A case study of MOIIN resins: 3D printed microfluidic devices for cell-based applications

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1. Introduction

The use of microfluidic devices has gained much attention with cell-based application such as tissue/cell culture and droplet encapsulation for single cell analysis. At present, most of these microfluidic device design and prototyping are reliant on PDMS soft lithography [1]. Due to the moulding nature of soft lithography, most designs of the microfluidic channels are restricted to a single plane with multiplanar channels requiring manual assembly by technicians. Therefore, the prototyping turnover rate of the microfluidic devices can be low [2]. The use of 3D printers is potentially effective in accelerating the prototyping turnover rate of microfluidic devices. In addition to 3D printing being capable of fabricating fluidic channels within the 3D space without requiring laborious assembly, the use of plastic materials also enables easier research translation into large scale manufacturing process. For the application of 3D printed devices in cell-based research, the device materials must minimally satisfy 3 main criteria: 1) Enable complex unibody printing of microarchitecture for cell trapping and flow manipulation; 2) Compatible to imaging platform; and, 3) compatible with imaging platforms such as microscopes.

2. Methods:

2.1. Materials and equipment

All chemical reagents are obtained from Sigma-Aldrich (Merck, Australia), unless otherwise stated. 2 resin formulations were used in this case study: MOIIN HighTemp resin and MOIIN TechClear resin (DMG Digital Enterprises SE, Germany). All printed devices are performed using ASIGA UV Max X27 DLP printers with slice thickness of 100 µm.

2.2. Device design, fabrication and post processing

The design of the microfluidic devices is done with AutoCAD (ver. 2020, AutoDesk) and is fabricated at 50 µm z-resolution. Modification of the print platform by attaching a glass slide is done prior to all device printing to create a smooth surface without polishing. After printing, the printed parts are immersed in isopropyl-alchohol (IPA) bath and sonicated at 480 seconds. Gentle flushing of the resin trapped within the microchannels were performed using a syringe. After flushing of the resins, the printed parts were then subjected to a separate IPA bath for 3 cycles of sonication at 480 seconds per cycle. The devices are then transferred to a clean IPA tank for 2 hours to leech out residual resin. The clean devices were then blown dry and subjected to 20 minutes of heat cure at 60ºC before placing in a UV curing chamber for 3 hours.

2.3. HepG2 Cell culture

Commercial HepG2 cells (ATCC, In Vitro Technologies) were cultured in T25 flask with high glucose DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin. Cells were

harvested at 70% confluences and seeded into the device at 1 million cells/mL density through a syringe pump. The seeding is stopped after 100 uL of cell suspension is pass through the microfluidic devices. The seeded devices are cultured in a humidified chamber at 5% CO_2 at 37ºC for 5 days. Daily change of fresh media is performed with manual pipetting. Post 5-day culture, the seeded devices is flushed with 1X PBS before incubating with 1 µM of propidium iodide in high glucose DMEM for 30 mins at 5% CO_2 at 37ºC. After incubation, the stained cells were washed with 1X PBS before imaging under fluorescent microscope (Nikon Eclipse, Nikon) at 555 nm excitation wavelength.

3. Results:

3.1 Establishing unibody printing of microfluidic devices for cell-based application

To improve the 3D printed devices' transparency to microscope platforms, we implemented a glass surface onto the ASIGA UV Max's print platform (Fig 1a). This is done by sandwiching a residual amount of intended resins between the build platform and the glass before exposing to UV chamber for curing. The incorporation of glass surface before printing ensures that the bottom surfaces are smooth to reduce light scattering during inverted microscope imaging. No sanding is performed in this work to reduce trapping of debris within the microchannels. The printing parameters were set as per manufacturer's instruction. As a case study to investigate the suitability of the resins for cell-based microfluidic device fabrication, we demonstrated three basic microfluidic designs that are commonly reported: 1) 2D monolayer culture devices (Fig 1b, e) , 2) Pillar arrays (Fig 1c, f) for 3D cultures, and, 3) constricting channels for droplet generators (Fig 1d, g).

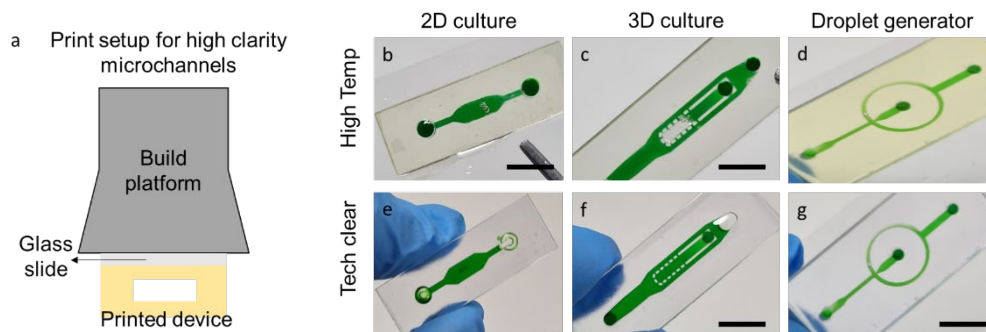

Figure 1: 3D printed microfluidic devices for cell-based applications using the MOIIN's Resin. (a) Printing strategies to enhance optical clarity of the 3D printing to ensure smooth surface. (b) 2D culture chip with MOIIN HighTemp, (c) 3D culture chip with pillar array with MOIIN HighTemp, (d) Droplet generator with MOIIN HighTemp. (e) 2D culture chip with MOIIN TechClear, (f) 3D culture chip with pillar array with MOIIN TechClear, (g) Droplet generator with MOIIN TechClear. Scale = 1 cm.

Both MOIIN HighTemp and MOIIN TechClear can support the fabrication of common microfluidic device channel geometries catered for cell-based experiments. Micro-architecture with the smallest dimension of 300 µm width at 1:3 aspect ratios (Pillar arrays for 3D culture devices) was achievable with high accuracies using both MOIIN's resin formulation (Fig 2a-b).

3.2 Device compatibility under microscope

For cell-based experiments in microfluidic devices, microscopy of the devices remains the go-to platform for tissue engineers and biologist. Therefore, to compatibilities of the MOIIN's resin in microscopy, we first flow in fluorescent dyes into our fabricated device channels and subject the devices to microscopy imaging work. Given that MOIIN HighTemp exhibited autofluorescence at 488 nm and 385 nm (data not shown), which severely limits its use in immunohistochemistry in the microfluidic devices, we focused our investigation on MOIIN's TechClear for its microscopy compatibility. For MOIIN TechClear, we noted that the resin does not exhibit autofluorescence at 488 nm (green) (Fig 2a -c) and 555 nm (red) (Fig 2d). With the

TechClear resin, we were able to achieve high resolution imaging of microchannels microarchitecture (Fig 2b, c) and also high-resolution particle flow (with particle size of 20 µm) down to 10X (Fig 2). This is likely due to the TechClear resins are primarily designed for transparent object printing.

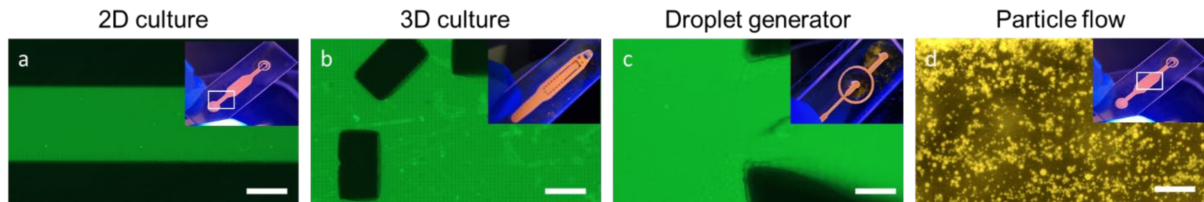

Figure 2:Microscopy images of microfluidic devices printed with MOIIN TechClear resin. (a) Green dyed microchannels in (a) 2D culture channels, (b) Pillar arrays for 3D culture, (d) Droplet generator. (d) microscopy flow field imaging with 20 µm particle size.

3.3 Resin biocompatibility with cells

We finally investigated the biocompabilities of the MOIIN TechClear and MOIIN HighTemp with tissue monolayer cultures. As a showcase, liver HepG2 cell line was used in this study. For this work, we 3D printed 2D culture channels with MOIIN HighTemp (Fig 1a) and MOIIN TechClear (Fig 1d, 2a) upon which the HepG2 cells will be seeded into the channels. Sterilisation of MOIIN HighTemp device is done with autoclaving at 121°C while MOIIN TechClear device was sterilised with soaking with 70% ethanol for 2 hours before flushing with copious amount of 1X PBS solution. All cell cultures were done *in situ* for 5 days under cell culture incubators. Leveraging on both resin compatibilities with microscopy (Fig 2), we demonstrated the ability of the resin in supporting cell monolayer formation (Fig 3a), visible under phase contrast microscopy. We subsequently labelled the dead cells within the cell monolayer using ethidium homodimer-1 (EthD-1) DNA stain. Post 5 days culture, we observed that there was limited cell death observed (Fig 3b, c). This evidence suggests that both MOIIN HighTemp and MOIIN TechClear are biocompatible and can support cell-based application within the 3D printed devices.

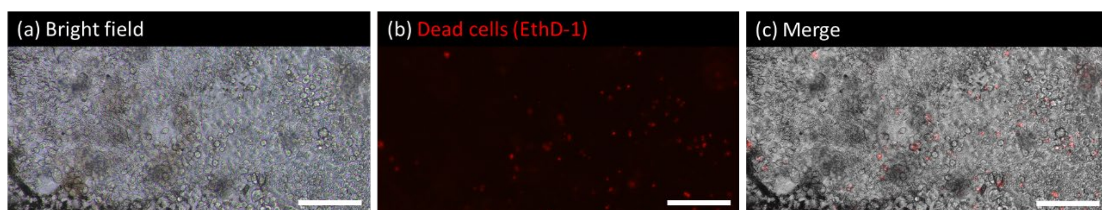

Figure 3: Biocompatibilities of MOIIN resins HepG2 monolayer culture. (a) Monolayer of HepG2 under phase contrast microscope. (b) Fluorescently labelled dead cells using ethidium homodimer-1 (EthD-1) DNA stain. (c) Merged microscopy images. Scale = 100 µm.

4. Conclusion:

In conclusion, our study revealed that with MOIIN HighTemp and MOIIN TechClear, the 3D printing of microfluidic channels for cell-based applications are feasible. Both resins are observed to be biocompatible and highly amendable for microscopy imaging. This observation potentially opens many opportunities for rapid prototyping of new generations of microfluidic channels for biomedical applications.

5. Acknowledgements:

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6. References:

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