Nanofountain Probes for Direct-Write Nanomanufacturing and *In Vitro* Single Cell Studies

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***Abstract*-We present a broadly-applicable nanodeposition tool, the Nanofountain Probe, for direct-write fabrication of functional nanostructures using liquid molecular "inks". Examples of nanopatterning of biomolecules, catalysts for subsequent nanostructure growth, and functional nanoparticle arrays for nanosystems fabrication and single cell studies will be presented. Recent developments in the use of the Nanofountain Probe as an *in vitro* single cell injection tool are also discussed.**

I. Introduction

Next-generation nanoscale devices will be built from functional nanoparticles (NPs) and nanostructures. Developing manufacturing tools with sufficient degree of control to apply these materials remains a critical challenge. For example, memory elements and sensors composed of nanowires (NWs) or carbon nanotubes (CNTs) will require precisely-aligned arrays of these nanostructures [1]. Similarly, next-generation biosensors and drug screening and delivery devices will require high-density arrays of bioagents [2, 3].

NPs have proven highly functional precursors for a variety of nanostructures. For example, patterned gold NPs may be sintered to form nanoscale conductive traces or photonic structures [4]. Diamond or other NPs may be used as seeding or catalyst for subsequent growth of diamond structures [5] or CNTs or NWs respectively [6, 7].

Using these applications as motivation, we demonstrate direct-write delivery of functional NPs and biomolecules using a broadly applicable Nanofountain Probe (NFP, [8-11]). The NFP is an atomic force microscope (AFM)-based delivery probe. Liquid molecular inks (e.g., NPs in solution) stored in an on-chip reservoir are fed through integrated microchannels (Figure 1a,b) to apertured dispensing tips (Figure 1a, inset), providing continuous delivery for direct-write nanomanufacturing (Figure 1c). The sharp, apertured tip geometry enables a unique combination of resolution and generality in its ability to pattern a broad range of chemical and NP inks. Beyond nanomanufacturing, the NFP tip geometry also enables *in vitro* injection into biological cells (Figure 1d).

This presentation begins with demonstrated nanopatterning capabilities of the NFP and a discussion of their applications. These include direct deposition of gold, diamond, and other catalytic NPs [9, 10, 12], DNA and proteins [8, 11], and drug-coated NPs [9]. We then show use of the NFP as an *in vitro* nanodelivery tool in single cell studies. The broad applicability of the NFP is emphasized throughout.

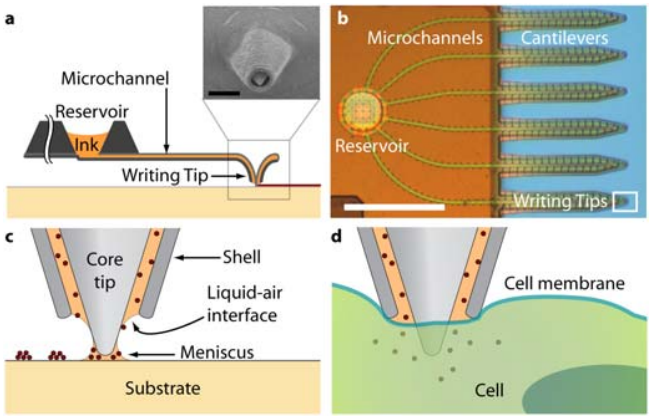

Fig. 1. Overview of the Nanofountain Probe. (a) Schematic of the NFP chip showing the integrated ink delivery system. Inset shows an SEM image of a dispensing tip (2.5 μm scale bar). (b) Optical image of an NFP chip (500 μm scale bar). Due to the unique dispensing tip geometry, the NFP can be used both for (c) direct-write nanopatterning, and (d) *in vitro* single cell injection.

II. Results and Discussion

A. Direct-Write Nanoparticle Patterning

Direct-write NP patterning enables creation of devices in a top-down bottom-up manner, in which NP precursors are first patterned, then treated to form or grow components of the final device. Toward this goal, the NFP was used to pattern gold and diamond NPs [9, 10], and catalysts for CNT growth [12].

Using the NFP, dot arrays of diamond [9] and gold [10] NPs were patterned. For example, negatively-charged gold NPs in aqueous solution were deposited directly on amino-terminated silicon dioxide substrates with sub-200-nm resolution. More recently, diamond NPs in aqueous solution were patterned with sub-100-nm resolution on glass substrates (Figure 2a). Feature size (dot diameter and height) depended strongly on the square-root of the dwell time (R^2>0.97), suggesting diffusive transport to the substrate [13]. This strong dependence enables precise control over feature size.

B. Direct Biomolecule Deposition

The ability to spatially orient and immobilize biomolecules on a substrate is valuable to the development of genomic and proteomic profiles of cells [14], drug screening, and biosensing [3], all of which require high-density arrays of biological

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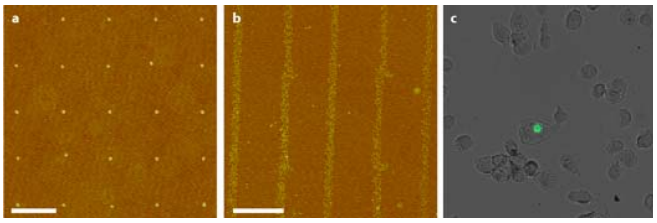

Fig. 2. Examples of NFP-based delivery. AFM images of (a) a dot array of diamond NPs patterned by direct deposition (4 μm scale bar), and (b) a linear protein array patterned at a rate of 80 μm/sec by direct-write electric field-assisted deposition (1 μm scale bar). (c) Individual RAW 264.7 murine macrophage targeted and injected with fluorescently-labeled diamond NPs.

material. Using the NFP, thiol-terminated single-stranded DNA (ssDNA) were patterned on gold substrates [11]. To test the preserved activity of the patterned ssDNA, they were hybridized first with a complementary linker strand, followed by a gold NP-terminated strand. The presence of gold NPs on the patterned features indicated successful hybridization, attesting to the preserved activity of the patterned ssDNA.

A variety of proteins were also successfully patterned using the NFP [8]. Initial attempts to pattern these proteins exhibited relatively slow and sporadic deposition. However, by applying an electric field between the on-chip reservoir of the NFP and substrate, transport of these charged biomolecules could be controlled. Immunoglobulin G (IgG) and bovine serum albumin (BSA) proteins were patterned on thiol-terminated gold substrates, as well as IgG- and BSA-coated substrates. Through appropriate control of the sign and magnitude of the applied electric potential, deposition of the molecules on the substrate could be controlled. Submicron dot and line molecular patterns were generated with the resolution dependent upon the magnitude of the applied voltage, the tip-substrate contact time, and writing speed. For example, line features 150 nm in width were patterned continuously at rates as high as 80 μm/sec (Figure 2b).

C. Direct In Vitro Single Cell Injection

Beyond nanopatterning for nanomanufacturing and biological studies, the ability to directly inject doses of functional NPs, viruses, and other bioagents into cells allows further study of the response of a single cell. The ability of the NFP to facilitate these studies was demonstrated through *in vitro* injection of fluorescently-tagged diamond NPs (Figure 1d). Here the positional accuracy and force sensitivity of the AFM are leveraged to guide the NFP during targeted cell injection. As an initial feasibility study, RAW 264.7 murine macrophages were injected with an aqueous fluorescein solution. Imaging of the cells after injection showed strong confinement of the fluorescent dose to individual cells, suggesting minimal damage to the cell membrane. Fluorescently-tagged diamond NPs were then injected into a variety of cancerous and wild type cell lines (Figure 2c). [9] Again strong confinement of the injected dose was observed, and the ability to target specific regions of the cell demonstrated.

III. Concluding Remarks

The Nanofountain Probe was presented as a broadly-applicable tool for nanomanufacturing. The ability to precisely pattern arrays of functional nanoparticles and other materials was demonstrated. Beyond nanomanufacturing, this was extended to single cell *in vitro* biological studies by using the NFP to inject nanoparticles into live cells.

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