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Integration of Three-Dimensional Silicon Microtip Arrays onto Flexible Substrate

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14. ABSTRACT
Vertically ordered Si nanotips, due to their nanoscale dimension and low cytotoxicity, serve as an ideal platform for minimally invasive penetration into biological cells for the measurement of important electronical and/or mechanical properties at the nanoscale. Currently available Si nanotip arrays are necessarily built on wafer-based substrates due to the requirement of compatibility with existing fabrication processes, leading to non-conformal interface between cells and Si nanotips. Here, we develop a novel fabrication method that enables the heterogeneous integration of vertically ordered Si nanotips with various kinds of mechanically flexible bio-related substrates.

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Final Report

Integration of Tectonic Silicon Nanotip Probes onto Flexible Substrates

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1. Objectives

Three-dimensional (3D) microelectrode arrays with vertical probes offer important opportunities for not only electrically recording electrical impulses in the electrically excitable cells or tissues but stimulating neurons with electrical impulses from an external source.[1,2] Recently, emerging micro/nano-fabrication techniques, such as photolithography, electron beam lithography, etc., enable to create precisely engineered nanoscale tip-based probes, allowing to efficiently access deep into tissues or cells to improve electrical coupling in a minimally invasive manner. Notable examples include the mushroom-shaped microelectrodes for intracellular recordings from *Aplysia* neurons and mammalian cell lines, pyramid-shaped micro-cones for intracortinal mapping and stimulating, and sharp needle-like arrays (often referred to as Utah arrays) for improved electrical coupling between electrodes and biosystems.[3-5] These arrays are built on rigid and inflexible wafer-based platforms, which are designed to be suitable for the existing micro/nano-fabrication techniques. These mechanical properties of such vertical sensor arrays yield non-conformal contacts to the curvilinear surfaces of the target biological systems, leading to low-fidelity electrical coupling and increased potential to cause damages to cells, especially when used chronically. A key challenge remains in the ability to overcome the mechanical mismatch between the rigid, planar platforms of the vertical sensor arrays and the soft, curvilinear surfaces of biological systems. This project aims to identify a collection of optimized structural designs and novel fabrication strategies that are capable of integrating arrays of vertical Si nanotip-based probes onto flexible, biocompatible substrates such as polyimide. We fabricated vertical arrays of nanotips on a Si wafer with nanoscale textures that can enhance the interfacial interactions with biological cells at the nanoscale. We developed a novel transfer printing technique based upon our previous similar approaches[6-12] that can physically separate the engineered Si nanotips from the fabrication wafer by using controlled cracks, allowing its heterogeneous integration with a flexible and biocompatible substrate. To illuminate its utility, prototype structures were introduced into various cells to evaluate its biocompatibility, invasiveness, and efficacy in delivery of genes.

2. Significance

The expected outcome represents flexible Si nanotip arrays that are capable of interfacing with the soft, curvilinear surfaces of biological cells in a conformable manner. The established principles, development strategies, and processes provide fundamental and practical insight into the development of arbitrary complexity to serve as a platform for functional nanosystems in clinical practices. We strongly believe that further studies using this type of such flexible Si nanotip arrays can open up new possibilities for highly flexible and soft interfaces in the bio/nano/3D electronics for nanoscale intracellular recording, which cannot be achievable by exploiting existing rigid nanosensor platforms.

3. Contents and Scope of Project

The scope of this study is to fabricate a vertical array of Si nanotips on a flexible bio-substrate for intracellular access and delivery. The main development contents of the technologies are as follows:

(1) We fabricated a vertical array of Si nanotips featured with sharp needle-like tips and high aspect ratio, allowing the non-invasive penetration of nanotips into biological cells. The Si nanotips were originally fabricated on a Si wafer using conventional photolithographic patterning and dry etching techniques.

(2) We developed a unique transfer printing technique that is capable of delivering the vertical Si nanotips from the fabrication Si wafer to an arbitrary flexible substrate. The mechanically soft and flexible substrate enabled the formation of conformal contacts to biological cells, facilitating non-invasive intracellular access. This transfer printing technique was enabled by using the swelling phenomenon of a silicone elastomer in organic solvent.

(3) We tested the prototype Si nanotips for intracellular access and delivery of genes to various kinds of biological cells. The results showed excellent biocompatibility and delivery efficacy through the conformal contacts with cells over large area.

4. Results

4.1 Fabrication of vertical array of Si nanotips with controlled undercuts

A vertical array of Si nanotips was fabricated on a Si wafer by using conventional photolithographic patterning and dry etching process (Fig. 1).[13] The diameter and pitch of the circular patterns used as a photoresist mask were about 3 um and 7 um, respectively. A deep reactive ion etch (DRIE) process was applied in repetitive cycles with the etching rate of 1 um/min. The dry etching time can be varied by adjusting the height up to ~20 um. The next step involved an isotropic etching to form undercuts at the bottom of each Si nanotip where the bottom parts of Si nanotips were underscored. This undercut area can serve as a cracking location where the maximum stresses can be concentrated under mechanical peeling.

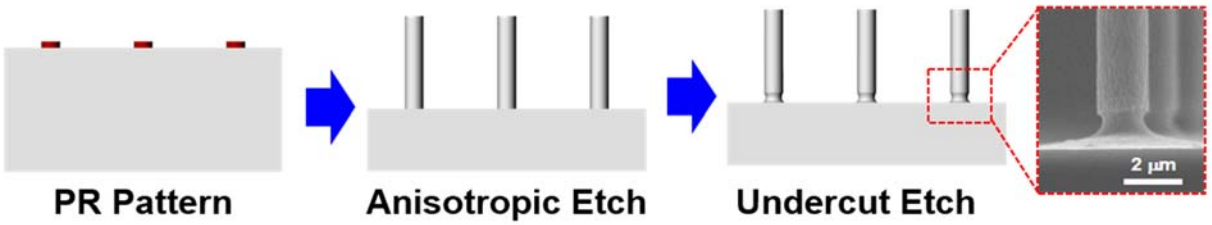

Fig. 1. Schematic illustration of fabrication process for Si nanopillars with undercut

A wet etching process by using potassium hydroxide (KOH) was followed to reduce the size of Si nanotips down to < 500 nm in diameter, which can provide minimally invasive intracellular access (Fig. 2).

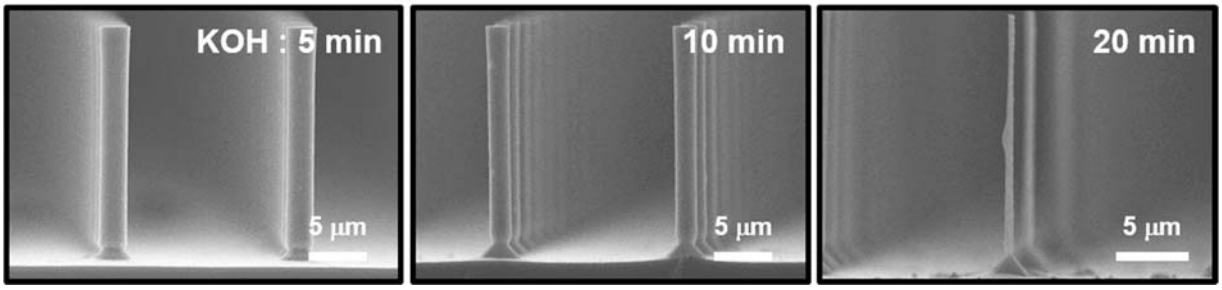

Fig. 2. SEM images of reduced size of Si nanotips

4.2 Engineering diverse shapes and surface textures of Si nanotips

We engineered the Si nanotips which had the diameter of 200 nm ~ 1 um and the height of 7 ~ 20 um by using conventional semiconductor processes on silicon wafer. The Si nanotips exhibited tapered sharp tips with adjustable pitch of 7, 10, and 15 um (Fig. 3).[14]

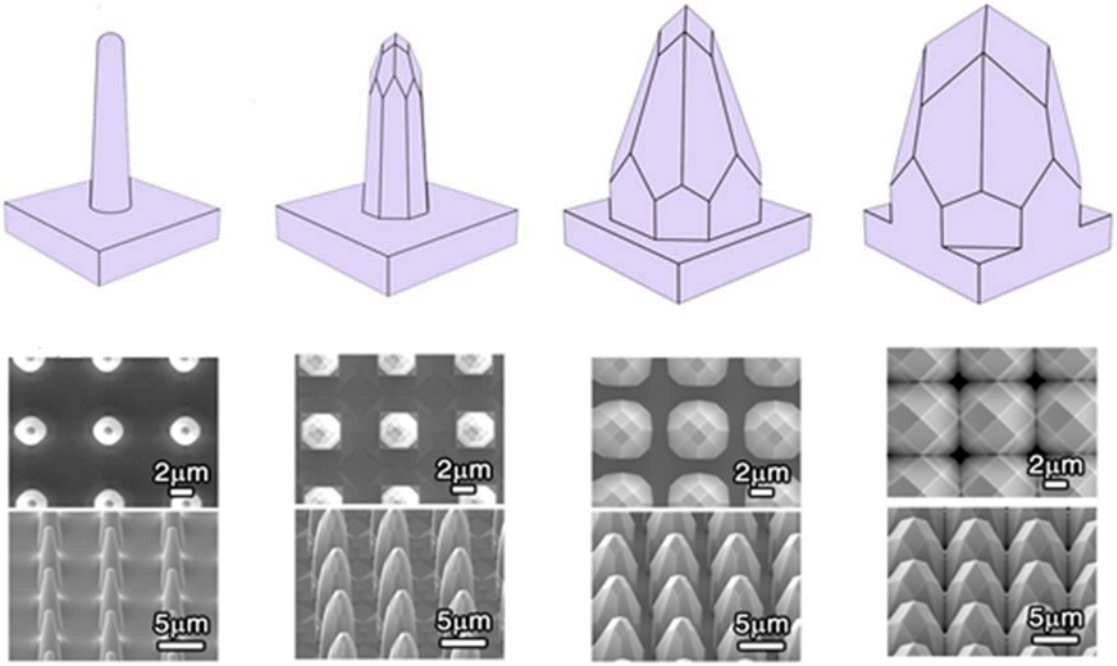

Fig. 3. Schematic illustration and SEM images of Si nanotips featured with diverse shapes

In order to maximize the surface area of Si nanotips, post-treatment by using wet etching processes such as metal assisted chemical etching (MACE) can be applied (Fig. 4).[15] The MACE process was conducted by immersing Si nanotips into a solution formed by a ratio of about 0.085 g to about 16.25 ml in $AgNO_3$ at room temperature in which the Ag ions were reacted with the Si nanotips in hydrofluoric acid (HF). This process allowed the surface of Si nanotips to become highly rough, thereby significantly increasing the surface area. An Ag etchant was used to remove agate crystals on the surface of the Si nanotips.

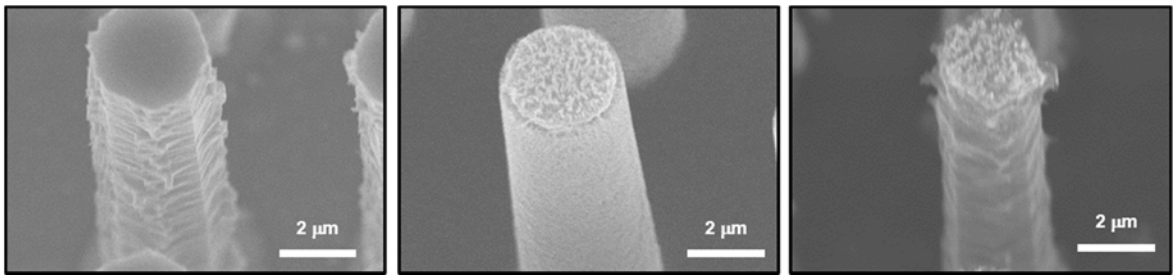

Fig. 4. SEM images of Si nanotips with nano-textured surfaces

4.3 Transfer printing of engineered Si nanotips onto flexible bio-substrates

Figure 5 shows schematic illustration of the transfer printing process that can deliver the pre-prepared Si nanotips from the fabrication Si wafer to a flexible, biocompatible substrate such as polydimethylsiloxane (PDMS). A layer of PDMS was spin-casted on top of the pre-prepared Si nanotips at the speed of about 100 RPM to have a thickness of 300 um and then cured at 150ºC. Another thin layer of PDMS was coated in order to create a glue layer on top. The Si nanotips were then placed on the surface of uncured PDMS layer as shown in the second schematic image. The entire structure was cured at 150ºC and then slowly cooled down to room temperature. Soaking the sample in hexane solution allowed the entire PDMS layer to swell in which the Si nanotips can be cracked at the under areas where the most significant mechanical strains were concentrated. This cracking process allowed the Si nanotips to be cleanly separated from the Si wafer. Drying the separated sample in air allowed the PDMS to de-swell and return into its original shape.

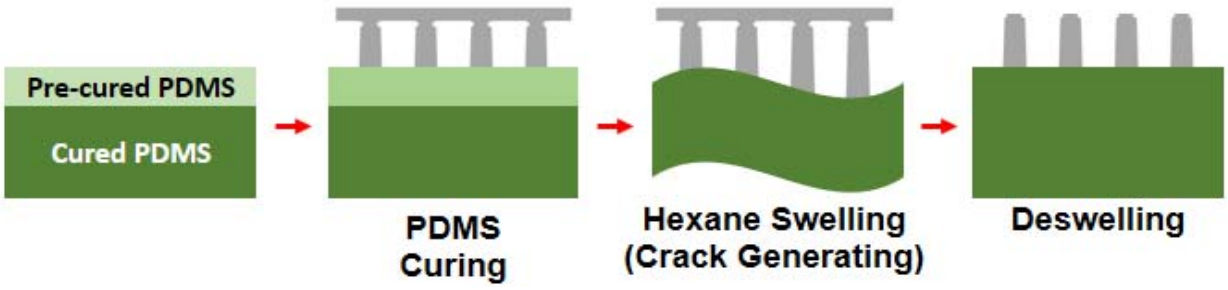

Fig. 5. Schematic illustration of transfer printing process

To understand the underlying mechanics at the undercut areas, computational analysis by using finite element method (FEM) was conducted. Figure 6 shows a representative result to highlight the concentrated mechanical strain at the undercut areas where the maximum strain can become large beyond the fracture limit of Si nanotip.

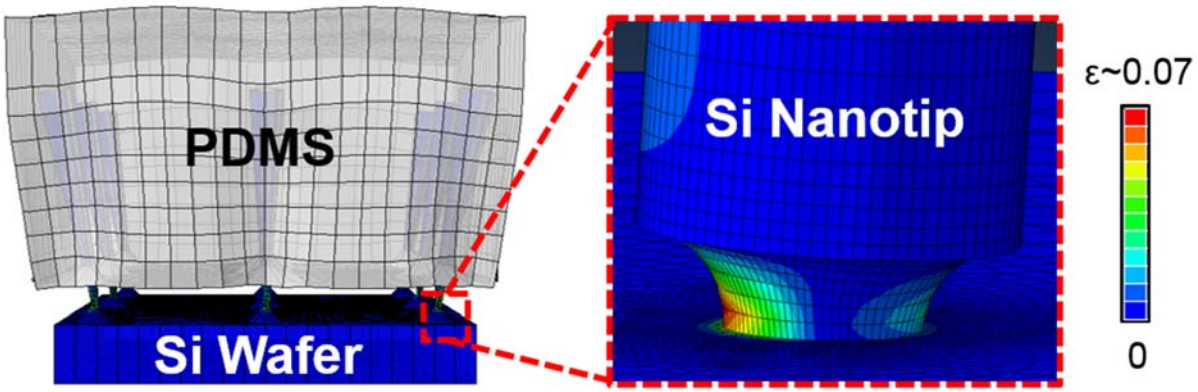

Fig. 6. Representative FEM results of Si nanotips under the swelling process of PDMS

Figure 7 shows schematic illustration and optical images of a representative transferred Si nanotip array on a PDMS substrate. The sample was very flexible and soft where the overall shape and size of the Si nanotips were uniform and consistent over the large area (1×1 cm). The sample can be bent in a manner that maintains the shape, geometry, and arrangement of the Si nanotips without any mechanical damages as shown in the second image.

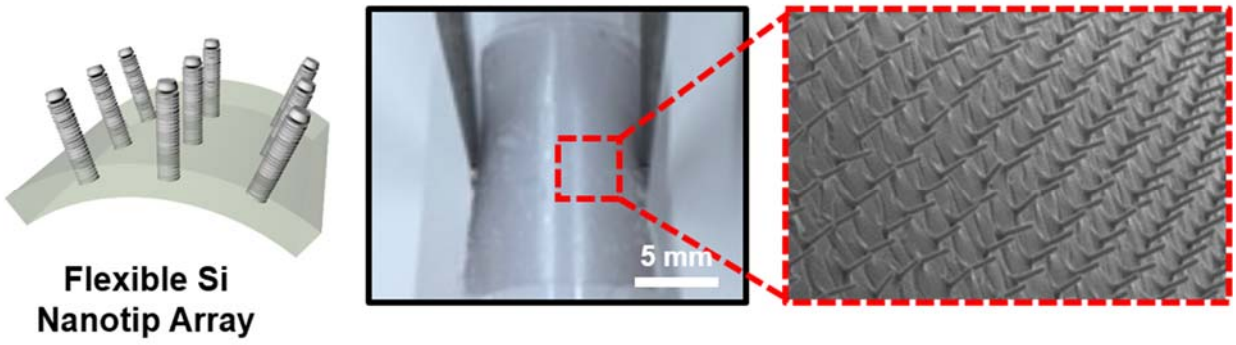

Fig. 7. Schematic illustration and optical images of a representative flexible Si nanotip array

4.4 Evaluation of the utility in intracellular access and gene delivery

The ultimate goal of this project was to utilize the flexible Si nanotip arrays for intracellular access and gene delivery. For this, we applied a siRNA gene material to the surface of Si nanotip arrays and then introduced into a cancer cell in a minimally non-invasive manner (Fig. 8). The red dots in the confocal microscope image (Fig. 8, right) indicate the siRNA gene material that is attached to the surface of the nanotip array. The blue and green colors in the image indicate the nucleus and the overall shape of the cancer cells, respectively. The tests also confirmed that the flexible Si nanotip array is biocompatible in which the cells were proliferated for 72 hours.

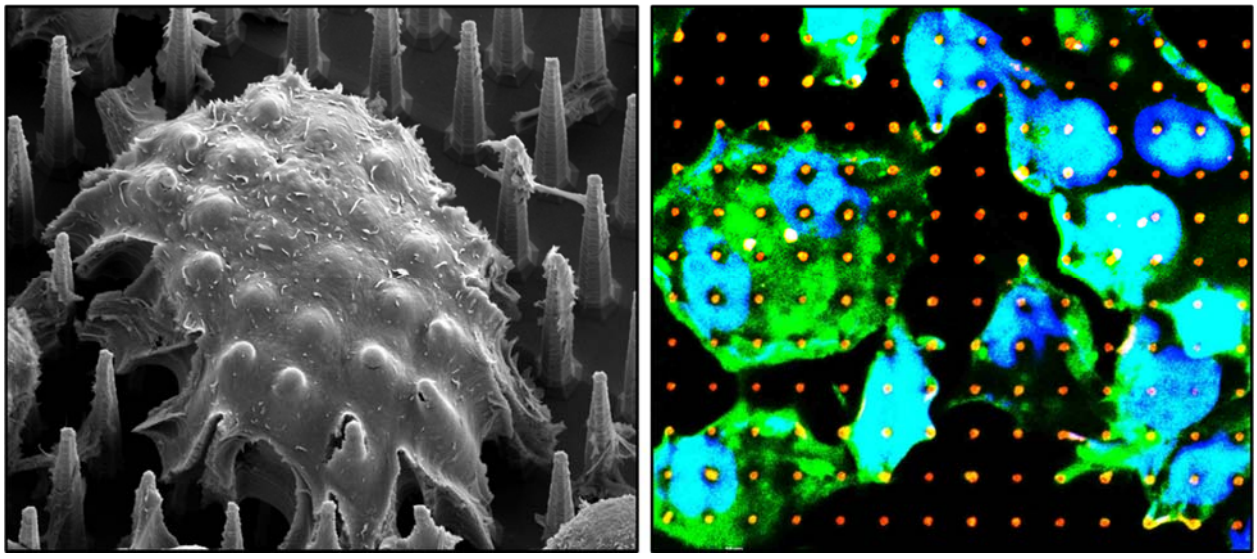

Fig. 8. Representative SEM and confocal image of intracellular tests on a cancer cell

A notable feature of the flexible Si nanotip array arises from its optical transparency because of the use of PDMS as the substrate, enabling us to obtain differential interference (DIC) images (Fig. 9). This allowed us to observe how the cells were interacted with the Si nanotips in a real-time manner. More tests will be needed to understand cell's behaviors affected by the Si nanotips incorporated with various drug molecules, genes, etc.

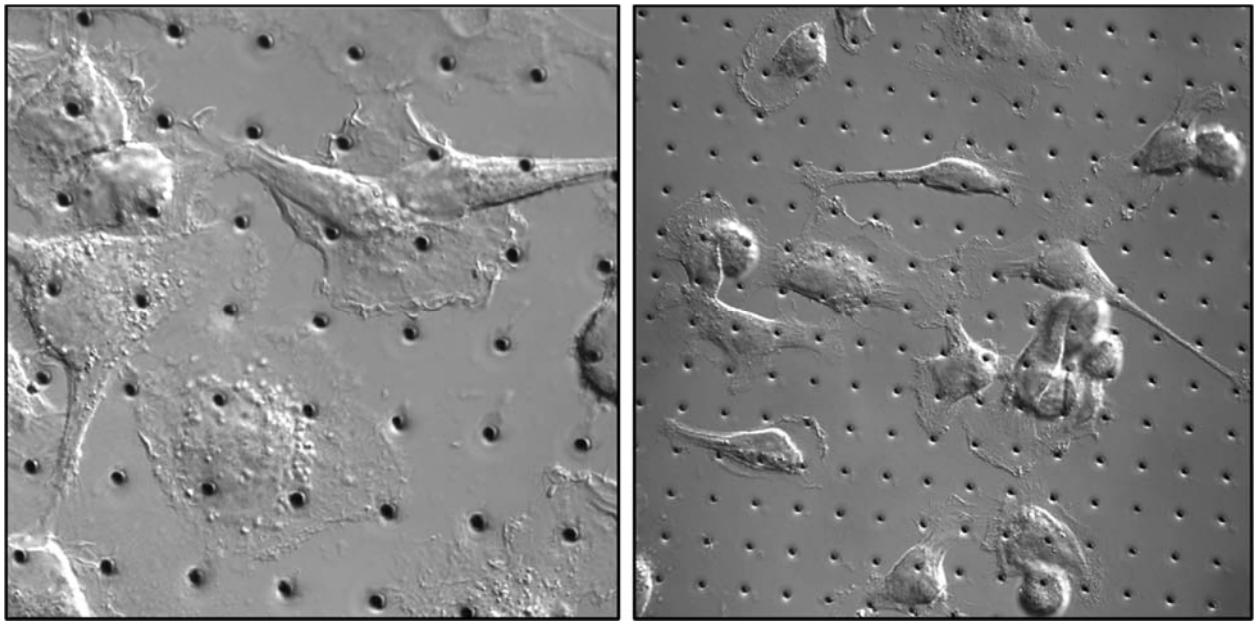

Fig. 9. Representative DIC images of Si nanotips interacted with cells

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