Development of a Scalable High-Throughput Platform for Plasmid Transfection in 3D Mammalian Cell Culture

Rosanna Jiang*[1] Ying Wang[2] Yufeng Zhao[3] Milica Radisic[1,2] Yimu Zhao[1]

[1]*[Acceleration Consortium, University of Toronto, Canada]* [2]*[Institute of Biomedical Engineering, University of Toronto, Canada]* [3]*[Leslie Dan Faculty of Pharmacy, University of Toronto, Canada]*

Correspondence to: [Yimu Zhao] yimu.zhao@utoronto.ca

1. Introduction

Mammalian cells are traditionally cultivated in 2 dimensions (2D) as adherent cultures for drug screening or shake flasks as suspension cultures for protein production. These culture conditions typically require high energy consumption while limiting biological relevance, miniaturization, throughput, and safety due to high volume and shaking requirements. To overcome these limitations, we propose a scalable high-throughput platform that combines 3-dimensional (3D) cell culture, machine learning and automation, which can be widely applicable to optimally transfect a variety of mammalian cells with plasmids to improve protein production and organoid or organ-on-a-chip (OOC) model development.
Plasmid transfections can be used for a wide range of purposes, including antibody production, stable cell line generation, gene therapy, and training data production for AI model development. Here, we present two use cases for automated plasmid transfection in a 3D culture improved by Bayesian Optimization (BO). The first case is optimization of antibody production in a miniaturized 384-well microfluidic plate format. The second case is optimization of differentiation conditions using a transposon system for stable cell line generation to produce high-content, non-invasive imaging data for training an AI model.

2. Bayesian Optimization of Antibody Production

For large-scale optimization of protein variants in a small footprint, we propose BO of plasmid transfection using an automated high-throughput bioprocess, where mammalian cells are encapsulated in a hydrogel to receive nutrients and oxygen, enabling efficient mass transfer with a reduction in infrastructure and energy requirements.

2.1 CHO Cell Growth in 3D Using Semi-Solid Alginate Enables 384-well Microfluidic Plate Cultures Without Shaking

Alginate was identified as an optimal hydrogel for mammalian cell encapsulation, preserving expected cell morphology and viability compared to other hydrogels, such as GelMA and GelMA/PEO [1]. Alginate enables simple protein harvesting by EDTA-mediated Ca^{2+} chelation, achieving up to 97 ± 16% yield, to facilitate downstream analysis. CHO cell cultivation was first established in alginate within a microfabricated, perfusable 384-well platform called the iFlowPlate™ (OrganoBiotech), where encapsulated cells occupy the middle well, while gravity-driven flow is maintained by plate tilting. To enhance mass transfer, cells were encapsulated in alginate with embedded gelatin particles. Gelatin was removed upon alginate crosslinking and temperature elevation, leaving behind a porous path. To fabricate the slab, the alginate solution with CHO cells was deposited into the middle well, allowing the solution to evenly spread across the well, followed by a rapid crosslinking using a $CaCl_2$ solution to yield an even distribution of live cells in 3D. After 10 days, CHO cells cultivated in alginate with rocking-induced flow, exhibited higher cell density and viability compared to those cultivated in a static alginate slab or a standard 384-well plate with cells grown in 2D at the bottom of the wells. Quantification of the IgG concentration using the Octet Biolayer Interferometry (BLI) platform further highlighted the superiority of the microfluidic platform, which showed significantly higher total amount of monoclonal antibody (mAb) under flow compared to static cultivation in alginate or a standard 384-well plate (Fig. 1).

2.2 Related work

The previously optimized manual 3D transfection protocol will be converted to an automation-friendly protocol that may be adopted by multiple liquid handling platforms. Further optimization will be accelerated by BO suggestions (compared to traditional design-of-experiment optimization) that can be

generated by the previously collected experimental data [1]. This plasmid transfection workflow will be broadly applicable to other types of mammalian cells or hydrogels and scalable across different plate formats.

2.3 Figures

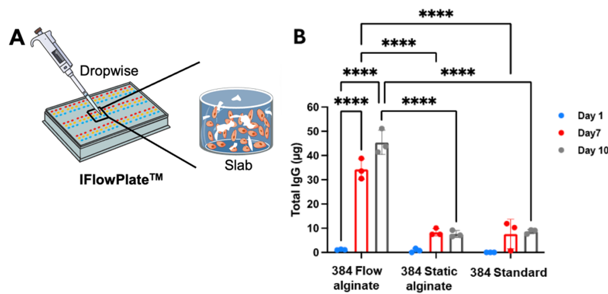

Fig. 1: Monoclonal antibody production in different 384-well formats.

3. Bayesian Optimization of Differentiation Conditions

Organ-on-a-chip (OOC) technologies and organoids recapitulate key physiological features of human organs and offer more clinically relevant readouts than traditional 2D cell cultures and animal models. However, the widespread application of these 3D platforms is limited by the lack of robust, non-invasive tools for real-time monitoring of molecular events within living tissues.

Current approaches to assess cell identity, differentiation, and function rely heavily on labour-intensive endpoint or invasive assays. While informative, these methods are destructive to living cells and can only provide static snapshots of cell states, resulting in long turnover times for iterative optimization. To overcome these limitations, we propose automated transfection of induced pluripotent stem cells (iPSCs) expressing custom reporter plasmids for BO of differentiation conditions in iPSC-derived organoid and OOC models.

3.1 iPSCs Expressing Custom Reporters Enable Label-Free Phenotypic Screening for AI Training Data

To enable multiplexed, non-invasive monitoring of live cell lineage markers and physiological signals, we constructed plasmids by traditional restriction enzyme cloning to insert custom reporter genes into transposable elements or transposon. These custom reporters serve as annotation tools to correctly identify differentiated populations at each stage of cell growth. For example, custom iPSCs expressing a green fluorescent protein (GFP) reporter can fluoresce when they are pluripotent. Reporter genes can be stably integrated into iPSCs using a transposase system, such as the PiggyBac transposase (Fig 2A). A pooled co-transfection of plasmids, which encode the transposase enzyme and multiple transposons, can insert multiple reporters into target sites in the iPSC genome.

3.2 Related work

An established protocol for efficient gene overexpression in iPSCs will be used to transfect our custom plasmids and converted to an automation-friendly workflow [2]. Antibiotic selection ensures that positive clones can be isolated as stably transfected cells. These newly generated stable cells will enable label-free phenotypic screening for AI training data before functional validation of OOC or organoid model development (Fig 2B). This platform can also be used for automated iPSC passaging and transfections by combining liquid-handling robotics with automated high-content imaging. The stable iPSCs will be differentiated into different cell types, such as cardiomyocytes, endothelial cells and epithelial cells, to generate data for AI-driven image analysis pipelines. BO will be used to rapidly refine differentiation conditions for maximal yield of successfully differentiated cells in high-fidelity tissue models.

3.3 Figures

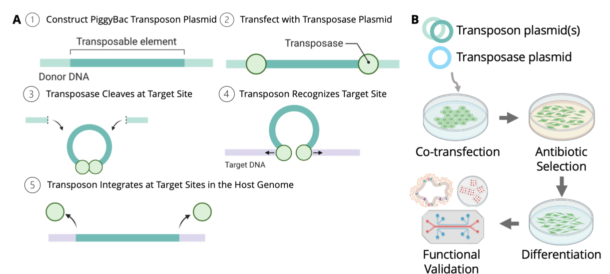

Fig. 2: PiggyBac transposase system to generate stable cell lines. Graphics created with Biorender.com.

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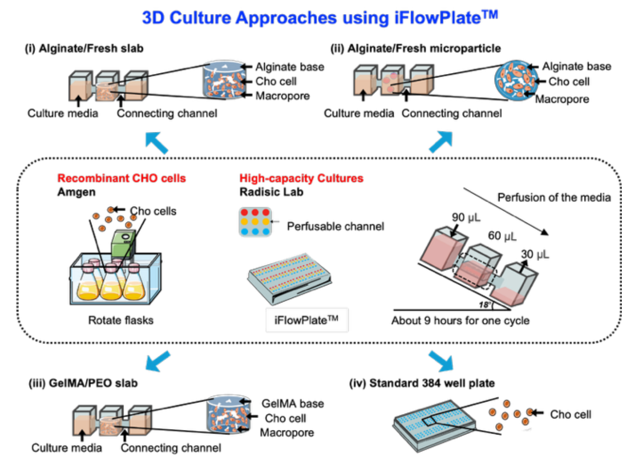
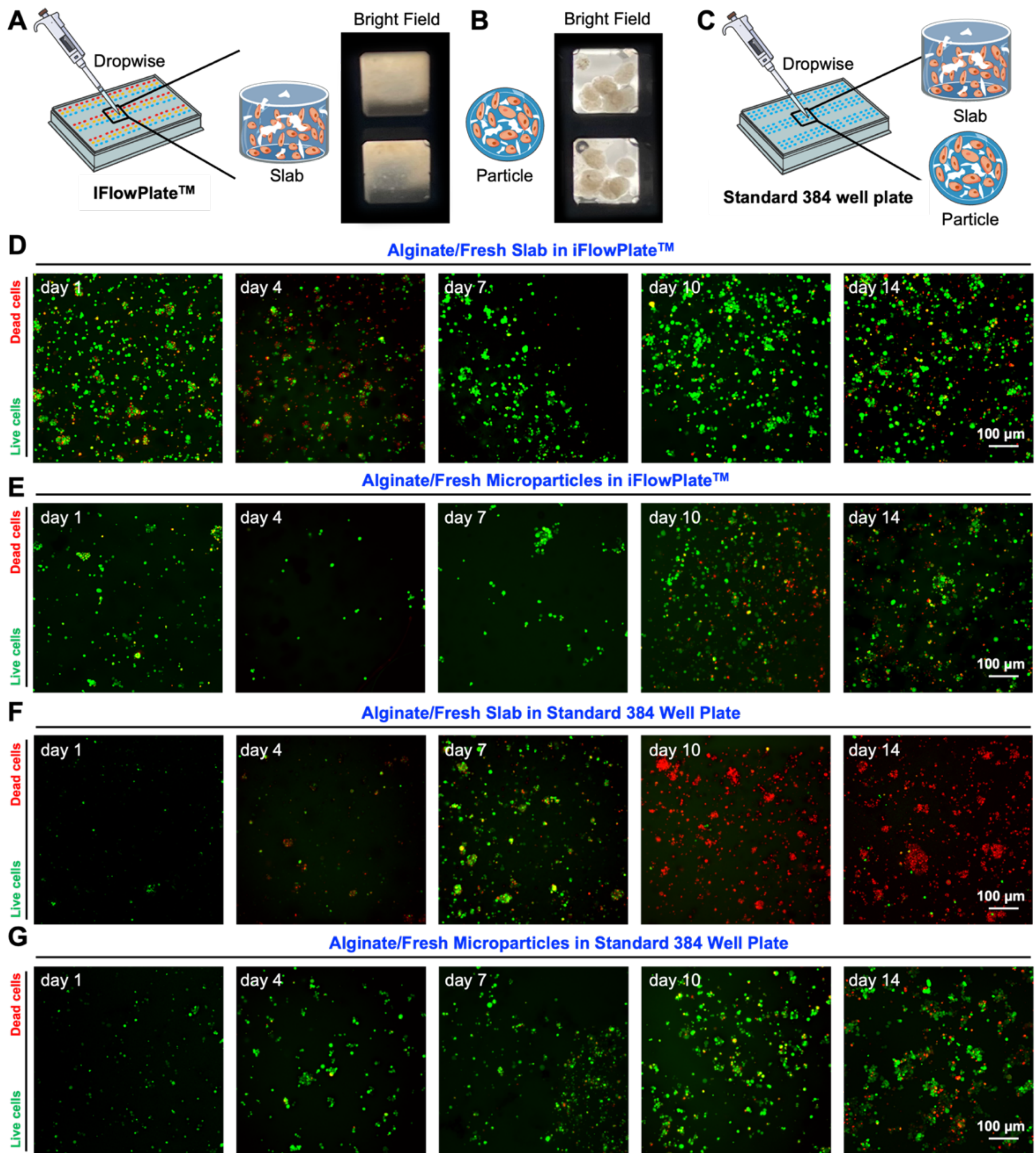
References

[1] Wang, Y. *et al. Unpublished.*
[2] Yang, J. *et al.* Protocol for inducible piggyBac transposon system for efficient gene overexpression in human pluripotent stem cells. *STAR Protocols*, 2026.

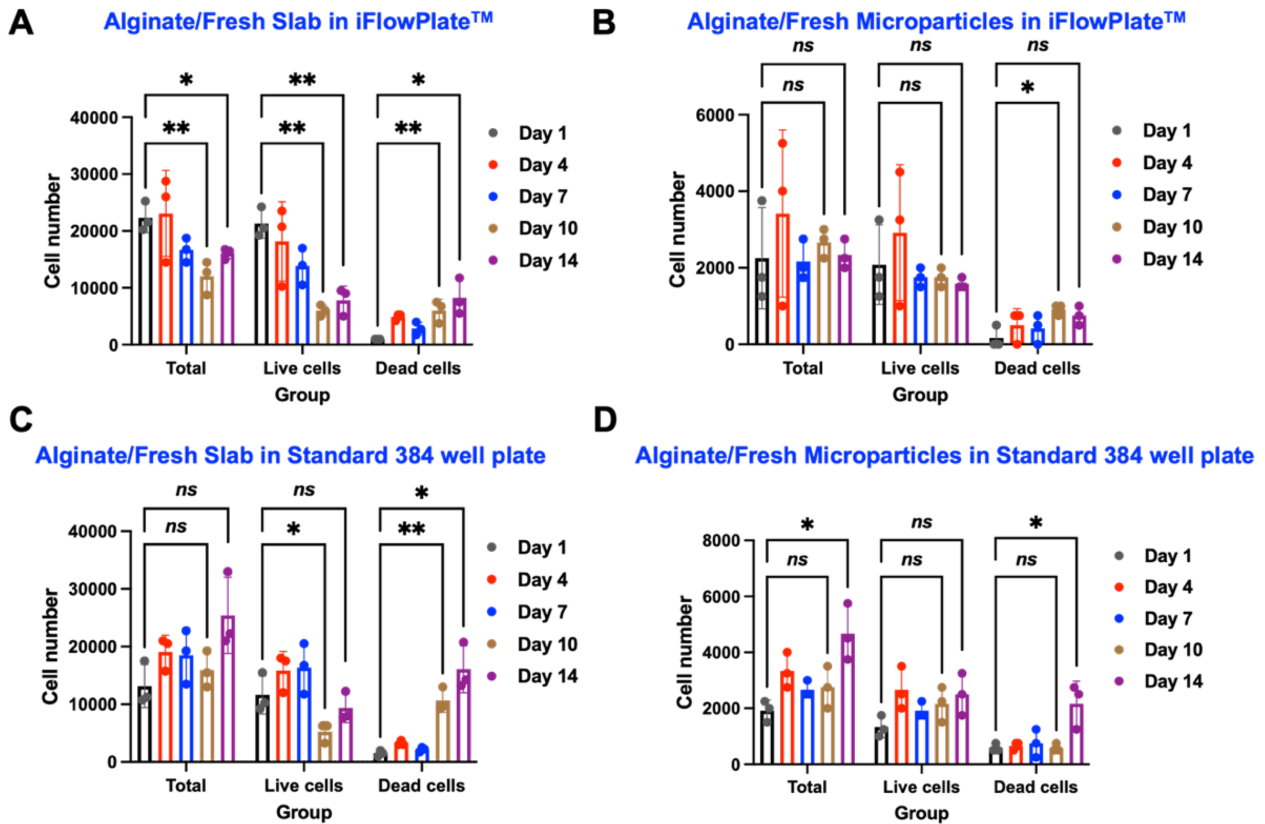
Appendix A. [Supplementary Material]



Supplementary Fig. 1: Open-well high-density microfluidic culture of antibody-expressing CHO cells. Schematic illustrating the fabrication of a porous alginate slab and microparticles for encapsulation of CHO cells for cell proliferation and protein production, along with the cells within the regular 384-well plate as the control group.



Supplementary Fig. 2: Preparation of alginate/FRESH slab and microparticles encapsulated CHO cells in iFlowPlateTM system on the rocker, along with the group of the samples in normal 384 well plate in static culture. (A-C) The fabrication and morphology of alginate/FRESH slab and microparticles in the bright field within the iFlowPlateTM system, along with the schematic showing the samples in normal 384-well plate. (D-G) The live/dead images show the cell viability in alginate/FRESH slab in iFlowPlateTM, alginate/FRESH microparticles in iFlowPlateTM, alginate/FRESH slab in normal 384 well plate, and alginate/FRESH microparticles in normal 384-well plate, respectively, at the desired time points (1, 4, 7, 10 and 14 days). The live and dead cells were stained in green and red, respectively. The cells in early apoptosis were stained yellow.



Supplementary Fig. 3: The recovery of CHO cells from alginate using Ca^{2+} chelators and cell viability analysis. (A-B) Quantification of CHO cell viability based on live/dead imaging in the group of alginate/Fresh slab and microparticles within the iFlowPlate™ system, (C-D) along with the samples in normal 384 well plate as control groups at various time points without changing the media.