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Electrochemical cell lysis on a miniaturized flow-through device

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ABSTRACT

A low-cost miniaturized flow-through device was fabricated using conventional photolithographic technique for lysis of whole cells using electrochemically generated hydroxyl groups. The device used low impedance Au-interdigitated electrode (IDE) fabricated on glass substrate to input DC potential to the overlaid PDMS based microchannel. The lysis of human cell line MCF-10A could be achieved between 2 and 5 V of DC input with optimum release of genomic DNA at 5 V for 5 min, which is the lowest potential range reported in any such study. The lysate was extracted to confirm release of genomic DNA and was successfully tested for PCR grade purity of DNA by amplifying a known tumor suppressor gene *SMAD4*. The proposed method was non-destructive for biocomponents due to absence of Joule heating and shall find use in miniaturized PCR analysis as well as where native protein extraction is required under aseptic conditions.

1. Introduction

Lysis of whole cells for extraction of intracellular components such as DNA, RNA, protein and metabolites is a routine procedure in most of the biological laboratories and diagnostic industries. The available methods [1] for this purpose are time consuming, laborious and require multistep chemical treatments, which are often quite expensive. For these reasons, miniaturization and automation of this technique is highly desirous [2]. The commonly used methods for lysis such as high voltage electroporation [3]; proteinase-K, detergents and lysozyme treatment [4]; laser induced lysis [5]; bead milling and sonication [6] or freeze–thaw in liquid nitrogen [7] are unsuitable for miniaturization and also require additional separation or neutralization steps.

Therefore, various groups have attempted in past to develop on-chip cell lysis protocol using different strategies. However, their methods lacked the aim of miniaturization. For example, a few groups have used extremely high voltage to the order of 1–10 kV [8,9] or laser induced cell lysis [10] and others needed sample pretreatment with addition of yet expensive reagents [11]. On the contrary, our goal in the present study was to develop a relatively inexpensive method for cell lysis that uses minimal reagents, power, and can be fabricated using common photolithographic techniques. The principle used for this purpose was based on previous reports of applying a DC voltage to electrochemically generate hydroxide ion inside the device [4]. The electrochemically generated hydroxide ions permanently disrupt the cellular membrane by cleaving fatty acid groups, thereby releasing intracellular material. In continuation to this idea, the microchip was completely redesigned in the present study for reagent-less and power efficient complete lysis of cells. Au-interdigitated electrodes were used to input DC potential across the cells flowing through the microchannel and lysate was examined for purity and denaturation.

2. Materials and methods

2.1. Chip design and fabrication

The microchip was developed on glass substrate. Gold interdigitated microelectrodes were fabricated over the glass for applying DC potential by using photolithography and evaporation method (Fig. 1). AZ-1512 was spin-coated on glass and patterned using photolithography. After photolithography process, gold electrode was deposited using evaporator. The electrode surface was cleaned with acetone and dried using N_2 gas. The microchannel was imprinted in PDMS mold using negative molding method (Fig. 1) [12]. For fabrication of microchannels, 40 μm thick negative photoresist (SU-8) was spin-coated and patterned on the silicon wafer. The degassed PDMS mixture of Sylgard 184 silicone elastomer along with curing agent (10:1) was poured on the SU-8 patterned wafer and cured for 4 h at 72 °C. The PDMS mold was then peeled

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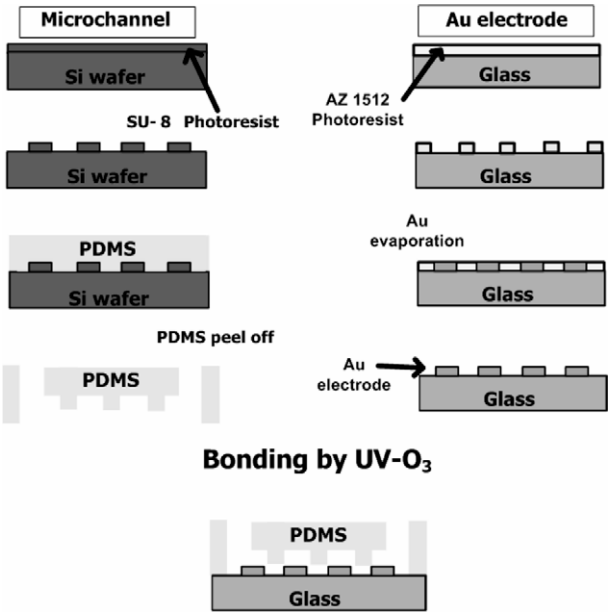

Fig. 1. Fabrication process for PDMS based microchannel and Au microelectrodes.

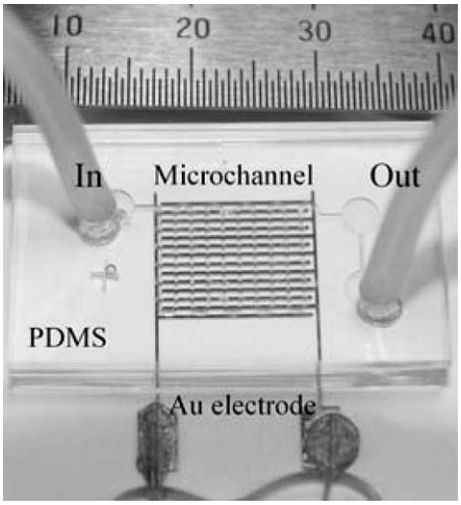

Fig. 2. The cell lysis device after fabrication; showing gold IDE, microchannel in PDMS and connected silicone tubing for injection of whole cell sample and collection of lysate.

off and manual drilling was performed to produce access holes of 3 mm diameter. The width and depth of the microchannel were 250 and 200 μm, respectively. The PDMS mold and Au-patterned glass substrate were subjected to UV–ozone treatment for 40 min and were bonded together.

2.2. Cultivation of cells

The MCF-10A cells were cultured in MEGM (Mammary Epithelial Growth Medium, Serum-free, Clonetics) supplemented with 100 ng/ml cholera toxin (Sigma) to 70% confluence as per method described by Caldas et al. [13]. The cells were detached from culture plate by trypsinization and then centrifuged, washed with pH 7.4 phosphate buffer saline (PBS) twice and re-suspended (to 2×10^7 cells/ml concentration) in pH 7.4 PBS.

2.3. Cell lysis

A suspension of 50 μl (10^6 cells/ml) human cell line MCF-10A in PBS was injected into the silicone tube carrying the same buffer in the microchannel using a precision syringe pump. The lysate was collected at the other end of the device (Fig. 2) and used in spectroscopic and agarose gel electrophoresis for confirmation of DNA release.

2.4. Lysate analysis

The cell lysate was analyzed for presence of released genomic DNA using conventional agarose gel (1%) electrophoresis. After electrophoretic separation, genomic DNA band was cut with a razor and redissolved in Mega Spin™ gel elution kit (Intron Biotech., Korea) for spectroscopic analysis and estimation of DNA concentration at 280 nm wavelength using an Eppendorf Biophotometer. For estimation of PCR readiness of the extract, cell lysate was centrifuged to remove cell debris and thereafter, cell free extract was analyzed for presence of *SMAD4* gene using conventional PCR (Applied Biosystems Thermal cycler model 2720) technique.

3. Results and discussion

In the present study, a low-cost cell lysis device was fabricated using conventional photolithographic technique. The glass substrate was used to make transparent devices and for its suitability in bonding with PDMS by UV–ozone method [12]. The electrochemical lysis of human cell line could be achieved between 2

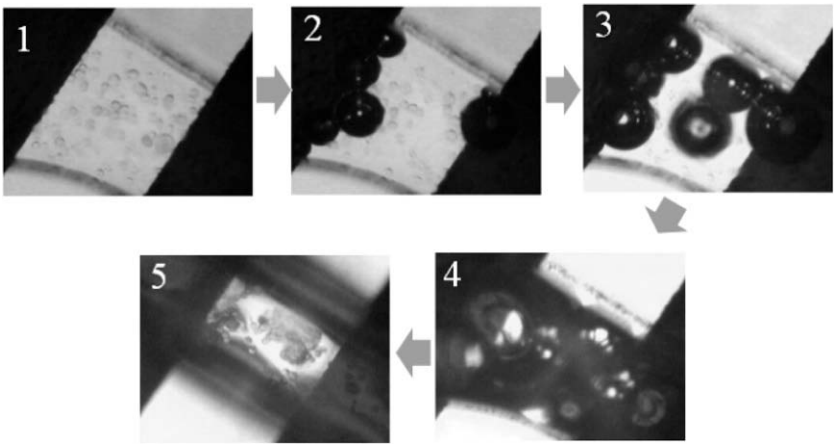

Fig. 3. State of MCF-10A cells flowing within microchannel: (1) before application of DC potential; (2) in-channel bubble formation at the onset and (3) next few second of application of potential; (4) after treatment of +5 V DC for 5 min and (5) condition of microchannel after stopping the reaction, where cell debris could be seen adhered to PDMS wall.

Fig. 4. Gel-doc showing DNA extracted from lysis of MCF-10A cell line on the chip. Lanes: M = 1.5 kb marker (6 μL loading); 1 = 0 V; 2 = 2 V; 3 = 5 V; 4 = 10 V (lanes 1–4 = 20 μL sample loading). A minimum potential of 2 V was required for lysis, whereas 5 V was ideal for effective genomic DNA extraction. The genomic DNA bands can be seen just below loading wells, whose respective concentrations were 0, 0.4, 0.7 and 1.0 μg/ml for 0–10 V treatment.

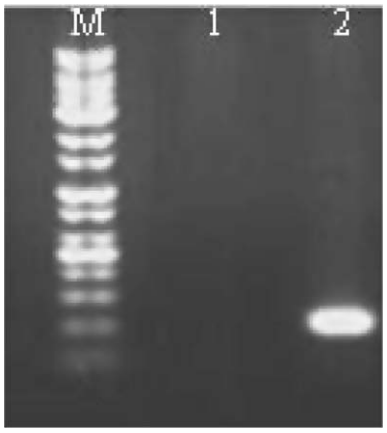

Fig. 5. Gel-doc showing conventional PCR for 30 cycles using cell free extract of MCF-10A lysate: lane: M: 10 kb marker, lane 1: MCF-10A pre-PCR cell free extract, lane 2: MCF-10A cell extract. PCR product yield was ~800 ng/10^6 cells. After PCR, 20 μl was loaded on gel compared to 6 μl of 10 kb marker.

and 5 V of DC input (Fig. 3) with optimum release of genomic DNA at 5 V for 5 min (Fig. 4), which is the lowest potential range reported in any such study. The reason for this success over similar experiments from other groups [8,9,11,14], where parallel electrodes were used along the microchannel [4] or the in-channel electrodes were separated by wide gap [9], could be attributed to the Au-interdigitated electrode geometry. The alternate +5 and 0 V DC across 200 μm gap between the Au electrodes was suitable for generation of hydroxyl ions desired for lysis and at the same time, electrode impedance was low enough to avoid generation of Joule heat [15] inside microchannel. The hydrogen bubbles released at the anode were indicative of this process and were required to quench the lytic hydroxyl ions at the downstream by recombination [4]. Therefore, the intracellular components, such as DNA, RNA etc. were supposed to be intact using this method. This was evident from Fig. 4, where no shearing of DNA could be observed. To further verify this fact, the cell lysate from MCF-10A cell line was analyzed for PCR grade purity of genomic DNA by amplifying a known tumor suppressor gene *SMAD4* [16] using conventional PCR method (forward primer: GTCTATGGCACATCAAACTATGCACAATGC; reverse primer: GTCTAACAATTTTCCTTGCAACG). The different temperature zones used were 92, 55 and 68 °C for melting, annealing and extension, respectively for 30 cycles and for 30 s each. The PCR reaction yielded desired 193 base pair product (Fig. 5) and thereby demonstrated the successful application of the cell lysis microchip.

4. Conclusion

A PDMS based microfluidic system was devised for electrochemical cell lysis. The device size was small enough and it required just 5 V for operation. Extraction of PCR grade DNA under asceptic condition was possible. The process was non-destructive because it did not generate Joule heat as in case of previous reports where as high as 10 kV was used to achieve cell lysis [14]. The proposed method shall find use in miniaturized PCR analysis as well as where native protein extraction is required.

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