SURFACE MICROMACHINED HOLLOW MICRONEEDLE ARRAY INTEGRATED ON A MICROFLUIDIC CHIP

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ABSTRACT
Fabrication of silica microneedle arrays on microfluidic devices has been studied in order to enable the direct extraction of organelles and biomolecules from single biological cells and/or direct injection of reagents into them. Self-standing and hollow needle structures have been attained using sacrificial etching of silicon and photoresist. The surface micromachined needle was demonstrated to be sharp enough to penetrate the membrane of single cells.

Keywords: microneedle array, microinjection and microsuction, microfluidics

1. INTRODUCTION
Intense focus has been recently seen on the application of nano/microfabrication technologies in the development of biological analysis or manipulation tools in the microscopic or mesoscopic scale world [1, 2]. In the present study, integration of the surface micromachined microneedle array on microfluidic devices has been studied aiming at the direct injection of biomolecules or reagents into living cells or direct suction of cytoplasmic organelles or nucleic acid from target cells or cell nuclei. Successful fabrication of transparent microneedle array on a glass substrate is reported here.

2. FABRICATION PROCESS
Figure 1 shows the developed process sequence for the fabrication of microneedle array on microfluidic chips. At the first step a Si film of 1.5 μm thickness, a SiO₂ film of 800 nm thickness and a Cr film of 300 nm thickness were sequentially deposited on a polished glass substrate using magnetron sputtering. Subsequently an array of microtrench pattern to define the inner wall of the microneedles was patterned using electron beam delineator on a spin-coated positive EB resist and then the microtrench array pattern was transferred to the thin Cr layer. Using this patterned Cr film as a hard metal mask, microtrench array pattern was etched into the SiO₂ layer to the depth of 500 nm in fluorine-based plasmas.
Then Cr residue was removed by wet chemical cleaning. At the fourth step, etched trench patterns were perfectly filled with photoresist that is used as a sacrificial structure to maintain the hollow feature of microneedles. Subsequently a SiO$_2$ film of 400 nm in thickness was deposited again to cover the photoresist as shown in Fig. 2, followed by the deposition of a thin Cr film of 10 nm. At the sixth step, needle features were patterned using a contact mask aligner so as to align the center of the microneedle feature with each microtrench pattern. The microneedle features patterned into photoresist were sequentially transferred into the Cr layer and the SiO$_2$ layer by using wet chemical etching and vertical etching in

**Figure 1:** Fabrication sequence of hollow microneedles on a glass substrate.

**Figure 2:** A cross sectional scanning electron micrograph of the buried photoresist in SiO$_2$. The width and depth of the channel is 500 nm.

**Figure 3:** A scanning electron micrograph of the etched microneedle patterns into SiO$_2$ layer deposited on a sacrificial Si layer.
Figure 4: Horizontal etched depth of Si in fluorine based plasmas plotted against the etching time. The characteristics of the horizontal Si etch rate was obtained to determine the process conditions for etching sacrificial Si layers to isolate self-standing microneedles from the glass substrate.

High-density plasmas, respectively. Figure 3 shows the scanning electron micrograph of the etched microneedle patterns into SiO$_2$ layer. After the removal of Cr residue, sacrificial resist structure remaining inside the microneedle was removed by chemical treatment to attain hollow structures. At the final step, a sacrificial Si film deposited as the bottom layer was isotropically etched in SF$_6$ plasmas to isolate the pointed end of microneedles from the glass substrate. Figure 4 shows the characteristic of horizontal etching of the sacrificial Si layer attained for deducing the optimum process conditions. Complex evolution of Si etch rate is possibly attributed to the rate limiting process of the transportation of etch products from the microstructures. In conclusion, surface micromachined hollow micro-needle array has been successfully fabricated as shown in the micrograph of Fig. 5. The bright area in the photo corresponds to the remaining Si layer on the substrate. Pointed ends of the microneedles are located in the shallow microchannel of 30 µm in width.

Figure 5: A microscopic photo of hollow microneedle arrays integrated on a microfluidic chip. Microneedles are transparent in visible light.
3. PENETRATION CHARACTERISTICS OF CELL MEMBRANES

The microfluidic chip integrated with micro needles was sealed with a thin cover glass plate to close microchannels. The glass substrate and the cover glass were soaked in gelatin veronal buffer (GVB) solution prior to the introduction of sample cells, and consequently nonspecific adsorption of cells on the microchannel wall was prevented. The samples mainly used in the present study were sheep red blood cells (SRBCs) of approximately 4 mm in diameter suspended in phosphate buffered saline (PBS). One of the SRBCs floating in the microchannel was trapped using a laser micromanipulation system (λ=1064nm) and was transported toward the microneedle. Figure 6 shows typical microphotographs of SRBC before and after penetration. Thus one of the important advantages of silica micro-needle arrays lies in the combined use with laser micromanipulator. Since silica hardly absorbs infrared laser light unlike silicon, the destruction of cells by heat generation never occurred.

4. CONCLUSIONS

Integration technology of the surface micromachined microneedle array on microfluidic devices has been developed. Such microfabricated devices for the direct manipulation and operation of single cells are expected to be applicable in the microscopic and molecular biological study of living cells.

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