Supporting Information

Development of super-dense transfected cell microarrays generated by piezoelectric inkjet printing

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1. Supplemental Results

NBT-L2b cells, a subpopulation of cells derived from NBT-II cells, tend to adhere strongly to surfaces coated with fibronectin or with type I collagen. Therefore, carefully defined conditions are necessary for micro-patterning a glass substrate with a hydrophobic or hydrophilic surface using PEG and ECM. Moreover, these cells migrate very rapidly (2 μm/min) and unidirectionally on surfaces coated with type I collagen.[7,13] Thus, the precise micro-pattern might be required to prevent migration of NBT-L2b cells among spots than HeLa cells.

Using NBT-L2b cells, we evaluated optimum concentrations of fibronectin (Fig. S1A) and type I collagen (Fig. S1B) in droplets for the preparation of spots for cell adhesion. We found that spotting of droplets that contained more than 0.01% fibronectin or 0.01% type I collage was necessary for adherence of cells to spots of ECM. We also confirmed that cells that had adhered to spots that contained more than 0.01% fibronectin or type I collagen failed to migrate across non-spotted

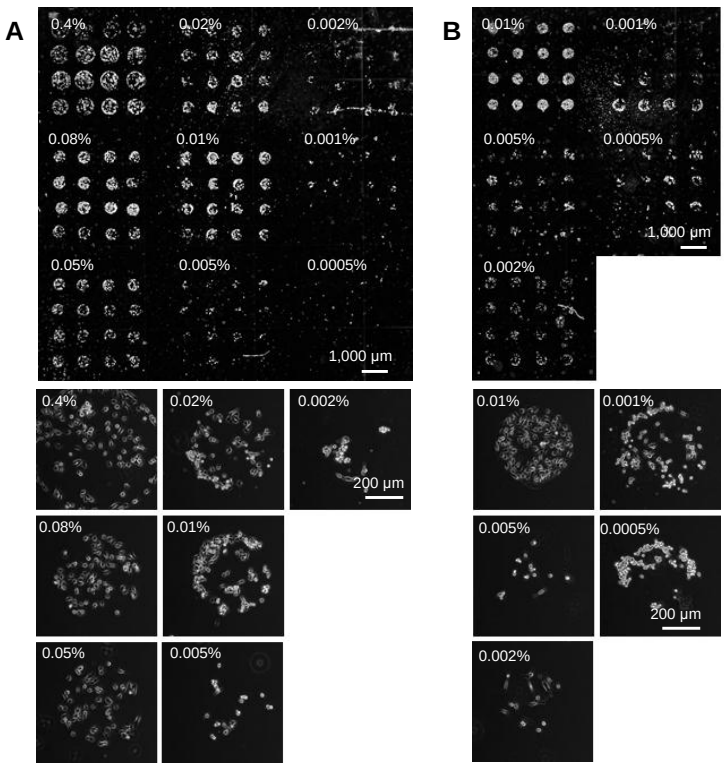

Fig. S1 Phase-contrast images of adherent NBT-L2b cells on arrayed spots that contained **A)** fibronectin and **B)** type I collagen on PEG-grafted glass surfaces. Ten-nl droplets of pure water, containing 0.0005-0.4% fibronectin or 0.0005-0.01% type I collagen, were spotted on the substratum and allowed to dry in air prior to cell seeding. Upper is 4x and lower is 20x magnification.

regions. These results were basically similar to the results for HeLa cells.

Next, we evaluated the rate of cell death and the efficiency of reverse transfection of NBT-L2b cells on the drying spots of droplets that contained various concentrations (0.01-0.08%) of fibronectin or type I collagen, the plasmid that encoded Venus fluorescent protein, and another materials required for the induction of reverse transfection on the TCM (Fig. S2). For NBT-L2b cells, 0.04% type I collagen provided the best conditions in terms of rate of cell death and the efficiency of reverse transfection. This result differs from the results obtained with HeLa cells and demonstrated that individual cell lines require specific and probably different ECM proteins.

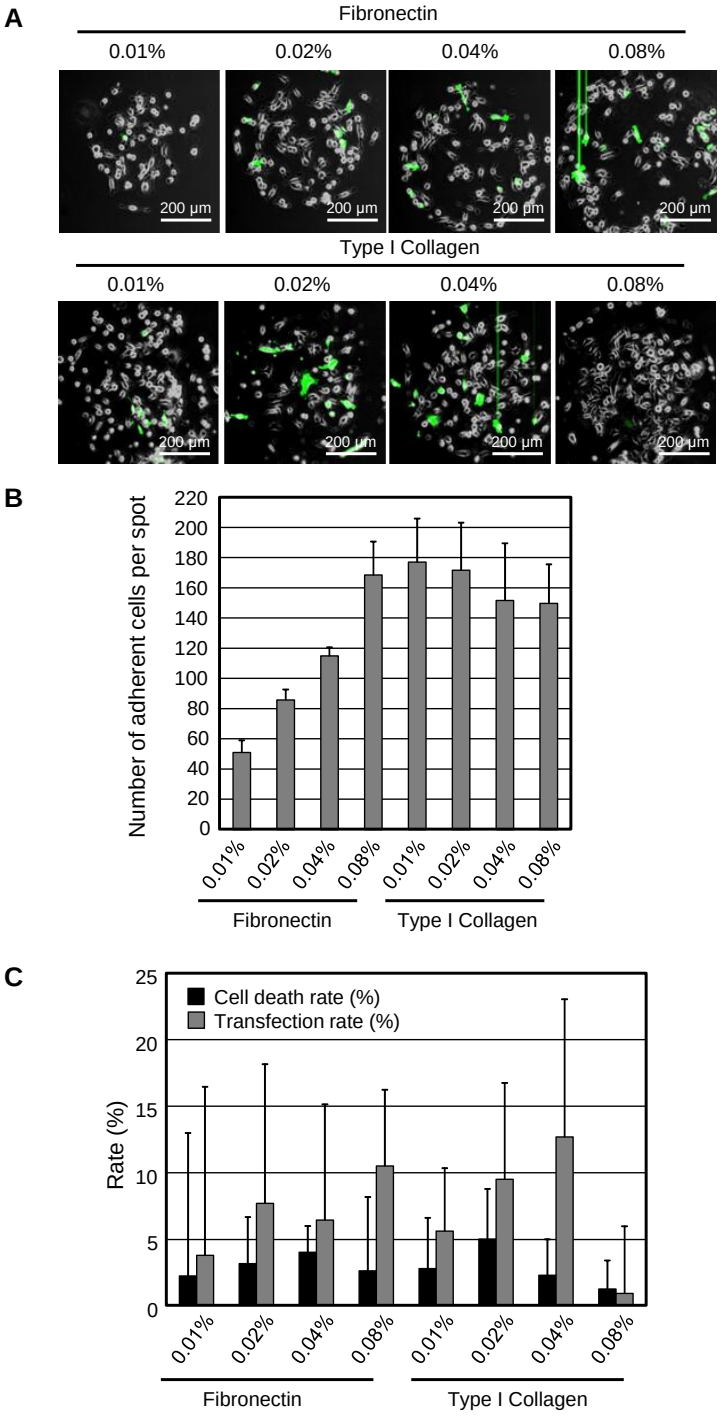

Fig. S2 Evaluation of the rate of cell death and the efficiency of reverse transfection of NBT-L2b cells. **A)** Merged phase-contrast and fluorescence images of NBT-L2b cells on spots that contained 0.01-0.08% fibronectin (upper) and type I collagen (lower) on PEG-grafted cover slips (20x magnification). Phase-contrast images and pseudo-color images of Venus protein obtained by fluorescence microscopy were merged by the image-processing program (ImageJ). **B)** Average numbers of adherent living cells per spot (n=8), as determined from phase-contrast images of NBT-L2b cells at 20x magnification. **C)** Average rates of cell death (black columns) and the efficiency of reverse transfection (gray columns) per spot (n=8). Results were derived from phase-contrast and fluorescence images of NBT-L2b cells at 20x magnification.

2. Experimental Procedures

a. Construction of cell microarrays for determination of optimum concentrations of fibronectin and type I collagen

Using a solenoid valve-type inkjet printer (KCS-mini; Kubota comps, Hyogo, Japan), we spotted 10-nl droplets that contained 0.0005-0.4% fibronectin (Life Laboratory Company, Yamagata, Japan) or type I collagen (Research Institute for the Functional Peptides, Yamagata, Japan) in Dulbecco's modified Eagle's medium (DMEM; Nacalai Tesque, Kyoto, Japan) onto hydrophilic PEG-grafted cover slips (CytoGraph®; 19 mm x 19 mm; Dai Nippon Printing Co., Tokyo, Japan) at 1-mm intervals. Each droplet was allowed to dry in air and, as a result, fibronectin and type I collagen were overlaid in circles on each glass surface. For example, in the case of the spotting of droplets that contained 0.08% fibronectin, after air-drying, spots of fibronectin of ~500 μm in diameter, with a coating concentration of ~60 ng/mm^2, were overlaid on the PEG-grafted surface. We suspended 2 x 10^4 HeLa cells (Riken Bioresource Center Cell Bank) in 1 ml of culture medium, namely, DMEM supplemented with 10% fetal bovine serum (FBS; ICN Pharmaceuticals, Costa Mesa, CA), 1% penicillin-streptomycin mix (Nacalai Tesque) and 1% kanamycin (Life Technologies), and poured the suspension over the spots for seeding. One hour later, we added and removed, twice, 4 ml of Dulbecco's phosphate-buffered saline (D-PBS[-]; without calcium and magnesium; Nacalai Tesque) to wash away non-adherent cells. Then, 24 h after seeding, we captured phase-contrast images of adherent HeLa cells on the microarrays with a customized IX81 fluorescence microscope (Olympus) as shown in Fig. 2.

b. Construction of TCMs and super-dense TCMs

First, we mixed 5 μl of 0.05-0.4% (w/v) ECM (fibronectin or type I collagen), 1 μl of a solution of plasmid (1 μg/μl) that encoded a fluorescent protein (Venus or mCherry), 2 μl of Lipofectamine™ 2000 reagent (Life Technologies) and 17 μl of DMEM in the appropriate order.[2,7,13] Then, using a solenoid valve-type inkjet printer for generation of normal TCMs (Figs. 3 and 4) and a piezoelectric inkjet printer for generating super-dense TCMs (Fig. 5), we spotted 10-nl or 90-pl droplets in arrays on the PEG-grafted glass cover slips. Each droplet was allows to dry in air and, as a result, these materials were overlaid as spots on each glass surface. Then we placed 700 μl of culture medium, in which 2 x 10^4 HeLa cells were suspended, on the glass surface of each TCM for reverse transfection of cells with the plasmid that encoded Venus or mCherry fluorescent protein. After 1 h, we added and removed, twice, 4 ml

of D-PBS[-] to wash away non-adherent cells. Two hours later, we started to record time-lapse phase-contrast and fluorescence images of cells for 21h (3-24 h after seeding) at 30-min intervals (Supplemental Movie 1-4). Finally, 24 h after seeding, we recorded phase-contrast and fluorescence images of adherent HeLa cells on the TCMs (Fig. 3). We calculated average numbers of adherent cells, rates of cell death, and the efficiency of reverse transfection using the image-processing program (ImageJ).

c. Seeding of NBT-L2b cells

We constructed cell microarrays and TCMs using NBT-L2b cells (Riken Bioresource Center Cell Bank) to evaluate the generality of our newly developed system (Figs. S1 and S2). As culture medium, we used MEM (Sigma), supplemented with 10% fetal bovine serum, non-essential amino acids (Life Technologies), 1% penicillin-streptomycin mix and 1% kanamycin, and we suspended 5 x 10^4 cells of NBT-L2b in 700 μl of medium for seeding.