HEPATOCYTE CO-CULTURE IN ALGINATE HYDROGEL FOR ANTI-CANCER DRUG ANALYSIS

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ABSTRACT

This paper presents the formation of double-layer cellular tumor spheroid for the co-culture of hepatocyte (HepG2 cancer liver cell and LO2 normal liver cell) using alginate hydrogel. With the microfluidic chip, double-layer cellular tumor spheroid can be formed by the hydrogel droplet to mimic the in vivo environment in which the cancer cell is surrounded by normal cell. A microchip with concentration gradient generation and tumor spheroid trapping site is designed for anti-cancer drug analysis.

KEYWORDS: Hydrogel, Hepatocyte, Drug analysis, Co-culture

INTRODUCTION

Hydrogels are commonly used for cell encapsulation and has been a common tool to form tumor spheroids [1]. Conventionally, hydrogel tumor spheroids are formed by hanging drops [2]. However, it has limited control over the droplet size and content. Microfluidic technology has demonstrated well-controlled cellular studies [3]. Recently, multiphase flow in microfluidic system has been designed to encapsulate cancer cells in hydrogel droplet and form a tumor spheroid [4]. Hydrogel droplets can be formed using a flow-focusing mechanism with highly uniform diameters (< 5%) [5]. By employing microfluidic technology, the droplet size and content can be easily controlled.

Previously demonstrated in vitro tumor spheroid formation schemes are either single cellular spheroid or multicellular spheroid [6]. However, for tumor spheroid under in vivo environment, the tumor cells are encapsulated by normal cells as shown in Fig. 1. By using this model for anticancer drug analysis, the drug effectiveness and specificity can be better analyzed.

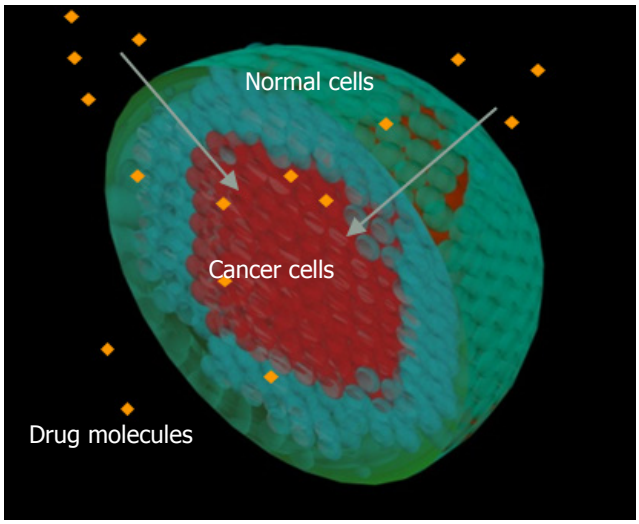

Figure 1: Schematic illustrations of double-layer cellular tumor spheroid for drug analysis.

CHIP DESIGN AND FABRICATION PROCESS

The schematic illustration of the double-layer cellular alginate droplet generation in the microfluidic environment as shown in Fig. 2(a). A 5-inlet junction is designed in which HepG2 cell (cancer liver cell) is injected in the central channel, LO2 (normal liver cell) is injected from the two side branches such that the HepG2 cells are focused in the centre by the LO2 cells, and mineral oil is injected in the two outer side branches to shape the cell flow streams into plugs. At the downstream, a sudden height expansion structure is designed to transform the cell plugs into cell droplets. Subsequently, the cell droplets are transferred to a calcium ion bath for gelation. After gelation, the double-layer cellular hydrogels are injected into the microchip as shown in Fig. 2(b). The hydrogels are trapped in the microchannel with the trapping site. Tumor spheroids are formed in the hydrogel after 5-day culture and the tumor spheroids can be tested with anti-cancer drugs, such as Etoposide, at different concentrations generated by the on-chip gradient generator.

The microfluidic chips are fabricated using standard soft-lithography techniques. For the hydrogel droplet formation microchip, the 5-inlet junction and the sudden height expansion are patterned using a 50-μm and 150-μm thick layer of SU8 master structure, respectively. Then, the mixture of poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning) prepolymer and curing agent (10:1) is poured over the masters, degassed, baked for 2 hours at 75 °C and then peeled off. After manually punching inlets and outlets, the PDMS slabs are exposed to air plasma for 15 s using a corona treater (BD-25, Electro-Technic Products), aligned and bonded. Similarly, the microchip used for tumor spheroid trapping and drug analysis is fabricated using the same process.

HepG2 and LO2 cells are maintained in DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 U/mL streptomycin. All cells were cultured in flasks for 3 days prior to microfluidic

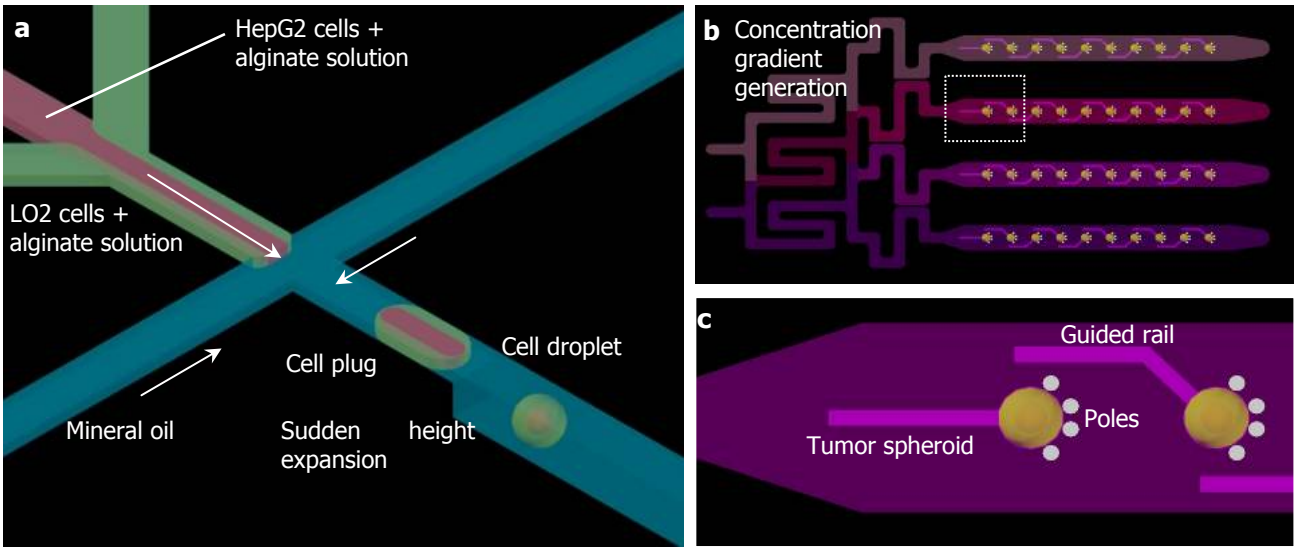

Figure 2: Schematic illustrations of (a) the chip design to form the double-layer cellular alginate hydrogel droplet, (b) the microchip for tumor spheroid trapping with concentration gradient generation for drug analysis, and (c) zoom view of the trapping site with trapping poles and guided rail.

experiments. Cells were detached from the culture flasks using 0.25% trypsin in PBS. Cell suspensions were prepared at a concentration of 10^7 cell/mL using DMEM medium mixed with 2.0 wt% alginate (Sigma-Aldrich). For gelation of alginate, 40 mM $CaCl_2$ solution (Sigma-Aldrich) is prepared as the buffer bath. For droplet formation, the continuous phase used in the experiment is mineral oil with 1.0 wt% surfactant Span 80. Mineral oil is chosen due to its biocompatibility and low cytotoxicity. The hydrogel droplets are collected externally in the calcium ion bath and gelled. The gelled droplets are then loaded into the microfluidic chip and trapped in the trapping sites for cell culture.

EXPERIMENTAL RESULTS AND DISCUSSIONS

Figure 3(a) shows the microphotos of the formation of double-layer cellular hydrogels. HepG2 and LO2 cells are injected into the core and the cladding of the dispersed phase and a stream of cell plugs are formed at the junction due to the mineral oil stream by shear. Fig. 3(b) shows a group of cellular hydrogel droplets in the calcium ion bath. When the calcium ion is diffused into the hydrogel droplet, the alginate hydrogel is gelled instantly. Fig. 3(c) shows the formation of the double-layer cellular hydrogel in which the HepG2 cells (red fluorescent) are surrounded by the LO2 cells (green fluorescent). The gelled hydrogel droplets are then loaded into the trapping microchip. Cellular hydrogel droplet is guided into the trapping site by the designed rail and trapped by the poles as shown in Fig. 3(d).

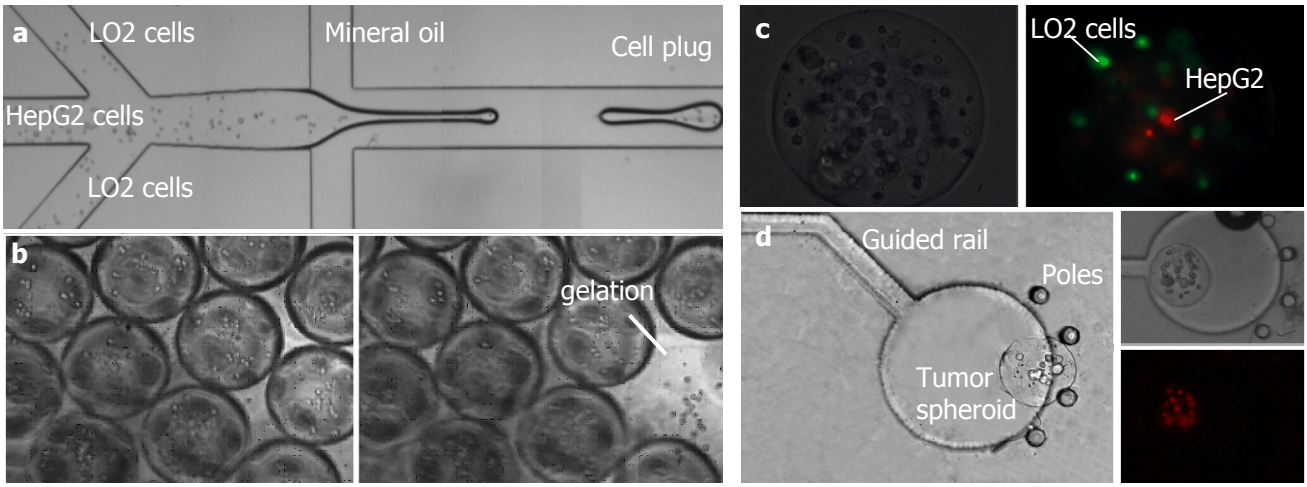

Figure 3: Microphotos of (a) alginate droplet formation, (b) alginate droplet gelation, and (c) double-layer cellular hydrogel, and (d) tumor spheroid trapping.

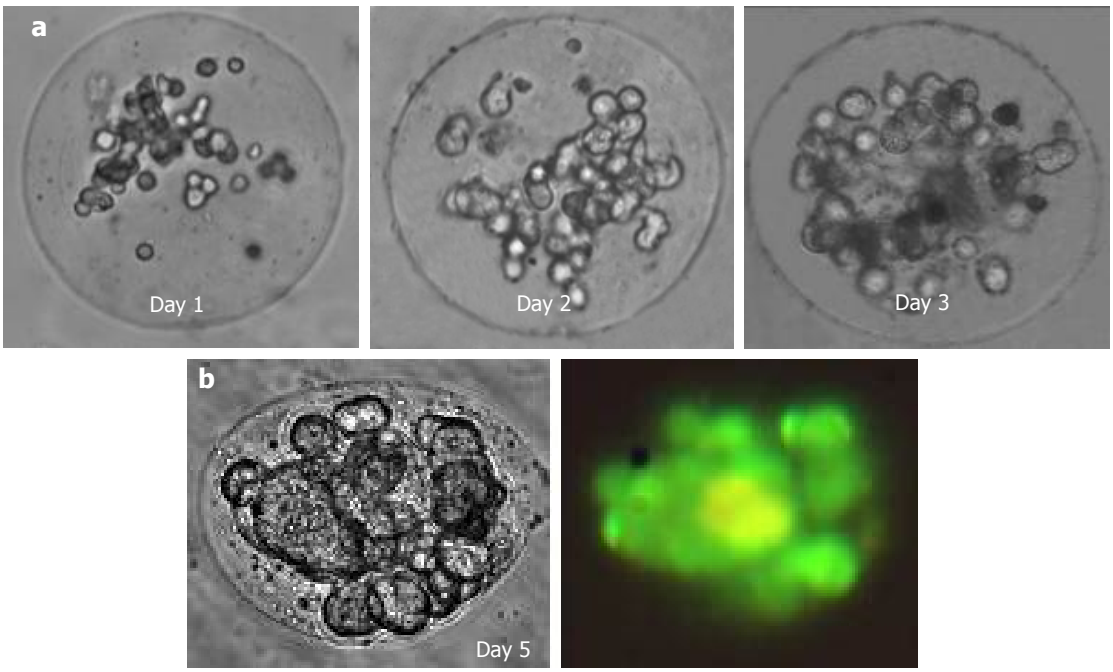

Figure 4: Microphotos of (a) cell proliferation and (b) cell viability test using Calcein-AM.

Figure 4(a) shows the 3-day culture of the cellular hydrogel. It shows that the cells proliferate in the hydrogel and a tumor spheroid is formed after 5 days. Viability test is also performed using Calcein AM in which live cells are green fluorescent as shown in Fig. 4(b). By ensuring cell proliferation and remains viable, the system can be used to generate double-layer cellular tumor spheroid for anticancer drug analysis.

CONCLUSIONS

In conclusion, a microfluidic chip is developed to form double-layer cellular tumor spheroid in alginate hydrogel and a microchip is designed for the culture and drug analysis of the tumor spheroid. HepG2 cells are surrounded by LO2 cells in the hydrogel to mimic the in vitro environment in which cancer cells are surrounded by normal cells. Cell proliferation and viability for the tumor spheroid in alginate hydrogel are investigated. The formed tumor spheroids can be tested with anti-cancer drugs, such as Etoposide. The double-layer cellular tumor spheroid model is a better model for anticancer drug analysis for different tumor spheroids.

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