PLANAR NANONEEDLES ON-CHIP FOR INTRACELLULAR MEASUREMENTS

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ABSTRACT

We present for the first time a functional planar SiN-nanoneedle system for intracellular mass transport and *in vivo* electrophysiological measurements on-chip. Though several micro- or nanoneedles for cell research have been described in literature, no needles of this small size equipped with nanosized inner channels or electrodes have been reported. A propidium iodide assay verifies the excellent penetration performance of the nanoneedles with diminished leakage from the cell after insertion and release of the needle from HL60-cells. Hollow needles connected to on-chip sub-picoliter electrochemical dosing systems are in development.

Keywords: Nanoneedles, intracellular, micro-machining, nanofluidics

1. INTRODUCTION

For intracellular research several techniques are used (e.g. patch clamping, electroporation), but mechanical hollow needles are superior for their simplicity and efficiency. In contrast with conventional pulled glass micropipettes the presented SiN-planar needles can be made by micromachine techniques. Micromachined transdermal drug delivery microneedles, presented at μTAS2003 by the groups of Horiike [1] and ours [2], are shaped as vertically standing pillars and can have microchannels inside, but due to the used dimensions (50 μm to 1 mm) these needles are not very suitable for intracellular use, and the pillar shape is a disadvantage for on-chip applications. Recently Obataya et al. presented nanoneedles that gave excellent results on penetration of the cell membrane without killing the cell [3], but their nanoneedles were not equipped with inner channels or electrodes.

2. FABRICATION

Three different needles are produced: hollow needles (figure 1), needles with electrodes of gold, silver or platinum (figure 2) or only plane (figure 3). In the hollow and electrode needles the channels or electrodes are encapsulated between a LPCVD-nitride bottom layer (300 nm) and a PECVD-nitride top layer (300 nm). Channel opening or electrode tip is placed at the needle tip. The plane needle only has the LPCVD layer. The needles are etched by reactive ion etching and the channels are made by sacrificial layer technique. A sacrificial chromium line is etched by ion beam etching, after deposition of the top layer the sacrificial material is removed. The electrodes are formed by wet etching. The planar needles are placed over a well, etched in silicon by KOH.

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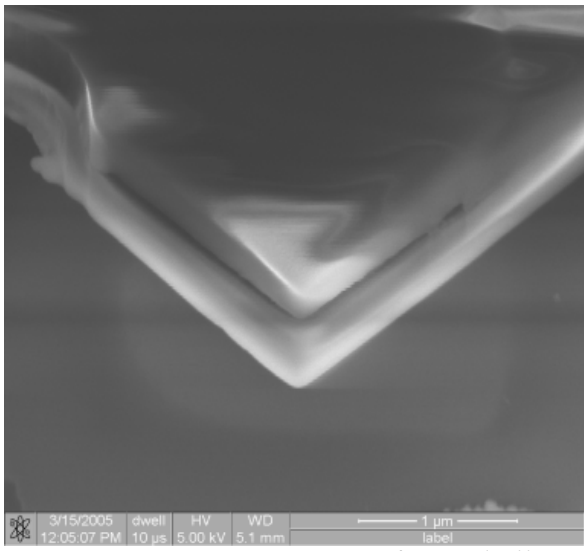

Figure 1. SEM-picture of a hollow nanoneedle. The channel is clearly visible in the middle of the needle. Inner channel dimensions: 150 nm high, 2 µm wide. Needle dimensions: 0.7 µm high, 3 µm wide and 5 µm long. The needle is sharpened by focused ion beam etching.

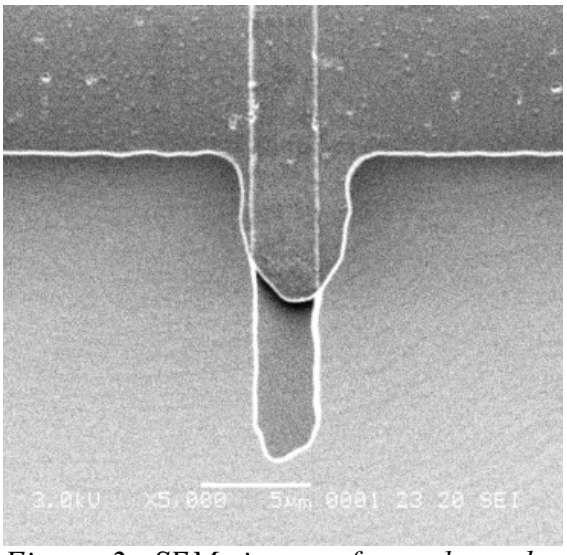

Figure 2. SEM-picture of an electrode needle. The electrode tip is 2 µm long, 2 µm wide and 100 nm high. Needle dimensions as in Figure 1.

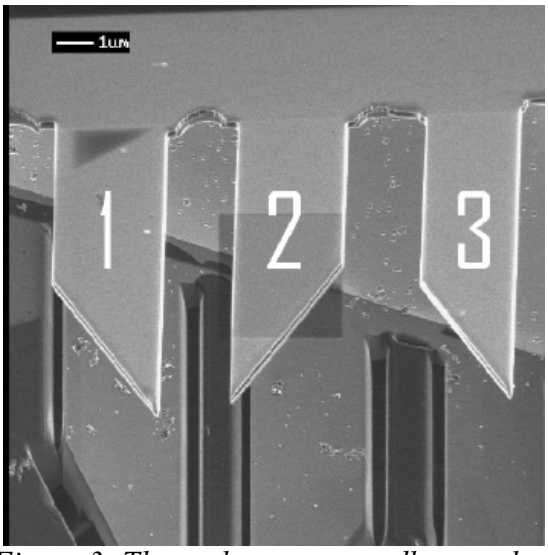

Figure 3. Three plane nanoneedles, marked with 1, 2, and 3. Below the nanoneedles, in the silicon bottom of the well, dicing lines are visible, produced during focused ion beam cutting of the sharp needle shape. Needle dimensions: 260 nm high, 3 µm wide and 10 µm long.

Figure 4. Microscopic picture of a HL60-cell punctured by a nanoneedle. The cell is positioned in the middle of the white square; the needle tip is the white tip in the top of the white square. In the lower left corner the external micropipette is shown.

3. RESULTS AND DISCUSSION

In figure 4 a HL60-cell is placed at the needle tip by an external micropipette, marked by the white square. After removing the cells from the nanoneedle a solution of propidium iodide was added to stain non-viable cells. After puncturing the cell membrane many cells were still viable. During these preliminary measurements no cell leakage was observed.

An electrochemical sub-picoliter dosing system is currently in development. This dosing system will be capable to dose volumes small enough not to rupture the cell membrane while injecting drugs, chemicals or biological samples into a normal sized cell and large enough to get a chemical analysis measurement from the cell contents after an extraction.

4. CONCLUSIONS

We demonstrated a nanoneedle suitable for biomedical cell research. Injection into and extraction from viable HL60 cells will be realized by means of integration of the demonstrated hollow nanoneedles with the sub-picoliter dosing system. *In vivo* electrophysiological measurements will be performed using the electrode needles.

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