illustrates the

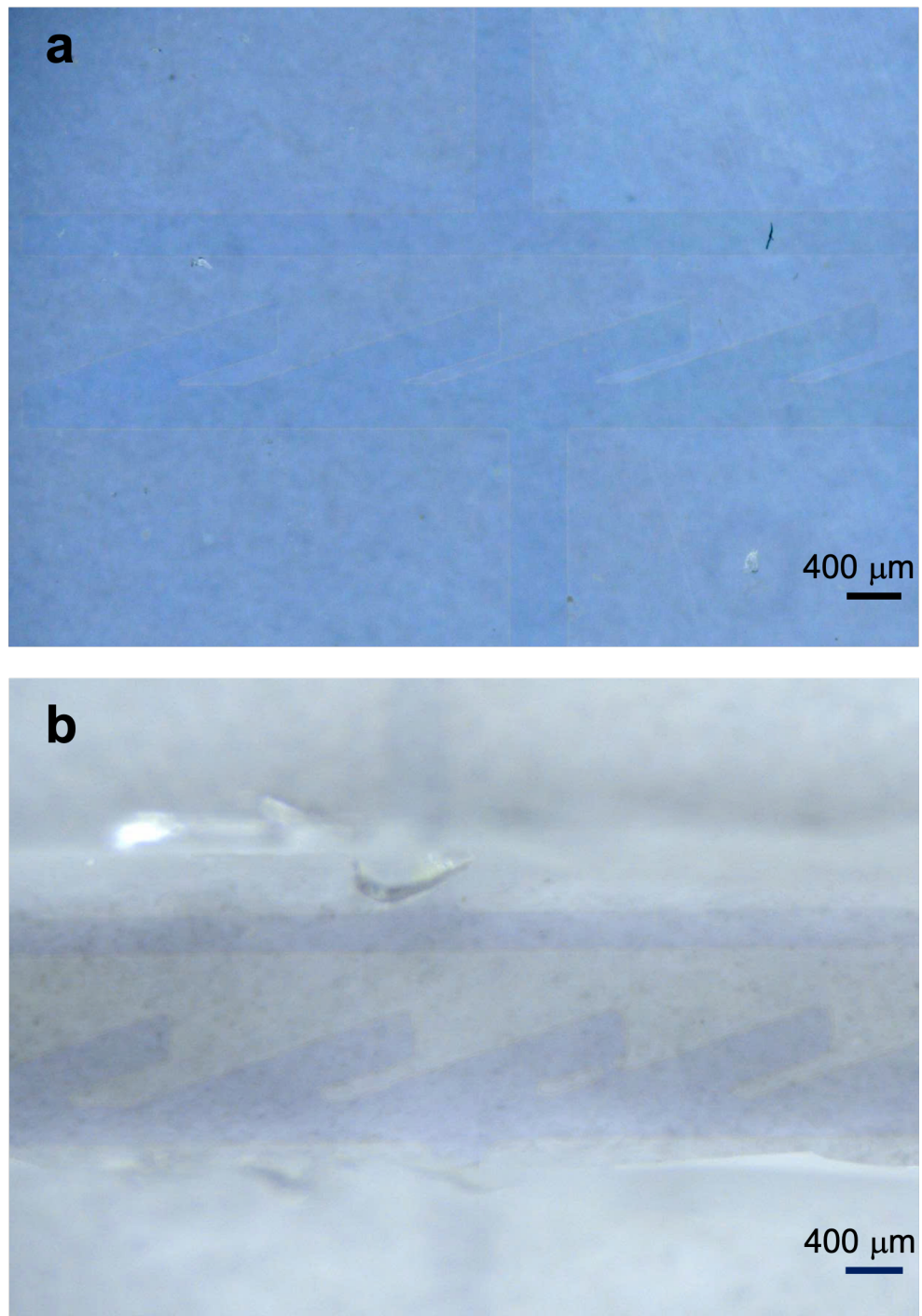


Figure 4.3: Fabricated microfluidic chip with slanted electrodes integrated at the bottom of the microchannel before (a) and after (b) microchannel bonding

velocity magnitude at the fluid interface at four different time points (0 s, 1.0 s, 1.5 s, and 3 s), describing the transition from free oocyte movement to successful capture within the trapping unit. As the oocyte approaches the trapping site, the flow field becomes increasingly non-uniform. At $t = 3$ s, the flow velocity reaches a peak of approximately $1500 \mu\text{m/s}$ in the narrow gaps surrounding the trap, while the velocity inside the trapping region significantly decreases. This pronounced velocity gradient creates favorable hydrodynamic conditions for oocyte immobilization. Once an oocyte is captured, subsequent oocytes are redirected toward a bypass channel, where flow resistance is lower and fewer obstructions are present.

The mechanical stresses acting on the cell include shear stress and Von Mises stress. Shear stress represents the frictional force exerted by the fluid flow on the cell surface and is typically expressed in dyne/cm^2 or Pascals ($1 \text{ Pa} = 10 \text{ dyne/cm}^2$). In contrast, Von Mises stress is an equivalent stress metric derived from multiple stress components in solid mechanics, commonly used to predict yielding or structural failure. In cellular studies, Von Mises stress is often applied in numerical simulations, such as finite element analysis, to characterize the internal stress distribution within cells under complex mechanical loading. Although cells are soft, viscoelastic biological materials rather than purely solid structures, the concept of Von Mises stress remains valuable for identifying regions of stress concentration, which may correspond to areas susceptible to deformation or potential damage.

Shear stress generated by fluid flow can induce deformation of the cell membrane, potentially affecting both the structural integrity and viability of oocytes. Oocytes and embryos are particularly sensitive to shear stress, especially during early developmental stages. Previous studies have shown that shear stress exceeding approximately 1.2 dyne/cm^2 ($\sim 0.12 \text{ Pa}$) can activate stress-responsive kinases (SAPK/JNK), leading to apoptosis in mouse embryos. Embryos at the 8–16 cell stage are more susceptible to mechanical damage compared to later-stage blastocysts. Furthermore, flow conditions reaching $2.06 \text{ dyne/cm}^2 \sim$ above the 1.2 dyne/cm^2 threshold have been reported to reduce embryonic development rates due to the adverse effects of mechanical stress [47].

To ensure the safety of oocytes, shear stress is typically maintained below biologically relevant thresholds. Advanced microfluidic designs for oocyte and embryo culture have therefore focused on optimizing channel geometry and flow rates to sustain very low shear stress levels. The zona pellucida, a protective glycoprotein layer surrounding the oocyte, acts as a mechanical shield against fluid-induced forces. Its elastic properties enable the oocyte to better withstand shear stress, thereby reducing direct mechanical impact on the cell membrane. Experimental observations indicate that under excessive mechanical stress, the first sign of damage is typically rupture of the zona pellucida; only afterward does the oocyte itself become compromised, often exhibiting darkening due to cell death. In microfluidic experiments, it has been observed that under designed flow

conditions, oocytes maintain normal morphology characterized by clear cytoplasm and intact zona pellucida even when exposed to localized flow regions reaching approximately 70 dyne/cm^2 for very short durations [139]. This suggests that oocytes can tolerate transient high shear stress pulses, provided the exposure time is sufficiently brief and most of the cell surface experiences low stress levels.

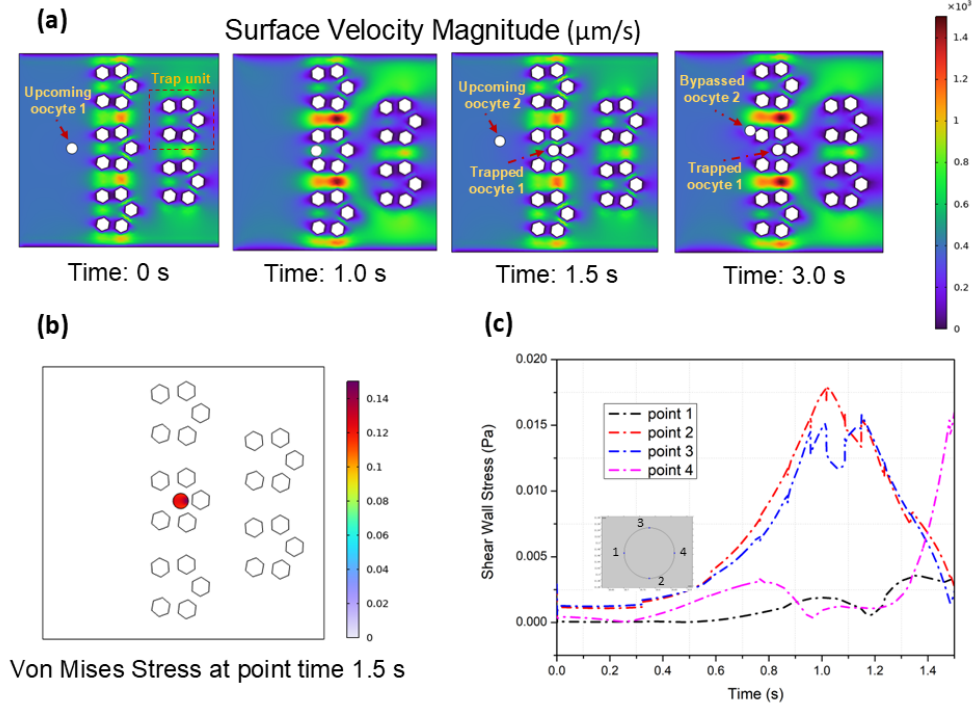


Figure 4.4: Computational fluid dynamics (CFD) simulation of the single-oocyte trapping mechanism in our FEM system. (a) Surface velocity magnitude showing flow distribution over time; the color gradient from blue (low) to red (high) indicates fluid velocity. Oocyte migration toward an empty trap unit 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

The simulation results indicate that the mechanical stresses exerted on the oocyte within the microfluidic platform remain much lower below levels generally associated with structural damage or loss of viability. Specifically, the maximum shear stress at the cell–fluid interface was about 0.018 Pa. These values are substantially lower than commonly referenced harmful thresholds, such as 1.2 dyne/cm^2 (approximately 0.12 Pa), which has been associated with stress-induced cellular responses in sensitive reproductive cells and embryos. Moreover, the simulation shows that only a limited region of the oocyte surface is exposed to elevated flow-induced stress, whereas most of the cell surface experiences very low shear levels.

In addition to fluid-induced stress, the intrinsic mechanical properties of the oocyte

should also be considered when evaluating the potential impact of microfluidic manipulation. Previous studies have reported that the Young's modulus of oocytes generally spans a wide range, from approximately 0.1 kPa to 10 kPa, depending on species, developmental stage, and measurement method [140]. This modulus reflects the stiffness of the oocyte and determines how it responds to external mechanical loading, including compression, deformation, and hydrodynamic stress during trapping, handling, or fertilization-related procedures. Compared with this stiffness range, the simulated stress values in the present system are several orders of magnitude lower, indicating that the imposed mechanical loading is very small relative to the oocyte's overall resistance to deformation. Taken together, these findings suggest that the proposed microfluidic design maintains a low-stress mechanical environment while still providing sufficient hydrodynamic resistance for effective oocyte immobilization. This combination is particularly important for applications requiring stable trapping and extended on-chip culture, where excessive mechanical loading could interfere with normal cellular function.

Regarding the on-chip denudation chip, Figure 4.5 presents simulation-based evaluation of the microfluidic device. The computational model includes three main functional regions: an inlet for introducing COCs, a denudation zone containing arrays of micropillars, and an outlet for collecting oocytes after separation from the surrounding cumulus cells. This configuration was designed to investigate how fluid-induced mechanical forces develop within the device and how these forces may facilitate progressive cumulus cell removal during flow through the micropillar region.

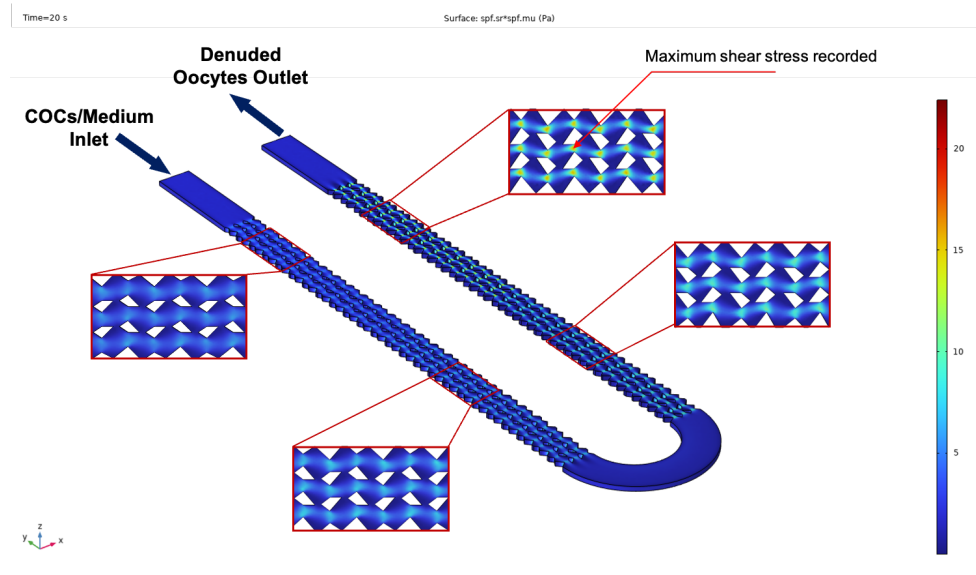


Figure 4.5: Shear stress distribution within the denudation chip

To improve the accuracy of the numerical analysis, a refined multi-element meshing strategy was applied. The final mesh contained 1,623,706 domain elements, allowing detailed resolution of local flow behavior, particularly within the narrow gaps between

adjacent pillars where strong velocity gradients were expected. Simulated inlet flow rates of 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mL/min were used to evaluate the resulting shear stress distribution throughout the microchannel. Fluid motion in the model was driven by the pressure difference between the inlet and outlet, reproducing the hydrodynamic conditions expected during actual chip operation.

The simulated shear stress distribution is summarized in Figure 4.5(b). As anticipated, shear stress increased markedly within the constricted spaces between micropillars and reached its highest values at the horizontal gaps near the pillar vertices, where the flow was locally accelerated. The results also revealed clear spatial differences in shear stress across the successive denudation regions of the device. As the fluid advanced downstream, both local flow velocity and the associated shear forces increased. Notably, the highest shear stress was observed in the final section of the micropillar array, indicating that this region provides the strongest mechanical action on the COCs and is therefore likely to contribute most effectively to complete denudation.

Quantitatively, the maximum shear stress acting on oocytes was estimated to be 5.31, 10.94, 16.95, and 23.09 Pa at flow rates of 0.25, 0.5, 0.75, and 1.0 mL/min, respectively, as shown in Figure 4.6. These values are substantially lower than those reported for several previously developed denudation strategies. For example, the jagged microchannel design reported by Weng *et al.* [126] produced a maximum shear stress of up to 163.5 Pa at a flow rate of 1.0 mL/min. Likewise, manual pipetting procedures have been estimated to expose oocytes to much higher mechanical loading, with calculated shear stress ranging from 149.8 to 392.0 Pa [126]. In comparison, the proposed chip generates a considerably milder hydrodynamic environment while still maintaining sufficient mechanical force for cumulus cell removal.

Overall, these findings indicate that the proposed microfluidic design enables a more controlled and gentle denudation process. By gradually increasing shear stress along the micropillar array and limiting the peak stress to levels far below those of conventional approaches, the device may reduce the risk of excessive mechanical damage to oocytes while preserving denudation efficiency. This controlled shear profile is an important advantage for applications in assisted reproductive technologies, where minimizing mechanical injury during oocyte handling is essential for maintaining developmental competence.

To elucidate the mechanism underlying DEP-based oocyte separation, the electric field distribution within the microfluidic channel was first analyzed. Figure 4.7 illustrates the spatial distribution of the electric field magnitude within the microfluidic channel. Regions located near the electrodes, particularly at the electrode edges, exhibit relatively high electric field intensity, while the field strength gradually decreases toward the central region, reaching values below 1×10^4 V/m. This electrode configuration establishes a strongly non-uniform electric field within the separation zone of the device.

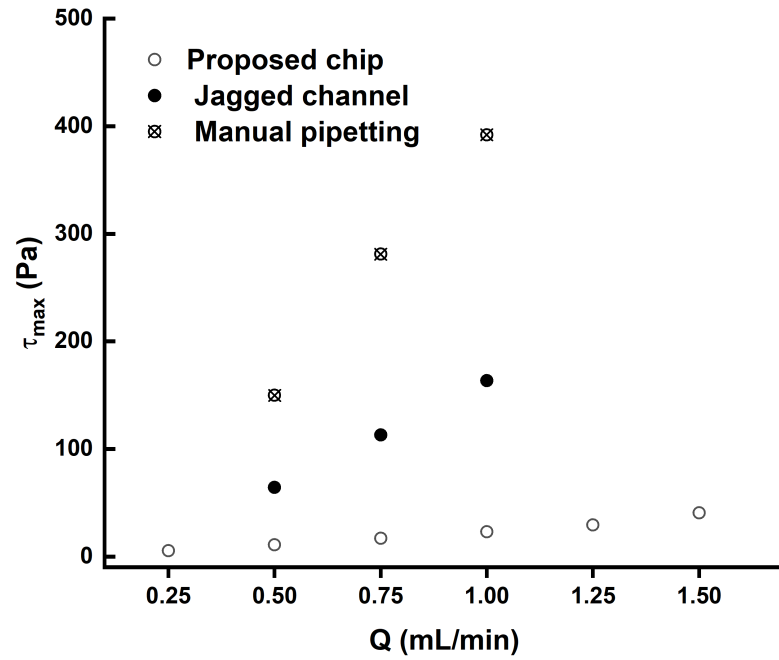


Figure 4.6: Comparison of simulated maximum shear stress generated in the proposed chip, the jagged-surface microfluidic chip [126], and standard manual pipetting [126]

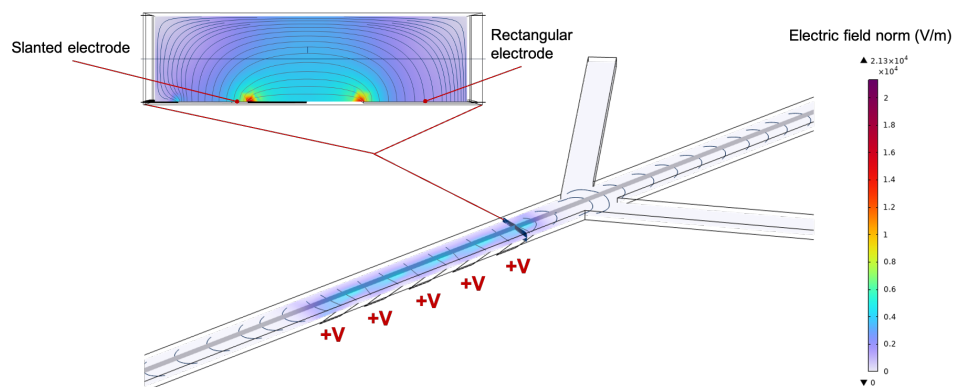


Figure 4.7: Distribution of the electric field norm within the microchannel

Based on this field profile, the system is expected to induce distinct dielectrophoretic responses depending on the dielectric properties of the oocytes. Specifically, good-quality oocytes are anticipated to experience pDEP and migrate toward high-field regions, whereas poor-quality oocytes are more likely to exhibit nDEP and move toward low-field regions. These behaviors result in trajectory deflections that enable spatial separation within the channel, as observed in Figure 4.8. Building upon this principle, the proposed electrode architecture serves as the basis for DEP-assisted oocyte classification within the microfluidic platform.

In the absence of an applied electric field, the motion of both good-quality and poor-quality oocytes is entirely governed by the hydrodynamic flow within the microfluidic channel. The flow velocity was set to $230\ \mu\text{m/s}$ and the applied voltage was set to $2.5\ \text{V}$ at a frequency of $1\ \text{MHz}$. Due to the channel design, where the main flow is directed straight toward the central outlet, both types of oocytes follow the streamlines and converge toward the same outlet. Under these conditions, no separation occurs, as there is no significant difference in their hydrodynamic behavior (Figure 4.8(a)). In contrast, when a non-uniform electric field is applied to induce DEP, differences in the dielectric properties between good- and poor-quality oocytes lead to distinct migration trajectories (Figure 4.8(b)). This distinct DEP response is attributed to the hypothesis adopted in the model that membrane damage associated with oocyte degeneration increases membrane conductivity. The resulting change in the effective complex permittivity alters the Clausius–Mossotti factor, causing good- and poor-quality oocytes to experience different DEP forces and therefore follow different migration trajectories.

To evaluate the performance of the DEP-based sorting device the value of separation efficiency was calculated. Separation efficiency was defined as the proportion of cells introduced at the inlet that were successfully recovered at their intended outlet. The results showed that under optimal conditions, 93% of abnormal oocytes were collected at the central outlets and were not detected at the two sides outlet, while 87% of normal oocytes were recovered at the two sides outlet. These findings indicate that the system achieved separation in accordance with the intended design principle. Abnormal oocytes, which were not significantly influenced by the DEP force under the applied conditions, predominantly followed the center flow paths, whereas normal oocytes experienced a sufficient DEP response and were guided toward the central outlet. However, when compared with the total number of oocytes introduced into the device, a fraction of the cells was either retained within the channel or lost during transport. Overall, these results are consistent with the DEP mechanism described above and support the feasibility of the proposed device for selective oocyte sorting.

Figure 4.9 illustrates the separation efficiency and cell trajectories obtained at different applied voltages ranging from 1.0 to $3.5\ \text{V}$. The electric field frequency and the

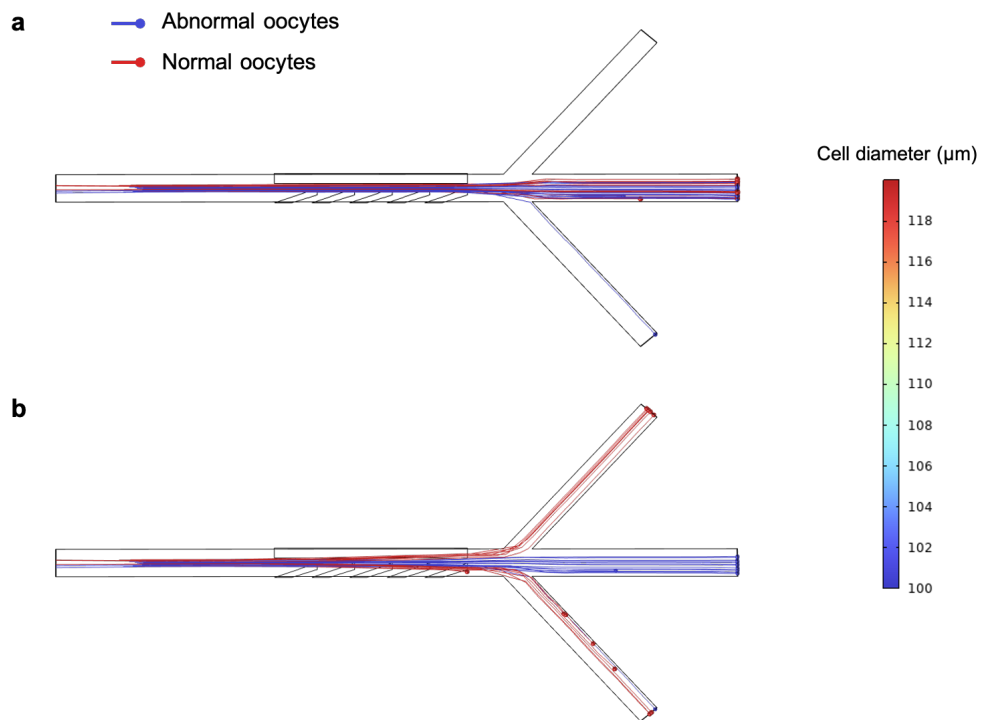
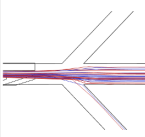
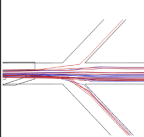
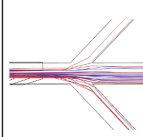
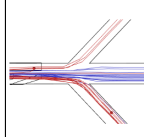
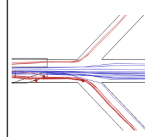
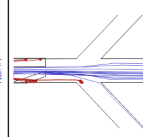


Figure 4.8: Cell trajectories in the microfluidic device in the absence (a) and presence (b) of DEP force; the electric potential distribution within the microfluidic chip is visualized using the color map in the x-y plane. Blue trajectories correspond to abnormal oocytes, while red trajectories indicate normal oocytes

V = 1 V	V = 1.5 V	V = 2 V	V = 2.5 V	V = 3 V	V = 3.5 V
					
Normal oocyte: 6.67%	Normal oocyte: 33.33%	Normal oocyte: 46.67%	Normal oocyte: 86.67%	Normal oocyte: 53.33%	Normal oocyte: 0%

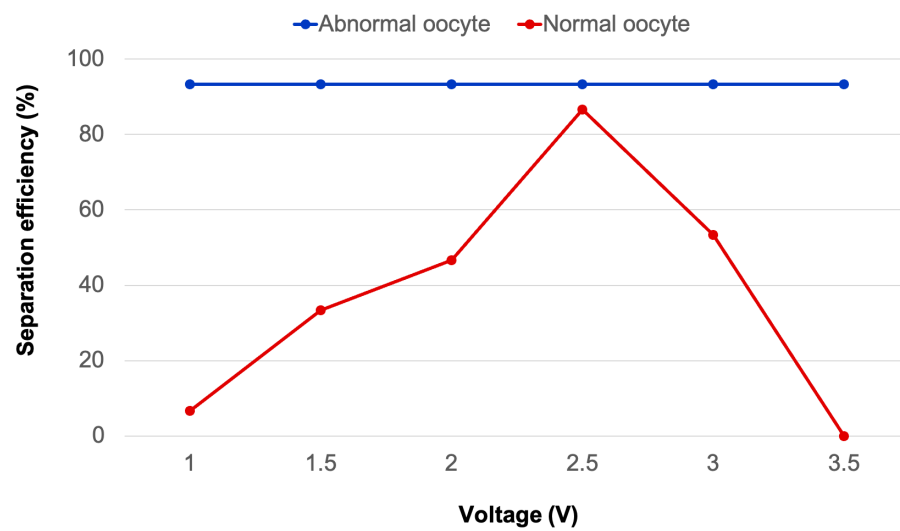


Figure 4.9: Dependence of the separation efficiency on the applied voltage magnitude when the voltage frequency remained 10 MHz, inlet velocity was $230 \mu\text{m/s}$

oocyte inlet velocity were fixed at 1 MHz and 230 $\mu\text{m/s}$, respectively. In the simulation, the dielectric properties of abnormal oocytes are found to be almost identical to normal oocytes, except for differences in the cytoplasmic conductivity. As the oocyte degenerates, the integrity of the cell membrane is compromised, leading to increased permeability. This change significantly reduces the DEP effect on the cell, making it either negligible or resulting in low nDEP forces. The nDEP forces push the abnormal oocyte toward the low-field region, typically the center of the channel where the electric field strength is weaker. Consequently, abnormal oocytes follow a single trajectory toward the central outlet, predominantly influenced by hydrodynamic drag rather than by DEP forces. As a result, their migration pattern remained nearly unchanged under all tested voltages. In contrast, the deflection of good-quality oocytes increased with increasing applied voltage, which is consistent with DEP theory, since the electric field gradient in the DEP force equation is directly related to the applied voltage. At 3.5 V, the DEP force became excessively strong, causing a considerable number of good-quality oocytes to deviate toward the main channel sidewall and become trapped there. By comparison, at lower voltages of 1.0 - 2.0 V, the DEP force was insufficient to induce significant lateral displacement, and most good-quality oocytes followed slightly deflected trajectories before being carried by the hydrodynamic flow toward the central outlet together with the poor-quality oocytes. At the optimal voltage of 2.5 V, good-quality oocytes were deflected along the desired trajectories toward the side outlets, resulting in the highest separation efficiency. In addition, this relatively low voltage is expected to generate negligible Joule heating, thereby helping preserve cell viability.

4.3 Experimental results

4.3.1 Single oocyte trapping and maturation on-chip

In the experimental procedure, a relatively high flow rate of 200–300 $\mu\text{L/min}$ is initially applied for approximately 30 seconds to generate sufficient hydrodynamic driving force, ensuring that oocytes are efficiently introduced into the microfluidic channel. Once the oocytes reach the main trapping region, the flow rate is reduced to 50–70 $\mu\text{L/min}$. This lower flow regime establishes a more controlled hydrodynamic environment, allowing for gradual oocyte movement and precise positioning into individual trapping units while limiting excessive mechanical stress that could adversely affect cellular integrity.

As illustrated in Figure 4.10, the fabricated device demonstrates a strong capability for single-oocyte trapping. However, in practice, oocytes frequently exhibit a tendency to aggregate into clusters due to extracellular secretions from surrounding cumulus cells. These secretions are rich in hyaluronic acid, a key component that enhances

cell–cell adhesion [141]. The formation of such clusters introduces several practical challenges, including reduced efficiency in sample loading, difficulty in guiding oocytes into designated trapping sites, and potential obstruction of microchannels. Moreover, clustered oocytes may lead to unintended multiple occupancy within a single trap unit, which is undesirable for applications requiring precise single-oocyte manipulation and analysis, as it may interfere with nutrient exchange and developmental assessment.

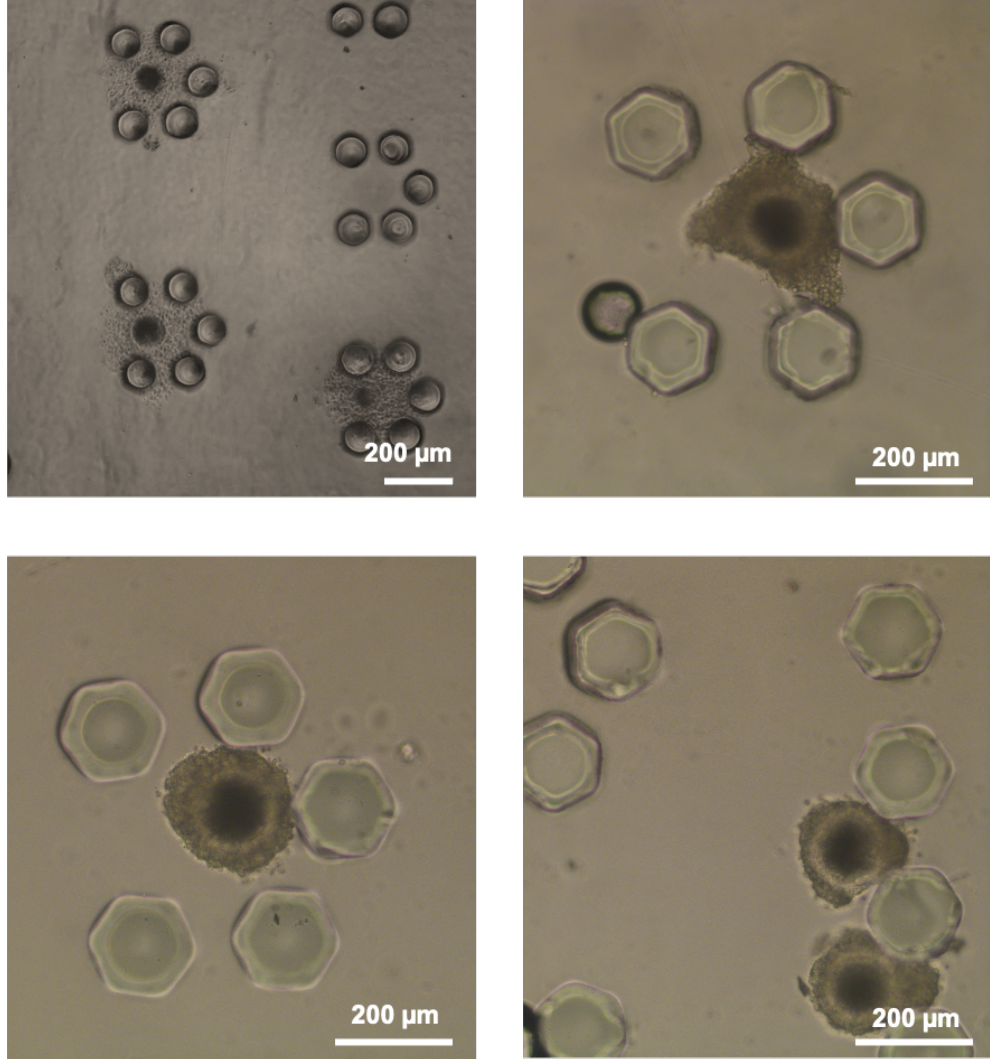


Figure 4.10: Single oocytes trapped within the trap units

To quantitatively evaluate the trapping performance of the proposed system, the key metric of trapping efficiency was defined. The trapping efficiency is calculated as the ratio of successfully immobilized oocytes within designated trap units to the total number of oocytes introduced into the device. Experimental results demonstrate a high average trapping efficiency of 88.37%, with a standard error of approximately 1.5%, indicating consistent and reliable device performance. Conversely, approximately 11.63% of oocytes were not successfully trapped. Among these, some oocytes bypassed the trapping sites due to variations in local flow dynamics or entry trajectories, while a

considerable portion exited the trapping region entirely.

Notably, the majority of these untrapped oocytes were smaller in size, typically ranging from 90 to 110 μm in diameter (including surrounding cumulus cells). Such oocytes are generally regarded as lower quality, as their reduced size is often associated with limited developmental potential and increased difficulty in downstream culture or ART applications [115]. This observation suggests that the microfluidic system inherently exhibits a passive size-selection effect. Specifically, the gradual reduction in trap dimensions along the channel may function as a screening mechanism, preferentially retaining oocytes of suitable size while allowing smaller ones to pass through.

In addition to its trapping capability, the proposed chip enables automated and precise handling of individual oocytes. The integrated microstructure also acts as an effective filtration system, removing cellular debris and impurities from the flow. This feature addresses a common limitation observed in conventional chamber-based single-oocyte trapping designs, where debris accumulation can disrupt flow patterns and compromise trapping performance [46], [47]. Overall, these results demonstrate that the proposed microfluidic platform not only achieves efficient single-oocyte trapping but also provides improved operational robustness and sample quality control.

To better mimic the *in vivo* microenvironment, the OvaOoC platform was operated under dynamic perfusion conditions and subsequently assessed for oocyte culture performance. In this system, continuous microfluidic perfusion was maintained at a flow rate of 20 nL/min, enabling a steady and well-controlled transport of soluble factors around the trapped oocytes. Such gentle perfusion is expected to improve the supply of nutrients and dissolved gases while simultaneously promoting the removal of metabolic waste products from the local microenvironment [142]. Compared with static culture, this dynamic exchange may help maintain a more stable biochemical milieu around each oocyte during prolonged culture.

For comparison, conventional static microdrop culture in Petri dishes was employed as the reference condition. After 2 days of on-chip culture, the COCs were retrieved from the device and denuded to allow subsequent assessment of nuclear maturation. Oocyte maturation was evaluated based on the presence of the PB1, which is a well-established morphological indicator of successful completion of the first meiotic division and reflects progression to the metaphase II stage, a prerequisite for fertilization competence [143]. By comparing maturation-related outcomes between the dynamic culture condition in the OvaOoC system and the conventional static culture method, this study aims to examine whether controlled microfluidic perfusion can provide a more favorable environment for oocyte maintenance and maturation. This comparison is particularly important because dynamic microculture may better reproduce the continuous fluid exchange that occurs *in vivo*, while also reducing the limitations of stagnant medium commonly encountered in static systems. The experimental results obtained from

both dynamic microfluidic perfusion and conventional static culture are summarized in Table 4.1.

Table 4.1: Comparison of oocyte survival, degeneration, and maturation rates between on-chip dynamic culture and conventional static culture

Culture system	Experiment	Number	Survival	Degeneration	Maturation
		of oocytes	rate (%)	rate (%)	rate (%)
On-Chip Culture	1	23	82.61	17.39	43.48
	2	30	96.67	3.33	76.67
	3	20	100	0.00	80.00
	4	39	97.44	2.56	89.74
	5	37	97.30	2.7	78.38
		Mean $\pm$ SEM	94.8 $\pm$ 3.1	5.2 $\pm$ 3.1	73.65 $\pm$ 7.88
Conventional Static Culture (Control)	1	103	51.46	48.54	40.78
	2	20	95.00	5.00	70.00
	3	47	97.88	2.12	68.09
	4	35	94.29	5.71	88.57
	5	48	95.83	4.17	77.08
		Mean $\pm$ SEM	86.89 $\pm$ 8.88	13.11 $\pm$ 8.88	68.9 $\pm$ 7.9

As summarized in Table 4.1, dynamic on-chip culture using the OvaOoC platform showed improved performance compared with conventional static culture. Figure 4.11 indicates that the overall maturation-rate patterns were generally comparable between the control and on-chip groups, although noticeable variability was observed among experimental replicates. Maturation rates ranged from 43.48% to 89.74% in the on-chip group and from 40.78% to 88.57% in the control group, suggesting that batch-to-batch differences in oocyte quality may have influenced the outcomes. When analyzed in a paired manner, both groups showed similar experimental trends: batches with higher maturation rates under static culture also tended to show higher maturation rates on-chip, whereas lower-performing batches remained relatively low in both conditions.

Despite this variability, oocytes cultured on-chip showed a higher survival rate than those maintained under static conditions ($94.8 \pm 3.1\%$ vs. $86.89 \pm 8.88\%$) and a lower degeneration rate ($5.2 \pm 3.1\%$ vs. $13.11 \pm 8.88\%$). Dynamic on-chip culture also resulted in a higher maturation rate than conventional culture ($73.65 \pm 7.88\%$ vs. $68.9 \pm 7.9\%$, $p = 0.04$, paired t-test). These results suggest that the platform can better support oocyte viability and maturation while reducing degeneration. Overall, the findings indicate that OvaOoC-based dynamic culture may provide a more favorable

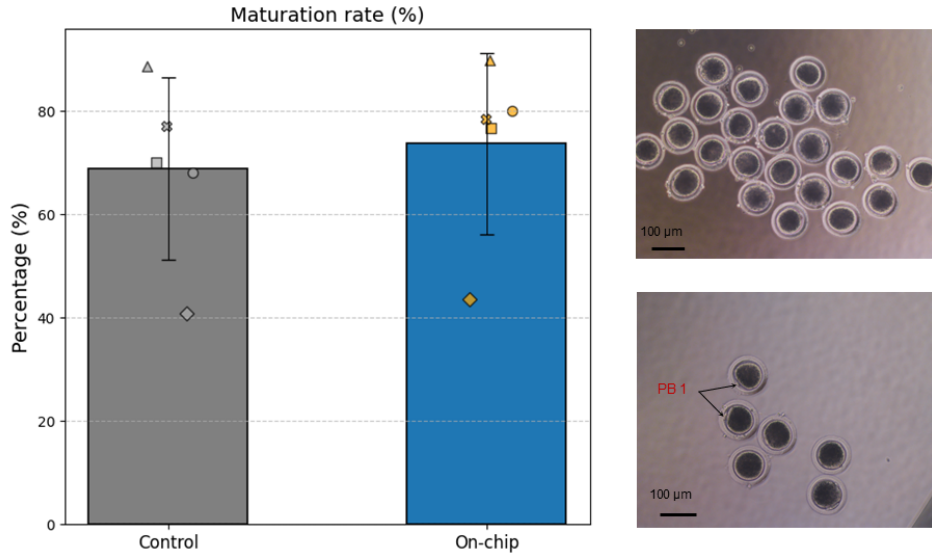


Figure 4.11: Comparison of oocyte maturation rate distributions under static and dynamic on-chip culture conditions, along with representative images of matured oocytes on the OvaOoC platform

microenvironment for IVM. During dynamic perfusion, the trapping units maintain each oocyte in a stable and well-defined position, thereby preventing displacement, rolling, or uncontrolled movement caused by fluid flow. This mechanical stabilization is important because it reduces unnecessary physical disturbance to the oocyte while allowing continuous medium exchange around it. Compared with previously reported microwell and microchamber designs [45], [46], [47], the proposed HMT structure accommodates COCs of varying sizes while maintaining open flow paths around each trapped sample. This configuration may reduce flow stagnation, promote more uniform transport of nutrients, dissolved gases, and soluble signaling factors, and facilitate the removal of cellular debris and metabolic waste. By partially reproducing key features of the *in vivo* environment, particularly continuous fluid exchange and localized microenvironmental control, the platform appears to better support both oocyte survival and maturation. In addition, because the device maintains oocytes at fixed and predictable positions, it is highly compatible with time-lapse monitoring and AI-based image analysis. This characteristic is particularly advantageous for continuous, non-invasive assessment of oocyte status throughout culture, and it further highlights the potential of the OvaOoC platform as a multifunctional tool for both mechanistic studies and future clinical applications in IVM.

Although the platform has reproduced several important aspects of the oocyte

microenvironment, it does not yet capture the full physiological complexity of the ovary. Further development should incorporate additional features such as three-dimensional follicular architecture, extracellular matrix components, interactions with other ovarian somatic cells, spatiotemporal hormonal regulation, and vascular-like nutrient and oxygen supply. These elements will be considered in future work to enable a more physiologically representative ovarian microenvironment.

4.3.2 Deep learning-assisted analysis of single oocytes

a. Segmentation model performance

The deep learning model for segmenting oocytes and cumulus cells was trained using approximately 480 images and evaluated on an independent validation set of 120 images. Although the training dataset was relatively modest in size, the highly standardized imaging conditions of the microfluidic platform, including fixed trapping geometries, consistent illumination, and minimal background variation, substantially reduced image heterogeneity. Under these controlled conditions, the available dataset was sufficient to support effective segmentation performance. The first two plots in Figure 4.12 illustrate the variation of loss functions as a function of training epochs, recorded on both the training and validation datasets. For the training set, it can be observed that during approximately the first 50 epochs, all loss functions rapidly converge, indicating an efficient learning process and effective feature extraction by the model. Among the different loss components, the box loss and classification loss reach the lowest average values, around 0.1, suggesting that the model achieves strong performance in object localization and classification tasks. In contrast, the segmentation loss and distribution focal loss remain at relatively higher levels, approximately twice as large. This indicates that although the model is capable of identifying object regions with reasonable accuracy, it still faces challenges in precisely predicting segmentation masks and exhibits a degree of uncertainty when handling objects with complex structures.

For the validation set, the loss functions exhibit a similar trend, with box loss and classification loss consistently maintaining the lowest average values and remaining significantly smaller than the other loss components. The fact that all loss functions remain stable and continue to gradually decrease beyond 100 epochs indicates that the model not only continues to learn effectively from unseen data but also shows no evident signs of overfitting or underfitting.

In terms of performance metrics, both Precision and Recall for the two output types—bounding boxes and segmentation masks—achieve average values exceeding 95%. This reflects the high overall accuracy of the model. This conclusion is further supported by the final evaluation plots of mean Average Precision (mAP), where mAP50 reaches near-perfect values, while mAP50–95 remains consistently high, approximately

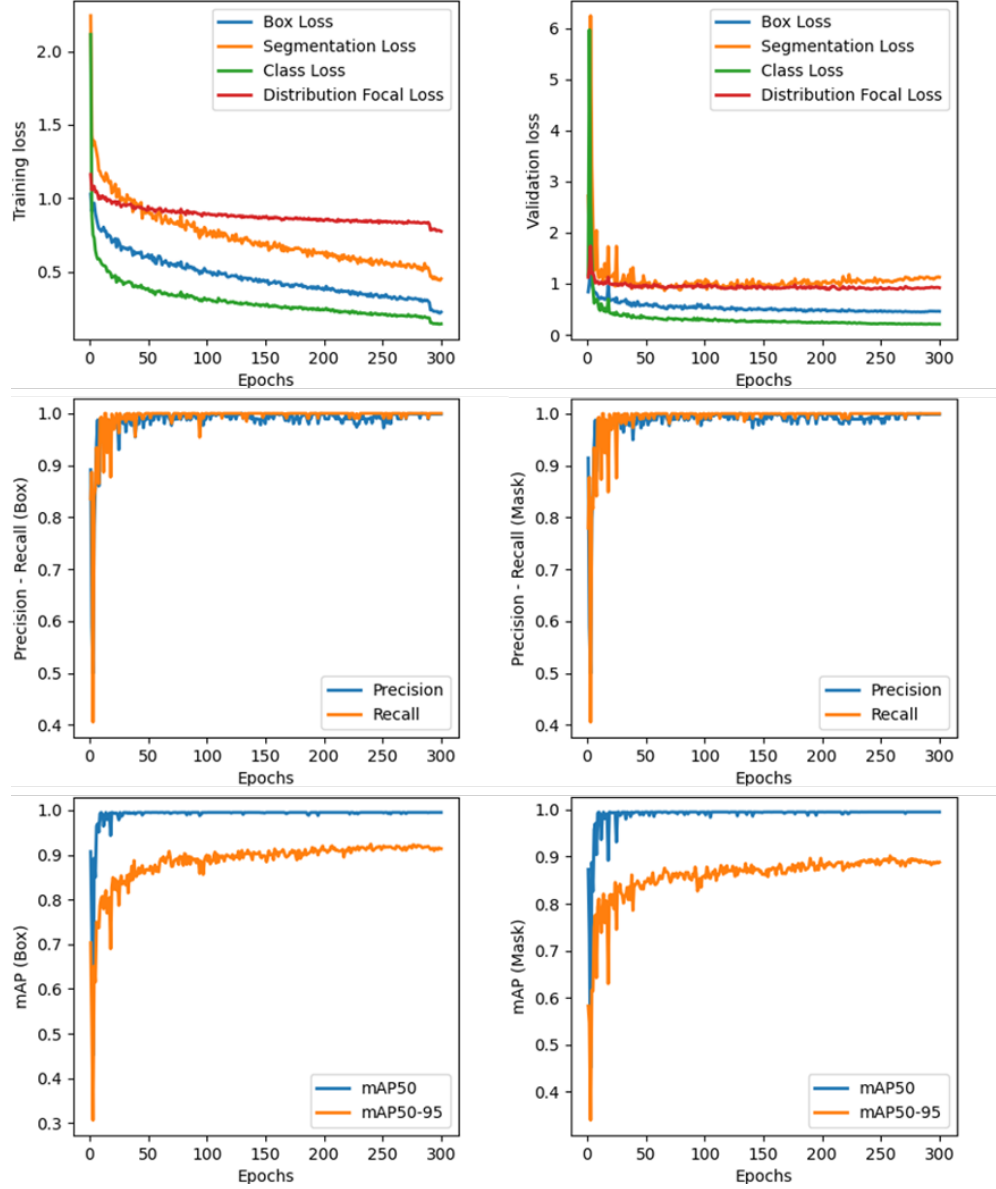


Figure 4.12: The model's training result after 300 epochs, with a batch size of 16 and a dropout of -0.1. The label "B" refers to the metrics for the bounding box segmentation, while "M" represents the metrics for the mask segmentation

90% for both bounding boxes and segmentation masks. The qualitative segmentation performance of the model is further illustrated in the figures below.

Experimental results demonstrate that the model is capable of accurately classifying and localizing objects with high confidence, even under varying imaging conditions. The predicted segmentation masks closely align with the ground truth, indicating that the model has effectively learned relevant morphological features. However, some limitations are still observed in regions with low cell density, where segmentation coverage is occasionally incomplete. This suggests that the model may not yet optimally capture complex boundary regions or subtle structural variations in sparse areas.

Notably, the model has demonstrated a certain level of robustness against common sources of noise in microscopic images. These include the presence of small, sparsely distributed cumulus cells in the surrounding medium, as well as structures with morphological features similar to the primary objects of interest. Such potential confounding elements were not misclassified by the model, indicating a relatively strong ability to distinguish between different morphological patterns and to focus on relevant target structures.

However, for applications involving the temporal monitoring of cumulus cell expansion, where high sensitivity to subtle changes in object area is required, further optimization of the model's accuracy is necessary. This is particularly important because cumulus cell clusters typically expand gradually, with only minor incremental changes over time. Accurately capturing these small variations requires the segmentation model to maintain consistently high precision, ensuring that no subtle morphological changes are overlooked during continuous microscopic observation.

b. Oocyte–cumulus cell matching performance

In simple cases such as the one illustrated in Figure 4.14, the true cumulus cell cluster corresponding to the oocyte can be identified relatively easily, since the other region recognized as cumulus cells is located too far from the oocyte to be considered a plausible match. At the same time, a cumulus cluster located near the center of the image does not adequately represent the more complex situations in which the proposed algorithm is actually needed. Therefore, to provide a more comprehensive evaluation of the algorithm's performance, further testing will be conducted on population-level data, where multiple cumulus cell regions may appear simultaneously in close proximity to different oocytes.

After the model inference step and extraction of coordinate data from the segmented masks, each oocyte is iteratively evaluated against all masks labeled as cumulus cell regions. A threshold parameter ε is defined as 1.5 times the thickness of the zona pellucida to determine spatial association. Based on this criterion, the pairing results between oocytes and their corresponding cumulus cell clusters are obtained as shown in Figure 4.16.

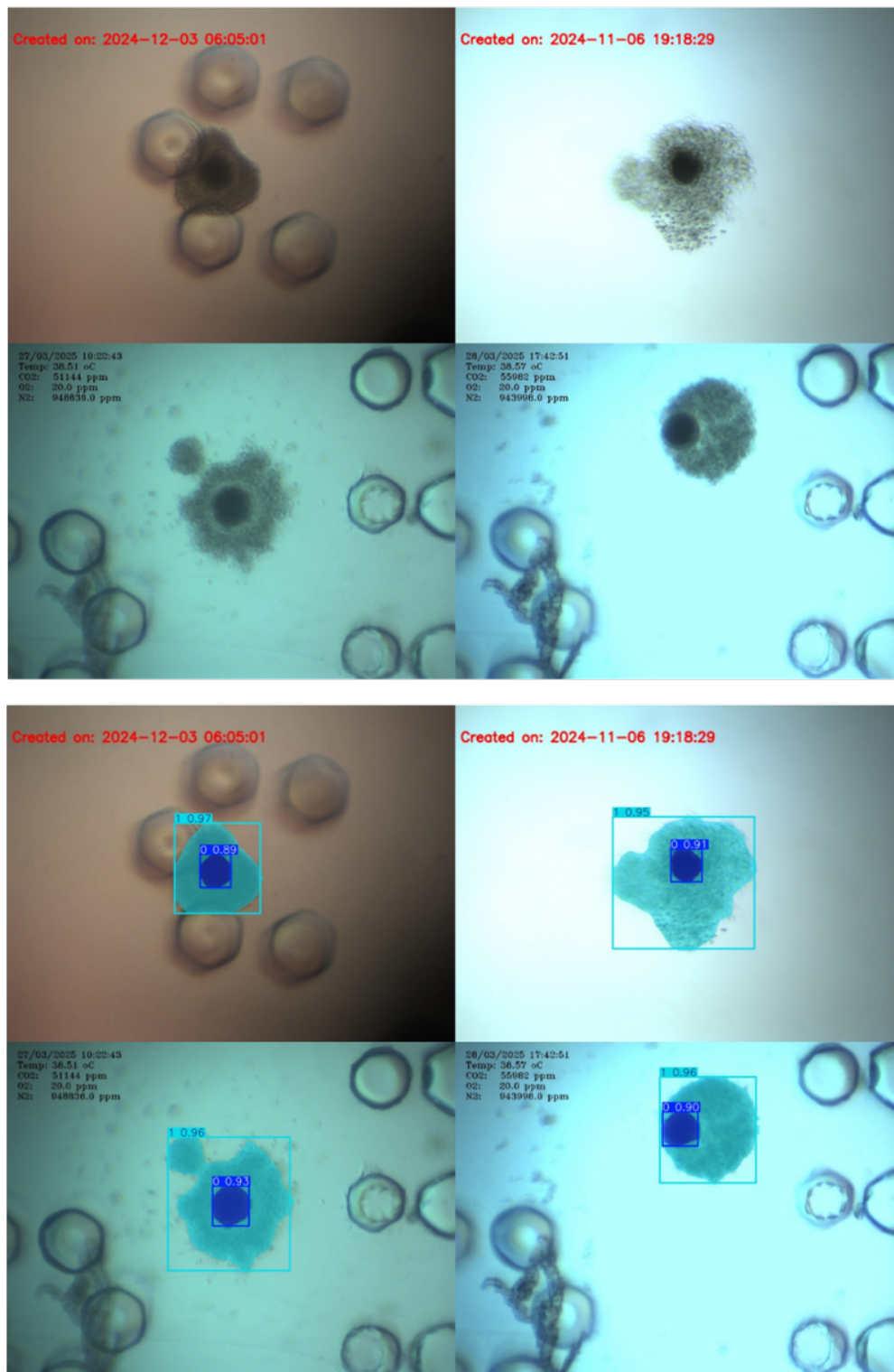


Figure 4.13: Original oocyte image (top) and the corresponding model prediction results (bottom), where class label 0 represents the oocyte and class label 1 represents the surrounding cumulus cell region

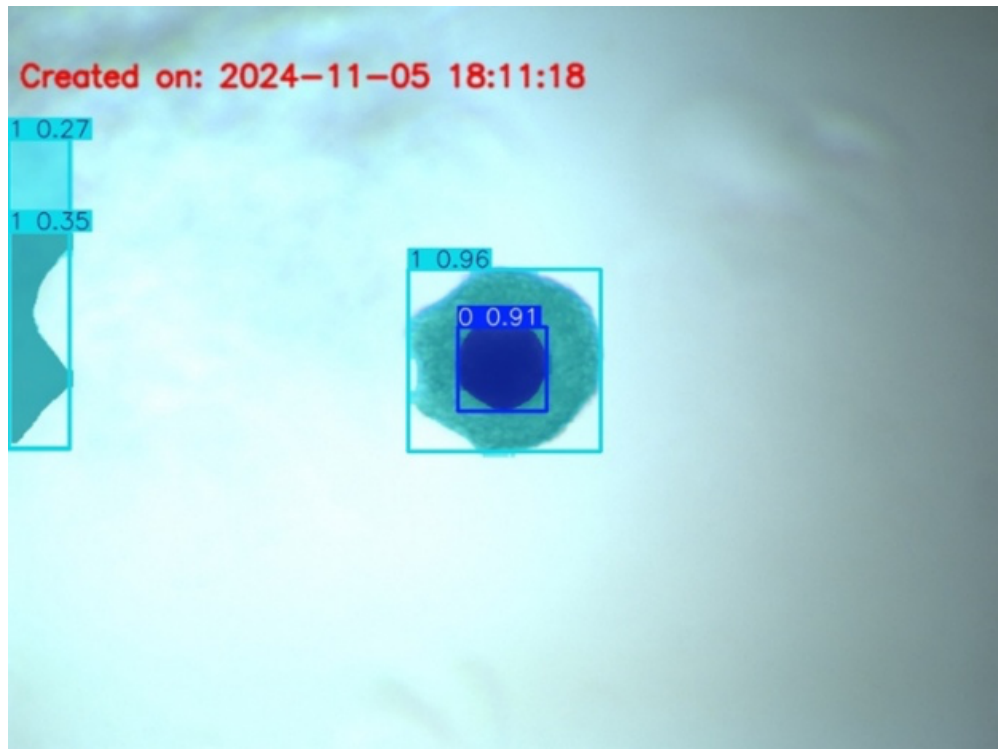


Figure 4.14: Illustration of a case with multiple predicted segmentation masks for the cumulus cell region

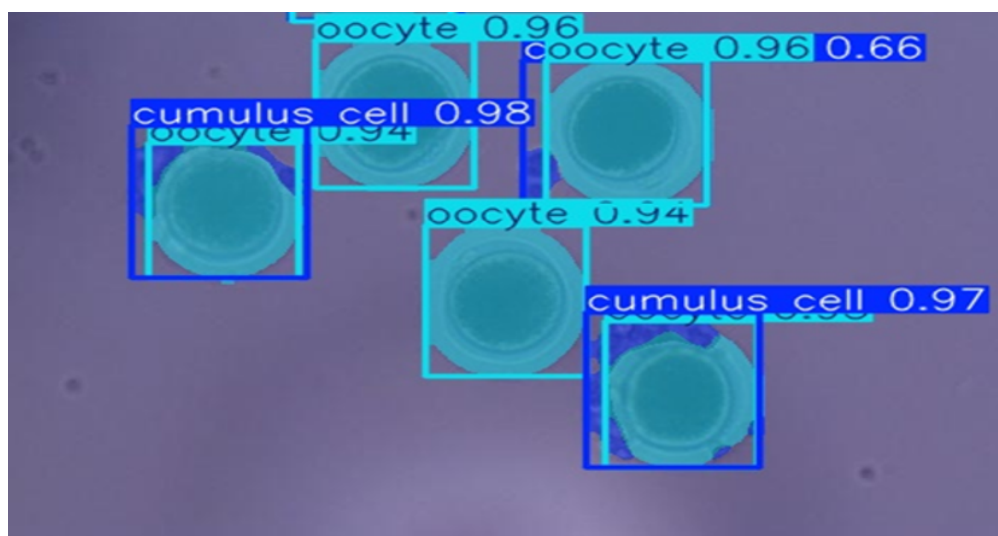


Figure 4.15: Image after model inference for oocyte-cumulus cluster association

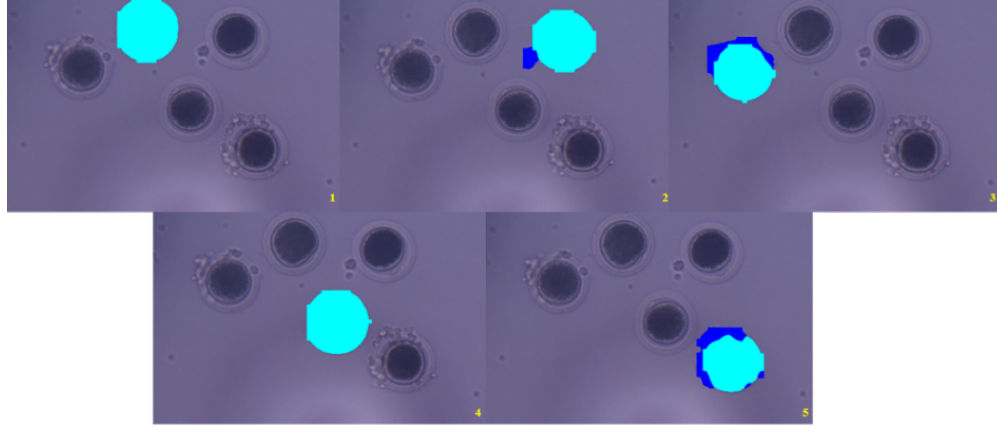


Figure 4.16: Cumulus cell–oocyte pairing results using the proposed algorithm

The figure above illustrates the outcome of applying the algorithm to identify and associate cumulus cell clusters with individual oocytes. The results demonstrate that the algorithm performs effectively, as reflected by its ability to correctly match oocytes with their corresponding cumulus regions. Even in relatively complex scenarios such as when a single cumulus cluster is located in close proximity to two neighboring oocytes, the algorithm maintains a high level of accuracy in determining the correct association.

Furthermore, the performance of the algorithm is influenced by the extent to which the cumulus cell cluster surrounds the oocyte. Specifically, regions with a higher coverage ratio along the oocyte perimeter tend to be paired more accurately. This can be explained by the geometric characteristics of the segmentation masks. Since these masks are typically non-convex polygons, their centroids often lie close to the centroid of the corresponding oocyte mask. As a result, a smaller Euclidean distance between the two centroids increases the likelihood of correct pairing, thereby improving the overall reliability of the algorithm.

c. Cumulus cell expansion assessment during oocyte maturation

Deep learning was employed to automatically evaluate the size distribution of COCs trapped within the OvaOoC chip. As presented in Figure 4.17, the histogram shows the distribution of COC sizes captured in the microfluidic chip. Since the introduced oocyte population was randomly collected without prior size-based selection, the observed distribution reflects the chip’s trapping performance across a broad size range. Most trapped oocytes were concentrated within the 150–210 μm interval, representing approximately 57.56% of the total population, indicating that this range corresponds to the predominant oocyte size in the examined samples. In contrast, the proportion of trapped oocytes was lower in the smaller size groups, such as 100–120 μm and 120–150 μm , while larger oocytes in the 250–350 μm range were rarely observed, accounting for only about 3.18%. These findings confirm that the microfluidic chip is

capable of accommodating oocytes with varying diameters, supporting its applicability for IVM-related studies.

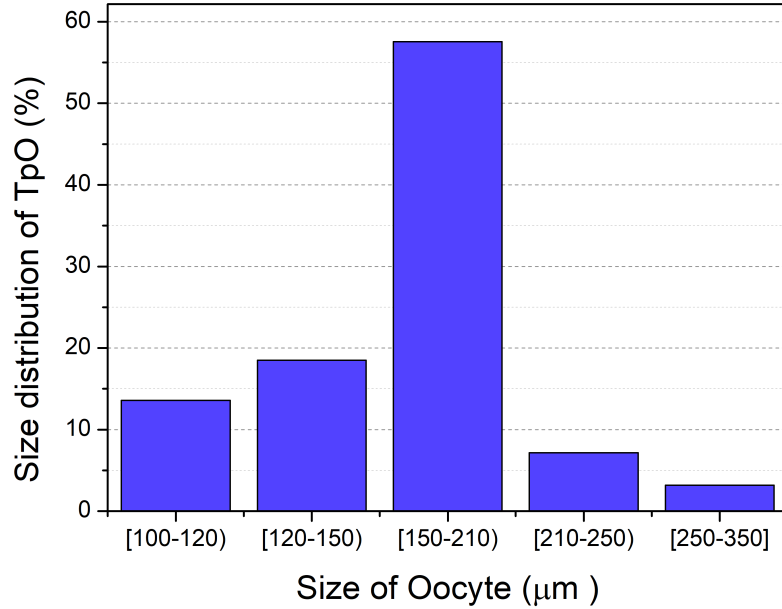


Figure 4.17: Size distribution of trapped COCs

To further monitor oocyte development, the culture platform was integrated with a real-time imaging system that continuously recorded morphological changes during culture. The acquired time-lapse images were used to construct a dataset for a deep learning model, which was trained to segment both the oocytes and their surrounding cumulus cells, thereby enabling quantitative assessment of cumulus expansion throughout development. Following segmentation, the cumulus area was calculated based on pixel-derived measurements. As an example, Figure 4.18 presents the morphological progression of one oocyte over an 18 h culture period, together with its corresponding segmented images. The model successfully identified the cumulus region and generated area measurements for each time point. The results showed an overall increasing trend in cumulus cell area during the 18 h observation period, with an expansion of nearly 25% relative to the initial state. This increase suggests progressive oocyte maturation, likely supported by effective nutrient exchange and a low-shear microenvironment within the chip.

Overall, the experimental results demonstrate the capability of the FEMI system to analyze and extract quantitative information on cumulus cell area and its temporal growth during culture. This parameter is considered one of the most reliable indicators for evaluating cumulus expansion and is closely associated with oocyte maturation as well as subsequent embryonic developmental potential [144]. Previous studies have investigated cumulus expansion as a non-invasive morphological indicator of oocyte

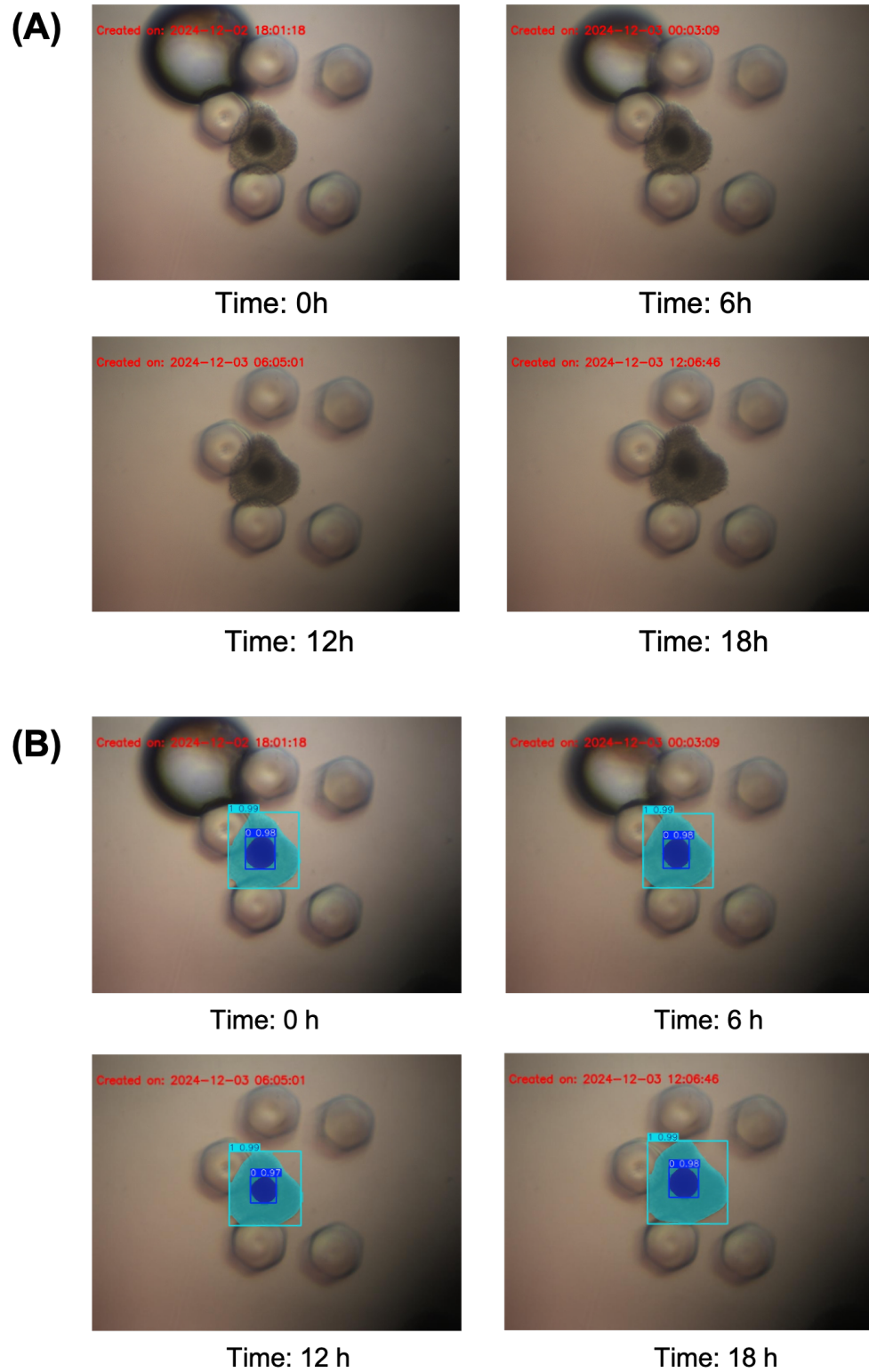


Figure 4.18: (a) Representative time-lapse images showing the oocyte maturation process over an 18-hour period, which were used as input for model prediction. (b) Predicted output images generated by the model, where Class 0 represents the oocyte and Class 1 represents the cumulus cells

competence, with reported associations with meiotic maturation and early embryonic development [145], [146], [147]. However, further studies are required to correlate these parameters with biological outcomes such as maturation rate, fertilization, cleavage, and blastocyst development.

4.3.3 Oocyte denudation on-chip

The migration behavior of COCs within the microfluidic device was captured using a high-speed camera operating at 100 frames per second, as presented in Figure 4.19. A notable event observed was the direct contact between an oocyte, surrounded by a compact cumulus cell layer, and the micropillars, as shown in Figure 4.19(a). At the points of contact, the mechanical impact generated sufficient force to detach a substantial portion of the surrounding cumulus cells, as shown in Figure 4.19(b). In addition, following collision, the oocyte exhibited rotational motion, which increased the contact area between the COC and the micropillars and thereby promoted further cumulus cell removal. As the oocyte continued to move through the structure, repeated interactions with subsequent pillars sustained the denudation process.

The pillar geometry was designed to localize mechanical and shear forces primarily at the cumulus cell layer surrounding the oocyte, enabling efficient cell removal while preserving the structural integrity of the oocyte. As shown in Figure 4.19(c), after passing through the micropillar array, the oocyte retained only a minimal amount of cumulus cells, indicating a high denudation efficiency. This denudation mechanism is advantageous because the controlled collisions with the micropillars reduce reliance on excessively high hydrodynamic shear stress, thereby providing a more targeted and controlled approach for cumulus cell removal. To ensure stable high-throughput denudation, only chips with fabrication deviations within $\pm 5\%$ of the design dimensions were used. In addition, the micropillar height ($200\ \mu\text{m}$) and minimum inter-pillar spacing ($120\ \mu\text{m}$) were maintained to provide consistent mechanical confinement and oocyte passage.

Figure 4.20(a) presents the COCs prepared for the denudation process following the IVM procedure. Mature oocytes were identified by the presence of the first polar body, which serves as a key indicator of successful meiotic maturation. After denudation using the proposed DENO chip, the oocytes were collected for subsequent assessment and *in vitro* fertilization, as shown in Figure 4.20(b). The system was further evaluated in a single run with 100 oocytes, and the collected samples confirmed the successful implementation of the denudation process. As illustrated in Figure 4.20(c), denuded oocytes were obtained within 90 s in one experimental run using the proposed chip, demonstrating its high-throughput performance compared with previously reported microfluidic platforms.

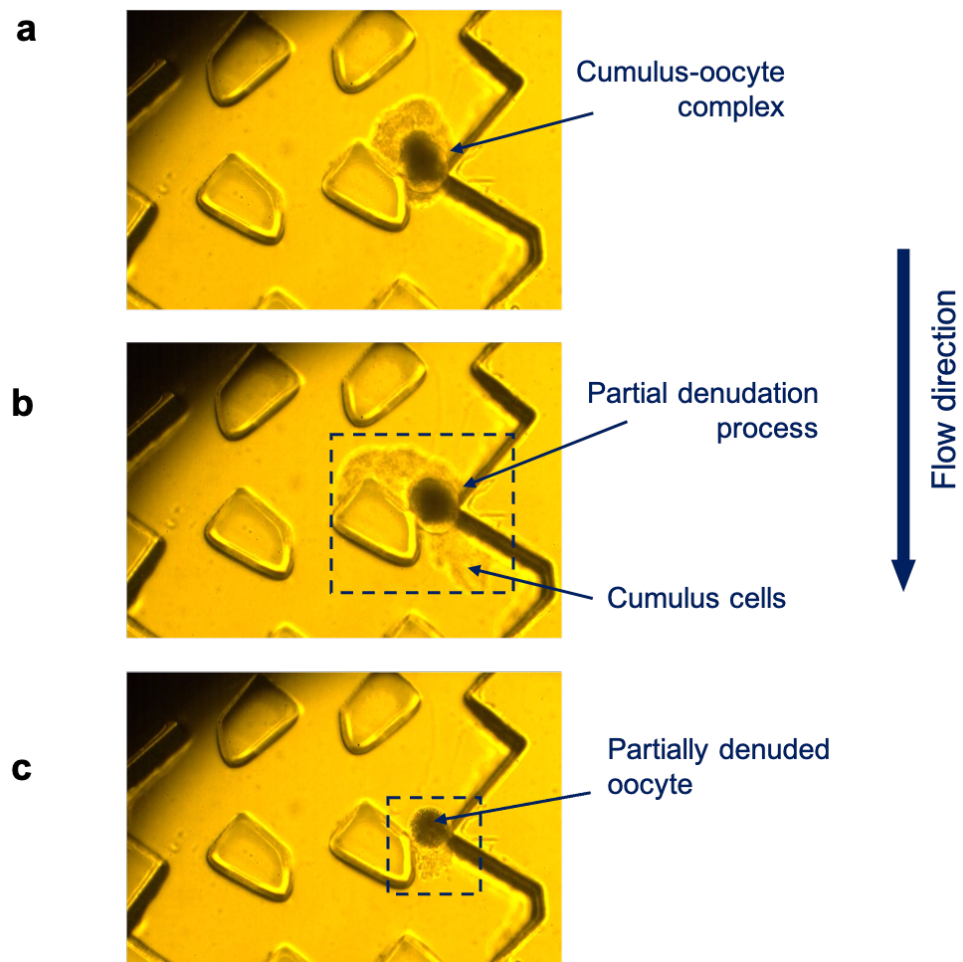


Figure 4.19: Partial denudation process captured by a high-speed camera at three time points: before (a), during (b), and after (c) the collision with a row of micropillars

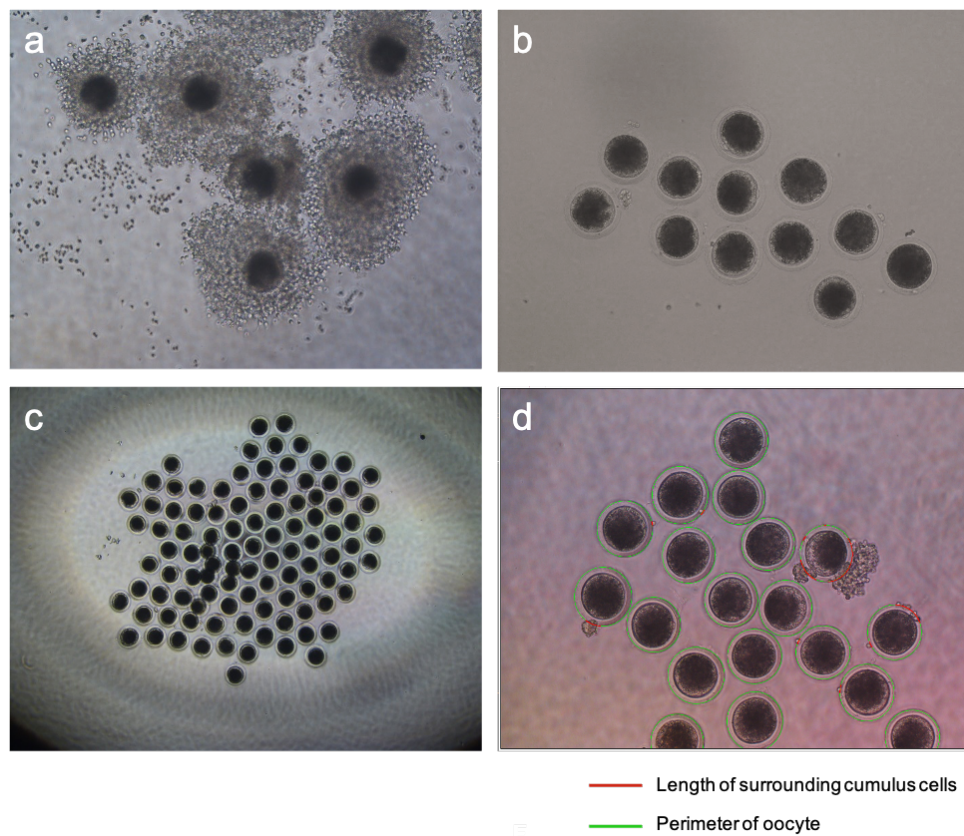


Figure 4.20: Morphology of oocytes before (a) and after the denudation process (b), 100 oocytes denuded in a single run (c), ImageJ processing of the denuded oocytes for evaluation of denudation efficiency (d)

To quantify denudation efficiency, all collected oocytes were analyzed using ImageJ. In this analysis, the green line represents the oocyte perimeter, while the red line indicates the cumulative length of the remaining surrounding cumulus cells, as shown in Figure 4.20(d). In this study, denudation was considered successful when the ratio of the non-cumulus-covered oocyte perimeter to the total oocyte perimeter exceeded 70%. This threshold was selected because a partial retention of cumulus cells may still be beneficial, as incompletely denuded oocytes have been reported to support improved embryo quality and blastocyst development [148], [149]. Previous studies have also highlighted the important role of cumulus cells in enhancing fertilization capacity and supporting the developmental competence of the oocyte cytoplasm [148], [149]. However, despite these beneficial effects, cumulus cells can also hinder sperm access to the oocyte, making their removal necessary for efficient IVF procedures. This is particularly important in ICSI, where the PB1 is typically positioned at either the 6 o'clock or 12 o'clock orientation to avoid damaging the meiotic spindle during sperm injection, thereby reducing the risk of aneuploidy [150]. The sperm is then commonly injected at the 3 o'clock or 9 o'clock position. Residual cumulus cells located at these positions may obstruct the injection process and compromise procedural success. Figure 4.21 presents representative examples of oocyte morphology after unsuccessful and successful denudation, as assessed using ImageJ.

The performance of the microfluidic device was quantitatively evaluated based on the oocyte yield rate and denudation efficiency. The yield rate was defined as the ratio of the number of oocytes collected at the outlet to the number of oocytes introduced at the inlet. The denudation efficiency was defined as the ratio of successfully denuded oocytes to the total number of collected oocytes. In addition, the damage rate was calculated as the ratio of damaged oocytes to the total number of collected oocytes. In this study, these performance metrics were assessed using COCs cultured for two days at the pre-fertilization stage, when cumulus expansion was more pronounced [151]. During the experiments, multiple operating conditions were investigated to optimize the denudation procedure, since complete on-chip denudation often required several processing cycles, together with flow-rate adjustment to achieve an optimal balance between denudation efficiency and oocyte integrity. The results demonstrated that the proposed platform achieved a consistently high throughput, with a yield rate of 100% under all tested conditions.

The effect of the number of processing rounds on denudation performance was first investigated. In this study, one round was defined as the transport of oocytes from the inlet to the outlet, followed by their return to the inlet for reprocessing. After a single round, the denudation efficiency remained low. However, as the number of rounds increased, the denudation efficiency improved markedly, as shown in Figure 4.22. After two rounds, the efficiency reached approximately 45%, with no observable oocyte

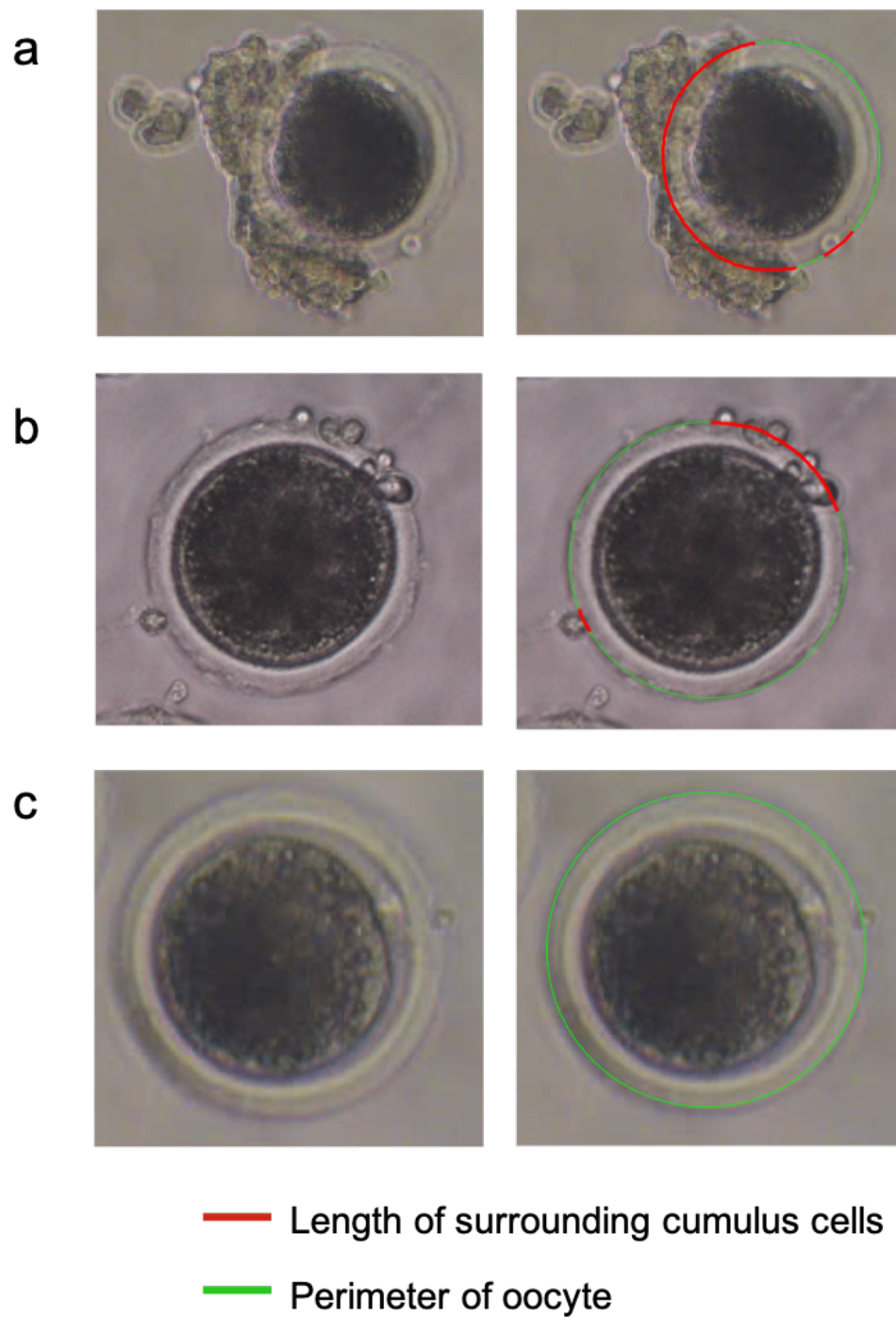


Figure 4.21: Morphology and ImageJ evaluation of (a) unsuccessful denuded oocyte, (b) successful denuded oocyte (exceeding the 70% threshold), (c) fully denuded oocyte

damage. When both denudation efficiency and damage rate were taken into account, three rounds were identified as the optimal condition, yielding a denudation efficiency of $95.94 \pm 0.94\%$ and a damage rate of $2.67 \pm 1.34\%$. Although four rounds resulted in a slightly higher denudation efficiency of $96.43 \pm 1.80\%$, the damage rate increased substantially to $10.33 \pm 2.91\%$. These results indicate that three rounds provide the most favorable balance between effective cumulus cell removal and preservation of oocyte integrity. While additional rounds may further improve denudation efficiency, they also substantially increase the likelihood of oocyte damage, thereby compromising the overall quality of the collected samples.

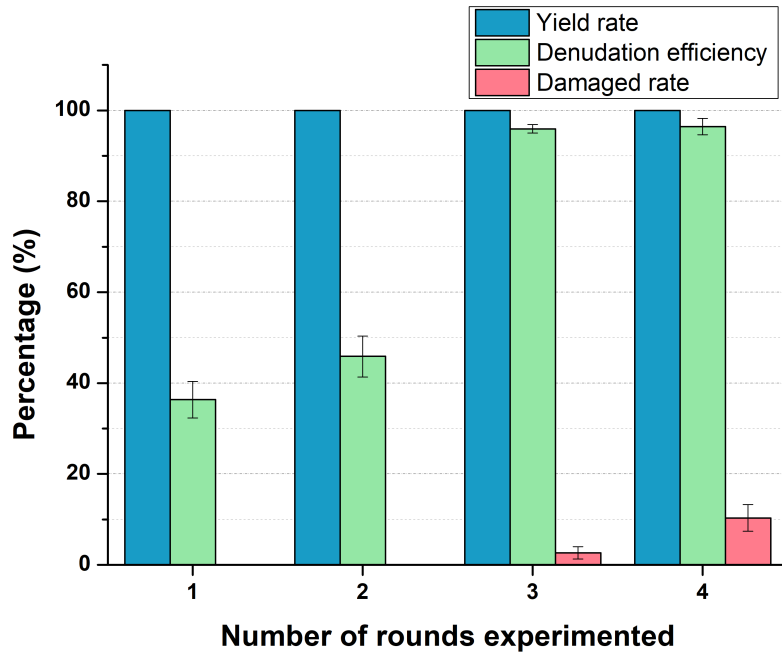


Figure 4.22: Effects of the number of rounds on denudation efficiency and damage rate

Although the proposed system demonstrated promising performance, the present results suggest that further refinement is still needed to achieve complete denudation in a single pass. Accordingly, future work will focus on optimizing the microstructure design and flow conditions to further enhance denudation efficiency while minimizing cellular damage.

Figure 4.23 further illustrates the effect of inlet flow rate on the performance of the proposed microfluidic chip over the tested range of 0.25–1.5 mL/min after three rounds of on-chip denudation, in agreement with the simulation results presented in the manuscript. The experimental results indicate that at relatively low flow rates (0.25–0.5 mL/min), the generated shear stress remains low, resulting in negligible oocyte damage, which is consistent with the simulation outcomes. However, under these conditions, the denudation efficiency also remains limited. In contrast, at higher flow rates (1.25–1.5 mL/min), the increased hydrodynamic forces lead to a higher damage rate. Moreover,

when the flow rate becomes excessively high, the COCs travel through the micropillar region too quickly, reducing the contact time required for effective cumulus cell removal. Based on these observations, the optimal inlet flow rate was determined to be 1 mL/min, which was therefore adopted for all experiments reported in this manuscript.

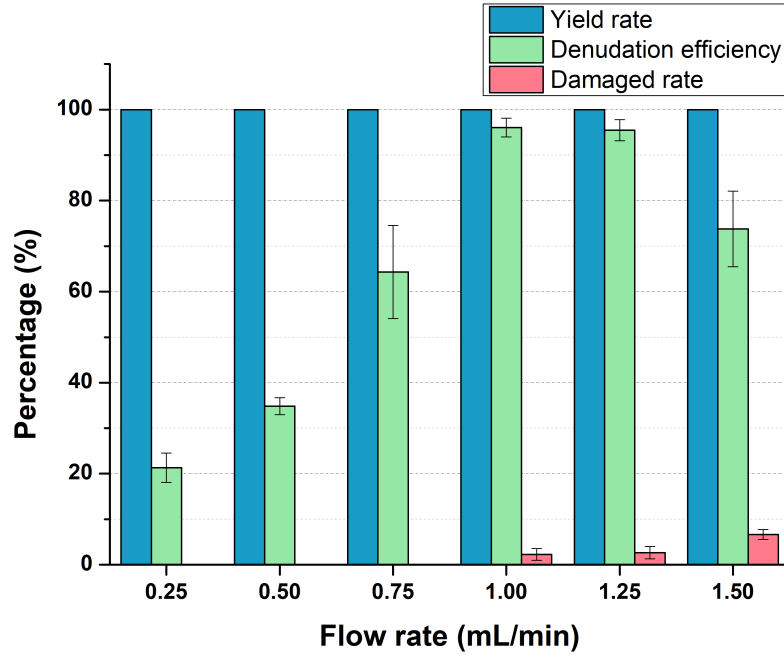


Figure 4.23: Effects of inlet flow rate on oocyte denudation efficiency

The slight differences observed between the results obtained from the flow-rate study and those from the investigation of denudation performance as a function of round number due to the two experiments being conducted separately. Nevertheless, the optimal operating conditions identified in both cases were highly consistent. In addition, the micropillar array offers further potential for scaling up processing throughput, while its geometric design may be further tailored to enhance denudation performance for different biological samples. The integrated micropillar structure promotes effective mechanical denudation under relatively low shear stress conditions, while the elimination of external actuation components simplifies both device fabrication and experimental implementation. Overall, the experimental results demonstrate the effectiveness of the chip and highlight several advantages over previously reported systems, including higher throughput, simpler operation, and reduced risk of thermal effects. A comparative summary of the key performance metrics is provided in Table 4.2.

Table 4.2: Comparison of current methods for oocyte denudation

	Refs	Denudation efficiency	Yield rate	Throughput/ Capacity	Shear stress	Time required	Enzyme required	Status	Limitations
Manual Denudation (pipette aspiration by embryologist) – Gold standard in ART labs.		Vary significantly with operator ~81.2% (± 2.7) fully denuded [152]	Vary significantly with operator ~86.9% (± 1.7) [152]	One oocyte at a time (serial processing)	High – 149.8 to 392.0 Pa [126] (flow rate generated by pipetting from 0.5 to 1 mL/min) (large due to manual force)	Vary significantly with operator ~5 min per oocyte [153]	Yes – hyaluronidase (standard pre-treatment)	Clinical standard	Labor-intensive; outcome varies with operator skill; exposes oocytes to high shear stress; one-by-one processing; requires enzymatic treatment
Robot-Assisted Denudation (automated micropipette system with vision control)	[152]	95.0% (± 0.8) completely denuded	97.0% (± 2.8)	One COC at a time (automated sequential handling)	High (similar to manual pipetting, but flow is precisely controlled to avoid extremes)	N/A (approximately similar per oocyte to manual)	Yes	Research	Requires complex and costly robotic hardware/software; still one-by-one (low throughput); requires enzymatic treatment
Microfluidic – Pilot Study	[154], [155]	100% (25/25 zygotes fully denuded)	100% (no oocyte loss)	One COC at a time (single device can only process one - three zygotes per run)	N/A	N/A (slow due to multi-step syringe operation)	N/A	Research	Low throughput (one-by-one) and required manual switching of flows and enzymatic treatment
Microfluidic – Jagged Constriction Chip	[126]	22.2%–98.6% (range depending on flow and design)	Maximum 98.9%	10 oocytes per run	64.1 to 163.5 Pa (flow rate increases from 0.5 to 1 mL/min)	30 s per experiment	Yes	Research	Low throughput (serial processing); requires enzymatic treatment
Microfluidic – Jagged Surface and Trap	[153]	80% (8/10 oocytes fully denuded)	100% (10/10 oocytes retained intact)	N/A (<i><10 oocytes</i>)	Not exceed 0.12 Pa at 0.16 mL/min flow	20 s per oocyte (denudation and holding)	Yes	Research	Low throughput (serial processing); Requires enzymatic treatment

	Refs	Denudation efficiency	Yield rate	Throughput/ Capacity	Shear stress	Time required	Enzyme required	Status	Limitations
Microfluidic – SAW-Based	[156], [157]	96.8% (area metric)	100%	Up to 30 oocytes per run	Low (non-contact denudation)	< 3 min per experiment	Yes	Research	Requires specialized acoustic transducers and precise setup; temperature fluctuation; complex fabrication; requires enzymatic treatment
This study: Microfluidic Micropillars-Based		95.94% (± 0.94) (length metric)	100%	High (can process 100 oocytes per run)	Low: 5.31 to 23.09 Pa (flow rates of 0.25 to 1.0 mL/min)	1 min 30 s per experiment	Yes	Research	Requires low concentration enzymatic treatment

To verify that the automated denudation process using the proposed chip does not compromise the fertilization capacity or developmental competence of denuded oocytes, IVF outcomes were evaluated and compared between oocytes denuded by the microfluidic device and those processed by the conventional manual method, as shown in Figure 4.24. In this study, the number of cleaved embryos refers to the total number of embryos that underwent early embryonic division, typically reaching the 2-, 4-, or 8-cell stage by day 2–3 of culture. The cleavage rate was calculated as the proportion of cleaved embryos relative to the total number of fertilized oocytes, representing the initial developmental competence of the treated oocytes. The number of blastocysts refers to the embryos that successfully developed to the blastocyst stage, characterized by the formation of a fluid-filled cavity and distinct cellular organization. The blastocyst rate was determined as the proportion of blastocysts relative to the total number of cleaved embryos, thereby reflecting the ability of embryos to continue development beyond the cleavage stage. The results showed that the cleavage rate of oocytes denuded using the DENO chip was comparable to that obtained with the manual denudation method under parthenogenetic activation.

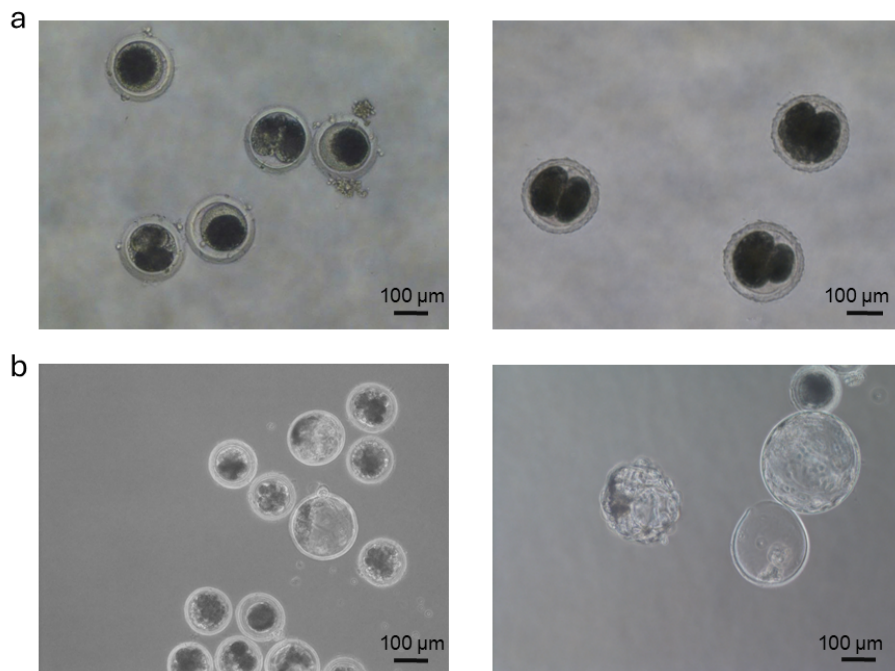


Figure 4.24: (a) Cleaved embryos and (b) Blastocyst-stage embryos obtained following the IVF procedure of oocytes denuded using the on-chip method

For the experimental comparison, oocytes in the on-chip group were denuded using the proposed microfluidic system developed in this study, in which the culture medium was supplemented with 0.01% hyaluronidase to enzymatically assist cumulus cell removal. In parallel, oocytes in the control group were denuded manually by trained embryologists

using the conventional protocol with 0.1% hyaluronidase. Mature oocytes obtained from both methods were subsequently transferred to a porcine IVF fertilization medium formulated to maintain ionic balance, support sperm activation, and facilitate sperm–oocyte interaction. Thawed sperm were introduced at two concentrations, 10^5 and 10^6 sperm/mL. Fertilization was carried out under controlled conditions at 38.5°C in an atmosphere of 5% CO_2 and 90% N_2 for 3–6 h. Presumptive zygotes were then collected and cultured in conventional culture dishes at 38.5°C and 5% CO_2 for 7 days to assess cleavage and blastocyst development.

As summarized in Table 4.3, the cleavage and blastocyst formation rates obtained with the proposed on-chip denudation system were comparable to those achieved using the standard manual procedure under IVF conditions. Notably, the on-chip group exhibited slightly higher average values for both cleavage and blastocyst formation ($p = 0.04$, paired t-test), suggesting that the proposed microfluidic system can preserve oocyte quality while offering potential advantages for IVF performance.

Table 4.3: Comparison of cleaved embryos and blastocyst rates using denuded oocytes from manual and on-chip oocyte denudation procedures

Denudation method	Experiment number	Sperm concentration	Number of oocytes in-seminated	Number of cleaved embryos (%)	Number of blastocysts (%)
On-chip	#1	10^5	50	39 (78%)	8 (20.51%)
	#2	10^6	45	39 (86.67%)	9 (23.08%)
	#3	10^5	40	34 (85%)	3 (8.82%)
	#4	10^6	39	35 (89.74%)	8 (22.86%)
Average				84.85%	18.82%
Manual	#1	10^5	67	57 (85.07%)	6 (10.53%)
	#2	10^6	58	51 (87.93 %)	11 (21.57%)
	#3	10^5	39	28 (71.79%)	0 (0)
	#4	10^6	40	36 (90%)	4 (11.11%)
Average				83.7%	10.8%

The comparison between on-chip and conventional manual cumulus denudation

demonstrated that oocytes processed using the proposed microfluidic system not only preserved a high cleavage rate following IVM and IVF, but also achieved a higher average blastocyst formation rate from cleaved embryos (18.82%) than those treated by the manual method (10.8%). This improvement may be attributed to the milder and more controlled conditions of the on-chip denudation process. In particular, the microfluidic approach required only one-tenth of the hyaluronidase concentration used in the conventional protocol (0.01% versus 0.1%), which may reduce enzymatic damage to the oocyte surface and zona pellucida and thereby support improved blastocyst development [158].

Moreover, cumulus removal in the microfluidic system was completed within a short processing time, substantially limiting oocyte exposure to both enzymatic treatment and mechanical stress. In contrast, conventional manual denudation involves repeated pipetting, which exposes oocytes to less controlled and operator-dependent mechanical shear forces. By comparison, the microfluidic chip generates fluid-induced shear stress in a controlled and reproducible manner over a brief period. Together with the reduced enzyme concentration and shorter exposure time, these factors likely contribute to better preservation of oocyte developmental competence.

It should also be noted that the relatively low overall blastocyst formation rates observed in both groups are consistent with previous reports on porcine IVF systems [159], [160], [161]. In this study, IVF was primarily performed to evaluate the biological compatibility of the proposed microfluidic cumulus denudation process. The comparable cleavage rates, together with the higher blastocyst yield observed in the on-chip group, indicate that the proposed system does not adversely affect fertilization or subsequent embryonic development and may serve as a promising alternative to the conventional manual method.

When compared with the standard denudation procedure currently used in clinical IVF laboratories, the proposed chip offers several notable advantages. Manual pipetting of COCs following enzymatic treatment remains the conventional approach for oocyte denudation [162]. As with many manual procedures in assisted reproductive technologies, this method is highly operator dependent, lacks standardization, is labor intensive, and its performance is strongly influenced by the skill and consistency of the practitioner [126], [163]. For example, Zhai *et al.* [152] reported that manual pipetting in mice performed by an experienced operator resulted in the recovery of only 86.9% of oocytes, with 81.2% being fully denuded, and the outcomes varied depending on flow control and manipulation technique. In contrast, the proposed microfluidic chip achieved a denudation efficiency of $95.94 \pm 0.94\%$ while maintaining a 100% oocyte recovery rate at the outlet.

Another advantage of the micropillar-based chip lies in its processing speed and throughput. Manual pipetting generally requires several minutes to denude a single

oocyte [153], making the procedure time-consuming and inherently limited in processing capacity. By comparison, the proposed chip was able to process approximately 100 oocytes within 90 s, demonstrating substantial improvement in both speed and throughput. This high-throughput capability markedly reduces hands-on time and improves procedural efficiency relative to manual denudation. In the conventional porcine oocyte denudation protocol, COCs are typically incubated in medium containing 0.1% (w/v) hyaluronidase for 2 min, followed by mechanical denudation of individual oocytes through gentle pipetting in enzyme-free medium [137]. Such a workflow may prolong the total processing time and increase the duration for which each oocyte is handled. In contrast, by integrating enzymatic treatment and physical cell removal within a continuous-flow process, the proposed chip enables efficient denudation while substantially reducing both the enzyme concentration required and the enzyme exposure time, using medium containing only 0.01% (w/v) hyaluronidase.

In addition, the proposed chip provides a mechanically gentler environment for oocyte handling. Manual pipetting has been reported to generate relatively high and inconsistent shear stresses, ranging from 149.8 to 392.0 Pa depending on the applied flow conditions at the pipette tip [126]. By contrast, the proposed chip generates considerably lower and more controlled shear stress levels, calculated to range from 5.31 to 23.09 Pa at flow rates of 0.25–1.0 mL/min. This more controlled hydrodynamic environment is expected to better preserve oocyte integrity during denudation. Taken together, these results highlight the major advantages of the proposed chip over manual pipetting in terms of processing speed, throughput, mechanical gentleness, and operational consistency. By reducing operator dependence and enabling the simultaneous processing of large numbers of oocytes, the platform represents a promising and scalable alternative to conventional denudation methods in assisted reproductive technologies.

Despite these advantages, several limitations of the current chip design should also be acknowledged. Under the present configuration, optimal denudation efficiency was achieved only after three processing rounds on the chip. Although the total processing time remained relatively short, the need for repeated cycles may reduce procedural simplicity and could present practical challenges for routine clinical application. Therefore, future work should focus on further structural optimization of the device, particularly through expansion and refinement of the micropillar array, to enable effective single-cycle denudation while maintaining oocyte integrity and developmental competence.

4.3.4 DEP-based oocyte classification on chip

In the sorting experiments, the DEP system was operated at 6 Vpp and 1 MHz, with the microfluidic chip placed on a warming stage maintained at 38.5°C, corresponding approximately to the physiological body temperature of pigs. These conditions were

selected to provide sufficient DEP force while maintaining a physiologically relevant thermal environment for the oocytes. Under this experimental configuration, increasing the applied voltage to approximately 12 Vpp resulted in visible bubble formation inside the microchannel, indicating excessive Joule heating effects at the electrode–medium interface. Therefore, 12 Vpp was considered the practical upper operating limit of the present device under the specified temperature, medium, and chip conditions. Under room-temperature conditions, where greater thermal margin is available, higher applied voltages may be used before visible bubble formation occurs. A mixed oocyte population was introduced into the microwell, consisting of three morphologically normal oocytes and five poor-quality oocytes. The poor-quality group included degenerated oocytes showing signs such as swelling, membrane disruption, nuclear damage, or other visible structural abnormalities. Before electric field application, all oocytes were randomly distributed over the electrode surface to better evaluate the intrinsic selectivity of the DEP-based sorting mechanism.

After the electric field was established, a short period elapsed before the dielectrophoretic response became clearly observable. After a while, the difference in motion between the two oocyte groups became pronounced. The three good-quality oocytes, which presumably retained more intact membrane structure and intracellular organization, exhibited a clear pDEP response. Under the influence of the non-uniform electric field, these oocytes gradually migrated along the field gradient toward regions of higher electric field intensity, eventually accumulating near the edges of the rectangular electrode, where the electric field was strongest. Their trajectories were consistent with the expected behavior of highly polarizable particles in a pDEP regime.

In contrast, the remaining five poor-quality oocytes displayed nDEP or negligible DEP response. Rather than being attracted toward the electrode edges, these oocytes moved away from the high-field regions and migrated toward the central axis of the microwell, corresponding to a relatively low-field zone. This opposite migration pattern suggests that their effective dielectric properties differed substantially from those of the normal oocytes, likely due to compromised membrane integrity, altered cytoplasmic composition, or structural degeneration affecting their polarizability under the applied AC field.

The experimental results showed good agreement with both the theoretical framework and the physical simulations established earlier. According to DEP theory, the force acting on a biological particle depends on the Clausius–Mossotti factor and the gradient of the squared electric field magnitude. As a result, oocytes with different dielectric properties are expected to exhibit distinct migration behaviors when exposed to a non-uniform electric field. Consistently, the simulations showed that oocyte trajectories followed the electric field distribution and separated near the channel outlets according to their dielectric properties. Experimentally, the good-quality oocytes were observed

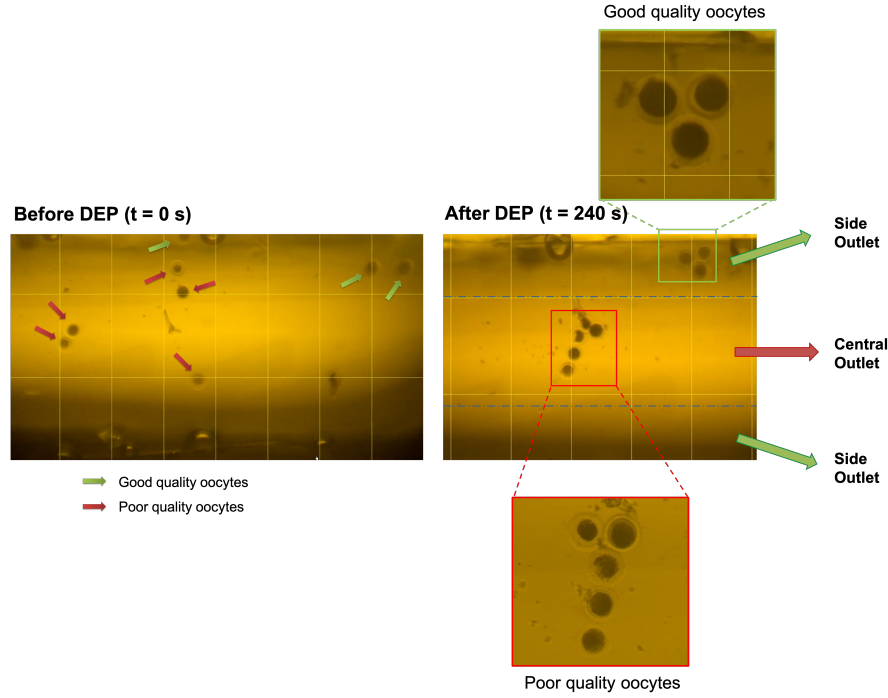


Figure 4.25: Dielectrophoretic-based separation of good quality and abnormal oocytes

to move toward the electrode edges after approximately $t = 240$ s, which is consistent with both the simulation results and the theoretical predictions. This agreement not only supports the validity of the electrohydrodynamic model developed in this study, but also demonstrates its capability to reliably predict oocyte behavior in a microfluidic system integrated with a non-uniform electric field. However, the variation in applied voltage between the simulation and experimental results can be attributed to factors such as differences in electrode material and alignment, variations in the culture medium and the dielectric properties of the oocytes, as well as ambient temperature effects on the DEP.

Overall, these preliminary results provide initial evidence of the potential of the proposed system for DEP-based oocyte sorting. Further work will include repeated experiments with larger sample sizes, clearly defined quantitative classification criteria, and systematic evaluation of sorting accuracy, sensitivity, and specificity. These studies will enable a more rigorous assessment of the reliability and reproducibility of the proposed system. In addition, the ability to electrically manipulate oocytes may support broader applications in assisted reproductive technologies, including IVF, ICSI, and other oocyte-handling procedures.

4.4 Summary of Chapter 4

In summary, Chapter 4 has presented and discussed the fabrication, simulation, and experimental results of the proposed microfluidic platform for assisted reproductive applications. The fabricated microfluidic chips, including the micropillar structures and integrated electrode systems, were successfully developed and experimentally validated, showing good agreement with the proposed designs and operational principles. The simulation results further provided insight into fluid flow behavior, shear stress distribution, trapping performance, and DEP-induced manipulation, thereby supporting the theoretical foundations presented in the previous chapters.

Experimental investigations demonstrated the feasibility and effectiveness of the developed platform for single-oocyte trapping, dynamic culture, on-chip denudation, maturation monitoring, and DEP-based selection. The experimental observations showed good consistency with the numerical simulation results and theoretical predictions. In addition, comparisons with existing studies highlighted advantages of the proposed system, including enhanced maturation performance, improved throughput and controllability, and the potential for automated and continuous oocyte handling within a single platform.

Despite these promising results, several limitations remain, including fabrication constraints, variability in biological samples. Although the PDMS-based devices showed comparable or potentially improved performance relative to standard methods for oocyte culture and denudation, several material- and operation-related factors remain to be examined. The possible adsorption of proteins and hormones by PDMS may alter the effective composition of the culture medium, particularly during prolonged use. Bubble formation within the microchannels may also disturb perfusion, create local variations in the culture environment, and affect process stability. Further experimental validation is needed to evaluate these potential effects more thoroughly.

At the current stage, the culture, denudation, and DEP modules are being evaluated separately to characterize their performance and optimize the operating conditions of each system component. Their subsequent integration into a common microfluidic platform will require a coordinated sequential workflow because each processing stage involves different requirements for flow rate, residence time, medium composition, and electrical conditions. Although oocytes will be introduced through a single main sample inlet, additional auxiliary inlets may be required to deliver stage-specific solutions. For example, the denudation module may require the controlled introduction of an enzymatic solution to facilitate cumulus-cell removal, followed by a washing medium to terminate enzyme exposure and remove detached cells. Similarly, the DEP module may require medium exchange to obtain a defined conductivity suitable for reliable dielectric manipulation and assessment. The integrated system must therefore incorporate

appropriate flow-routing structures, washing regions, and waste outlets to control fluid delivery, prevent reagent backflow and cross-contamination, remove cellular debris, and minimize oocyte loss during transfer between modules. Future work will focus on optimizing these physical and operational interfaces and validating the complete workflow on a single integrated chip.

Conclusions

This thesis presents the development of a modular microfluidic platform aimed at streamlining key processes in the IVM workflow, including single-oocyte trapping, dynamic culture, maturation monitoring, denudation, and quality-based selection. By combining these functions within a microfluidic framework, the proposed system addresses the need for more controlled, standardized, and automation-compatible approaches in assisted reproductive technologies. At the center of this platform is the organ-on-chip module, OvaOoC, which enables precise immobilization and manipulation of individual oocytes and supports controlled on-chip culture under controlled microenvironmental conditions. This configuration provides an advantageous basis for monitoring single-oocyte development over time while maintaining a more consistent and reproducible culture environment. The simulation and experimental results demonstrate that the proposed platform achieves a high single-oocyte trapping efficiency with stable positioning, which supports reliable longitudinal monitoring and minimizes variability during culture. The implementation of dynamic perfusion and co-culture with cumulus cells contributes to improved mass transport and microenvironmental regulation, which may enhance oocyte maturation outcomes compared to conventional static systems. In addition, the integration of deep learning-based image analysis enables objective and automated evaluation of oocyte development, reducing reliance on subjective morphological assessment.

For post-culture processing, the microfluidic denudation module demonstrated high efficiency in removing cumulus cells while maintaining high processing throughput. Compared with conventional manual denudation, this approach reduces operator dependency, shortens handling time, and offers a more controlled mechanical process. These characteristics are particularly meaningful for applications requiring the processing of large numbers of oocytes, where manual methods may become labor-intensive and difficult to standardize. The denudation results obtained in this study suggest that microfluidic systems may have potential as scalable alternatives to conventional laboratory procedures.

Furthermore, the incorporation of DEP provides an effective, label-free strategy for oocyte selection based on intrinsic dielectric properties. The observed DEP responses indicate a clear distinction between high-quality and abnormal oocytes, highlighting the potential of this technique as a non-invasive and objective alternative for oocyte

classification within ART workflows.

The current OvaOoC platform supports the co-culture of oocyte and surrounding cells under a dynamic microfluidic environment with controlled medium exchange and hydrodynamic stimulation. This configuration reproduces selected physical and functional features of the oocyte microenvironment more effectively than conventional static culture. However, it does not yet capture the full physiological complexity of the ovary, particularly three dimensional follicular architecture, extracellular matrix components, spatiotemporal hormonal gradients, vascular support. Future work will focus on further optimizing the microfluidic platform to better support oocyte culture, selection, and downstream fertilization processes. In particular, incorporating additional biochemical and biomechanical cues through optimized channel geometry, flow regulation, and culture design may help more closely recapitulate the *in vivo* reproductive microenvironment and further enhance IVM performance.

An important direction for future work is the physical and operational integration of the dynamic oocyte culture, denudation, and DEP-based selection modules on a single microfluidic platform. This integration will require coordinated control of sample transfer, flow rate, processing time, medium composition, enzymatic exposure, washing steps, and electrical conditions across the three stages. In parallel, additional analytical functions, including multimodal sensing and data-driven assessment, may be incorporated to provide a more comprehensive and objective evaluation of oocyte quality. The DEP module should also be further optimized to improve selection accuracy, throughput, and compatibility with automated operation. In the longer term, the proposed platform could be extended to include biomimetic sperm selection, on-chip fertilization, and embryo culture or assessment, thereby contributing to the development of more physiologically relevant, efficient, and standardized ART workflows.

Although the results obtained with porcine oocytes are promising and this biological model is well established in reproductive research, translation of the platform to human applications will involve several important challenges. Major challenges include interspecies differences, limited access to donated human oocytes, ethical and regulatory requirements, and the need for long-term evaluation of fertilization, embryo development, pregnancy outcomes, and the health and development of resulting offspring. These issues should be carefully addressed before the platform can progress toward clinical evaluation.

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List of Publications Concerning the Dissertation

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”, (2026), *Biomicrofluidics* 1 May 2026; 20 (3): 034102. <https://doi.org/10.1063/5.0328820>. (**Q2 Journal, Editor’s Pick**)
Scilight: “Enhancing in vitro maturation with microfluidics and artificial intelligence”, *Scilight* 2026, 231107 (2026). <https://doi.org/10.1063/10.0044135>.
2. **Thu Hang Nguyen**, Hung Anh Nguyen, Y-Van Tran Thi, Hoang Trung Nguyen, Amber Cao, Quang Loc Do, Thanh Tung Bui, Duc Trinh Chu, Hung Cao, “Emerging Bioengineering Technologies in Female Reproduction: Preclinical Advances, Translational Challenges, and Future Outlook”, (2026), *Biotechnology Journal* 21, no. 7 (2026): e70274. <https://doi.org/10.1002/biot.70274>. (**Q2 Journal**)
3. **Thu Hang Nguyen**, Hoang Trung Nguyen, Nam Anh Ngo, Mai Chi Nguyen, Hang Bui Thu, Jens Ducr  e, Trinh Chu Duc, Thanh Tung Bui, and Loc Do Quang, “Numerical study on a facing electrode configuration dielectrophoresis microfluidic system for efficient biological cell separation”, (2024), *Scientific Reports*, Vol. 14, Article 27627. <https://doi.org/10.1038/s41598-024-78722-7>. (**Q1 Journal**)
4. **Thu Hang Nguyen**, Mai Chi Nguyen, Hoang Trung Nguyen, Y Van Tran Thi, Ngoc Trung Vu, Hang Bui Thu, Trinh Chu Duc, Tung Thanh Bui, Chun-Ping Jen, and Loc Do Quang, “Numerical Analysis of Dielectrophoresis-Based Microfluidic Chip with a Facing-Electrode Design for Cell Separation”, (2024), *Journal of Biosystems Engineering*, Vol. 49, 29–40. <https://doi.org/10.1007/s42853-024-00211-6>. (**Q2 Journal**)
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6. **Thu Hang Nguyen**, Hung Anh Nguyen, Y-Van Tran Thi, Donna Hoang Tran, Hung Cao, Trinh Chu Duc, Tung Thanh Bui, and Loc Do Quang, “Concepts, electrode configuration, characterization, and data analytics of electric and electrochemical microfluidic platforms: a review”, (2023), *Analyst*, Vol. 148, 1912–1929. (*Q1 Journal*)
 7. **Hang Nguyen Thu**, Anh Nguyen Thi Ngoc, Trung Nguyen Hoang, Junhan Xiao, Tung Le Thanh, Hang Nguyen Thi, Hanh Nguyen Van, Hoang Anh Phan, Thien Nguyen Duy, Tung Thanh Bui, William C. Tang, Hung Cao, Trinh Chu Duc, and Loc Do Quang, “A deep-learning-based approach for in vitro maturation monitoring utilizing hexagonal micropillar trap microfluidic platform”, (2025), *2025 23rd International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers)*, Orlando, FL, USA, 1279–1282. doi: [10.1109/Transducers61432.2025.11111665](https://doi.org/10.1109/Transducers61432.2025.11111665). (*Scopus Conference*)
 8. **Hang Nguyen Thu**, Trung Nguyen Hoang, Anh Nguyen Thi Ngoc, Mobina Malekifar, Tung Le Thanh, Mai Anh Tran, Hanh Nguyen Van, Ha Tran Thi Thuy, Tung Thanh Bui, Hoang Anh Phan, Hung Cao, Trinh Chu Duc, and Loc Do Quang, “Experimental evaluation of multi-quadrilateral-pillar array microfluidic platform for oocyte denudation utilizing a deep-learning approach”, (2025), *2025 23rd International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers)*, Orlando, FL, USA, 1260–1263. doi: [10.1109/Transducers61432.2025.11111282](https://doi.org/10.1109/Transducers61432.2025.11111282). (*Scopus Conference*)
 9. **Thu Hang Nguyen**, Hoang Trung Nguyen, Mai Chi Nguyen, Loc Do Quang, Jens Duccrée, Trinh Chu Duc, and Thanh Tung Bui, “Circulating Tumor Cell (CTC) Separation from Blood Constituents Using A Dielectrophoresis-Based Microdevice”, (2024), *2024 Tenth International Conference on Communications and Electronics (ICCE)*, Danang, Vietnam, 167–171. doi: [10.1109/ICCE62051.2024.10634638](https://doi.org/10.1109/ICCE62051.2024.10634638). (*Scopus Conference*)
 10. **Thu Hang Nguyen**, Ngoc Anh Nguyen Thi, Nam Anh Ngo, Hoang Trung Nguyen, Linh Nguyen Thi, Quang Loc Do, Jens Duccrée, Duc Trinh Chu, and Thanh Tung Bui, “Development of a Micropillar-Based Concept for Hydrodynamic Trapping and Long-Term Culture of Single Oocytes”, (2024), in *10th International Conference on the Development of Biomedical Engineering in Vietnam. BME 2024*, IFMBE Proceedings, Vol. 122, Springer, Cham. https://doi.org/10.1007/978-3-031-90194-2_58. (*Scopus Conference*)