Nanoneedles for Intracellular Measurements

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Primary CNF Tools used: Odd/even evaporators, Oxford 81 and 100 reaction ion etchers, ABM contact aligner, AJA sputter tool

Abstract:

The ability to measure a cell's membrane potential is crucial to understanding many cellular characteristics such as excitability, intracellular kinetics, and networking behavior. We report on the fabrication of releasable nanoneedle devices for insertion into a cell.

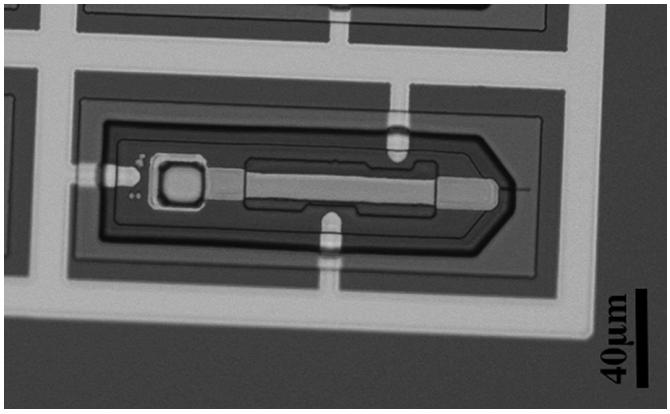
Summary of Research:

Sharp electrode intracellular recording is a standard technique involving piercing the cellular membrane with a micropipette filled with conductive fluid; this micropipette typically has a sub-micron diameter [1].

To more readily investigate the size-scale at which needle insertion can damage or kill cells, we have produced nanoneedle devices that can be released from a substrate, picked up with a micromanipulator, and inserted into cells. Each fabrication layer in the process uses standard photolithographic techniques and all layers are exposed with the ABM contact aligner. The final device before release is depicted in Figure 1.

Devices were fabricated on silicon-on-insulator wafers (SOI) allowing for release of the completed unit after fabrication. The nanoneedles protruding out of the end of the device were made by e-beam evaporation of platinum with a titanium adhesion layer (Ti-Pt) at a 70° angle, to allow for a nanoneedle width smaller than the minimum pattern width achievable with the contact aligner. A scanning electron microscope (SEM) image of a nanoneedle is shown in Figure 2.

After patterning of aluminum release tabs, the Xactix xenon difluoride etcher was then used to etch the silicon handle, resulting in suspended devices. The aluminum was then selectively etched to release the nanoneedle devices into solution. Free-floating devices could then be pipetted up using a standard hand pipette, and dispersed into fluid.



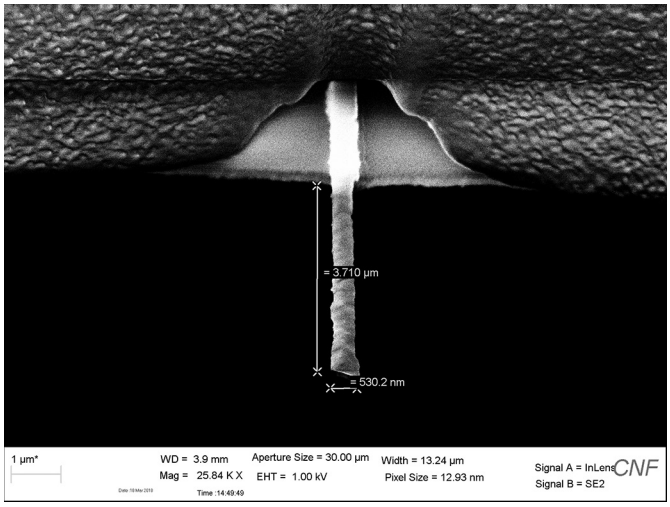


Figure 1, top: *An optical microscope image of a completed nanoneedle device.* ***Figure 2, bottom:*** *An SEM image of a typical nanoneedle after evaporation.*

Although devices survive the full process with remaining nanoneedles, the xenon difluoride etching the oxide under the needles and the pipetting force used cause many needles to break off.

To test the ability of our devices to penetrate the cellular membrane without causing cell death, we cultured HL-1 cardiomyocytes in Petri® dishes. When the cells were approaching confluence, we used a pipette to disperse the nanoneedle devices into the cell media. To monitor cell health while performing experiments, a green fluorescent protein (GFP, Cal-520 AM) was used for concurrent calcium imaging. If the cell membrane is punctured irreparably, the GFP will cause the cell to fluoresce as the calcium present in the surrounding fluid enters the cell.

The nanoneedles devices were then manipulated either by poking into the SU-8 encapsulation layer with a microprobe or using a small micropipette under vacuum to create enough suction to lift the device. Using a micromanipulator, the devices' protruding nanoneedle could then be controllably forced into a cell. In Figure 3, we show a nanoneedle device being manipulated by micropipette suction. The white regions of the cell indicate fluorescence of the GFP. The cell continues to spontaneously blink indicating that the cell has not died; however, the white region near the nanoneedle indicates that membrane penetration may have taken place. The fabrication of an exposed platinum pad at the end of the device opposite the nanoneedle in theory allows for electrical contact to determine whether the nanoneedle is inside, but the large ratio between the surface area of the pad and the surface area of the nanoneedle means that the nanoneedle must have an excellent seal with the membrane to detect the penetration electrically. Further experiments and improvements to the nanoneedle fabrication are being investigated.

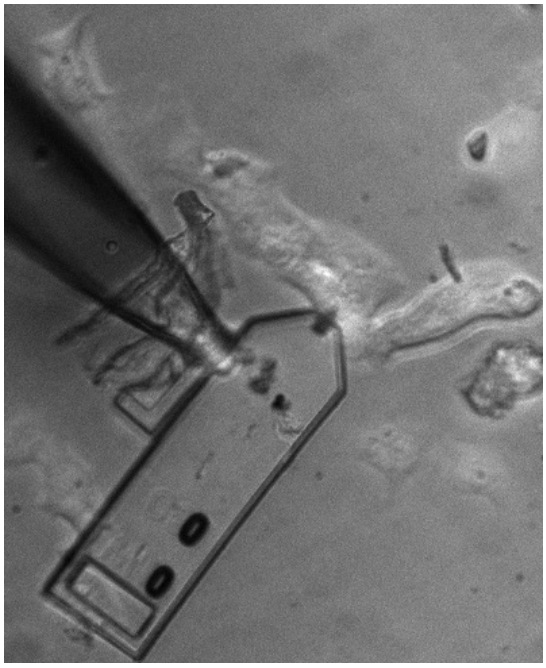


Figure 3: A nanoneedle device being manipulated to puncture a cardiomyocyte.

References:

[1] Yang, R., et al. Investigation of penetration using atomic force microscope: potential biomarkers of cell membrane. IET Micro and Nano Letters 10 (2015).