# ON-PLATE AND ON-DEMAND REMOVAL OF ADHERENT CELLS USING PHOTO-ACID-GENERATING SUBSTRATE AND MICRO-PROJECTION SYSTEM

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## ABSTRACT

In order to provide a powerful tool to process cell culture systems on a culture substrate, we developed the methods to kill and remove adhrent cell by using photo-acid-generating (PAG) -polymer-functionalized culture substrates and micro-patterned light irradiation. We synthesized a poly(methylacrylate) functionalized with visible-light-responsive PAG group (pPAGMMA), and fabricated a photoresponsive culture substrate by spin-coating the polymer solution on polystyrene culture surface. We seeded CHO-K1 cells on the substrates uniformly, cultured them for 1 day, and then irradiated the substrates locally with the blue light (wavelength : 436 nm) using a PC-controlled micropattern irradiation system. As a result, we observed that the cells were killed only in the micro-patterned irradiated area. By checking the cell viability using the Live/Dead assay kit, we confirmed that only the cells in the irradiated region were totally dead while the other cells maintained the viability well. At constant irradiation dosage having no lethal effect, the lethal rate increased with the increase of pPAGMMA density. Based on the results, we demonstrated selective killing of targeted cells was achieved without critical damage to the neighboring cells without using expensive pulse laser or deep UV light which cannot transmit through usual optical systems of microscope. Further, we investigated the photo-induced cell detachment from the other substrates functionalized with PAG polymer.

## KEYWORDS

photo-selective cell killing, photo-acid generator, light irradiation, adherent cell, cell patterning, cell purification

## INTRODUCTION

Corresponding to the development and widespread use of "imaging cytometry" which analyzes cultured adherent cells as they are on a culture substrate, there is a growing need for individual processing of those cells based on the result of the analysis. In such circumstances, a method to kill cultured cells selectively by applying high-energy pulsed laser to cell nuclei was developed and has been commercialized [1]. However, laser beam is focused to a point to gain the peak intensity enough to affect the cells, and cannot be applied to more than one point at a time. Therefore, point scanning is necessary to process many cells even in the case that they all make up one colony.

As a tool to screen the adherent cells by the mild light irradiation with high efficiency, herein we report a novel method to remove the cells using a photo-responsive culture substrates functionalized with a polymer having photo-acid-generating (PAG) groups (Fig.1). In clear contrast to existing laser-based methods requiring point scanning, many cells can be managed at a time by areal projection of light in this scheme [2, 3]. We examine the efficiency of the photo-induced cell killing and investigate its application to on-plate screening and purification of adherent cells by means of precisely patterned projection of visible light. Also we attempt photo-selective detachment of the cells from a photo-responsive culture substrate through the similar process.

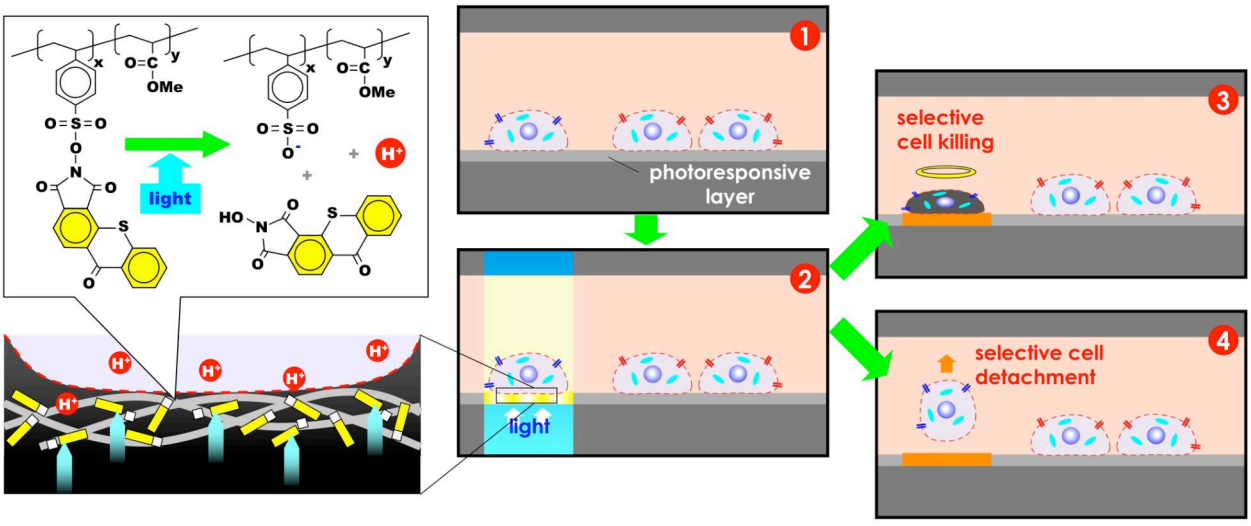


*Figure 1: Schematic illustration showing on-demand removal of adherent cells through photo-induced cell killing/detachment on culture substrates functionalized with PAG polymer.*

## EXPERIMENT

We synthesized a poly(methylacrylate) functionalized with visible-light-responsive PAG group (pPAGMMA) through radical polymerization, and fabricated a photoresponsive culture substrate by spin-coating the polymer solution on polystyrene culture surface [4]. We coated the surface with fibronectin and seeded CHO-K1 cells, and cultured them for a day until they reach confluence. Then we irradiated the blue light (wavelength: 436 nm, dosage: 11 J/cm$^2$) in a micro-pattern onto the surface under microscopic observation using a PC-controlled micro-projection system (DESM-01, Engineering System Co.) installed in an inverted research microscope (IX70, Olympus Co.). Schematic illustration of the system is shown in Fig.2. To check the cell viability, we applied LIVE/DEAD reagent (Invitrogen) and observed the cells using a confocal scanning laser microscope system (FV-300, Olympus Co.)

Further, we fabricated another type of culture substrate by introducing the pPAGMMA and poly (4-vinylpyridine) on the culture surface of a polystyrene dish in order to implement photo-induced cell detachment. NIH/3T3 cells were cultured to reach confluence on this surface, and blue light (wavelength: 436 nm, dosage: 24 J/cm$^2$) was irradiated locally within a circular area of 5 mm diameter by using a light source with a fiber light guide (LC6, Hamamatsu Photonics). Bright field images of the culture surfaces were taken with a cooled CCD camera system (VB-7000, Keyence Co.) installed on the same microscope described above. Viability of detached cells was checked by Trypan Blue assay.

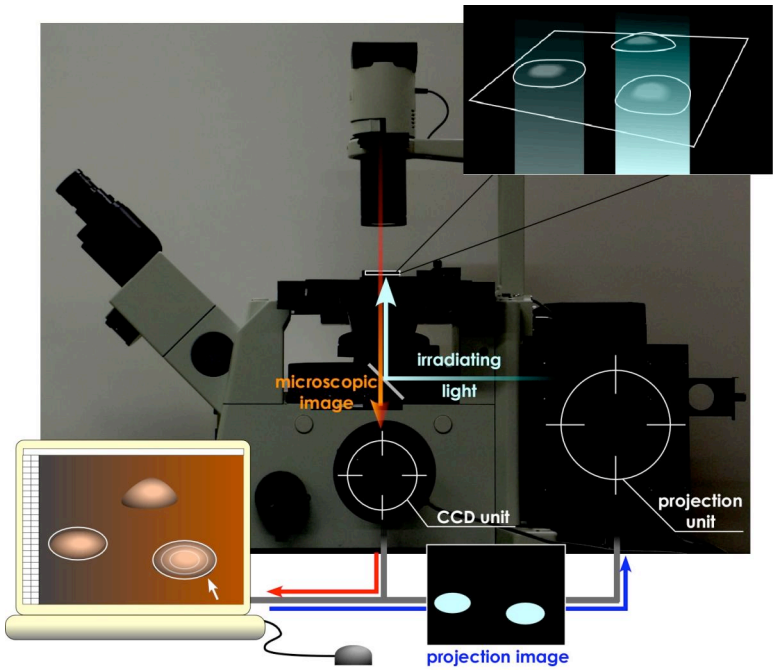


*Figure 2: Schematic illustration showing a micropatterned light irradiation under microscopic observation using a PC-controlled micro-projection system installed in an inverted research microscope.*

## RESULTS AND DISCUSSION

Figure 3 shows the fluorescence image of CHO-K1 cells on pPAGMMA-functionalized substrate after micro-patterned light irradiation and subsequent application of LIVE/DEAD reagent. The PAG group with the structure shown in Fig.1 was reported to produce proton efficiently through its photolysis induced by blue light irradiation, and its applications to photolithography were demonstrated [5, 6]. The distribution of dead cells (red) was corresponding to the irradiation pattern suggesting strongly the lethal effect of pH drop brought by the photolysis of the PAG group. Such photo-induced lethal activity of the substrate was observed also for all other cell lines we attempted, such as MDCK, NIH/3T3 and HeLa. While the cells in the irradiated area were killed efficiently, the cells in the unirradiated area maintained their viability even if they were surrounded by large irradiated area. The result indicated that the pPAGMMA was not toxic without light and that the lethal activity of the photolysis of the pPAGMMA was confined within the vicinity of irradiated culture surface as expected above. In addition, we observed that the light irradiation of the same condition killed no cell without the PAG polymer.

Further, we fabricated another type of culture substrate by introducing the PAG polymer and poly (4-vinylpyridine) on the culture surface of a polystyrene dish in order to implement photo-induced cell detachment. NIH/3T3 cells were cultured to reach confluence on this surface, and blue light was irradiated locally within a circular area of 5 mm diameter. 3 hours after irradiation, we observed that most of the cells became detached from the irradiated area while no detectable change was found for the cells outside of the area (Fig.4). As a result of Trypan Blue assay, about 85 % of the cells were estimated to maintain their viability even after photo-induced detachment. Beside NIH/3T3, also HepG2 cells were effectively detached from the substrate by the light irradiation.

As a result, photo-induced killing of the cell after photo-induced patterning was demonstrated successfully. The compatibility with electrically controlled micro-projection system is advantageous in the automation of on-plate cell screening especially in such cases that many cells belonging to a population or a colony are processed all together.

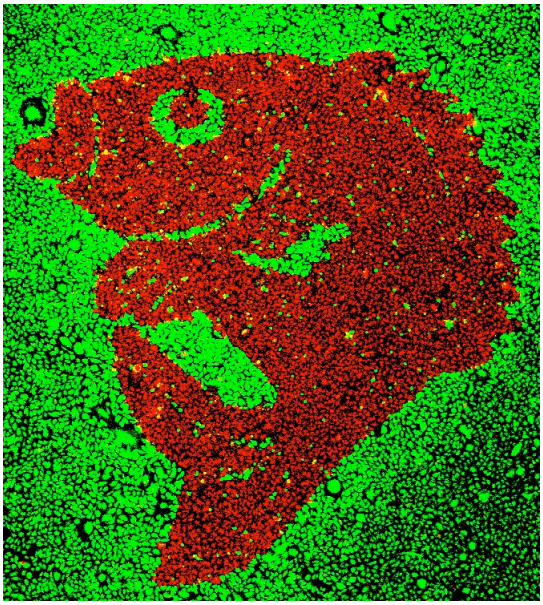

*Figure 3: CHO-K1 cells cultured on a pPAGMMA-functionalized substrate after micro-patterned blue-light irradiation and application of LIVE/DEAD reagent (green: alive, red: dead).*

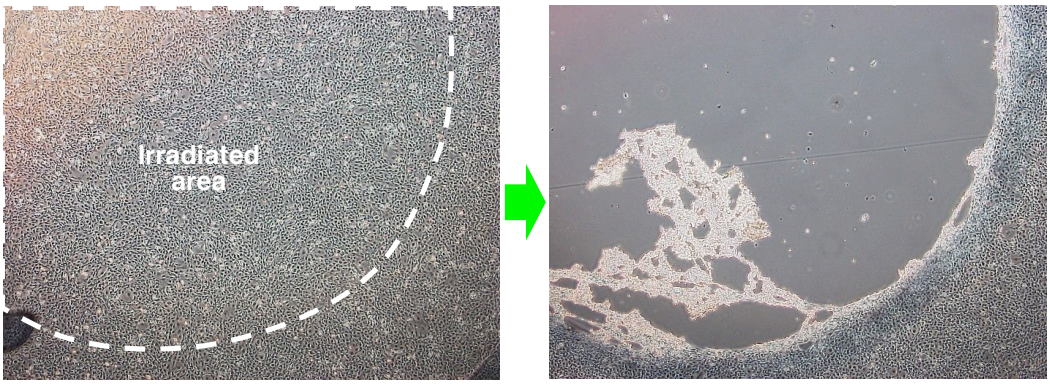


*Figure 4: Photo-induced detachment of adherent NIH/3T3 from a culture substrate functionalized with pPAGMMA and poly (4-vinylpyridine).*

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