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APPLICATION NOTE

SMALL PROJECTILES FEATURING TRIPLE-LAYER METAL CLADDING WITH POTENTIAL APPLICATIONS IN THE FIELD OF CELL TRANSFECTION

by

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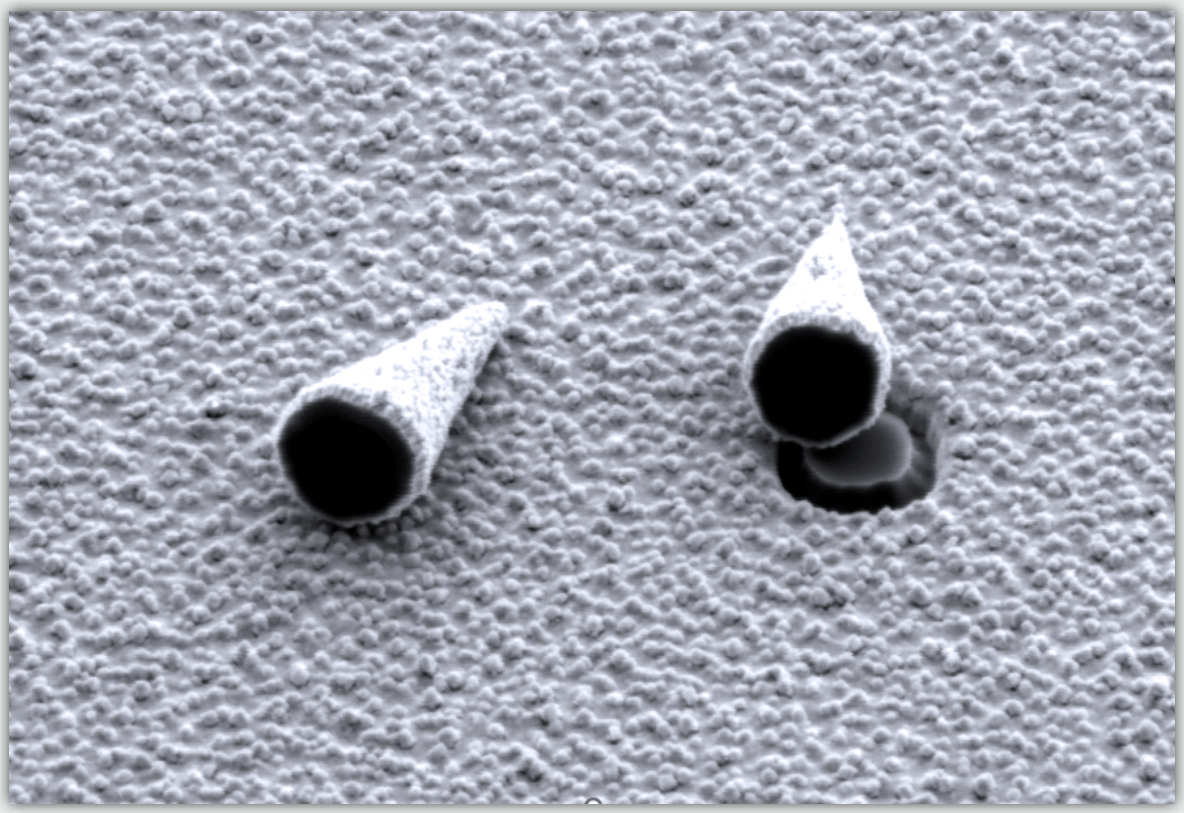


Conical projectiles with a tip apex radius well below 100 nm were produced using a relatively simple and reproducible process flow (Figure B)) based on UV lithography using a MLA 150 maskless aligner from Heidelberg Instruments to fabricate the starting cones in the photoresist layer. After the resist development, a finely tuned O_2 plasma descum process reduced the size of the obtained conical shapes. The following low etch rate isotropic dry etch of silicon, created a topography step that effectively isolated the resist cones on top of silicon pedestals. Finally, a metal deposition step covered the produced cones with a triple layer cladding consisting of Ni, Cr and Au. The metalized cones were detached from the substrate using mild sonication in a water bath.

The resulting micro-projectiles can be guided magnetically for transfection, the process of introducing e.g. nucleic acids into eukaryotic cells. The precious Au layer on the cones allows for functionalizing the outer surface of the projectiles with e.g. foreign nucleic acids (DNA/RNA) which can be introduced inside eucaryotic cells (sizes of app. 5-10 µm) or bacteria (sizes of app. 0.5-5 µm) in order to alter their properties. Using the magnetically actuated projectiles allows for selectively transfecting individual cells or an ensemble of cells in real time by controlling the projectiles with an external magnetic field.

DETAILED PROCESSES

This process allowed for obtaining 9 million projectiles at the proof-of-concept stage. Single side polished <100> Si substrates with a thickness of 525 µm, were used as carrier substrates. Prior to resist deposition, the Si wafers were HMDS primed and subsequently coated with a 1.5 µm thick positive photoresist layer (AZ®5214 E). The GDSII design file featured a 2D array of 3000 x 3000 circular holes with a diameter of 1 µm, covering an area of 16 x 16 mm^2. Being unexposed, the holes remained in photoresist. Maskless UV exposure was conducted using the 375 nm laser of the maskless aligner MLA 150, with real-time optical auto-focus and a pre-optimized dose of 45 mJ/cm^2. The focus position which can impact the resist side wall taper angle was set to -4 on a unitless range. This focus offset was chosen to obtain conical resist structures after development. To achieve the best results, the imaging DMD width was set to 400 pixels and the high-quality mode (associated with an address grid of 40 nm) was activated for the exposure. The full field was exposed within minutes. After the maskless UV exposure, the irradiated resist was developed with a slightly prolonged single puddle of TMAH developer for 90 seconds.

The final resist structures (seen in Figure A)) exhibited the desired conical shape with a height of approximately 1.2 µm and a base diameter close to 750 nm. To further sculpt the shape and reduce the size of the produced projectiles a mild O_2 dry etch process was employed on an Advanced Silicon Etcher. The implemented process features a resist etch rate of approximately 5 nm/s which allowed for controlling the final size of the conical projectiles. The plasma process was terminated when the base diameter was approximately 400 nm.

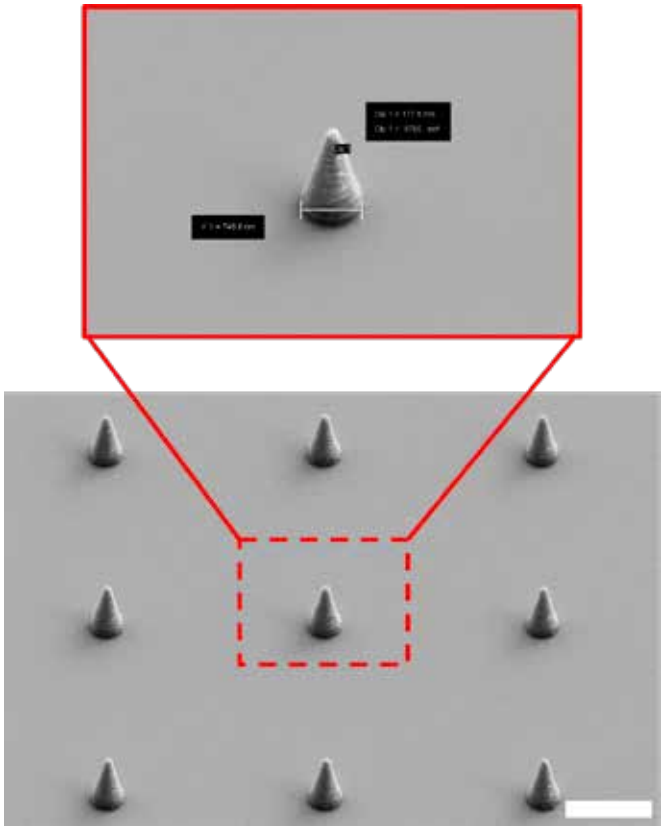


Figure A) SEM image and zoom-in on the conical resist structure(s) after MLA exposure and subsequent prolonged development. As evident, the base diameter is approximately 750 nm. The scalebar corresponds to 2 µm.

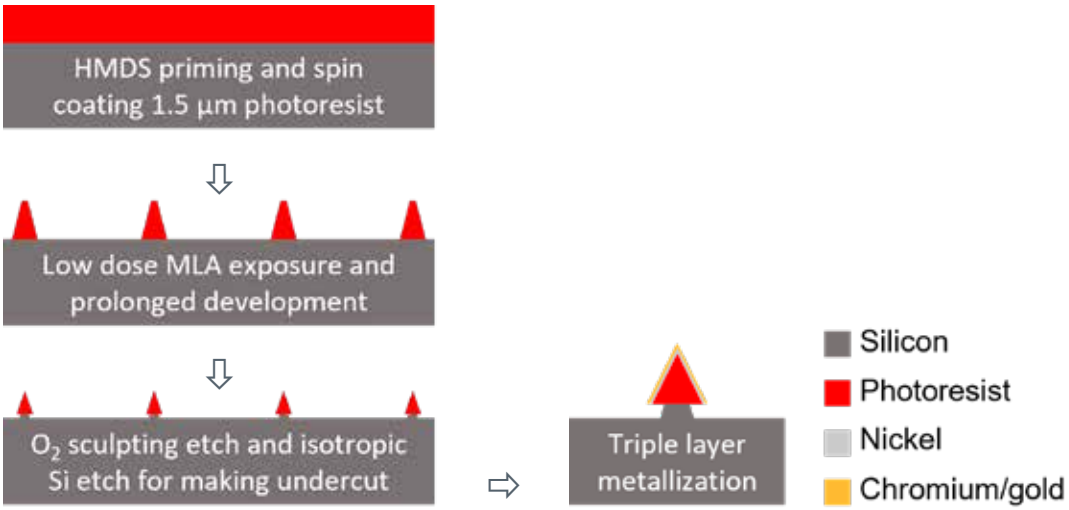


Figure B) Schematic outlining the process flow for making nanoscale projectiles for magnetically actuated cell transfection. The flow simply consists of a UV lithography step, followed by O_2 and SF_6 based dry etching for sculpting the conical projectiles and making a topography step which alleviates release after metal deposition.

After this dry etch targeting the organic resist material, the ASE was used to initiate a flash isotropic silicon etch using SF_6 gas to achieve a substantial undercut in the silicon near the base of the resist cones. This effectively created a topography step (evident in Figure C)) which enabled smooth release of the projectiles after the metallization step. In order to facilitate magnetic actuation as well as enabling surface functionalization of the produced projectiles, a triple layer metal deposition was employed using a dedicated batch system. The metal cladding layer consists of 70 nm ferromagnetic Ni, a 5 nm thick Cr adhesion layer and finally a 25 nm thick layer of the precious metal Au (final structures can be seen in Figure D).

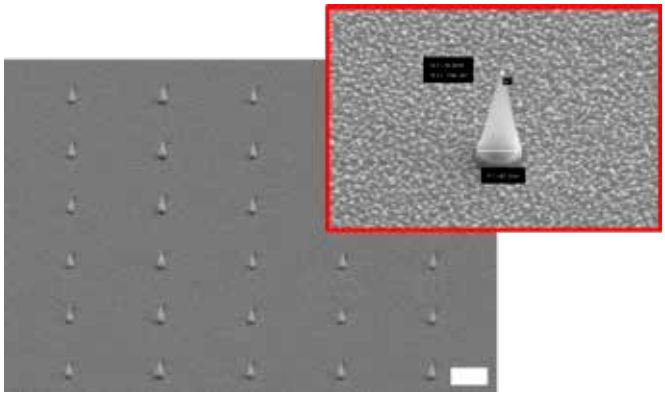
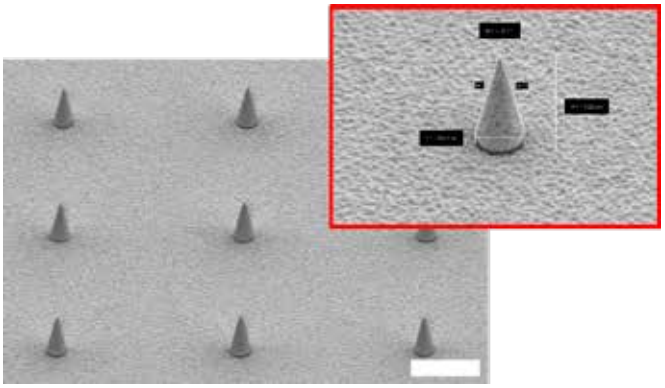
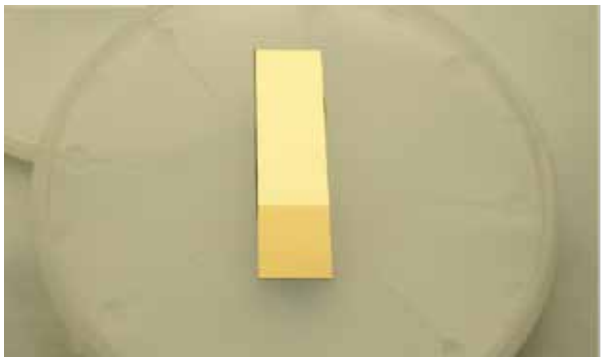


Figure C) SEM image and magnified view of a single projectile after the dry etching steps. The base diameter has been reduced to 430 nm and the tip apex has a radius of curvature of around 40 nm. The scalebar corresponds to 2 µm.



(D) SEM image and magnified view of a single projectile after the triple layer metallization. It is evident that the topography step effectively causes a clear discontinuity in the metal layer on the projectiles and the base silicon substrate. The base diameter has been increased by approximately 100 nm but the tip apex remains sharp. The scalebar corresponds to 2 µm.

The metals were deposited without breaking vacuum and the deposition was initiated at a chamber base pressure of $4x10^{-7}$ Torr. After metallization, the silicon substrates were scribed, and broken into rectangular pieces. A mild sonication in a water bath (Figure E)) released a substantial number of structures: around 9 million projectiles in 10 mL DI water inside a small glass vial. Owing to the ferromagnetic Ni in the cladding layer, the projectiles could easily be manipulated using a conventional NdFeB power magnet with a diameter of 10 mm and a thickness of 2 mm. The manipulation was carried out in a small petri dish and the movement of the projectiles was recorded using a dark field microscope equipped with a 50X objective. The movie shows hundreds of projectiles moving around deterministically in response to the externally applied magnetic field from the power magnet. Individual projectiles can be distinguished but larger clusters are also clearly evident.



Scan the QR-code to view the video clip "Dynamic manipulation of small projectiles – exploring potential applications in cell transfection" on the Heidelberg Instruments YouTube channel.

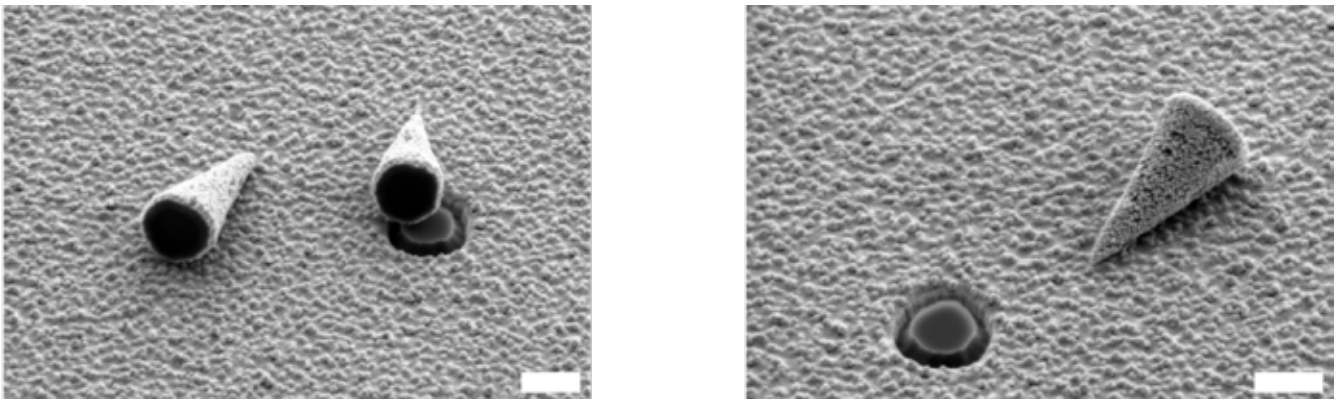


Figure E) Camera picture (above) of the sample stripe prior to projectile release by means of ultrasonication in a water bath. The SEM images (below) show a few of the projectiles remaining on the substrate after the release. On these images, the formed silicon pedestal is visible, and the perfect conical shape of the projectiles is also noted. The scalebars correspond to 400 nm.

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