Additive Manufacturing Enabled Monolayer Chambers for Immune Cells' Communication Studies

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1 INTRODUCTION

Microfluidic devices have become essential tools for biomedical research; particularly for elucidating interactions between various types of cells. There is a growing demand for microfluidic features such as monolayer chambers (MCs) to enable the capture and analysis of cellular signals during time-lapse microscopy and provide insights into cellular behaviour over extended periods. Cells such as macrophages and fibroblasts create a cell-cell communication network based on reciprocal PDGF- and CSF1-dependent signalling that provides each cell population with resilience to perturbations [1]. Recently, computational models of tunable intercellular communication parameters (i.e., gene type and activity) have been developed to understand the better mechanisms by which fibroblasts and macrophages achieve varying coordinated multicellular behaviours via paracrine signalling during immunological response [2]. Due to challenges in simulating paracrine signalling in tissue culture, little is known about the major parameters and possible rate-limiting steps underlying paracrine signalling mechanisms in these cells, either via intrinsic factors, such as a particular gene activity in the network, or by extrinsic factors, such as the environment or cell contact configuration. Integrating advanced microfluidics and microscopy opens new horizons for studying immune cell interactions with unprecedented detail. Conventional methods like photolithography have been the gold standard for fabricating microfluidic devices. However, these methods are expensive and time-consuming, often requiring specialized laboratory personnel and cleanroom environments. Over the past few years, 3D printing has emerged as a promising alternative for producing microfluidic devices, offering a comparatively faster, cost-effective, and user-friendly approach [3]. Despite significant progress in the 3D printing industry, making MCs in dimensions of tens of microns to study immune cells' communication dynamics remains a challenge [4, 5]. However, most of them fall short of providing the optical transparency and spatial-temporal resolution needed for long-term studies. The integration of advanced 3D printing techniques such as stereolithography (SLA) and digital light processing (DLP) with soft lithography to produce polydimethylsiloxane (PDMS)-based microfluidic devices has shown promise for the rapid prototyping of immune cell environments that enable the study of spatial-temporal dynamics over extended periods. In this study, we leverage the precision of DLP to develop MCs for studying immune cell communication.

2 METHODS

Design and fabrication of microfluidic molds

We designed microfluidic molds in Autodesk Fusion-360 and fabricated them using FormLabs' Forms3+ 3D printer and NanoDimensions' Tera-250. Molds from the Forms3+ were washed in 70% isopropyl alcohol for 10 minutes using FormsWash, then cured at 65°C overnight in FormsCure. Tera-250 molds were prepared per manufacturer guidelines. For PDMS replica molding, Sylgard 184 elastomer (1:10 base agent) was mixed, degassed, poured into molds, and cured overnight at 70°C. PDMS was released from molds, bonded to a glass slide using oxygen plasma, and cured overnight at 70°C. Surface characterization was performed using a KLA Zeta-20 optical profiler, and data were analyzed with ProfilmOnline and plotted in OriginPro2024b.

Cell culture and analysis

Murine macrophages (RAW 264.7, ATTC) were cultured in T-25 flasks with complete culture media (Dulbecco's modified eagle medium, with 10% fetal bovine serum, and 1% penicillin/streptomycin) at 37°C with 5% supplemental CO_2. At 80% confluency, fresh media was introduced, and the RAW cells were lifted from the surface of the flask using a cell scraper with a pivoting blade. The contents of the T25 were transferred to a 5 mL conical tube and centrifuged at 1000 rpm for 5 minutes. After centrifugation, the pellet was resuspended in 200 mL of culture media. Trypan blue stain and hemocytometer were utilized to determine the approximate

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concentration of cells in 10 mL of the suspension (10,000,000 cells/mL).

Using a 100 mL syringe, the microfluidic channel was pre-flushed with 10 mL of culture media and loaded with approximately 5ul of cells into the device fabricated with the DLP method. The chip was centrifuged at 2000 rpm for 5 minutes to transfer cells into monolayer chambers. A syringe

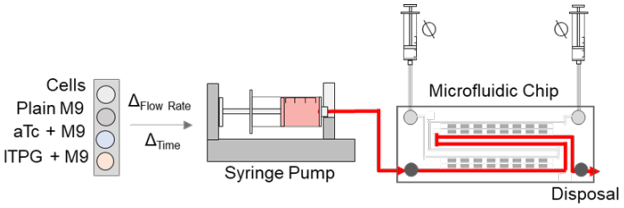

Figure 1: Experimental setup of the microfluidic chip. We used syringe pumps for the administration of the prepared culture media at 10L/min for 14 hours.

pump containing fresh culture media was used to administer media to the device (Figure 1) . The chip was then studied under the microscope (Nikon Eclipse Ti2) at 37°C overnight (1̃4 hours).

3 RESULTS

Fabrication of monolayer chambers

We designed immune cell microenvironments to study the interactions of immune cells and produced microfluidic molds using SLA and DLP technologies. The microfluidic platform contains 80 monolayer chambers (MCs) with 20 MCs per pathway, each Monolayer Chamber (MC) measuring 500 µm wide, 500 µm long, and 30 µm high to trap immune cells (Figure 2).

The optical profiling of microfluidic molds produced via SLA and DLP indicated significantly superior performance of the DLP approach. Printer technology and resolution were the most significant factors determining the quality of the microfluidic molds produced.

As the mold produced via DLP was superior (MC-DLP) to that produced via SLA (MC-SLA), we used MC-DLP for soft lithography to create a PDMS replica. The surface analysis of MC-DLP demonstrates the successful production of individual MCs. SLA offers a cheaper alternative with a quick turnaround time. However, it limits the users to produce more minor features. On the other hand, the DLP approach is comparatively precise and requires significantly more cost.

Cell trapping ability of the DLP PDMS microfluidic device

After centrifugation, the cells were trapped within the monolayer chambers. The syringe pump administered 10 mL/min of media continuously throughout the night. Images were

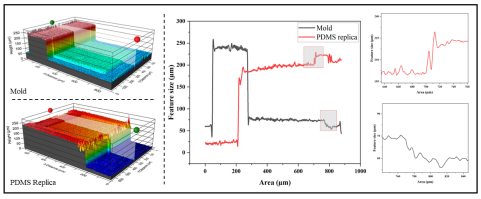

Figure 2: Optical profiling of the cross-section of an MC on both the microfluidic mold and the PDMS replica shows the superior and precise production of MCs via DLP.

obtained every five minutes to track if there were changes in how the cells behaved under continuous flow. From the beginning of the microscopy study to the end 14 hours later, there was no observable change concerning how the cells were trapped (Figure 3).

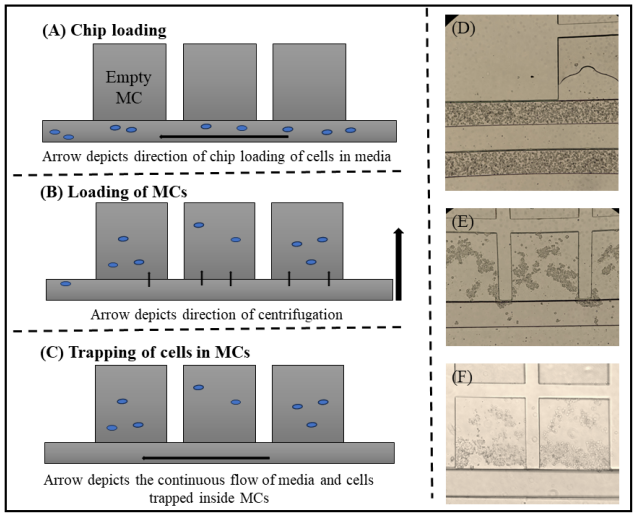

Figure 3: MCs were loaded with immune cells using centrifugation (A-C). Microscopic images of the cells in the microfluidic device obtained (D) after chip loading and before centrifugation, (E) after centrifugation, and at the end (F) of the overnight study with cell adhesion and cell growth.

4 DISCUSSION

The liquid volume the microfluidic device could hold is approximately 2 µL; prompting the usage of a slower flow rate. A high concentration of cells demonstrated the DLP device's ability to trap mammalian cells, with residual cells flushed out. The chip enables multiple experiments, with capabilities to enable studies of immune cell interactions. Future studies may explore two chambers with a filter for selective biomolecule filtering, requiring precise 3D printing techniques like two-photon polymerization (2PP). This innovative approach will allow us to capture the dynamics of

immune cell communication in real time, visualizing molecular signals and cellular responses with unparalleled precision. The platform will provide a generalizable framework for studying paracrine signalling mechanisms in various cell types and physiological contexts in the future.

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