Microfluidic Devices for Cellular Manipulation and Analysis on a Chip

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Abstract
This paper reports the micromachining technology developed for the fabrication of microfluidic devices for the biological research and also our recent development of on-chip manipulation and analysis of cells. Development of micromachining technologies such as deep glass etching using high-density plasmas has enabled us to fabricate highly functional microfluidic devices with precise microstructures and has appreciably increased the potential of the microchip technology for realizing innovative biological analysis tools and methods. As an instance, an innovative cell sorting system that can sort each biological cell based on the biologically significant information about their state has been developed using a microfluidic device and a microscopic image processing system. Damage-free selective collection of a single biological cell becomes possible owing to the combined use of the laminar sheath flow and the repulsive dielectrophoretic force in the well-designed microfabricated channels.

1. Introduction
Transparent glass or plastics are preferably used as substrate materials for biochemical analysis chips since light is often used for detection and observation of samples. Especially borosilicate glass is a material frequently used for micro analysis chips as well as conventional lab wares. One of technological challenges in the fabrication of microfluidic devices on borosilicate glass chips lies in precise microfabrication technology. Recent development in deep dry etching of silicon and silica glass has enabled us to fabricate precise microstructures of μm or sub-μm scale so as to realize highly-functional devices [1]. On the other hand, deep dry etching of borosilicate glass with smooth surface has been a difficult issue since borosilicate glass contains metal elements which produce nonvolatile fluorides [2]. In the former half of this paper, deep dry etching technology of borosilicate glass using high density plasmas and a rapid bonding method of glass plates using modified anodic bonding have been described [3,4], and fabrication of multilayer microfluidic devices has been
demonstrated using these technologies. In the latter half of the paper, self-aligned electrode fabrication process for nanofluidic devices has been established using ion implantation into non-doped poly-Si thin layer and has been applied to the fabrication of on-chip sorter devices with sub-micrometer scale channels, which is named “nanosorter”. It has been reported that microfluidic devices with microelectrodes can be utilized as a powerful tools for sorting and/or operating biological cells or microparticles of micrometer size [5]. And furthermore, nanofluidic devices are expected to be applicable to sorting and/or operating biomolecules or organelles of sub-micrometer or nanometer scale size. In practice, however, a severe technical issue such as alignment of electrodes to the nanochannel still remained in the fabrication of nanofluidic devices.

2. Dry Etching and Anodic Bonding of Borosilicate Glass Plates

In order to satisfy both smooth etched surface and the high mask selectivity, i.e., necessary requisites for deep etching, etching characteristics of borosilicate glass (Corning 7740) in SF₆ based inductively coupled plasmas have been investigated using Cr metal masks. As a result of the comprehensive experimental study, Ar addition has been revealed to be useful for the improvement of the surface roughness. High-flux Ar ion bombardment removes nonvolatile fluorides on the etched surface even under low-energy ion bombardment conditions. As demonstrated in Fig. 1, 30-μm-deep anisotropic etching has been achieved at the etch rate of 0.6 μm/min. Another important issue in the fabrication of microfluidic devices is the sealing method of microchannels since bonding of glass plates is usually time-consuming process. Then we attempted to bond two borosilicate glass plates by anodic bonding method via sputter-deposited thin Si films. Bonding was carried out at higher heater temperatures than 300 °C because 7740 glass becomes conductive at temperatures above 300 °C due to the increase of the mobility of alkaline ions. The voltage around 2 kV was found to be necessary for bonding borosilicate glass plates of 1 mm thickness in a few minutes, though it is rather higher than the voltage value around 500 V that is commonly used for anodic bonding between glass plates and Si wafers. It is ascribed to the high resistivity of the upper glass plate which exists between an anode electrode and a Si layer (see Fig. 2).

3. Fabrication Sequence of Multilayer Microfluidic Devices

Feasible and reliable fabrication process of microfluidic devices on borosilicate glass chips has been established as illustrated in Fig. 2. At first a thin Si film of 50-500 nm thickness and a Cr film of a few μm thickness were sequentially deposited on an optically flat glass plate of 1 mm thickness by magnetron sputtering. Subsequently the Cr film was patterned using photolithography and wet chemical etching. Using this Cr film as a hard mask, the microcapillary patterns with 20 μm depth were etched into a glass plate coated with a Si film in SF₆/Ar plasmas. After removal of the Cr residue by wet chemical cleaning, the top surface of the glass plate covered with Si appeared. The processed borosilicate glass plate was superposed on another glass plate with reservoir and/or interconnect holes opened by ultrasonic machining so that it might face across the side covered with Si, and it was set on the equipment for anodic bonding. These glass plates were
quickly bonded by anodic bonding method to close microchannels. Figure 3 shows an example of the multilayer microfluidic device fabricated using anodic bonding process twice. It is noteworthy that only the portion where microchannels exist is transparent and this self-aligned slit is desirable for optical observation and measurements.

4. Design of Cell Sorter Devices

As shown in Fig. 4, a nanosorter chip consists of channels for sample introduction, sheath liquid introduction, sample collection and sample waste, and microelectrodes necessary to induce repulsive dielectrophoretic (DEP) force at the entrance of the sample collection channel. Samples are transported from the injection channel toward the central collection channel owing to the laminar sheath flow. Repulsive DEP force, induced by applying ac voltages to a pair of electrodes on both sides of the collection channel, enables selective drive of unnecessary samples to the waste channel. The width of the sample collection channel was designed to be 0.6 μm in the present study.

5. Fabrication Sequence of Sorter Devices with Self-Aligned Microelectrodes

The fabrication sequence of the nanosorter chip is schematically shown in Fig. 5. At first 500-nm-thick non-doped silicon films were sputtered on an optically flat silica glass plate of 1 mm thickness and 30 by 30 mm area by magnetron sputtering, followed by the crystallization anneal in an electric furnace at 1000 °C for a certain time. Subsequently a thin Cr film of 100 nm thickness was deposited on the Si layer, and the fluidic channel pattern was transferred to the Cr film using an electron beam delineator and precise dry etching in downstream chlorine plasmas [6]. Using this Cr film as a hard mask, the fluidic channel pattern of 1.5 μm depth was etched into a silica glass plate coated with Si in SF₆ inductively coupled plasmas. After removing Cr residue by wet chemical cleaning, the top surface of the silica glass plate covered with Si appeared. Next the
A resist mask for ion implantation was patterned using a mask aligner with the alignment precision of 0.5 μm as schematically shown in Fig. 7. Boron ions were implanted at 100 keV and 5×10^{15} dose/cm² into the poly-Si layer in the neighborhood of the branch channels, followed by the activation anneal of 10 min at 1000 °C. Figure 6 shows the effect of the crystallization annealing time on the crystalline size and the electric resistivity of the boron-doped poly-Si electrodes. A sufficiently low resistivity of 0.01 Ω·cm was attained when crystallization anneal was carried out for 180 min. Such process sequence enables one to form a pair of electrodes just at the edge of the branch channels in the self-aligned manner. Figure 8 clearly shows the shape of self-aligned electrodes that appeared after ion implantation. Thus, it is suitable for fabricating fine electrodes.

Fig. 5 Fabrication process of the on-chip sorter device. Microelectrodes are formed using self-aligned process via ion implantation.

Fig. 6 The crystalline size and the resistivity of sputtered poly-Si films are plotted as a function of the crystallization anneal time.

Fig. 7 The mask pattern for ion implantation. Electrodes are formed just at the edge of the branch channels in the self-aligned manner.

Fig. 8 The photograph clearly shows boron-doped microelectrodes formed just beside the channel.
electrodes beside the nanofluidic channel without the severe alignment tolerance. Finally the silica glass plate with engraved capillary patterns was bonded with another glass plate with four reservoir holes opened by ultrasonic machining to seal nanofluidic channels. As seen in the photo of Fig. 9, the whole chip might appear to shut out the visible light due to the sandwiched Si thin layer. However, the portion where the fluidic channel exists is completely transparent, and hence there is no problem in optical detection and image observation.

6. Summary
A new sophisticated fabrication process of microfluidics devices on a borosilicate glass chip using deep dry etching and anodic bonding has been established. By adopting these technologies, a prototype of multilayer microfluidic device has been fabricated. Moreover, by employing precise dry etching and the self-aligned electrode fabrication process, an on-chip sorter device with sub-micrometer scale fluidic channels and self-aligned microelectrodes has been fabricated as a core element of the sorting system for cytoplasmic organelles and sub-micrometer particles.

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