TEMPERATURE-FLEXIBLE CELL MICROCONTAINERS FABRICATED WITH A PHOSPHORYLCHOLINE POLYMER HYDROGEL ON CHIP

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ABSTRACT

A cell microcontainer which was fabricated with a phosphorylcholine polymer hydrogel on a microchip exhibited capability to flexibly preserve living cells for more than one week, not only at 37°C in a cell culture incubator but also at room temperature (RT) in a common indoor condition or at 2-6 °C in a household refrigerator. The use of the hydrogel microcontainer could facilitate various miniature cell-based tools to be used onsite or at home.

KEYWORDS

Cell microcontainer, Hydrogel, Preservation, Room temperature, Refrigeration temperature

INTRODUCTION

A major impediment to the widespread use of cell-based chips for onsite and portable applications is the lack of feasible approaches to maintain cell viability and activity in the field without highly equipped cell culture facilities and highly trained personnel. Herein, we address a cell microcontainer fabricated with a phosphorylcholine polymer hydrogel, which is capable of long-term maintenance of cell viability in temperature-flexible conditions without conventional perfusion culture.

EXPERIMENTS

The phosphorylcholine polymer hydrogel is composed of polyvinyl alcohol (PVA) and a water-soluble phosphorylcholine polymer, poly (2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-*n*-butyl methacrylate (BMA)-*co*-*p*-vinylphenylboronic acid (VPBA)) (referred to as PMBV, Figure 1). The PMBV was synthesized by a conventional radical polymerization of monomers, MPC, BMA and VPBA. By mixing the PMBV and the PVA aqueous solutions, the PMBV/PVA hydrogel forms spontaneously, via a covalently cross-linking process between the phenylboronic acid groups of the VPBA units in the PMBV and hydroxyl groups in the PVA (Figure 2). A cell-containing PMBV/PVA hydrogel can be simply prepared by mixing the cell suspension polymer solutions either in bulk [1, 2] or in a microchip chamber [3], the latter format called by us as a cell microcontainer (Figure 2).

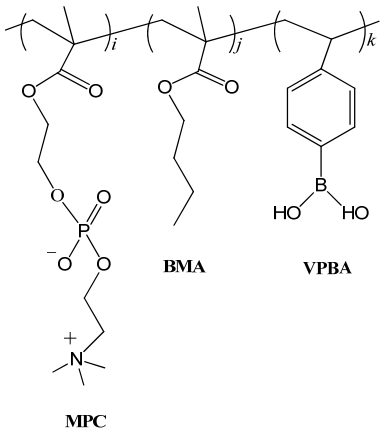

Figure 1. Chemical structure of poly (MPC-*co*-BMA-*co*-VPBA) (PMBV); *i* / *j* / *k* = 60/30/10.

The microchip as depicted in Figure 2 was fabricated on glass substrates using a photolithographic wet etching technique [1]. It is composed of two sets of cell microcontainer chambers and accessorial introducing channels, and thereby two independent experiments can be performed at one time under the same conditions. The chambers are 5.0 mm in radius and approximately 400 μm in depth, and each is approximately 30 μL in total volume. All channels are 700 μm in width and 200 μm in depth, which are sizes designed to facilitate the transportation of cells. When introducing a solution to an objective chamber, the chip is positioned vertically by a chip holder so that all accessorial inlets/outlets lie on the upper side of the objective chamber. By taking advantage of the chip design and operation, the liquid effluence from the outlet can be effectively avoided, and a fully filled chamber can be realized. The chip is positioned horizontally during the incubation process. To fabricate the cell-containing hydrogel in the microchip chamber (i.e., cell microcontainer), 5 μL of 2.5 wt% PVA solution was first injected into the chamber through the introducing microchannel using a microsyringe pump with a withdraw mode. Then, 15 μL of cell suspension in 5.0 wt% PMBV was introduced at a high flow rate of approximate 5 μL s^{-1}. The cell-containing hydrogel then spontaneously formed in the chamber. With all inlets/outlets open, the microchip was put in a cell

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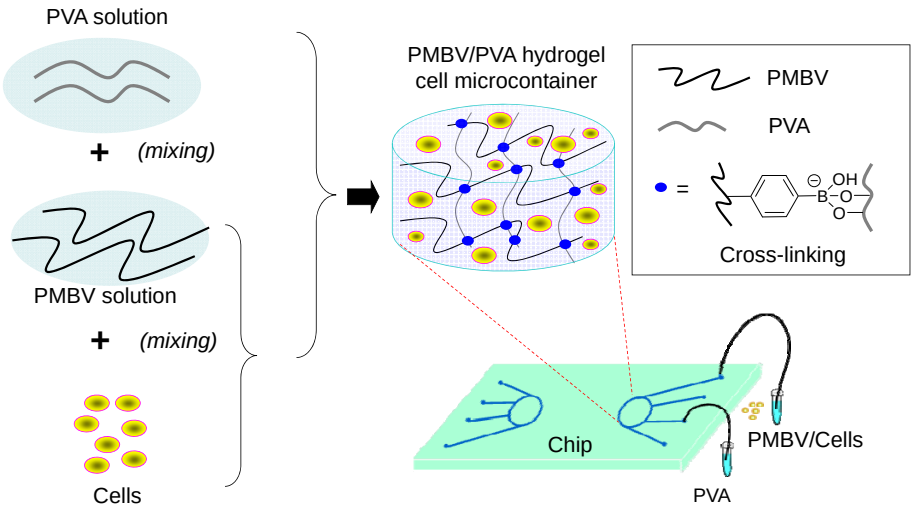

Figure 2. Mechanism of PMBV/PVA hydrogel formation and fabrication of PMBV/PVA hydrogel cell microcontainer.

culture incubator (37 °C), in a common indoor condition (RT), or in a household refrigerator (2~6 °C) without any additional operation.

The viability of the cells preserved in the PMBV/PVA hydrogel cell microcontainer was investigated using live/dead assays (live/dead viability/cytotoxicity kit, Molecular Probes) according to a protocol described previously [3]. For each sample, after calcein-AM (2 μM)/ethidium homodimer-1 (4 μM) reagent mixture solution (10 μL) was introduced to the chip, the chip was incubated at room temperature for 45 min, and then observed and analyzed using the fluorescence mode of an inverted microscope (IX 71; Olympus) with a charge coupled device (CCD) camera (Retiga EXi; QImaging). Great care was taken to minimize the photobleaching because of excessive exposure. In a live/ dead assay, green fluorescence (calcein, excitation (Ex) : emission (Em) = ~494 nm : ~517 nm) is an indicator of live cells (live image), while red fluorescence (ethidium homodimer-1, Ex : Em ~528 nm : ~617 nm) is an indicator of dead cells (dead image). The viability was estimated by counting the number of live and dead cells in more than 3 different areas of the chamber (i.e., more than 3 merged live/dead images) using an image processing program, ImageJ 1.40g, developed by the National Institutes of Health (NIH). The viability (or the percentage of live cells) is defined as the number of live cells/number of cells in total (%). The viability of cells preserved in medium in a 96-well microplate was investigated using the same protocol. Cell suspension (100 μ L) was first seeded in each well. Before live/dead assays, the cells were washed 5 times with 1 × PBS (100 μ L per well), and then 1 × PBS (100 μ L) was added to each well. After that, the live/dead assay solution (50 μ L) was added to each well for the following viability assays. The same initial cell densities were applied in comparative investigations of two formats.

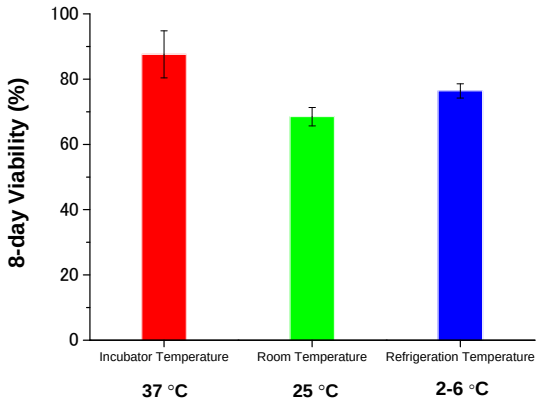

Figure 3. Viabilities of the L929 cells preserved in the PMBV/PVA hydrogel cell microcontainers for 8 days at 37°C in a cell culture incubator (red column), at room temperature (RT) in a common indoor condition (green column), and at 2-6 °C in a household refrigerator (blue column).

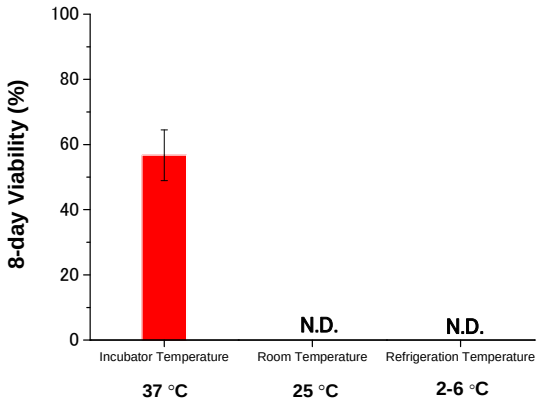

Figure 4. Viabilities of the L929 cells preserved in DMEM cell culture medium in 96-well microplates for 8 days at 37°C in a cell culture incubator (red column), at room temperature (RT) in a common indoor condition, and at 2-6 °C in a household refrigerator. (N.D.: not detected, i.e., viability = 0%)

RESULTS

The mouse fibroblast cell line L929 cells were used as model cells considering their wide applications in cell-based assays. The 8-day viabilities of L929 cells preserved in the PMBV/PVA hydrogel cell-microcontainer at 37°C in the cell culture incubator, at room temperature in a common indoor condition, and at 2-6 °C in a household refrigerator are shown in Figure 3. For a comparison, the 8-day viabilities of L929 cells preserved in Dulbecco's modified Eagle's medium (DMEM) in a 96-well microplate at 37°C in the cell culture incubator, at room temperature in a common indoor condition, and at 2-6 °C in a household refrigerator are shown in Figure 4. In comparison with cells in cell culture medium exhibiting low viability or totally dead in the corresponding conditions, cells in the hydrogel microcontainers in all conditions exhibited quite high viability (90-70%) after 8 days. This indicates that the PMBV/PVA hydrogel cell microcontainer has capability to flexibly preserve living cells for a relatively long period which can meet the requirement for cell-based chips for onsite and portable short-term applications.

CONCLUSIONS

A cell microcontainer capable of preservation of living cells in temperature-flexible conditions was developed with a phosphorylcholine polymer hydrogel on a microchip. The developed the cell microcontainer could make it possible to perform cell-based assays in miniature format under flexible conditions without highly equipped cell culture facilities and highly trained personnel.

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