LONG-TERM STORAGE OF LIVING CELLS ON CHIP FOR CELL-BASED ASSAYS

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ABSTRACT

Long-term storage of living cells on chip by a spontaneously formed phospholipid polymer hydrogel is reported. The technique can conquer the shortcomings of recent cell-based chips needing preparation of cells off chip before assays, and would finally make cell-based assays really on chip.

KEYWORDS: Hydrogel, Cell Storage, Cell-Based Assays, Cytotoxicity

INTRODUCTION

There are numerous advantages of using microfluidic systems for cell based assays(i.e., cell-based assays on chip), some of which are abilities to recreate in vivo-like microenvironments and even to modulate the microenvironments of single cells. However, recent cell-based assays on chip usually need to prepare cells off chip before assays. To realize all processes of a cell-based assay on chip, recently we have developed a technique for a long-term storage of living cells on chip by taking advantage of a spontaneously formed hydrogel.

Cell suspension in a culture medium containing a dissolved polymer was firstly delivered into a chip. Then via a spontaneously cross-linking gelation of polymers, the cells were encapsulated in the formed hydrogel on chip without any physical process such as photo- and electro-treatments. Cells stored in hydrogel can keep alive for at least a week, and can be further used for cell-based assays (e.g. cytotoxicity assays), either in a gel encapsulated format or after released by dissociating the hydrogel.

EXPERIMENTAL

The hydrogel can be prepared by mixing a solution of poly(vinyl alcohol) (PVA) with a solution of poly(2-methacryloyloxyethyl phosphorylcholine(MPC)-*co*-*n*-butylmethacrylate(BMA)-*co*-*p*-vinylphenylboronic acid (VPBA))(PMBV)(Figure 1), a water-soluble phospholipid polymer reported previously[1]. Human arterial endothelial cells (HAECs) cultured in an EC culture medium were used. A chip with a cell-container chamber was designed and fabricated on glass substrates (7 cm × 3 cm) (Figure 2). Firstly, 25 μL of cell suspension (1.6 × 105 cells/mL) in the culture medium containing 5 wt% PMBV flowed through the introducing microchannel into the cell-container chamber by using a microsyringe pump with a withdraw mode[2]. Then 5 μL of 5 wt% PVA (in D-PBS) solution was introduced. Five minutes later,

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the cell encapsulated PMBV/PVA hydrogel was spontaneously formed. With a state of all inlets/outlets open for life support, the chip was incubated at 37 °C in 5% CO_2 for a week. After that, 10 µL calcein-AM/ethidium homodimer-1 solution (LIVE/DEAD Viability/Cytotoxicity Kit, Molecular Probes) was introduced to investigate cell viability. A cytotoxicity assay was also performed off chip. Firstly, hydrogel encapsulated HAECs were prepared in a 96-well microplate. After a seven-day incubation period, the hydrogel encapsulated cells were exposed to 7% (V/V) and 70% (V/V) methanol and evaluated with a LIVE/DEAD assay.

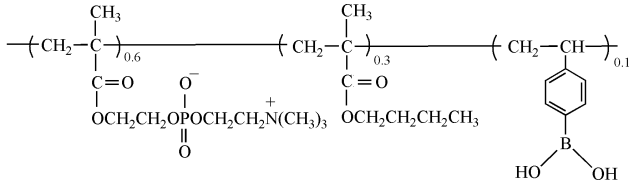

Figure 1. Chemical structure of poly(MPC-co-BMA-co-VPBA)(PMBV). The compositions of MPC, BMA and VPBA are 0.6, 0.3 and 0.1, respectively.

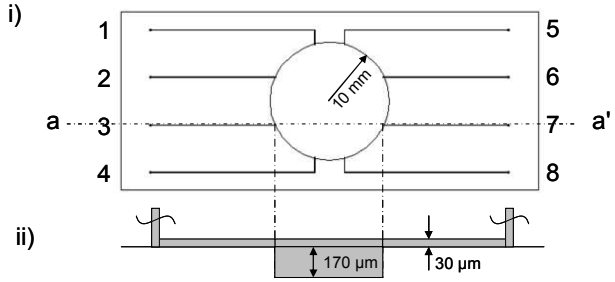

Figure 2. Schematic diagram of the cell-container chip. i) whole image of the chip; and ii) a-a' cross section. All introducing channels are 30 µm in depth and 100 µm in width. The cell-container chamber is 10 mm in diameter and 200 µm in depth. The PMBV/cell suspension was introduced through inlet 1 by withdrawing force from outlet 8. The PVA solution was introduced through inlet 4 by withdrawing force from outlet 5. Other inlets and outlets were used to introduce toxins and LIVE/DEAD agents for viability and cytotoxicity assays.

RESULTS AND DISCUSSION

In a LIVE/DEAD assay, green fluorescence (calcein, Ex/Em ~ 495 nm/~ 525 nm) is an indicator of live cells(live image), while red fluorescence(Ethidium homodimer-1, Ex/Em ~ 495 nm/~ 635 nm) is an indicator of dead cells(dead image). As Figure 3 demonstrated, most HAECs encapsulated in PMBV/PVA hydrogel on chip kept alive after seven days' storage. This is well consistent with the result of the off-chip experiment (Figure 3). Furthermore, the cells encapsulated in hydrogel can be directly used for further cell-based assays, such as a cytotoxicity assay. Figure 4 shows the cytotoxicity results of PMBV/PVA hydrogel encapsulated HAECs exposed to methanol. In the case of lower concentration (7%) methanol exposure, most cells were

still live, while in the case of higher concentration (70%) methanol exposure, most cells were dead. This reveals that the encapsulated cells kept functional activity and high resolution to different toxins. In addition, as reported previously [1], the encapsulated cells can be released after dissociating the hydrogel by adding D-glucose, so researches on cell releasing on chip and following assays are ongoing recently.

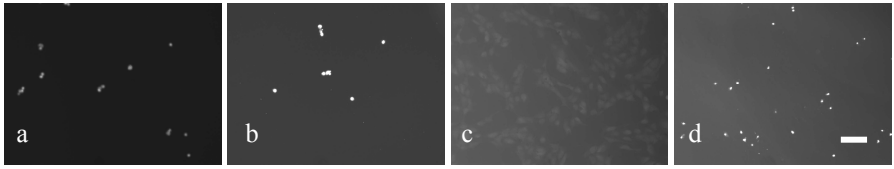

Figure 3. Fluorescence images of LIVE/DEAD viability assays of HAECs encapsulated in PMBV/PVA hydrogel for seven days: a) live image and b) dead image of cells encapsulated in a cell-container chip; c) live image and d) dead images of cells encapsulated in a 96-well microplate. Scale bar is 100 μm.

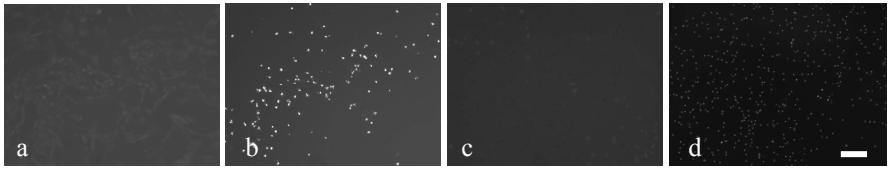

Figure 4. Fluorescence images of a cytotoxicity assay of HAECs encapsulated in PMBV/PVA hydrogel in a 96-well microplate for seven days. After toxin exposure and LIVE/DEAD staining, a LIVE/DEAD Viability/Cytotoxicity assay was performed. a)Live image and b) dead image of encapsulated cells exposed to 7% (v/v) methanol; c) live image and d) dead image of encapsulated cells exposed to 70%(v/v) methanol. Scale bar is 100 μm.

CONCLUSIONS

Long-term storage of living cells on chip was realized by a spontaneously formed phospholipid polymer hydrogel. The technique would finally make cell-based assays really on chip, because it can conquer the shortcomings of recent on chip cell-based assays needing preparation of cells off chip before assays.

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