

# Fabrication of PEG Hydrogel Microstructures in Microfluidic System for Multicellular Spheroid Formation, Culturing and Analysis

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## ABSTRACT

This study presents a novel microfluidic device for 3-dimension cell culturing and analysis. Through defining the exposure to ultra-violet light by a photomask, localized photopolymerization of poly (ethylene glycol) diacrylate hydrogels form biocompatible and permselective microstructures within a microfluidic device. The designed geometry of microstructures allowed cells to be trapped into structures and to form spheroids *in situ*. Subsequent analysis and long-term three-dimension cell culture can achieve within the same device. This technique may apply as a microscale bioreactor or a platform for spheroid-based drug screening and study.

**Keywords:** multicellular spheroids, photoresponsive hydrogel, microfluidic systems, microscale bioreactor, and drug screening

## 1 INTRODUCTION

Because of their structural and functional resemblance to real tissues, multicellular spheroids (MCS) have been widely used in the last decade as 3-dimensional cell culture models for basic cell biology studies and cell-based drug screenings [1-2]. Traditionally, MCS can be generated in suspension culture using the hanging drop method, spinner flasks, culturing on nonadhesive surface, rotary cell culture systems or other engineering techniques [3]. However, many spheroids-based studies require spheroids of a specific size and transportation of the pre-formed spheroids to the assay chambers for analysis. These procedures are usually labor intensive and time consuming. In addition, growing multicellular spheroids in fix-sized multiwell dishes also suffer from problems such as inadequate nutrient supply and poor waste removal. These limitations prohibit the utilization of MSC for drug screening and application on tissue engineering. Recent advancement of microfluidic technologies has provided scientists many new capabilities in performing cell culture including continuous dynamic perfusion and automatic fluidic control. All these

functions are beneficial for growing cells in a long term culture and their subsequent treatment and analysis [4-6].

The photoresponsive hydrogels have drawn much attention recently in cell culture and tissue engineering. This photopolymerization technique applies light to generate free radicals from a photoinitiator that react with the active end groups on hydrogel pre-polymers to form covalent crosslinks [7]. The Poly(ethylene glycol) diacrylate (PEG-DA) is a promising photoresponsive hrogels with numerous advantages, such as good biocompatibility and high mass transfer efficiency. The PEG-DA hydrogel has already been applied on scaffolds fabrication, cells encapsulation as well as *in vitro* 3-D reconstructions. Previous studies have shown that, using photolithography, PEG-DA hydrogels can generate precise microscale scaffolds or microstructures easily [8].

In this study, we describe a microfluidic device integrated with U-shaped PEG hydrogel microstructures that allow cell trapping, spheroid formation through self-assembly, long-term culture of the spheroids as well as preventing cells from shear force damage while allowing free diffusion of nutrients and wastes. This hydrogel-based microfluidic device can be easily fabricated by using the *in situ* photopolymerization technique [9-10].

## 2 MATERIAL AND METHODS

### 2.1 Materials

All tissue culture media, antibiotics, sera were purchased from Invitrogen Corp. HepG2 cells, the human hepatoma cell line, (American Type Culture Collection) was maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a 5% CO<sub>2</sub> humidified incubator at 37°C. Other chemicals were purchased from Sigma-Aldrich unless indicated otherwise. Polydimethylsiloxane (PDMS) was purchased from Dow Corning. Photomasks were designed using the L-Edit v10 software (Tanner Research) and printed on emulsion films (Taiwan Kong-King) with the resolution of 20,000 dpi.

## 2.3 Microfluidic devices fabrication

The PDMS microchannels were fabricated using the conventional PDMS casting method, mixing A and B reagents (10 : 1) and pulling into acrylic molds [11-12]. After backing, PDMS microchannels were peeled off from molds and bonded to glass slides to form PDMS growth microchambers with oxygen plasma treatments. To enhance the bonding ability between PEG-DA hydrogels and glass slides, 3-ethacryloxypropyltrimethoxysilane (MPTMS) was used to introduce reactive acrylate groups on the glass and PDMS surface.

## 2.4 PEG-hydrogel microstructures generation

PEG-hydrogel microstructures were generated in PDMS microchambers using the *in situ* photopolymerization technique. The 70% PEG-DA (Mw = 575 DA) precursor solutions were prepared by dissolving PEG-DA in phosphate saline buffer (PBS) containing at least 1% (v/v) photoinitiator (2, 2-dimethoxy-2-phenylacetophenone). To form microstructures, the PEG-DA solutions were first injected into the device and then exposed to UV light through the photomask. The light source in this study were used the mercury lamp on a fluorescence microscope (BX-51; Olympus). After photopolymerization, non-reactive PEG-DA polymers and photoinitiators were removed by washing devices with DI water. (Figure 1A-B)

## 2.5 Cell trapping and spheroid formation

Cells were directly injected into a vertically PEG-based growth microchamber and trapped into U-shaped structures by applying the combined forces between gravity and the fluid flow. The flowrate was controlled using a syringe pump. Parts of cells were settled down into U-shaped structures and the other non-trapping cells were washed away through the flow (Figure 1C). After cell trapping process, cells were cultured and formed spheroid in the continuous perfusion system (Figure 1D).

# 3 RESULTS AND DISCUSSION

## 3.1 Microfluidic device generation

The PEG-based growth microchamber were consist of a PDMS microfluidic channel and PEG-DA hydrogel microstructures, the accomplished device is shown in Figure 2. The PDMS microchannel are fabricated by the standard molding technique, and then bonded to a glass slide (75 mm × 25 mm). The channel length is 27 mm, the width is 3 mm, the height is 0.7 mm and the total volume is about 56.7  $\mu$ l. The PEG-DA hydrogel microstructures were generated using *in situ* photopolymerization technique and the optimal exposure time was between 4 - 6 s. Each U-shaped structure had an inner space of 250  $\mu$ m × 200  $\mu$ m × 700  $\mu$ m. These PEG-DA microstructures could be maintained in the microfluidic device for at least 2 weeks.

This U-shaped were designed for the purpose of trapping cells, controlling spheroid as well as reducing the shear force during the perfusion culture. Using photoresponsive hydrogel material provides high mass transfer efficiency and the various structures and sizes could be generated easily through using different pattern of photomasks.

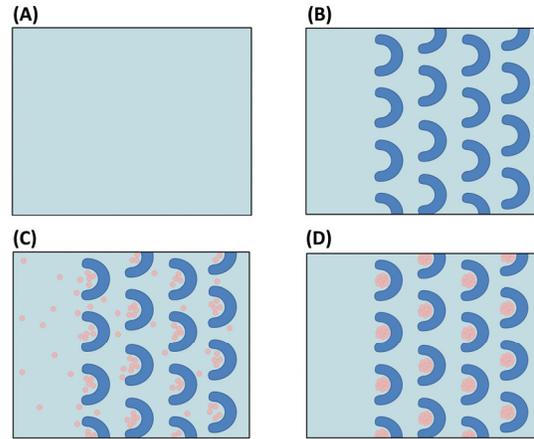


Figure 1: Processes of PEG-hydrogel microstructures generation and spheroid formation. (A) PEG-DA loading (B) Hydrogel microstructures generation (C) Cell trapping (D) Spheroids formation and culturing.

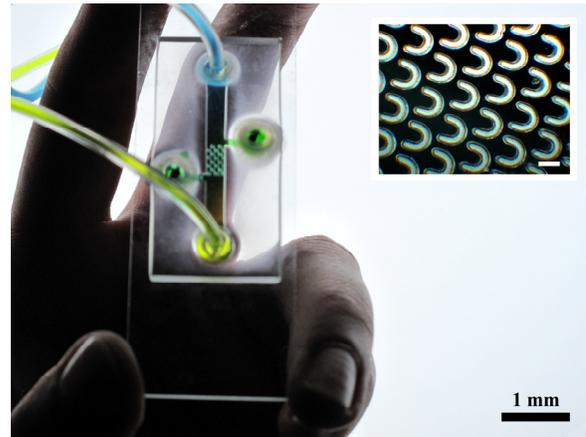


Figure 2: The accomplished PEG-based growth microchamber. The insert picture shows the U-shaped microstructures with the scale bar 200  $\mu$ m.

## 3.2 Cell trapping and spheroid formation

HepG2 cells were continuous loaded into a vertical microfluidic device with the cell density at least of  $2.8 \times 10^7$  cells/ml. As shown in Figure 3, after 3-5 min, each U-shaped microstructure was filled with cells about half to two-third volume of U-shaped structure. After cell trapping process, the device was set up horizontally and cells were cultured with perfusion system. The trapped cells were formed spheroids between 9 to 24 h under a proper flowrate control and higher degree of compactness spheroids could

be observed after several days of culture. The sizes of spheroids were according to the sizes of U-shaped structures we designed. Cell culturing could perform at least one week and numerous biofunctional assays could be accomplished easily with this device.

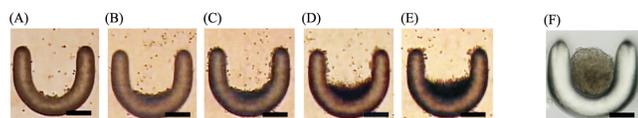


Figure 3: Cell trapping and spheroid formation. (A-E) Cell trapping between 0 - 5 min (F) After one day

## 4 CONCLUSIONS

In conclusion, we generate a novel PEG-based, simple-to-fabricate, U-shaped micro-scale growth chamber, which allows cell trapping and forming into spheroids as well as culturing in the presence of a continuous perfusion flow, a long-term 3D cell culture can be achieved. We believe that it can provide a good platform for many spheroid-based studies and applications in the future.

## REFERENCES

- [1] F. Pampaloni, E. G. Reynaud and E. H. Stelzer, *Nat. Rev. Mol. Cell Biol.* 8, 839, 2007.
- [2] J. Friedrich, C. Seidel, R. Ebner and L. A. Kunz-Schughart, *Nat. Protoc.* 4, 309, 2009.
- [3] R. Z. Lin and H. Y. Chang, *Biotechnol. J.* 3, 1172, 2008.
- [4] D. Di Carlo, L. Y. Wu and L. P. Lee, *Lab Chip* 6, 1445, 2006.
- [5] L. Y. Wu, D. Di Carlo and L.P. Lee, *Biomed. Microdevices.* 10, 197, 2008.
- [6] L. Yu, M. C. Chen and K. C. Cheung, *Lab Chip* 10, 2424, 2010.
- [7] F. Yang, G. W. Christopher, D. A. Wang, H. Lee and J. Elisseeff, *Biomaterials*, 26, 5991, 2005
- [8] A. Revzin, R. J. Russell, V. K. Yadavalli, W. G. Koh, C. Deister, D. D. Hile, M. B. Mellott and M. V. Pishko, *Langmuir* 17, 5440, 2001.
- [9] D. Dendukuri, S. S. Gu, D. C. Pregibon, T. A. Hatton, P. S. Doyle, *Lab Chip* 7, 818, 2007.
- [10] S. A. Lee, S. E. Chung, W. Park, S. H. Lee and S. Kwon, *Lab on a Chip* 9, 1670, 2009.
- [11] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. Schueller and G.M. Whitesides, *Electrophoresis* 2000, 21, 2002.
- [12] J. C. McDonald and G. M. Whitesides, *Acc. Chem. Res.* 35, 491, 2000.