FABRICATION OF HOURGLASS-SHAPED MICROAPERTURE VIA TWO-STAGE LASER PULSES AND ITS APPLICATION

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ABSTRACT

This work presents the fabrication of hourglass-shaped aperture by applying two-stage CO₂ laser pulses on glass substrate to control the size of the aperture to 1-10 μm, and applies the fabricated aperture to measure ion-channel activities and to trap single cells in array configuration. First stage laser pulses with longer duration was utilized to generate apertures larger than 10 μm, and a series of shorter duration laser pulses was added for maintaining the glass reflow to reduce its diameter. This easy fabrication procedure can be operated without complicated micro-fabrication process for applications of single-cell analysis.

KEYWORDS: Single-cell analysis, laser drilling, microaperture, patch-clamp

INTRODUCTION

Single-cell analysis has known to constrain cells in a certain confined area or fixed location for studying their morphological or physiological behaviors under environmental stimuli. Recently varies approaches have been presented to locate single cells in place. Rettig et al. showed large-scale single cells trapped by seeding of cells in PDMS microwells [1]. Besides, micro-openings were created in varies ways to trap a single cell for measuring ion-channel responses [2]. However, most of these techniques for creating microapertures are time-consumed, or need to be proceeded through complicated micro-fabrication processes.

In our previous research [3], a fast and easy method was developed to fabricate an hourglass-shaped microaperture by CO₂ laser drilling on glass material. The size of the apertures typically ranged between 5-15 μm, and smallest size could be attained to 1-3 μm under strictly control of laser machine. In order to create apertures with more stable and controllable size, a new laser drilling strategy was proposed. The size of the aperture was much stable and predictable by this method. The fabricated aperture could be used in planar patch-clamp application or single-cell trapping in microarray configuration.

THEORY

A two-stage laser pulses command illustration is demonstrated in Fig. 1A. The first stage is composed of a number of pulses with longer duration than the second stage pulses. These laser pulses drill through the glass substrate and form an hourglass-shape aperture with diameter typically larger than 10 μm. The second stage comprised a series of shorter duration pulses is designed to maintain the reflow of melted glass back to the core of the aperture to reduce its diameter to 1-10 μm.
Figure 2 and 1B and 1C shows side-view micrograph and isometric-view SEM image of the hourglass-shaped aperture respectively.

**EXPERIMENTAL ASPECT**

A 25W CO₂ laser (Synrad 48-2) was driven by LabVIEW program via data acquisition card (NI-6251) to execute the drilling process. Borosilicate cover glass (Matsunami Glass) with typical thickness of 150μm was used as substrate. The size of the fabricated aperture was measured under 500× microscope.

HEK 293T cells were grown in DMEM supplemented with 10% FBS at 5% CO₂ incubator. Electrophysiological recording was performed with conventional Axon 200B amplifier in whole-cell configuration. Cells were stained with calcein AM 15 min before trapping on microaperture array, and the fluorescent image was taken by Olympus DP70 CCD on IX71 microscope.

**RESULTS AND DISCUSSION**

SEM image of fabricated apertures with different number of second stage pulses is showed in Fig. 2. The apertures correspond to different number of pulses between 0 to 10 in 2 interval revealed gradually reduced aperture diameters. The smallest size of the aperture in this demonstration was around 3μm. Figure 3 shows the relationship between the number of second stage laser pulses verses aperture diameter. Results show the more the second stage pulses, the smaller the aperture diameter. This result verifies the concept of the two-stage laser pulses approach. The error bars represent the standard deviations which located in the acceptable range.
Planar patch-clamp application with fabricated aperture in the range of 1-3μm was presented. Whole cell current recording of endogenous channels in HEK 293T cell without leak subtraction is shown in Fig. 4A. Voltage steps were elicited between -100 and +80 mV in 20 mV interval from holding potential of -80 mV. Strong ion channel activation appeared at positive membrane potential. Seal resistance at whole cell configuration was around 1 GΩ. Figure 4B indicates the current-voltage relation obtained from the whole cell current traces.

A 3x3 micro-aperture array fabricated via the two-stage laser drilling technique was described. Figure 5A shows the SEM image of microarray with uniform size of the apertures smaller than 8μm. HEK 293T cells stained with calcein AM were trapped as a microaperture array by application of suction, as shown in Fig. 5B.

CONCLUSIONS

The two-stage laser pulses approach proves feasible for fabricating 1-10μm apertures. We can acquire a decreasing aperture size by increasing the number of second stage laser pulses. The fabricated aperture can be used in single-cell analysis such as planar patch-clamp application or microarray for single-cell trapping.

REFERENCES