Nanofountain Probes for the Delivery of Molecular Inks

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Principal Investigator(s): Horacio D. Espinosa
User(s): Owen Loh, Nicolaie Moldovanan

Affiliation(s): Department of Mechanical Engineering, Northwestern University
Primary Research Funding: Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award EEC-0647560
Contact: espinosa@northwestern.edu, o-loh@northwestern.edu, n-moldovan@northwestern.edu
Web Site: http://clifton.mech.northwestern.edu/~espinosa/

Abstract:

Nanofountain probes (NFPs) are atomic force microscopy (AFM) probes designed for direct-write delivery of liquid molecular “inks” with sub-100 nm resolution. Liquid inks’ stored in an on-chip reservoir are fed through integrated microchannels to apertured dispensing tips by capillary action. This allows continuous delivery either to a substrate for direct-write nanopatterning, or to a cell for in vitro injection. Recently demonstrated applications focus on direct delivery of chemotherapy drug-coated diamond nanoparticles for single cell studies. The nanodiamonds were delivered by both nanopatterning and nanoinjection method enabling drug dosing and kinetics studies.

Summary of Research:

The Nanofountain Probe (NFP [1-3]) functions as a highly miniaturized fountain pen that can be used to deliver a variety of materials with precision in the 50 nm to 1 µm range. As in a conventional fountain pen, the liquid material to be delivered (the “ink”) is contained in a reservoir and flows through a channel to an apertured dispensing tip (Figure 1). Past demonstrations of direct-write nanopatterning include proteins [4] and DNA [5] in buffer solution, gold nanoparticles in aqueous suspension [6], thiols [1,3], and drug-coated nanodiamonds [7].

Piezoelectric positional control of the NFP by an AFM enables ultra-precise prescription of pattern geometry. The precision of the NFP combined with the broad range of molecular delivery capabilities enable studies at a truly single cell level through two modes of delivery: direct write nanopatterning, and direct in vitro injection (Figure 2).

Based upon these capabilities, the NFP was used to study nanomaterial-mediated drug delivery at the single cell level. In this study, both modes of delivery were leveraged to deliver drug-coated diamond nanoparticles (NDs). First, to study dosing, dot arrays of NDs dots coated with doxorubicin HCl (DOX), a commonly used chemotherapy drug, were patterned on a glass substrate on sub-100 nm resolution [7]. Cells were then cultured on the patterned substrates to observe their response to a given dose. Here the dose depended both on the size of the patterned dots and the pitch between them. To create the dot features, the NFP was brought into contact with the substrate for a

Figure 1: Schematic of the Nanofountain Probe. Liquid ink is stored in an on-chip reservoir and fed through enclosed microchannels to apertured writing tips by capillarity (inset, scale bar: 2 µm) [7].

Figure 2: Schematic of the two modes of delivery for the Nanofountain Probe. (left) For direct-writing nanopatterning, the tip is brought into contact with the substrate where an ink meniscus forms (right) for in vitro cellular injection, the tip is introduced to the cell membrane [7].
prescribed dwell time, then lifted and translated to the next point in the array. The feature size (both dot diameter and height) depend strongly on the square root of the dwell time (Figure 3). Various cell lines were subsequently cultured on the nanopatterned glass substrate and the preserved activity of the drug was verified by a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The assay successfully detected the DNA fragmentation and apoptosis; two key inducing mechanisms of the DOX drug. Beyond nanopatterning, the ability to directly inject doses of drug bound nanoparticles (e.g., NDs) into cells was demonstrated. This allows further study of the response of a single cell to a given dose. Fluorescently-tagged NDs were injected into various cancerous cell lines [7]. The positional accuracy and force sensitivity of the AFM are leveraged to guide the NFP during targeted cell injection. As a preliminary study, the spreading and kinetics of the injected nanoparticles were further investigated by an integrative optical imaging technique. The spread in fluorescent intensity of the injected dose in an MCF-7 cell (Figure 4) was captured in a series of images over time. A diffusion coefficient of \((11.8 \pm 0.2) \times 10^3 \, \text{µm}^2 \, \text{s}^{-1}\) at 25ºC was estimated using a Gaussian fit function.

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**References:**


