ABSTRACT

We demonstrate a lateral patch-cramp device structure, made of fused silica, whose patch pipettes were fabricated by using femtosecond laser irradiation and wet etching. The pipettes were an oval-shaped cross-section that was 200 nm \( \times 4 \) \( \mu \)m, and the minor axis can be controlled on the nano-scale. It is expected that a Giga-seal resistance and its observation using a high magnification microscope can be simultaneously achieved. We confirmed that fluids such as buffer solutions can flow through the nano-pipettes, and some bacteria (B. Subtilis) were successfully trapped at the inlet of a nano-pipette at a given moment.

KEYWORDS: Femtosecond Laser Machining, Patch-cramp, Fused Silica, Nano-Channel

INTRODUCTION

The patch-cramp device has been an important research tool in the field of electrophysiology. It aids in the functional analysis of ion channels and transporters that exist in a cell membrane. A high throughput performance is required in the drug discovery process, and planer patch-cramp arrays, which enable for multi-simultaneous and automatic measurement to be taken, are essential. Pipettes of conventional planner patch-cramp devices are formed vertically. Therefore, it is difficult to observe them using a high magnification microscope, because the overall device is too thick, and pipettes obstruct the observation of the sample. Furthermore, there is also a limitation on the density of the pipettes placement due to the complexity of the channel configuration. So, a lateral patch-cramp device, which is made of polydimethylsiloxane (PDMS), was demonstrated [1]. It allows for the observation of cells from the opposite end, and a >300 M\( \Omega \) seal resistance for the whole cell patch-cramp measurement [1]. The seal resistance should be more than a G\( \Omega \)-order, which is called a Giga-seal in order to measure the ionic current with a high accuracy, and a Giga-seal has been achieved by using a device that is made of glass and quartz pipettes.

We present a lateral patch-cramp device structure made of fused silica, whose patch pipettes were fabricated by using femtosecond laser irradiation and wet etching. Nanometer-scale lateral pipettes (nano-channels) were implemented inside a fused silica substrate, and each end was connected to micrometer-scale channels that were fabricated using conventional photolithography and dry etching. Since the lateral pipettes were made of fused silica, it is expected that a Giga-seal resistance and its observation using a high magnification microscope can be simultaneously achieved for the first time.

MECHANISM OF NANO-CHANNEL FORMATION

The fabrication of lateral nano-channels is based on a self-assembled periodic nanostructure technology [2]. As shown in Fig. 1 (a), focused, ultra-fast, short-pulse laser irradiation in fused silica generates self-assembled periodic nanostructures, whose nanometer-scale periodicity is along the direction of the laser’s electric field vector. The laser pulse generates a periodic modulation of the oxygen concentration in the silica, with areas of high and low oxygen concentration alternately appearing. The low-oxygen-concentration regions, shown as gray planes in Fig. 1, can be selectively etched using an aqueous solution of hydrofluoric acid (HF) to fabricate periodic nano-grooves [3]. Such periodic nanostructures are generated as a result of interference between the electric field of the laser beam and the plasmon-polariton wave [2]. By carefully
controlling the pulse energy, it is possible to modify the oxygen content in a single (Fig. 1(b)), nanometer-scale region, where the interference is maximized, so that the oxygen concentration is below the threshold for silica removal using wet etching [4]. Therefore, using this technique, lateral nano-channel can be fabricated inside a fused silica substrate.

FACTORICATION OF LATERAL NANO-CHANNEL FOR PATCH-CRAMP

Figure 2 shows a schematic of a lateral nano-channel device. Channel A is for cell transport and channel B is for suction. Lateral nano-channels are connected to the two micro-channels, and they are placed inside the fused silica substrate. It is designed so that a cell, which flows in channel A, is trapped at the inlet of the lateral nano-channel due to the suction coming from channel B. There are four steps in the fabrication process for this structure. First, a focused femtosecond laser is scanned on the positions of the nano-channels (Fig. 3(a)), and a single low oxygen content is formed. Second, micro-channels are fabricated using lithography and an anisotropic dry etching method (Fig. 3(b)). Third, the area scanned by the laser is selectively etched using hydrofluoric acid, and the lateral nano-channels are formed inside the fused silica substrate (Fig. 3(c)). Finally, the fused silica and PDMS slab are combined (Fig. 3(d)). In our study, a synthetic fused silica substrate was irradiated with a Ti-sapphire laser (wavelength of 800 nm, pulse time width of 280 fs, and reputation of 200 kHz). An objective lens (N.A. 0.5) was used to focus the laser pulse, and during the laser irradiation, the sample stage was moved at 1 mm/s to draw lines. During the irradiation, the electric field vector of the laser beam was set vertical to the direction of the laser scanning.

Figure 4(a) shows an optical microscope image of our device. This image was observed before the junction of the PDMS slab. The nano-channel was 55 µm long, and the pitch was 20 µm. Figure 4(b) shows a scanning electron microscope (SEM) image observed at an angle of about 45 degrees from the surface normal. The lateral nano-channel was located at about 5 µm below the surface, and the size of the oval hole was about 200 nm in width and about 4 µm in height.

OPERATION CHECK

We attempted to trap a bacterium (B. Subtilis), which had a minor axis of about 500 nm to determine the lateral nano-channel device characteristics. Since the minor axis of a nano-channel is about 200 nm, it is small enough to trap the bacterium. Figure 5 shows an optical microscopic image of trapped bacterium at the inlet of a nano-channel. We confirmed that fluids such as buffer solutions can flow through the nano-pipettes, and some bacteria were successfully trapped at the inlet of a nano-channel at a given moment.
CONCLUSION
We demonstrated a lateral nano-channel for a patch-cramp fabricated in fused silica. We successfully fabricated a device with a lateral nano-channel using a femtosecond laser machinery and conventional micro-TAS formation processes. We confirmed that fluids such as buffer solutions can flow through the nano-channels, and a some bacteria (B. Subtilis) was successfully trapped at the inlet of a nano-channel at a given moment. This proves the great possibility of application of patch-cramps.

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