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Sectioning of cultured cell monolayer using photo-acid-generating substrate and micro-patterned light projection

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ABSTRACT

A novel cell processing tool to cut and section a cultured cell monolayer was implemented by the micro-projection of grid pattern onto a substrate functionalized with photo-acid-generating polymer. In adequate conditions of irradiation dosage and line width of grid pattern, Madin-Darby canine kidney cell monolayer was cut into cell clumps with similar size corresponding to the projected grid pattern. The sectioned monolayers were collected as cell clumps with the similar size from the substrate maintaining their viability enough to outgrow after subculture to the other dish. Also the influence of cutout size on the growth rate of collected cell clumps after subculture was also investigated. Further, passage of human induced pluripotent stem cells with size uniformity of the colonies was achieved by using this sectioning method without critical influence on their viability and undifferentiated state.

1. Introduction

Video projection mapping is an artistic technology to project moving image onto the surface of real objects such as constructions, and has been used increasingly in recent years. Simultaneous and parallel control of areal light irradiation implemented by projection technology has provided huge amount of freedom to the special effects lighting in spectacular ceremonies and events, in which laser beam show had once been a standard lighting production. Also in microscopic world of cell culture system, the technologies utilizing the light as a control means have been in a wave of innovation. Laser microdissection has been widely used as a standard method to clip specific sections from a tissue specimen under microscopic observation [1,2]. As means of processing of living cell culture, the ablation and shock-wave generation by pulsed laser has been examined to remove selectively unwanted cells from cell culture [3,4], and the technology has been already commercialized [3]. Further, Elliott et al. reported the application of scanning pulsed laser to sectioning of human induced pluripotent and embryonic stem cell (iPSC, ESC) colonies [5].

As the other methodology to manipulate cells by light, technologies based on areal projection of micro-pattern has been examined by several research groups [6–12]. In this scheme, the light is projected to the cells in pattern, as is mapped onto the objects in the video projection mapping. Compared with the methods to scan a focal point of laser beam, many cultured cells can be processed in a parallel manner at a time by areally projected light in principle. Further, this scheme is more compatible with image-based technologies including imaging cytometry on a substrate [13]. This feature is advantageous in the automation of cell processing on a substrate, which will be desired in the increasing needs for the mass-production and standardization of model cell systems [14]. Actually, the importance of utilizing human cell culture has been recognized more than ever not only in tissue engineering but also in drug screening and investigation of disease mechanism [15–17]. However, the light available from the general set up of

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microprojection systems is too mild in intensity and wavelength to affect the cells directly [8,18].

We examined a substrate functionalized with a photo-acid-generating (PAG) polymer as a light transducer, and found the condition that the anchorage dependent cells cultured on substrate can be killed effectively by irradiating the visible light at low intensity. Based on this finding, we demonstrated to kill the cells selectively in an individual and parallel manner by areal projection of micro-pattern [18]. Recently, we observed that the cells collapsed in certain conditions of light irradiation and PAG polymer density. In this study, we investigate the application of this phenomenon to the "surgical" processing such as cutting and sectioning of adherent cell monolayer [5,19,20]. As well as the irradiation dosage, the projected grid patterns with different line widths are examined to optimize the operational condition without constraint of point scanning. Further, we attempt to apply this sectioning method to the process of passaging human iPSC culture.

2. Materials and methods

2.1. Materials

A Madin-Darby canine kidney (MDCK) cell line and a human iPSC line (201B7) were purchased from RIKEN BioResource Center and repeatedly subcultured under feeder-free condition. Minimum Essential Medium Eagle (Sigma-Aldrich Co.) supplemented with 10% fetal bovine serum (Gibco BRL) was used as a culture medium for MDCK cells. ReproFF2 (ReproCELL Inc.), supplemented with 5 ng/mL human recombinant FGF-2 (bFGF, ReproCELL Inc.), was used as a culture medium for human iPSCs. BD Matrigel solution (hESC-qualified, Becton, Dickinson & Co.) was diluted 1:1000 and used for extracellular matrix (ECM) coating for human iPSC culture. Rho-associated coiled-coil forming kinase (ROCK) inhibitor (Y-27632, used as an apoptosis suppressor) was purchased from Wako Pure Chemical Industries, Ltd. and used to prevent the excessive death of human iPSCs in subculture process. Collagenase type IV (#17104-019, Life Technologies Co.) was dissolved in Hank's balanced salt solution (Sigma-Aldrich Co.), and used in the collection of the cells after photo-induced sectioning. For the currently adopted process to obtain cell clumps, a CTK solution (ReproCELL Inc.) was used to detach human iPSC colonies from the culture substrate. LIVE/DEAD (Life Technologies Co.) was used for the examination of cell viability. As a probe which is specific to human iPSC or ESC, lectin protein rBC2LCN labeled with Cy3 [21,22] was provided kindly by Drs. Tateno and Hirabayashi in Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Japan, and used to check the undifferentiated state of human iPSCs. Polystyrene petri dish (#351008) and tissue culture dish (#353001) with 35 mm diameter were purchased from Becton, Dickinson & Co.

2.2. Fabrication of PAG substrates

We synthesized a photo-responsive poly(methyl methacrylate) (pPAGMMA) functionalized with near UV–visible light responsive PAG moiety [23,24] at 1 mol% through the radical copolymerization of methyl methacrylate and styrene derivative having PAG group as we have already reported [18] (see Supporting Information). PAG substrates were prepared by spin-coating of the pPA GMMA solution on culture surfaces of polystyrene petri dish and tissue culture dish [18]. The densities of pPAGMMA on the culture surfaces used for MDCK cells and human iPSCs were 0.8 and 2 $\mu g/cm^2$, respectively.

2.3. Apparatuses

Light irradiation onto the photo-responsive culture substrates was carried out by using a PC-controlled micro-projection system (DESM-01, Engineering System Co.) installed in an inverted research microscope (IX71, Olympus Co.) [7,8,25]. Near UV and blue light with wavelength range from 320 nm to 450 nm supplied from Hg lamp was projected in arbitrary micro-pattern to the substrate at the intensity of 200 mW/cm^2 through a 4X objective lens. Bright field images were taken with a cooled CCD camera system (VB-7000, Keyence Co.) installed on the same microscope. Fluorescent observation was carried out with a confocal scanning laser microscope (CSLM) system (FV-300, Olympus Co.) installed in an inverted research microscope (IX70, Olympus Co.) using Ar and HeNe lasers with the wavelengths of 488 nm and 543 nm, respectively, which hardly induce the photolysis of pPAGMMA.

2.4. Demonstration of principle

Fig. 1 shows the scheme of sectioning of a cell monolayer on plate by the micro-projection of grid pattern onto the PAG substrate. Upon the light irradiation, PAG moiety at pPAGMMA generates protons through the photolysis as shown in an inset of Fig. 1 [23]. The protons can reach and affect the superjacent cells while by-products including sulfonate ion covalently attached to water-insoluble poly(methyl methacrylate) remain on the substrate due to their poor water-solubility [18]. In order to obtain sufficient cell-cell bonding, MDCK cells were seeded onto the PAG substrate at the number density of 1×10^4 cells/cm^2 (1×10^5 cells/dish) and incubated in the culture medium for 66 h at 37 °C to over confluent. The light was projected in a honeycomb grid pattern composed of 35 hexagons with the area of 0.17 mm^2 (cutout size) partitioned with 42 μm wide line, to the cell monolayer for 14 min. Two hours later, the culture medium was replaced with 50 U/mL collagenase type IV buffer solution, and left for 5 min at room temperature. After removal of the cell monolayer outside the grid-irradiated area by peeling off with the tip of a pipette, the medium was sprayed to the remaining cells gently, and the resultant cell clumps were collected from the substrate.