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High-aspect ratio submicrometer needles for intracellular applications

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Abstract

A processing technology is presented to produce high-aspect ratio submicrometer silicon needles suited for intracellular interfacing with living cells. Pillars are created using deep reactive ion etching, and the sharpening of the pillars is achieved by reactive ion etching. A simple polyimide-based micro-fabrication approach was developed to integrate the silicon needles with a larger silicon base designed to carry elements such as amplifier, battery or memory. The current design allows convenient handling of the device during implantation and minimal mechanical load on the implanted region. Prototype devices were tested for usability and animal compatibility.

1. Introduction

Intracellular sensing probes have a great potential in understanding the physiology and processes of single living cells. While the information gathered by extra-cellular sensing is an average over several cells located at the vicinity of the probe, intra-cellular probing can be used to identify the behavior of single cells and, in the case of neuronal recording, it provides the critical information about the DC state of a cell, which is associated with synaptic interactions. Therefore, intracellular recording is of great advantage for understanding heterogeneous living cell populations that make up dynamic systems such as neural networks.

A major challenge in producing probes for intracellular sensing is the tip geometry. Based on parameters of conventional intracellular probes, such devices must have tips in the submicrometer dimensions and a length greater than $\sim$100 μm (assuming cell diameters in the hundreds of micrometers). These characteristics are necessary for effective bending and penetration of the flexible cell membrane (figure 1).

Standard techniques for intracellular recording consist of glass capillaries [1] and fine-insulated conducting wires [2]. Pulled glass micro-capillaries can be transformed into intracellular neuronal probes by filling them with an electrolyte and placing an Ag/AgCl electrode in the electrolyte. The glass walls provide ionic insulation and ensure low leakage current.

Despite their widespread use pulled micro-capillaries lack in a number of categories. Among them are their overall large dimensions and the limited ability to integrate them with other components, such as amplifiers, memory and power source in a compact manner that will allow measurements from freely behaving animals. Therefore, a very worthwhile task is the development of implantable tools for intracellular sensing (electrical or chemical) from single living cells.

Micro-electro-mechanical system (MEMS) technology is a likely candidate to achieve this task. The advantages of MEMS devices are the small dimensions, the ease with which multi-site devices can be produced and the ability to integrate the probes with additional components. While numerous extra-cellular MEMS devices for neuronal recording have been presented [3–7], only a single MEMS approach for intracellular recording has been presented so far [8, 9]. In [8, 9], we have shown that by using silicon micro-fabrication techniques it is possible to realize needles with geometry close to the conventional pulled glass capillaries mentioned above. Measurements obtained with these micro-needle devices using isolated brains of a sea slug (*Tritonia diomedea*) confirm the qualities of the electrodes as extremely localized bio-sensors and provide evidence that silicon needles can be used to measure signals from the interior of living cells.

The overall aim of our project is to record intracellular signals from freely behaving animals; therefore a major remaining challenge is to integrate sharp silicon needles, such

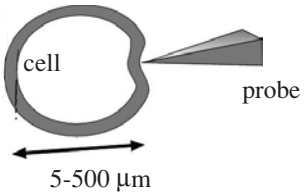

Figure 1. Schematic drawing of the bending of the cell membrane during insertion of a sharp probe.

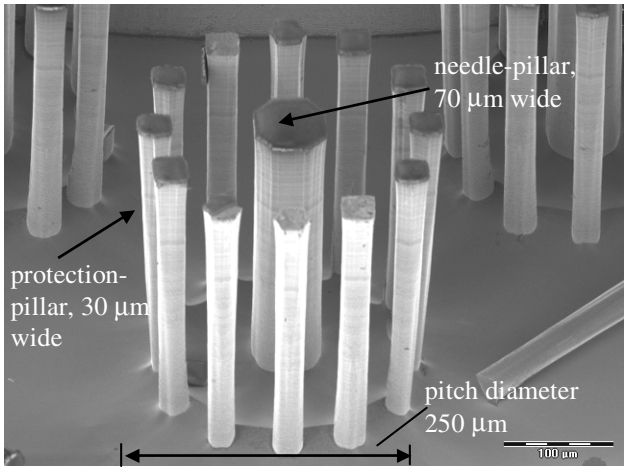

Figure 2. DRIE-etched pillars; pitch diameter 250 μm. The pillars are about 300 μm tall. The use of protection pillars ensures a straight profile.

as those described in [8, 9], with more elements needed for a complete system for signal recording.

In this paper we describe a new DRIE- and RIE-based micro-fabrication process that allows us to build systems that include both high-aspect, submicrometer needles and additional large, silicon components that provide support for additional elements, such as amplifier, battery or memory. The micro-fabrication process also includes a flexible polyimide-based interconnect scheme to produce a passivated metallic line as a flexible electrical connection between the needles and the silicon base [10].

2. DRIE process

The needle fabrication consists of DRIE process to form high-aspect ratio pillars followed by a RIE sharpening step. To fabricate needles suited for intracellular recording, highly conducting (p-type, 0.01–0.06 Ω cm, 500 μm thick) silicon wafers were used. The masking material is a 12 μm thick positive photoresist (Clariant AZ4620). The resist was softbaked before exposure at 70 °C for 5 min and at 95 °C for 5 min on a hotplate. The wafer is then placed in an Oxford Instruments DRIE to etch 300 μm tall pillars as shown in figure 2. The etching rate is ∼3.5 μm min^{-1}.

In the DRIE process the sidewall angle depends on the mask geometry and loading effects. Single free-standing pillars tend to develop a more negative profile than densely spaced structures. Since our subsequent needle sharpening etch fails on pillars with negative profile, we have devised a

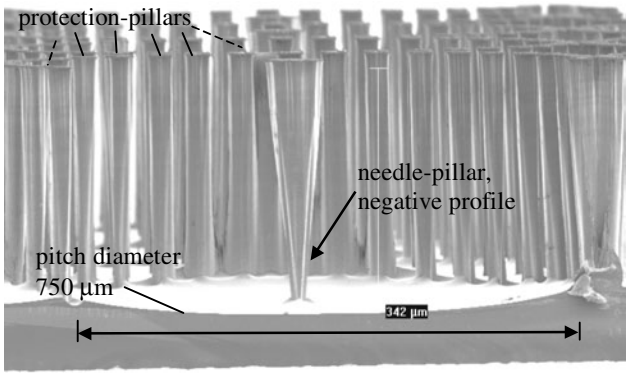

Figure 3. DRIE-etched pillars; pitch diameter 750 μm. The pillars are about 340 μm tall. Large distances between needle pillar and protection pillars result in a negative profile.

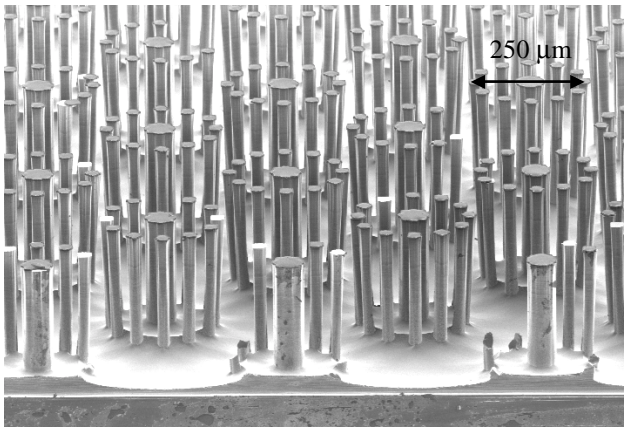

Figure 4. Array of DRIE-fabricated pillars. The pillars are about 300 μm tall. The distance between the center pillars is 400 μm.

simple method, based on protecting pillars, to produce more positive profiles. This method works independently from adjustments in the etching and deposition cycles of the Bosch process.

For the DRIE (Bosch process), the distances of the needle-pillar (∼70 μm) to the protection-pillars (∼30 μm) have been varied. Pitches of 250 μm (figure 2) and 750 μm diameter (figure 3) of protection-pillars with the needle-pillar in the center have been designed. The results after etching show that the pillars within the small pitch have a vertical sidewall, whereas the needle-pillars with a greater distance from the protection-pillars have a negative profile as can be seen in figure 3.

When the process parameters were changed to favor a more positive profile for the 750 μm pitch design, the unwanted formation of silicon-grass (black silicon) begins. This is due to polymer build-up during the deposition stage, which is not completely etched away during the etching stage.

3. RIE process

After the DRIE process the resist is stripped with acetone. Then the wafers were cleaned with EKC830 (EKC Technology) to remove the DRIE residual polymer. Without EKC830 cleaning, a white residue would remain after the

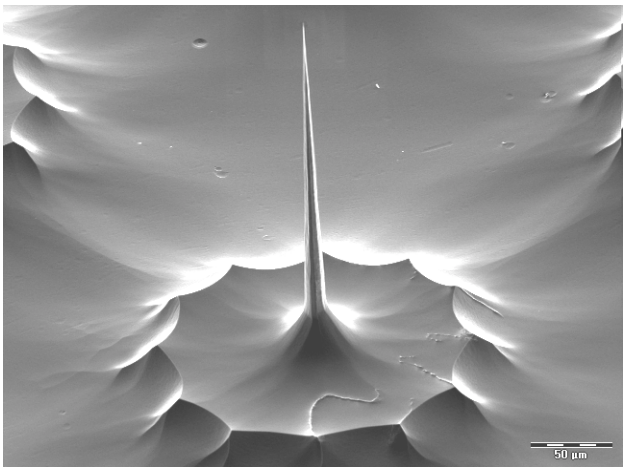

Figure 5. RIE-sharpened silicon needle. The needle is 230 μm tall and etched from the needle shown in figure 2. The protecting pillars are eliminated during the sharpening process.

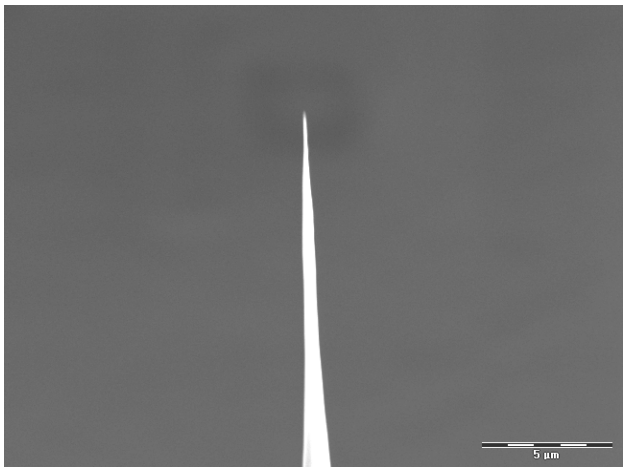

Figure 6. Submicrometer tip of an RIE-sharpened needle. The tip is 200 nm wide.

RIE process. The wafer is then placed into an RIE chamber (Trion) to form needles out of the 70 μm diameter pillars (figure 5). This is a robust, self-sharpening process using SF_6, which is optimized in order to obtain long tips with high-aspect ratio. At the same time the 30 μm protection-pillars are completely etched away leaving a $\sim$230 μm tall single needle per pitch (figure 5). The processing time is about 25 min. The tip of the sharpened needle has 100 nm radius of curvature (figure 6), which reaches the goal of producing long submicrometer silicon micro-needles.

4. Flexible polyimide-based interconnection

A flexible polyimide-based interconnect scheme was developed to connect isolated needle-like microelectrodes [10]. A simple fabrication approach allows the integration of micro-machined silicon needles with a larger silicon base designed to carry elements such as amplifiers, battery or memory (figure 7). The interconnecting scheme uses two polyimide (PI) layers to sandwich a metallic layer. The metal

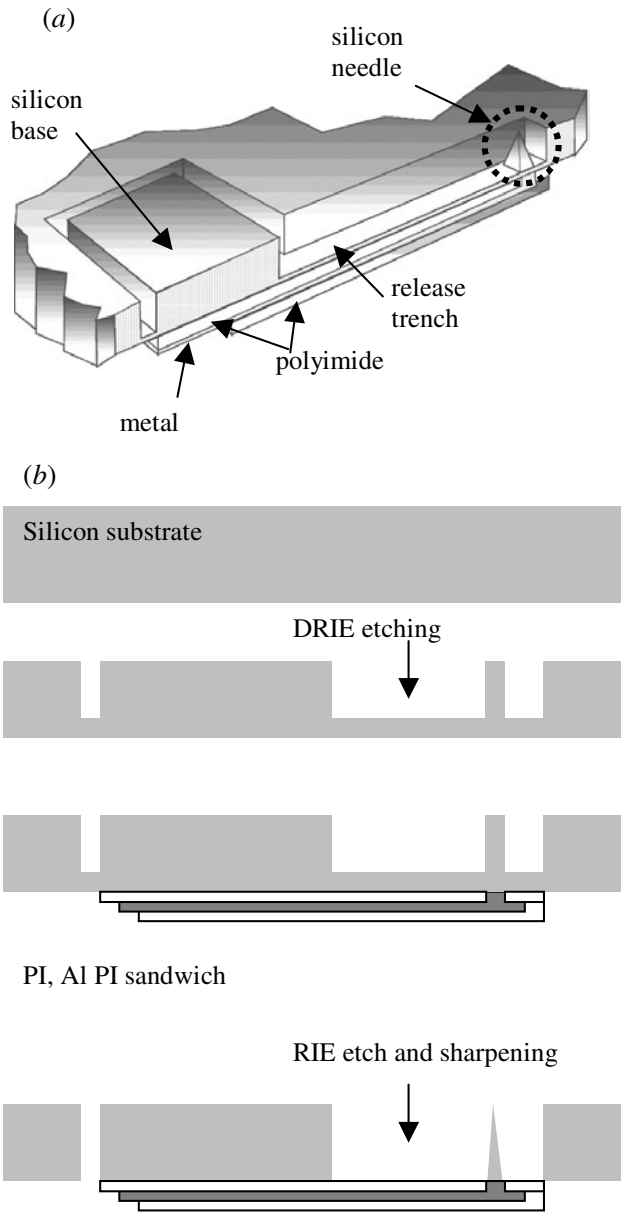

Figure 7. Schematic of the flexible neural implant device. The device consists of a large silicon base, a metallic wire encapsulated between two polyimide layers and silicon needle.

layer forms the electrical connection between the silicon base and the needle, while the polyimide layers provide flexible insulation.

The realization of such integrated devices is achieved by a process schematically presented in figure 7. The first step in this process consists of deep trenches in the silicon substrate. These trenches define the shape of the silicon parts in the final device (silicon base and silicon needles) and are used to release the final device from the supporting wafer after the final needle sharpening. A thin silicon channel floor (20–30 μm) provides enough support to hold the wafer together and to allow planar processing of the polyimide and metal films. During the final release step, the silicon channel floor is etched away. Simultaneously, the small silicon pillar is sharpened into a needle. As a result the base and the needle

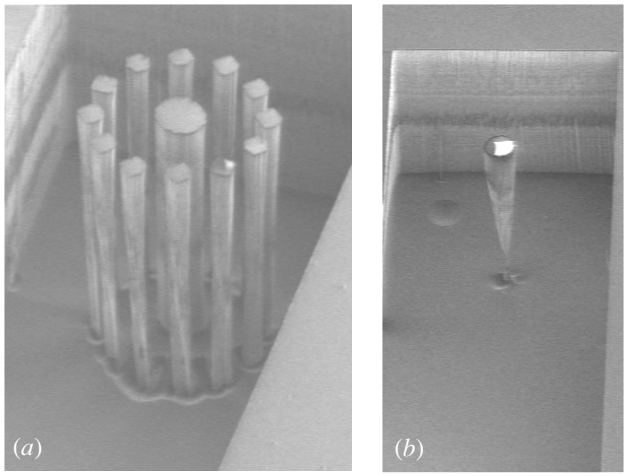

Figure 8. DRIE fabricated 480 μm tall pillars, which are part of the flexible polyimide-based interconnection scheme. The trench is 500 μm wide. (*a*) A straight profile is obtained with protecting pillars around the center pillar. (*b*) Using the center pillar only results in a negative profile.

are connected via a metal layer which is encapsulated by two polyimide layers.

Figure 8 shows an SEM image of a 480 μm long pillar isolated from the silicon substrate by a trench. The center thick pillar forms the needle after RIE sharpening. Protecting pillars are used (figure 8(*a*)) to avoid negative profile as seen in the single pillars result shown in figure 8(*b*).

Following the DRIE process a polyimide (PI2721, HD MicroSystems) layer was patterned and cured (10 μm after curing) on the opposite side of the silicon wafer. The alignment to the backside trenches was accomplished using an IR ABM aligner. It was critical to fully cure the first PI film prior to any further processing to ensure good strength and adhesion of the PI layer. Also, since multiple films were processed a relatively low final curing temperature was used. Following the first PI layer, a metal was deposited (Ebeam, 5 μm Al) to form Ohmic contact to the base of the silicon needle. To ensure continuity, a blanket metal deposition was used followed by photoresist patterning. The unmasked Al regions were etched by Al etch (Almeda Chem). A linear contact resistance of 25 Ω was measured using test samples with Al line dimensions and Al-silicon contact size comparable to typical device parameters (needle base of 70 $\times$ 70 μm).

A final RIE process, as described in section 3, concludes the fabrication process. The parameters of this process are tuned to achieve both the release of the devices from the substrate and the final sharpening of the needles. At the end of this step the devices can be gently nudged out to be separated from the substrate (figure 9). By combining the release and the sharpening process, we ensure that the needles are not damaged as they are produced only at the very last step of the fabrication.

To optimize the devices for intracellular recording, it is essential to metalize the electrode tip, in order to enhance the electrical properties of the metal–electrolyte interface, and to insulate the stump of the needle to reject electrical signals form outside the cell [8]. These features are currently under development.

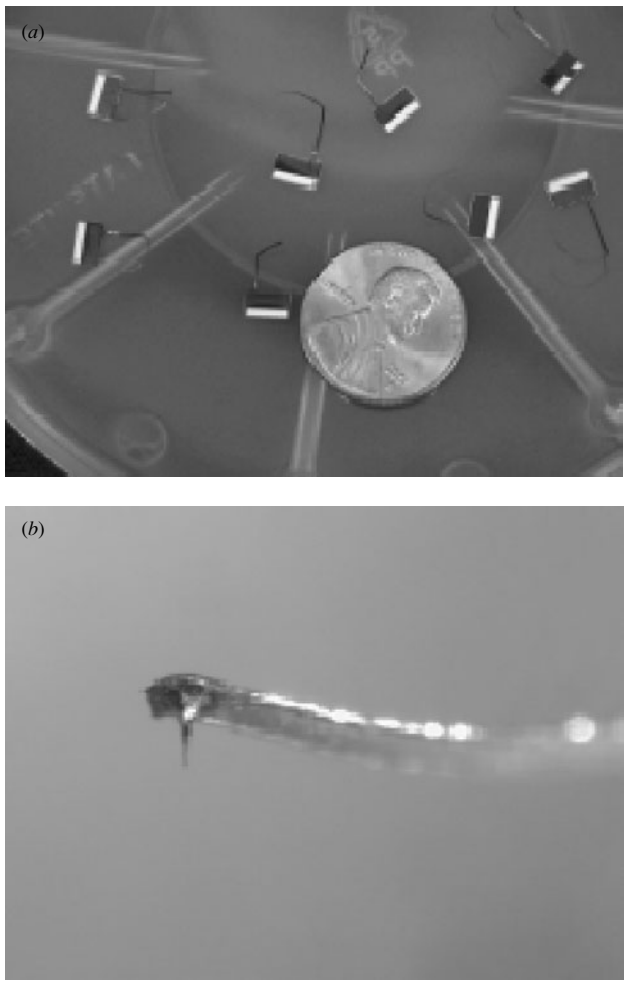

Figure 9. (*a*) Released devices with PI support. (*b*) A needle with PI support. This device was produced using a single pillar protected by narrow side walls.

5. Fabrication verification studies

To test the fabrication process described above both sharpened silicon needle and bare metal patch devices (same devices as describes in figure 7 but without the silicon needle) were fabricated (figure 9). We confirmed that both types of devices were electrically connected to the base contact area. We also validated that the needle's adhesion to the PI support is sufficient to withstand standard manipulation. Preliminary experiments with sea slugs (*Tritonia diomedea*) show that the fabricated devices are suited for implantation as they allow convenient handling as well as full recovery of the slugs from surgery and survival of a minimum of two weeks with normal behavior.

6. Conclusion

We have described a new scheme to fabricate and electrically interconnect high-aspect ratio submicrometer silicon needles using DRIE, RIE and lithographically defined flexible polyimide encapsulated metallic wires. Using this technique arrays of 230 μm long and 200 nm wide needles were fabricated. 480 μm long single needles have been integrated

into flexible interconnection devices. Initial usability tests demonstrate the effectiveness of the presented method to manufacture devices which are easy to use and to handle. Device design can be modified to meet varied user requirements such as number or locations of electrodes. Future efforts will focus on further optimization and testing of the described scheme.

Acknowledgments

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