A HIGHLY EFFICIENT MICROFLUIDIC U–WELL ARRAY DEVICE FACILITATING HIGH-THROUGHPUT METASTATIC TUMOR SPHEROID CULTURE AND DRUG EVALUATION

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ABSTRACT

Establishing a cost-effective and easy-to-use preclinical model that can mimic the tumor microenvironment and be applied in drug evaluation has been highly sought-after for decades. The available preclinical platforms are not effective in predicting human clinical trial outcomes. Therefore, there is a need for developing an efficient platform to mimic the tumor environment. Here we present a microfluidic U-well array device integrated with biomimetic nano-cilia that contributes to highly efficient tumor spheroid formation within one day. For the first time, the device can not only recapitulate the in vivo-like microenvironment but also achieve hallmarks of tumor-based bioassays.

KEYWORDS

Microfluidics; In-vitro Model; 3D Cell Culture; Drug Evaluation

INTRODUCTION

3D tissue cultures have emerged as invaluable cancer models for a wide range of clinical issues that exhibit microenvironmental heterogeneity as well as for tumors in vivo. 3D tumor cell cultures have been revealed to possess characteristics – in terms of cellular heterogeneity, mass transport, and complex cell–matrix/cell–ell interactions – compared with conventional 2D cell cultures [1, 2]. To develop systems that better mimic in vivo cellular microenvironment, researchers are increasingly looking to 3D cell culture. However, the usage of currently existed techniques for assessing the key hallmarks of caner, including cell motility and matrix invasion, remains poorly accessible [3, 4].

Over the past several decades, microfluidic devices have been widely used for cell culture and drug screening [5-7]. These devices offer a cell culture environment that closely resembles the in vivo conditions. Compared to traditional cell culture tools, microfluidic devices allow for better regulation of chemical and physical surroundings. Specifically, when cells are situated in micro-channels or chambers, they can be exposed to flowing media, making the culture conditions more like in vivo conditions than static cell culture in Petri dishes. However, there are still challenges that need to be addressed, such as intricate operational control and chip design, and non-standard culture protocols [8].

Herein, we present a new microfluidic U–well array device to address the above issue. Upon the combination of round bottom U–well, microfluidic U–channel, and F108 nano-cilia [9], the parallel formation of a unique tumor spheroid array could be achieved. In addition, we demonstrated that this device could be of a superior tumor spheroid formation within one day compared to the convention hanging droplet approach typically required for larger than 7 days.

EXPERIMENTAL

Fabrication of the microfluidic U-well array device

Figure 1 illustrates the design of the microfluidic U–well array device, which integrated a U–well array and a microfluidic U–channel. For the fabrication of the U-well, we followed the ParaStamp approach [10]. As shown in Figure 1a, a commercial Parafilm was sandwiched between a foil and a patterned Polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning) master. The whole set was transferred to a hot plate at 120 °C for 30 s. Afterwards, the micro-patterned PDMS absorbing the liquid-phase wax was peeled off and its corresponding patterns were stamped on a glass slide. Each pattern was then sprayed with 1.5 L of DI water droplets. PDMS prepolymer was poured into the mold and cast at 75 °C for 2 h. We further adopted a 3D printer equipped with PLA (Prusa Research; Original Prusa i3 MK3S+) to fabricate the U-channel (Figure 1b). The final device was achieved following alignment of the top channel and the bottom U–well (Figure 1c).

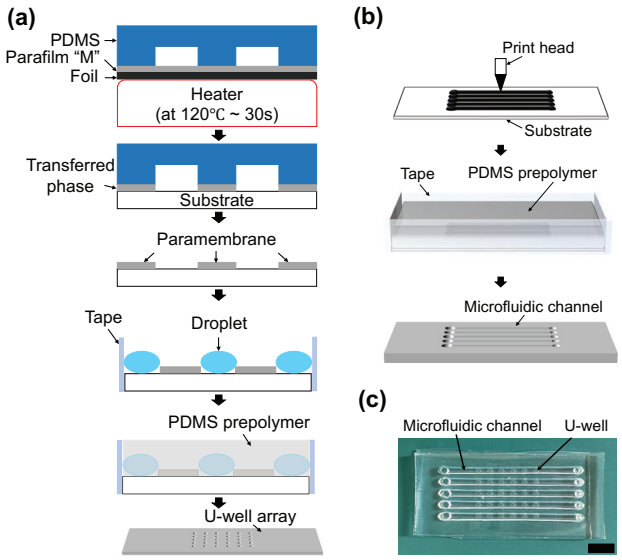

Figure 1: Design of the microfluid U–well array device. (a) Fabrication of the U–well array. (b) Fabrication of the microfluidic channel by 3D printing. (c) The final device integrated with U–well array and microchannel. Scale bar: 5 mm.

Cell culture

Human ovarian cancer cell line SKOV3 (HTP-77, ATCC) was cultured in RPMI-1640 medium (31800-022, GIBCO) supplemented with 10% fetal bovine serum (FBS; SH30396.03, Hyclone) and 1% penicillin/streptomycin (P/S; P3032-25MU, SIGMA). The cells were kept at 37 °C in a humidified chamber with 5% CO2.

Before the cells were loaded, a 1 mL type I collagen solution (~4.26 mg/mL; C3867, SIGMA) was prepared by adding cell culture medium and 1 N sodium hydroxide for pH adjustment. Before each individual experiment, the pH of the collagen solution was measured to be around 7.4. The cells were resuspended in solutions with collagen (500 g/mL). Pipettes were used to dispense 1.5 L of cellular drops into the U-well array. And the procedure of cell culture with the hanging drop technique was followed by a PDMS-based HDA approach [11]. Briefly, each cellular drop with a volume of 1.5 µL was dispensed onto the PDMS-HDA device by pipettes. Then the device was flipped and placed in a 6-cm cell culture dish, which had been pre-filled with medium of 300 µL and sealed with parafilm to prevent evaporation of the cell-containing drops. After cells were loaded, both devices were transferred to a 5% CO2 incubator at 37 °C immediately to allow tumor spheroid formation.

Preparation of triblock copolymers for cell culture

In this work, we designed a microfluidic U-well array (MUWA) chip, shown in Figure 2, to meet the following requirements: (1) rapid tumor spheroid formation; (2) controllable distribution of tested cells and drugs; and (3) convenient operation. Each microchamber was 800 m deep and housed six 1.5 µL U-wells.

Figure 2 also depicts the design procedures for 3D cell culture and thus in-situ drug evaluation using the microfluidic U-well device. To reduce the complexity of the operation, all the solution was loaded by pipettes instead of by other expensive instruments. At first, the U-well surfaces were coated with nano-cilia (triblock copolymer; 1% Pluronic F108 in DI water, SIGMA) for 1h, which would promote the locomotion of suspended cells down to the bottom of wells, thereby increasing the modeling efficiency of multi-cellular spheroids. The cells were then loaded into the wells, and the cell density in each well was adjustable. After 1 day of culture, the cells self-aggregated into a spheroid.

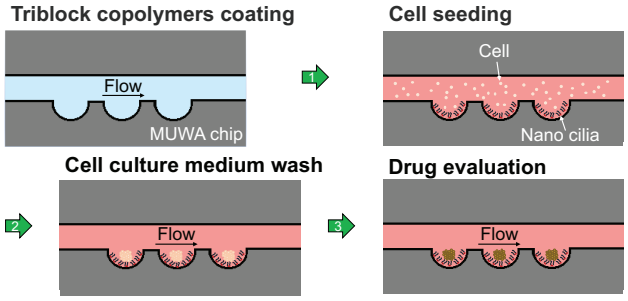

Figure 2: Schematic showing the procedures for 3D cell culture as well as drug evaluation by the microfluidic U–well device.

Imaging and Quantification

The image of U-well was taken with an Olympus CKX53 inverted microscope (Olympus, Tokyo, Japan) and cells were taken using a Carl Zeiss Axiovert 25 microscope (Carl Zeiss, Thornwood, NY) equipped with iPhone SE2 (Apple; Cupertino, CA, USA) and analyzed by ImageJ.

RESULTS AND DISCUSSION

Characterization of the device

Figure 3a shows a representative cross-sectional view of the U-well. It impacts a relatively smooth and concave surface, enhancing the efficiency of 3D cell cultivation. Since the curvature of the microfluidic channel would be affected by the printed source, different reflow conditions of the printed PLA lines were examined (Figure 3b). There were six different reflow temperatures tested to conduct the fabrication of the device. We conducted different settings to fabricate the device five times in each group to optimize the working parameter. The higher the coefficient of variation, the greater the dispersion. Notably, an optimized reflow temperature of 200 °C was conducted.

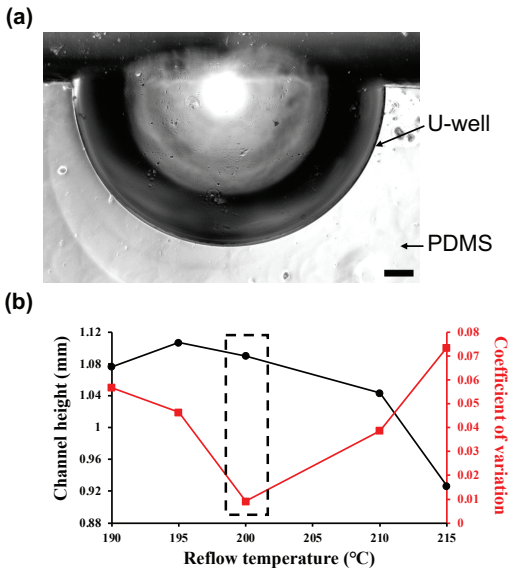

Figure 3: Characteristics of the microfluidic U–well device. (a) Cross-sectional view of the U–well. Scale bar, 100 µm. (b) Fabricated channel heights versus reflow temperature by 3D printing. Dash rectangle indicates the optimized parameter for the microchannel design.

Spheroid culture

Figure 4 shows two representative time-sequenced observations of ovarian SKOV3 tumor spheroids performed in the microfluidic U–well device. With the optimized conditions, namely, 1.5 µL droplets, 650 cells per well, and a 5-day incubation without changing culture medium, 3D cell culture could be performed in the U-well array. In the experiment, cell spheroids of human ovarian cancer cells, SKOV3, we successfully formed. Remarkably, the time required for performing an unique tumor spheroid was as quick as less than one day.

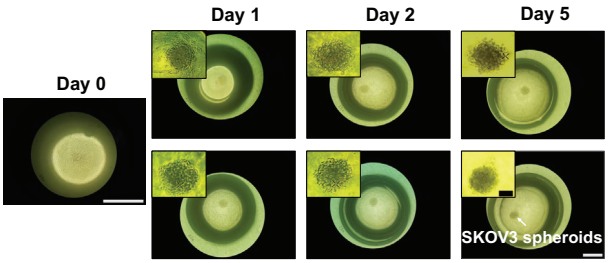

Figure 4: Time-sequenced images showing the growth of ovarian SKOV3 tumor spheroids over a 5–day culture in the U–well. Scale bars, 500 µm; insert scale bar, 100 µm.

Comparison with conventional device

To further demonstrate the efficiency of generating tumor spheroids, we compared our proposed microfluidic U–well device and the conventional hanging droplet approach (Figure 5). Our results clearly showed that the usage of our microfluidic U-well device, in combination with a collagen scaffold of 500 µg/mL, was able to significantly boost the tumor spheroid formation within just one day. In contrast, using the conventional approach, no tumor spheroids could be generated even after 3 days. This further validates the efficiency of our device in generating tumor spheroids for various applications, including drug screening and cell migration and invasion assays.

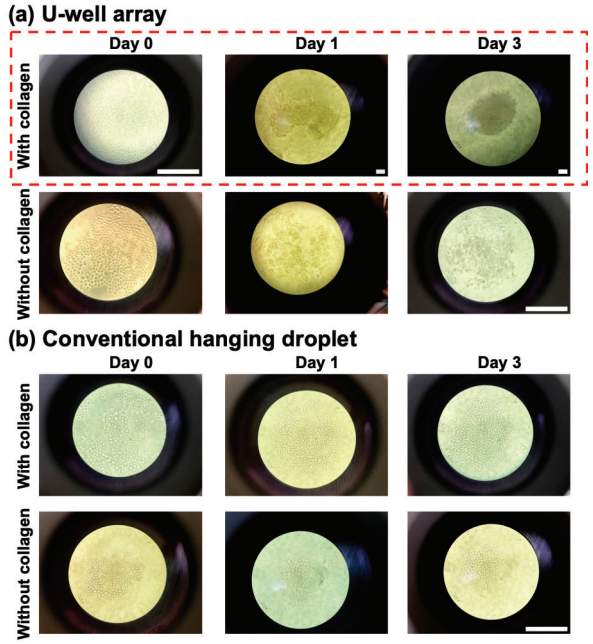

Figure 5: Comparison of SKOV3 tumor spheroids generated between (a) the microfluidic U-well device and (b) the conventional hanging droplet approach. The microfluidic U–well (denoted as red dash) impacts the tumor spheroid formation within 3 days. Following the conventional approach, in contrast, no spheroid was performed even after 3 days. Scale bars, 100 µm.

CONCLUTION

In conclusion, an integrated microfluidic device with non-planar microchannels and U–well arrays has been successfully demonstrated. Our results demonstrated that the device could boost the cell spheroids formation within just one day. Also, we compared our device with conventional HDA device, our device had shown the higher efficiency of generating tumor spheroids. The device may facilitate tumor spheroid culture and drug screening as well as cell migration and invasion assays on demand.

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REFERENCES

[1] Derda, R., et al., *supported 3D cell culture for tissue-based bioassays.* Proceedings of the National Academy of Sciences, 2009. **106**(44): p. 18457-18462.

[2] Yamada, K.M. and E. Cukierman, *Modeling tissue morphogenesis and cancer in 3D.* Cell, 2007. **130**(4): p. 601-610.

[3] Jensen, C. and Y. Teng, *Is it time to start transitioning from 2D to 3D cell culture?* Frontiers in molecular biosciences, 2020. **7**: p. 33.

[4] Ravi, M., et al., *3D cell culture systems: advantages and applications.* Journal of cellular physiology, 2015. **230**(1): p. 16-26.

[5] Feng, J., et al., *Microfluidic trends in drug screening and drug delivery.* TrAC Trends in Analytical Chemistry, 2022: p. 116821.

[6] Mehling, M. and S. Tay, *Microfluidic cell culture.* Current opinion in Biotechnology, 2014. **25**: p. 95-102.

[7] Kwapiszewska, K., et al., *A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening.* Lab on a Chip, 2014. **14**(12): p. 2096-2104.

[8] Halldorsson, S., et al., *Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices.* Biosensors and Bioelectronics, 2015. **63**: p. 218-231.

[9] Kuo, C.-T., et al., *Configurable 2D and 3D spheroid tissue cultures on bioengineered surfaces with acquisition of epithelial–mesenchymal transition characteristics.* NPG Asia Materials, 2012. **4**(9): p. e27-e27.

[10] Kuo, C.T., et al., *ParaStamp and its applications to cell patterning, drug synergy screening, and rewritable devices for droplet storage.* Advanced biosystems, 2017. **1**(5): p. 1700048.

[11] Kuo, C.-T., et al., *Three-dimensional spheroid culture targeting versatile tissue bioassays using a PDMS-based hanging drop array.* Scientific Reports, 2017. **7**(1): p. 4363.

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