CELL-BASED TOXIN SCREENING INTEGRATED WITH A CELL-SUSTAINABLE HYDROGEL ON CHIP FOR ONSITE AND PORTABLE APPLICATIONS

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ABSTRACT

We demonstrate a microfluidic cell-based toxin screening with integration of a cell-sustainable hydrogel, which is capable of long-term maintenance of cell viability and biological sensitivity without conventional perfusion culture. The use of the cell-sustainable hydrogel would bring about an onsite and portable resolution for cell-based applications to be performed on microfluidic chips.

KEYWORDS: 2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer, Hydrogel, Cell sustainability, Toxin screening, Cell-based assays

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 sensitivity in the conditions without highly equipped cell culture facilities and highly trained personnel. Herein, we address a microfluidic cell-based toxin screening with integration of a cell-sustainable hydrogel, which is capable of long-term maintenance of cell viability and biological sensitivity without conventional perfusion culture. The use of the cell-sustainable hydrogel would bring about an onsite and portable resolution for cell-based applications to be performed on microfluidic chips.

EXPERIMENTAL

The cell-sustainable hydrogel is composed of poly [2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA)-co-p-vinylphenylboronic acid (VPBA)] (PMBV, Figure 1) and polyvinyl alcohol (PVA), which have been used for cell encapsulation both in bulk [1] and in chip [2]. The major component of the hydrogel, PMBV is one of the MPC polymers which are well known for their excellent biocompatibility and high ability to suppress nonspecific protein adsorption and subsequent bioreactions [3]. PMBV (Figure 1) was synthesized by a conventional radical polymerization according to previously reported method process [1].

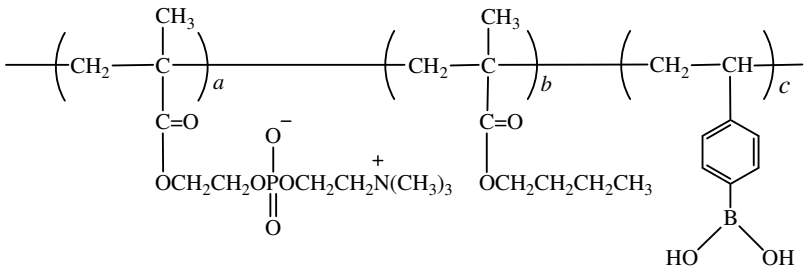

Figure 1. Chemical structure of poly(MPC-co-BMA-VPBA), referred to as PMBV. a/b/c = 60/30/10.

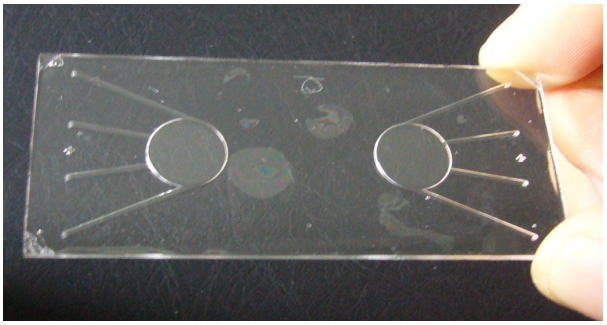

Figure 2. A picture of the two-chamber chip fabricate on glass substrates (70 mm × 30 mm) for cell-based toxin screening experiments on chip.

For cell-based toxin screening experiments, a chip (Figure 2) was fabricated on glass substrates. It is composed of 2 sets of cell-container chambers and accessorial introducing channels, and thereby 2 independent toxin screening experiments can be performed at one time in the same condition. The chambers are 5.0 mm in radius and approximately 400 μm in depth (D), 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. By simply introducing and then mixing 5 μL of 2.5 wt% PVA solution (prepared in PBS) and 15 μL of PMBV solution (prepared in the cell culture medium) suspended with cells in the chamber (Figure 2), cell-encapsulating hydrogel spontanesouly formed in the chip without inflicting any adverse physical effects.

RESULTS AND DISCUSSION

Without perfusion culture, the cells in the hydrogel in the chip maintained viability as high as that of cells conventionally cultured in a medium, over a period of days and weeks. For example, in the case of L929 cells with an initial cell density of 1.0×10^6 mL^{-1} in the hydrogel in the chip, the viabilities after 4 days and 8 days were 88.3% and 87.6%, respectively (Figure 3).

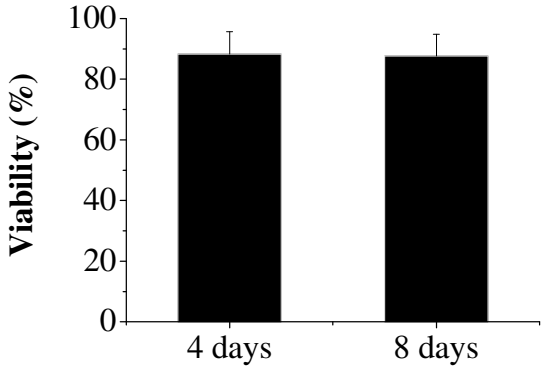

Figure 3. Viabilities of the L929 cells with an initial density of 1.0×10^6 mL^{-1} preserved in the PMBV/PVA hydrogel in the chip for 4 days and8 days. The viability, defined as number of live cells/number of cells in total, % , was estimated by a protocol of Live/Dead assay using calcein-AM (2 μM)/ethidium homodimer-1 (4μM). Data are mean ± SD, n > 3. Scale bar is 100 μm.

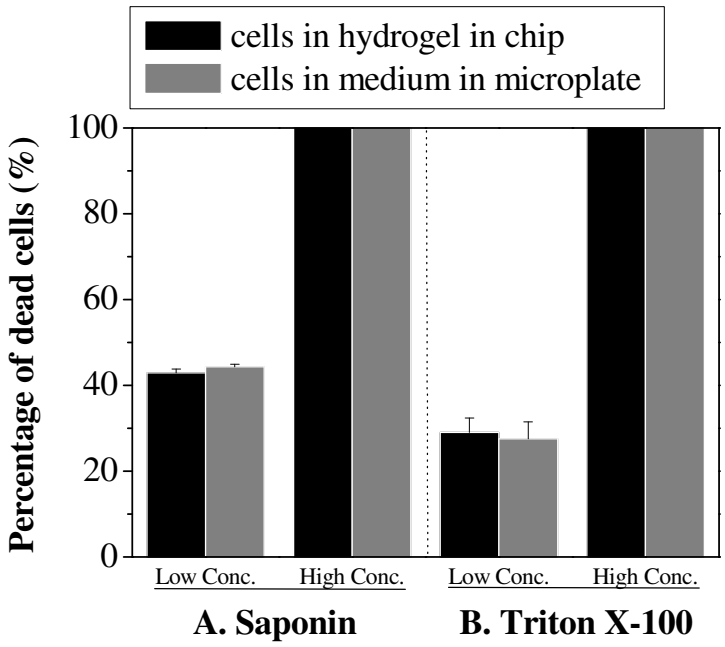

Figure 4. Comparative toxin screenings using L929 cells preserved in the hydrogel in the chip (initial density 1.0×10^6 mL^{-1}) for 8 days (black columns) vs. L929 cultured in medium in a 96-well microplate for 2 days with a density of

approximate 1.0 × 10^6 mL^{-1} (grey columns): (A) Saponin (0.01% and 0.10%, w/w) and (B) Triton X-100 (0.01% and 0.10%, w/w). Low Conc. and High Conc. notations on the x-axis represent high and low concentrations for each toxin.

In addition, toxin screenings were performed using the cells preserved in the hydrogel in the chip (initial density: 1.0 × 10^6 mL^{-1}, 8-day preservation) in comparison with the cells cultured in medium in a 96-well microplate (2 day culture to a density of 1.0 × 10^6 mL^{-1}). High-resolution toxin screening on chip was achieved as shown in Figure 4, suggesting that the cells preserved in the hydrogel in the chip maintained toxin sensitivity as the cells conventionally cultured in medium.

CONCLUSION

Therefore, we believe that the integration of the microfluidic chip with the cell-sustainable hydrogel would establish a revolutionary flexibility for cell-based screening for onsite and portable applications.

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