PHOTO-IMMOBILIZATION OF CELLS FOR *IN SITU* DNA ANALYSIS

Naoki Sasaki, Anri Isu, Reina Ishii, Kae Sato*

Japan Women's University, Japan

ABSTRACT

We present a simple and robust technique to immobilize cells onto a glass substrate. Immobilization of cells at specific location onto planar substrates is useful for biomolecular analysis within cells. Our technique enables on-demand immobilization of cells by irradiating photoresponsive substrate that lies underneath the cells. Cells located at the irradiated area were almost completely immobilized under optimized conditions. This technique was applied to *in situ* DNA analysis by target-primed rolling circle amplification. Mitochondrial DNA in the immobilized cells was successfully detected. This technique will be a powerful tool for biomolecular analysis in cells on microfluidic devices.

KEYWORDS

Benzophenone, Crosslinking, Rolling circle amplification, Mitochondria, Single cell analysis

INTRODUCTION

Immobilization of cells at specific location onto planar substrates is useful for biomolecular analysis within cells. Light-assisted cell immobilization can achieve spatial and temporal control of the immobilization by controlling the light. However, conventional techniques require patterning of the substrate surface prior to immobilization [1, 2]. In addition, *in situ* DNA analysis in the immobilized cells has never been reported. In contrast, our technique enables on-demand immobilization of cells by irradiating photoresponsive substrate that lies underneath the cells. Both adherent and nonadherent cells were immobilized to the irradiated area. Cells located at the area were almost completely immobilized under optimized conditions. This technique was applied to *in situ* DNA analysis by target-primed rolling circle amplification (RCA) [3].

PRINCIPLE

Figure 1 shows the schematic illustration of the cell immobilization procedure. Cells are disseminated on the glass substrate modified with benzophenone (BP) molecules via silane coupling reagents. BP is a well-known photoresponsive molecule that can form covalent bonds with a wide range of biomolecules. Then, the substrate is irradiated with an UV light (λ = 365 nm) through a photomask. By the irradiation, the membrane of the cells is crosslinked with BP on the substrate. After washing, only immobilized cells remain on the substrate.

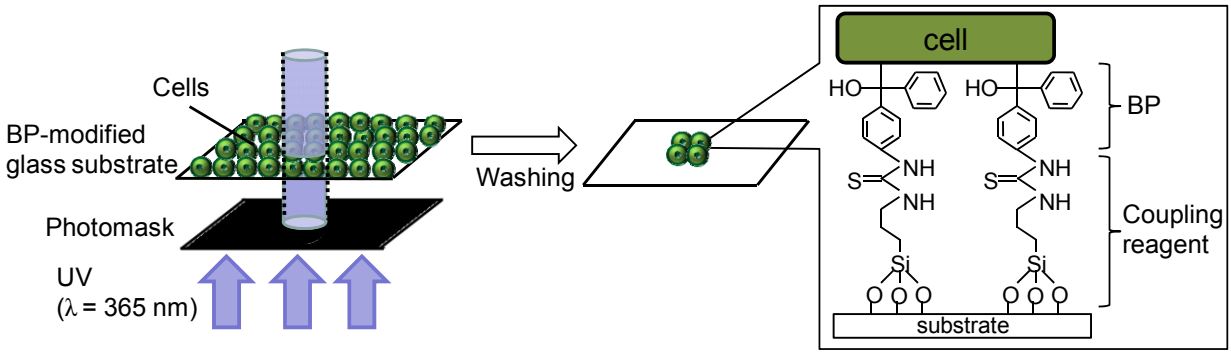

Figure 1. Schematic illustration of the cell immobilization procedure.

EXPERIMENTAL

A polydimethylsiloxane (PDMS)-glass device with a BP-modified bottom surface was fabricated as follows. Benzophenone-4-isothiocyanate was immobilized on a cover slip (24 × 60 mm) via 3-aminopropyltriethoxysilane [4]. The cover slip was bonded to a PDMS substrate possessing a through-hole (5 mm diameter, 2 mm depth). A suspension of human K562 erythroleukemia cells was poured into the well, which was then irradiated with UV light (λ = 365 nm) passed through a photomask under an inverted microscope. Phase-contrast images of cells were taken as required. For the evaluation of immobilization, the cell density on the substrate was estimated from the images. Mitochondrial DNA within immobilized cells was detected using target-primed RCA according to the literature [3]. Sequences of DNA probes are shown in Table 1. Fluorescence images were taken as required. Similar experiments were also conducted in a microchannel (1 mm width, 0.5 mm depth, 10 mm length).

Table 1. DNA probes used in this study.

Probe	Sequence
Padlock probe	5'-phosphate-TAAGAAGAGGAATTGCCTTTCCTACGACCTCAATGCACATGTTTG GCTCCTCTTCCCATGGGTATGTTGT-3'
Detection probe	5'-Alexa Fluor™ 555-CCTCAATGCACATGTTTGGCTCC-3'

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

RESULTS AND DISCUSSION

Figure 2 shows the demonstration of the immobilization. Nonadherent K562 cells were immobilized to the whole of the irradiated area (1 mm diameter), both in an open well (5 mm diameter, 2 mm depth, Fig. 2(b)) and in a microchannel (1000 µm width, 500 µm depth, Fig. 2(c)). The use of adherent HeLa cells yielded almost the same results (data not shown). Complex patterns of cells can be formed as shown in Figs. 2(d) and 2(e). Spatial resolution of the technique will be discussed in the presentation.

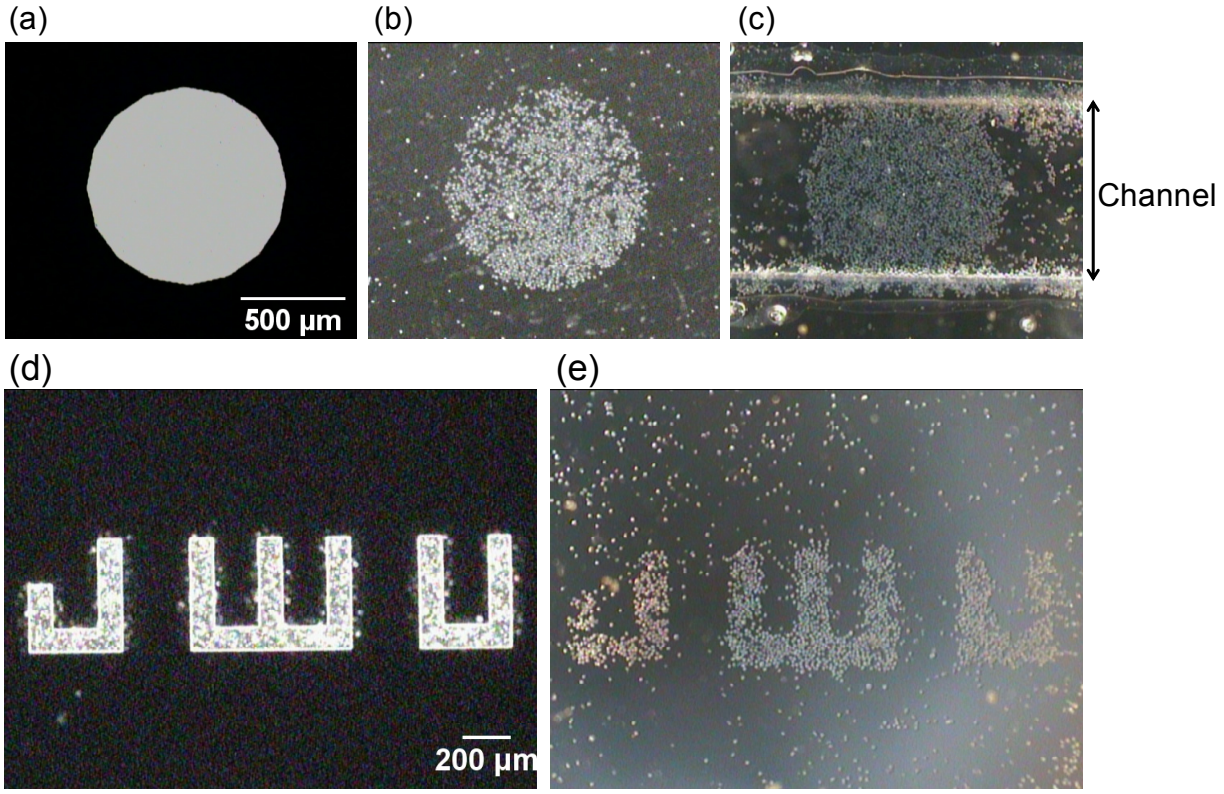

Figure 2. (a-c) Micrographs of (a) a circular pattern on a photomask, (b) immobilized K562 cells on the BP-modified substrate in an open well, and (c) immobilized K562 cells in a microchannel (1000 µm width, 500 µm depth). (d,e) Micrographs of (d) complex pattern on a photomask and (e) corresponding pattern of immobilized cells.

Figure 3 shows time course of density of immobilized cells with different light power density. The cell density increased with increasing the irradiation time, although the over-irradiation with a power density of 12.6 mW mm^{-2} resulted in a decreased cell density. Under optimized conditions, 98% of cells located at the irradiation area prior to the UV irradiation were immobilized.

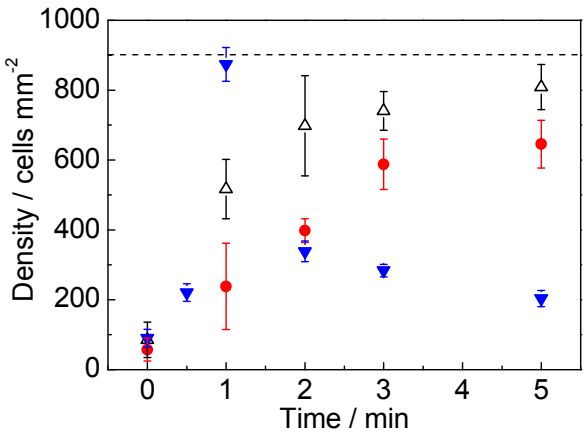

Figure 3. Timecourse of cell density following UV irradiation and rinsing with different light power densities: 0.70 (●), 2.54 (△), and 12.6 mW mm^{-2} (▼). The dashed line shows the cell density prior to UV irradiation.

Figure 4(a) shows schematic illustration of *in situ* detection of mitochondrial DNA by target-primed RCA. Immobilized cells are fixed with ethanol, treated with pepsin-HCl, and then reagents for RCA are added. A target sequence in mitochondrial DNA is recognized by a Padlock probe, and a long single-stranded DNA molecule is

obtained by RCA. The long DNA molecule is hybridized with detection probe, and we can detect the long DNA molecule as a fluorescent dot. Since the long DNA molecule is produced from a sequence in a single DNA, we can know both the number and the location of DNA molecules that possess target sequence by imaging the dots. Figure 4(b) and 4(c) show fluorescence images showing the K562 cells after RCA. Bright dots around the nuclei of the cells were clearly observed, and RCA was successfully performed in the immobilized cells. Mitochondrial DNA in K562 cells immobilized in the microchannel was also successfully detected, and details will be discussed in the presentation.

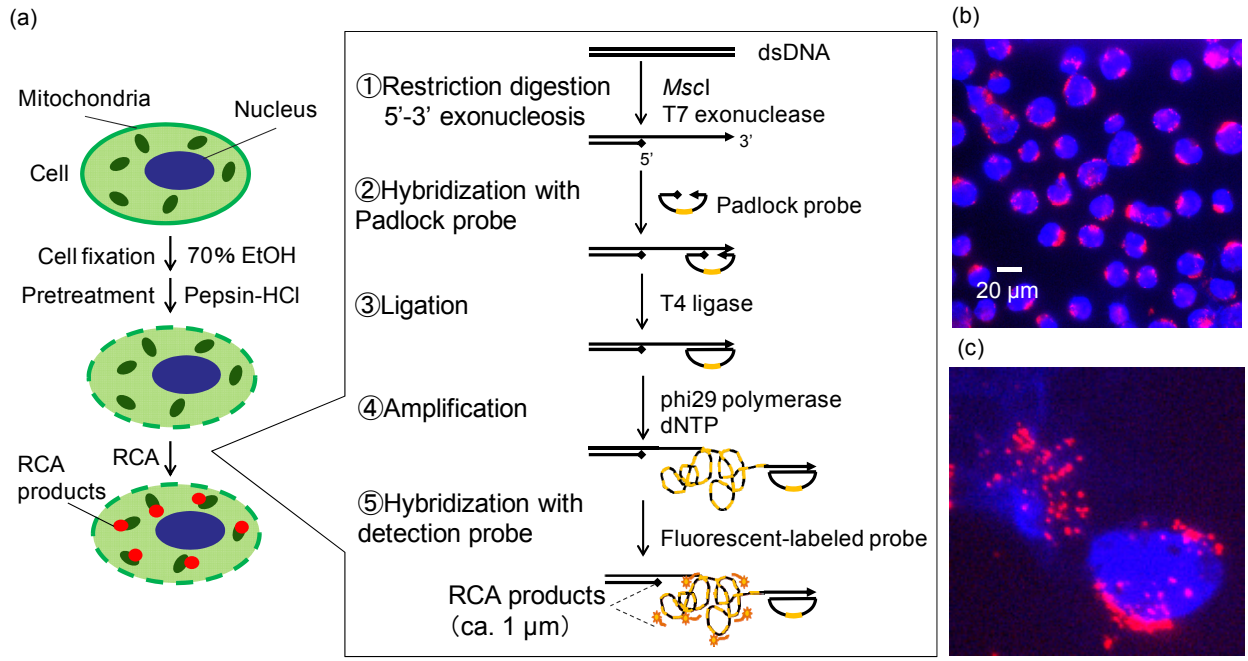

Figure 4. (a)Schematic illustration of in situ detection of mitochondrial DNA by target-primed RCA. (b, c) Fluorescence images showing the K562 cells after target-primed RCA. (b) Overall image. (c) Magnified image. RCA products were hybridized with detection probes (red). Cell nuclei were counterstained with Dapi-Fluoromount-G (blue).

CONCLUSION

We have demonstrated a simple and robust technique to immobilize cells onto a glass substrate. Mitochondrial DNA in the immobilized cells was successfully detected by target-primed RCA. This technique will be a powerful tool for biomolecular analysis in cells on microfluidic devices.

ACKNOWLEDGEMENTS

This work was partially supported by the NEDO Industrial Technology Research Assistance Project.

REFERENCES

[1] J. Nakanishi, Y. Kikuchi, T. Takarada, H. Nakayama, K. Yamaguchi, M. Maeda, Photoactivation of a Substrate for Cell Adhesion under Standard Fluorescence Microscopes, Journal of the American Chemical Society, 126, 16314-16315 (2004),

[2] S. Yamaguchi, S. Yamahira, K. Kikuchi, K. Sumaru, T. Kanamori and T. Nagamune, Photocontrollable Dynamic Micropatterning of Non-adherent Mammalian Cells Using a Photocleavable Poly(ethylene glycol) Lipid, Angewandte Chemie International Edition, 51, 128-131 (2012).

[3] C. Larsson, J. Koch, A. Nygren, G. Janssen, A. K. Raap, U. Landegren, M. Nilsson, In situ genotyping individual DNA molecules by target-primed rolling-circle amplification of padlock probes, Nature Methods, 1, 227-232 (2004).

[4] M. Y. Balakirev, S. Porte, M. Vernaz-Gris, M. Berger, J.-P. Arié, B. Fouqué, F. Chatelain, Photochemical Patterning of Biological Molecules Inside a Glass Capillary, Analytical Chemistry, 77, 5474-5479 (2005).

CONTACT

*Kae Sato, tel: +81-3-5981-3661; satouk@fc.jwu.ac.jp