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Nano fountain probe technology for *in vitro* single cell studies

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We present a broadly-applicable Nano fountain Probe technology for single cell delivery and analysis using biomolecules and functional nanoparticles. The nano fountain probe is a scanning probe nano delivery tool which makes use of on-chip fluid reservoirs and integrated microchannels to deliver liquid solutions to sharp-aperture dispensing tips. The unique tip geometry allows for both sub-100nm nanopatterning on substrates for subsequent cell culture, as well as direct biomolecular delivery inside cells with minimum invasiveness. The spatial and force resolution of the atomic force microscope are leveraged to control the probe with nanometer and nano newton precision during nanopatterning and *in vitro* transfection experiments. We begin by describing nanopatterning capabilities and their application to cell adhesion and nanomaterial-mediated delivery studies, followed by *in vitro* single cell transfection of biomolecules (DNA, RNA, plasmids). In this presentation, an emphasis is placed on the broad utility of the nano fountain probe as a nano delivery tool with the goal of motivating future studies in cell biology. Direct-write nanopatterning of several biomolecules and functional nanoparticles using the nano fountain probe will be presented. Examples include DNA and protein, as well as gold and drug-coated diamond nanoparticles. Models of the deposition process describing the effects of probe geometry, liquid properties and patterning parameters on resolution will be discussed. These models enable optimization of the patterning process, resulting in sub-100nm resolution. The need for high resolution delivery arises in nanoscale studies of protein and cell functions such as the creation of adhesion templates, where for example, protein clustering in cell focal adhesion occurs at 5 to 200nm length scales. Similarly, this resolution allows extremely precise spatial control of dosing in nanomaterial-mediated drug delivery studies. As an example, patterning drug-coated diamond nanoparticles, in which the dosing is controlled with yoctogram precision, will be presented. Direct *in vitro* transfection of functionalized nanoparticles and biomolecules will be discussed. The transfection of fluorescently-labeled diamond nanoparticles, on multiple cancerous and normal cell lines, will be illustrated. Likewise, the temporal delivery of proteins and RNA molecular beacons will be discussed in the context of non-destructive cell analysis.

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