HIGH-THROUGHPUT CELL CULTURE CONDITION SCREENING BY MICROENVIRONMENT ARRAY

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ABSTRACT

We report a microenvironment array: perfusion culture microchamber array with different cell culture conditions. The microenvironment array provides the 4 x 4 combinatorial cell culture conditions composed of four different soluble factors and four different extracellular matrixes (ECM). We present the high-throughput cell culture condition screening by the microenvironment array. The cultivable condition for Chinese hamster ovary (CHO) cells without the serum was successfully detected by the microenvironment array.

KEYWORDS: Cell-based assay, Perfusion culture microchamber array, Microenvironment,

INTRODUCTION

Cellular events (e.g., extension, proliferation, apoptosis and differentiation) depend on extracellular stimuli from surrounding environment composed of soluble factors and scaffolds, i.e. ECMs [1]. Especially, the screening for the appropriate differentiation environment of the stem cells is urgent issue in the fields of regenerative medicine and drug discovery.

Recently, microfluidic cell culture chips have been expected to be applied to rapid and reproducible assays of small-volume samples without labor-intensive routine works and expensive robotics. We and other researchers have previously reported perfusion culture microchamber array chips, which can perform the parallel on-chip cell-based assays in the array-formatted microchambers [2-5].

In this paper, we developed the "microenvironment array chip" as a new-type of the perfusion culture microchamber array chip, where cells are cultured in the discrete microchambers with the different environment composed of combination of ECMs and soluble factors. We demonstrated the screening of the cell culture environment for cultivating CHO cells without serum by using the microenvironment array chip.

EXPERIMENTAL

Microenvironment array was fabricated by assembly of a "microchamber array chip" and an "ECM array chip" (Fig. 1). The microchamber array chip has an 8 x 8 array of 64 microchambers and provides different four types of growth factors in each two rows through the perfusion microchannels (Fig. 1a). Culture media containing growth factors are supplied through the macroscopic medium-stock chambers on the left side. Cells are loaded into all microchambers through the cell-inlet/medium-waste chamber on the right side. The microchamber array chip was fabricated by replica molding of PDMS.

ECM array chip has an 8 x 8 array of ECMs and provides different four types of ECMs in each two columns (Fig. 1b). To immobilize the ECMs on a PDMS, ECM array chip was prepared by using a "microfluidic patterning chip". The microfluidic patterning chip has an 8 x 8 array of through-holes, which is correspondent with the 8 x 8 array of the microchambers on the microchamber array chip, four ECM-inlet/ chambers, the four ECM-outlet chambers and the connecting microchannels through the ECM solutions. The microfluidic patterning chip was attached to the ECM array chip with the alignment and fastened by a microchip holder. Four different solutions of the ECMs including one blank milli-Q water were introduced by apply-

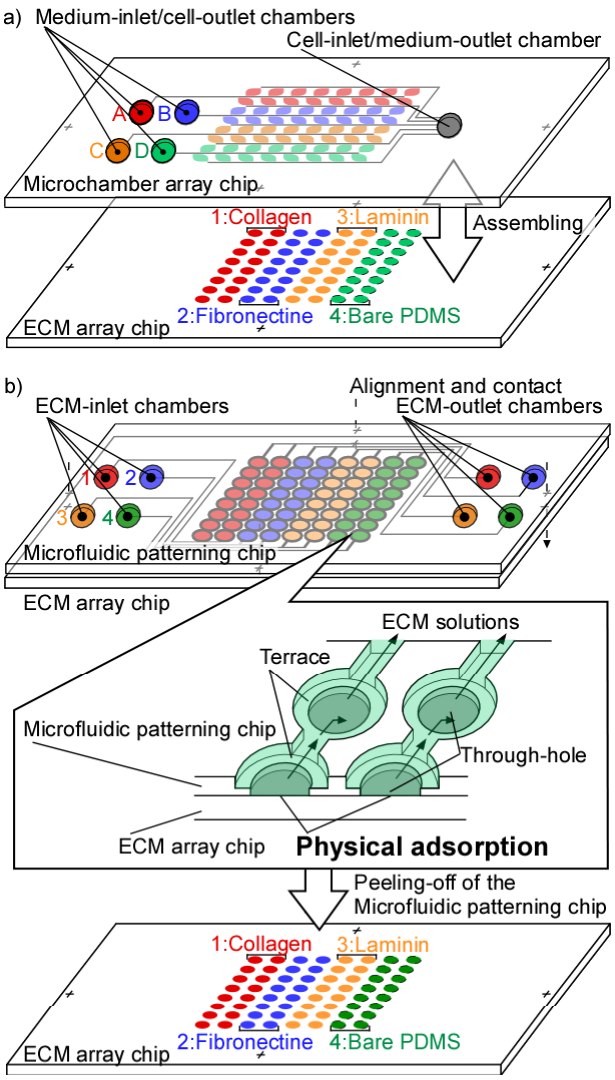

Fig. 1 Fabrication of microenvironment array chip and ECM array chip. (a) Fabrication of microenvironment array chip by assembling microchamber array chip and ECM array chip. (b) Fabrication of ECM array chip by using microfluidic patterning chip.

ing pressure. The ECMs were then immobilized on the ECM array chip by physical adsorption.

To form microenvironment array chip, the microchamber array chip and ECM array chip were attached after alignment with mutual alignment keys "+". Both of the chips were covalently bonded by O_2 plasma with ECM array protected by a mask [6].

CHO cell was used as a model cell and cultred in the microenvironment array. The cell suspension (6.4 x 10^5 cells/mL) was loaded into the microenvironment array. The cell-loaded microchip was firstly incubated under static culture conditions to induce the cell adherence. After 12 h, four culture media containing different soluble factors (Table 1. A-D) were added into the medium-inlet/cell-outlet chambers and continuous perfusion culture was carried out for total 2 days. The viability of the CHO cells was fluorometrically analyzed by staining with Calcein-AM.

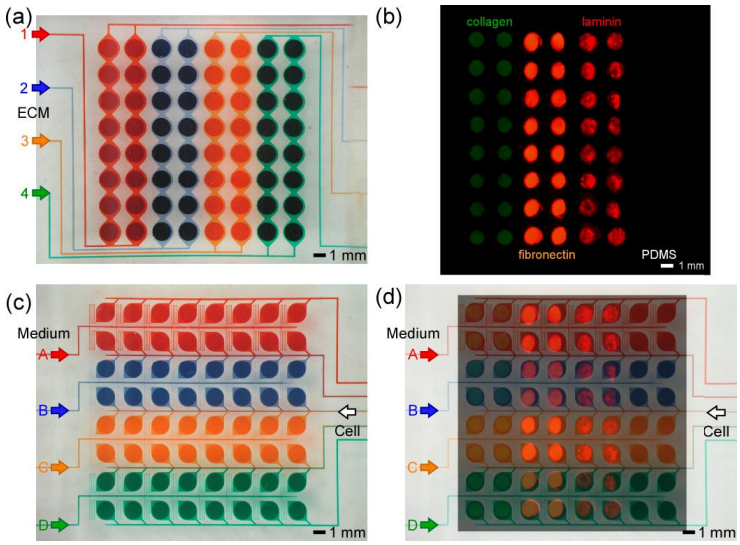

Fig. 2 Microscopy images of (a) Micropatterning chip, (b) ECM array chip, (c) Microchamber array chip, (d) Microenvironment array chip.

RESULTS AND DISCUSSION

To visualize the microfluidic networks of the microfluidic patterning chip, dye solutions were introduced into the microchip by applying pressure to the ECM-inlet chamber. (Fig. 2a). During introduction of solutions, no leakage of the solutions was observed, which indicates that microfluidic patterning chip and ECM array chip were sufficiently sealed by the microchip holder. The ECM microarray was visualized by the fluorescently labeled ECMs (Fig. 2b). All of ECM spots were individually formed with approximately 1.2 mm diameter and 2.25 mm pitch by the microfluidic patterning chip. The microfluidic networks of the mictrochamber array chip were visualized with dye solutions (Fig. 2c). The microenvironment array can provide the 4 x 4 combinatorial microenvironments composed of four different soluble factors and four different ECMs in the microchamber array (Fig. 2d).

The perfusion culture was successfully performed in the microenvironment array chip and cell viability was analyzed (Fig. 3a). CHO cells grew in the microenvironment array with the serum, and cell growth depended on the ECM type. The growth of CHO cells was better in the microchamber array with laminin, fibronectin and collagen in this order. On the other hand, most of the cells died in the microenvironments without serum. However, CHO cells in the microenvironment array of B-1 and B-3 were survived without the serum (Fig. 3b). The amount of living CHO cells was larger in the microchamber array with the laminin than in the microchamber array with the collagen. These results indicate that the viability of the CHO cells depended on the extracellular stimuli from the microenvironment composed of the combination of soluble factors and ECMs. Furthermore, the cultivable environments for the CHO cells without the serum were successfully detected from 4 x 4 combinatorial microemvironments.

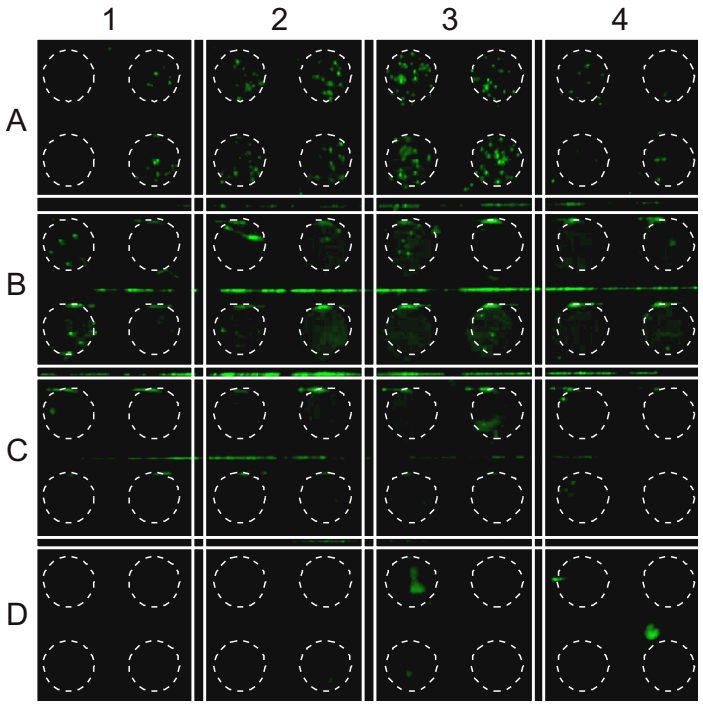

Fig. 3 Screening of the cell culture environment from 4 x 4 combinatorial microenvironment array chip.

Table1. Scafolds and soluble factors in the microenvironment array.

	Scaffold			Soluble factor
1	collagen		A	fetal bovine serum, non-essential amino acids, penicillin, streptomycin
2	fibronectin	x	B	human transferrin, bovine insulin
3	laminin		C	bovine insulin
4	PDMS		D	-

CONCLUSION

In this study, the microenvironment array chip was successfully demonstrated to create combinatorial cell culture environments composed of ECMs and soluble factors. The microenvironment array chip is expected to be applied to high-throughput screening of the cell culture environment for cultivation of primary cells and stem cells.

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