Lipid Dip-Pen Nanolithography for functional biomimetic membrane systems

S. Lenhert*, H. Fuchs**

*Forschungszentrum Karlsruhe GmbH, Institut für NanoTechnologie
76344 Eggenstein-Leopoldshafen, Germany, steven.lenhert@kit.edu
**Physikalisches Institut, Universität Münster, D-48149 Münster,
Germany, fuchsh@uni-muenster.de

ABSTRACT

Dip Pen Nanolithography (DPN) is uniquely capable of integrating of multiple materials (or inks) with both high resolution and high throughput. Phospholipid-based inks are particularly well suited for this purpose for several reasons. First, their lyotropic liquid crystalline nature enables reproducible tip-coating using multiplexed inkwells for humidity controlled delivery of different inks to different tips in an array, for multiplexed patterning. Second, their chemical diversity allows a variety of different functional groups to be directly integrated into the ink. Third, since the ink transport from the tip to the substrate is based on self-organization and adhesion just about any surface can patterned in this way, including insulating glass or hydrophobic polymer surfaces such as polystyrene. Finally, being a major structural and functional component of biological membranes phospholipids are compatible with the vast amount of molecular resources provided by nature, for instance lipophilic materials and membrane bound proteins.

Keywords: dip pen nanolithography, phospholipid, membrane, liquid crystal, interface

1 INTRODUCTION

Dip-Pen Nanolithography (DPN) is a versatile tool for nanotechnology by using the tip of an Atomic Force Microscope (AFM) as an ultrasharp pen.[1-3] Being a constructive method of scanning probe lithography, DPN can be carried out in a massively parallel fashion, in principle enabling the resolution of electron beam lithography, integration capabilities typical of inkjet printing, and a high throughput comparable to microcontact printing.[3-15] In particular, DPN makes it possible to integrate different materials on scales (both in size and complexity) that appear impossible to reach by any other direct-write fabrication method. Such a method is especially desirable for the fabrication of biomolecular arrays, and opens entirely new possibilities in the study and development of nanobiotechnology.[16-21]

Phospholipids are ubiquitous biological molecules that self-assemble under physiological conditions to form the bilayer structure of biological membranes. Established methods for generating supported lipid bilayer membranes include as vesicle fusion, Langmuir-Blodgett transfer or self-spreading.[22] Self-spreading from dehydrated lipid multi-layer stacks is particular applicable here as it will be shown that it is directly compatable with lipid DPN.

Several methods have been used for the patterning of supported lipid bilayers, for instance vesicle fusion onto pre-patterned substrates,[23] direct photolithography,[24] microcontact printing,[25] microarraying,[26] stensiling,[27] and molecular editing by Atomic Force Microscopy (AFM).[28, 29] These and other methods for generating heterogeneous phospholipid arrays, are severely limited either in their lateral resolution or in the ability to integrate multiple lipids on a single surface, which is desireable for creating surfaces that mimic biological membranes.[30-32]

2 PHOSPHOLIPID-BASED DPN

Here an approach to DPN is used that is based on non-covalent adhesion and humidity control of the liquid crystalline phase of phospholipid inks. This makes it possible to pattern phospholipids on a variety of substrates. The chemical structure of a typical phospholipids ink (DOPC) is shown in Figure 1 along with a schematic illustration of the lipid DPN process. In contrast to the transport behavior of most other inks, where the ink transport can be controlled by the tip-contact time and scan speed, respectively, as well as humidity, phospholipid inks tend to stack into multilayer structures where the thickness of the film can be controlled by those same parameters.[4]

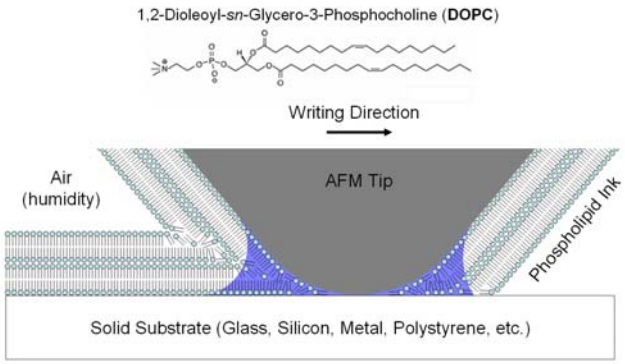

Figure 1: Chemical structure of a typical phospholipid ink (DOPC), and schematic illustration of the writing process.

A crucial step in the DPN process is to coat the tip with the ink to be patterned. Typically, AFM tips are coated for DPN either by thermal evaporation onto the tips or by dip-coating, i.e. immersing the tip into a solution containing the material to be patterned and then allowing the solvent to evaporate from the coated tip. Phospholipids (and related materials) provide a third alternative for tip coating because their fluidity depends on their degree of hydration. In the case of DOPC at room temperature, the bulk material behaves like a solid below 40% humidity, but becomes viscous fluid above that humidity. At 75% humidity, the pure (hydrated) phospholipids material readily flows onto tip. The tip-coating process using microfluidic inkwells (commercially available from the company NanoInk) is drawn in Figure 2.[33]

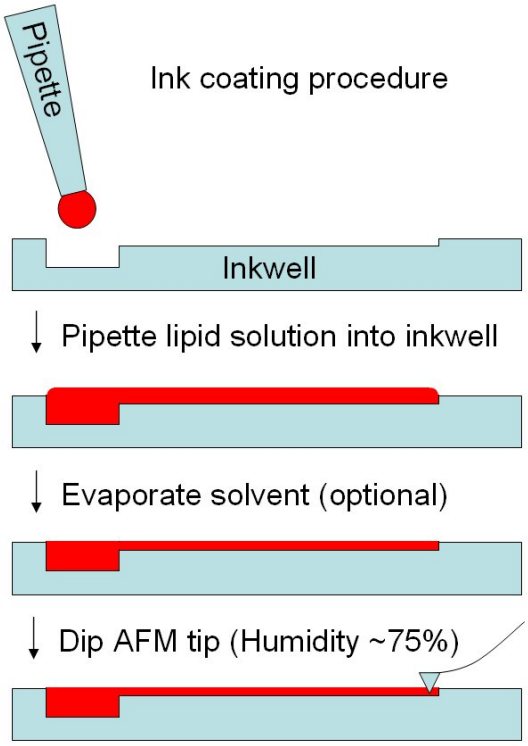

Figure 2: Schematic illustration of the method for coating the AFM tips using inkwells.

The ability to adjust the fluidity (or viscosity) of the ink in this manner facilitates the delivery of different ink materials to different tips in an array for multiplexed patterning. In comparison, it is difficult to get a thick coating of solution based inks onto two or more neighboring tips of an AFM tip array primarily for two reasons. First, the tip is coated mostly by solvent, and upon evaporation of the solvent much of the ink volume disappears. Second, solution based inks that have low viscosity and surface tension can wick out of inkwells when the AFM tip contacts them, thus coating the entire chip and contaminating the tips. Phospholipid based inks provide a general solution to these problems and an example is shown in Figure 3. In this case, small amounts (1 Mol%) of fluorescently labeled lipids were dispersed in the DOPC ink.

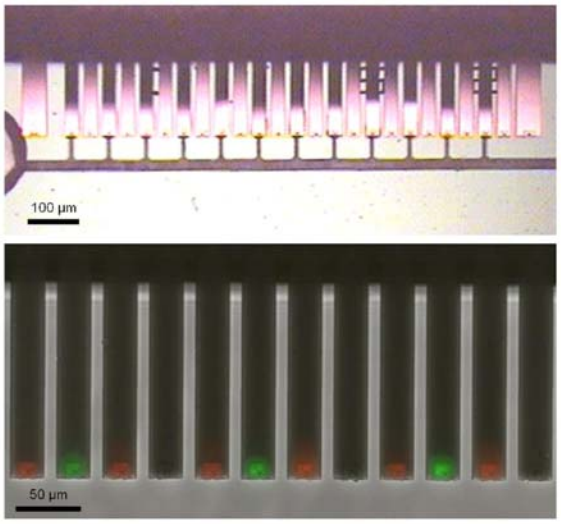

Figure 3: Top: optical micrograph of a one dimensional tip array having a phospholipid ink selectively delivered to every second tip in an array. Bottom: Fluorescence image of different fluorescently doped lipids (Rhodamine DOPE in red and FITC-DOPE in green) selectively applied to different tips on a parallel array. The 2 channel fluorescence signal is overlayed with the brightfield image of the cantilever array.

The rate of ink transport during the writing process can then be precisely controlled by lowering the humidity within a range of 40-75% for DOPC. In contrast to the transport behavior of most other DPN inks, where the dot and line width could be controlled by the tip-contact time and scan speed, respectively, as well as humidity, phospholipid inks tend to stack into multilayer structures where the thickness of the film can be controlled by those same parameters. Careful adjustment of the humidity and scan speed has enabled line widths down to 100 nm and thickness as low as a single bilayer.[4]

3 SUBSTRATE DEPENDANCE

In order to generate biomimetic lipid membranes from the lipid multilayer patterns it is necessary to immerse the patterns into aqueous solution. As biological membranes are fluid, it is generally undesirable to covalently stabilize the patterns for this purpose. However, depending on which substrate is used (and which type of biomembrane one wishes to mimic), the multilayer patterns can be immersed into water while retaining their multilayer structure, or they can be spread to form supported lipid bilayer membranes.

3.1 Supported Lipid Multilayers

An example of a heterogeneous lipid multilayer pattern that was written in parallel and immersed in water without loss of lateral resolution is shown in Figure 4, and a hypothetical structure of the multilayer patterns is drawn. From a physical chemistry perspective, the stability of the multilayer patterns can be understood in terms of substrate wettability. That is, the interfacial tensions involved (solid/water, solid/lipid, water/lipid) must be such that spreading is thermodynamically unfavorable, thus resulting in a non-zero contact angle.[34] The multilayer structures under water provide the possibility of reducing the friction of membrane bound materials with the substrate, as typically the goal of polymer supported lipid membranes.[35] Furthermore, the entirely new possibility of encapsulating materials within the multilayers becomes accessible, further characterization is necessary in order to test these capabilities as well as to determine the precise supramolecular structure of the multilayer patterns under water.

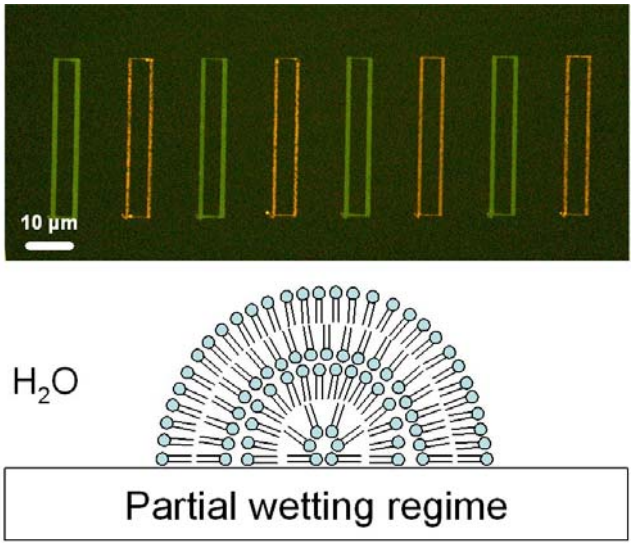

Figure 4: Top: 2 channel fluorescence image of 2 different fluorescently labeled lipids patterned in parallel on a glass surface (used as received without further treatment) while immersed in water. The fine lateral structure of the patterns (visible down to ~200 nm by fluorescence) is preserved. Bottom: hypothetical structure of the phospholipid multilayers on a partially wetting surface under water.

3.2 Spread Supported Lipid Bilayers

A better characterized example of an artificial biomimetic surface is the supported lipid bilayer formed by self-spreading. When a dehydrated multilayer stack is printed onto a hydrophilic surface, and immersed into water, it can spread to form a homogenous and fluid lipid bilayer.[36-42] The formation of a supported lipid bilayer in this way can be confirmed by fluorescence microscopy. The spreading of the small multilayer spots can be watched in real time, providing insights into dynamic membrane organization processes. Upon equilibration, the resulting thin films then show a homogeneous fluorescence intensity indicating the bilayer has been formed. A final test of the function of the bilayer is through fluorescence recovery after photobleaching experiments (FRAP), in order to determine the fluidity of the membranes as shown in Fig 5 (A-E). Once the bilayers have spread, the spreading stops and the bilayer patterns remain stable in water for at least several weeks. As a variety of functional lipids are readily available both from biological and synthetic sources, the combination of DPN with self-spreading therefore provides a reliable method for generating multi-component biomimetic membrane systems.

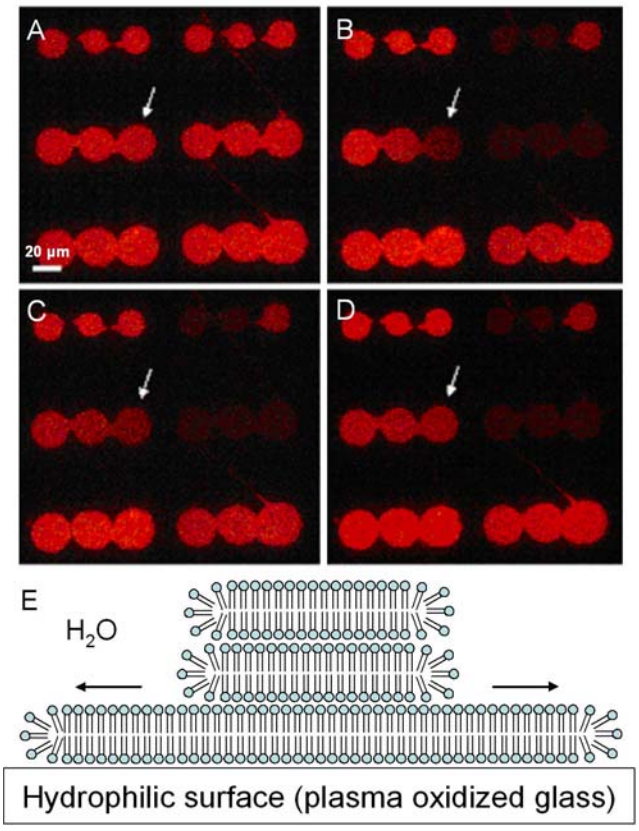

Figure 5: Supported lipid bilayer patterns formed by spreading DPN deposited multilayers onto a hydrophilic surface. Top: shows fluorescence recovery after photobleaching. A) image before bleaching. B) image after bleaching. C) image after 10 minutes, and D) image after 20 minutes. The arrow shows the bleached and recovered part of the patterns. E) shows a hypothetical structure of the phospholipid multilayers spreading on a hydrophilic surface under water.

4 CONCLUSIONS

The use of phospholipids as an ink for DPN opens several new possibilities both for materials integration as well as the fabrication of biomimetic surfaces. The non-covalent interactions with the substrate make the ink generally applicable to a variety of surfaces. The humidity dependant nature of the inks fluidity makes it ideal for both multiplexed tip coating as well as writing. Furthermore, the supramolecular structure of the resulting micro and nanostructures can be dynamically modulated by the underlying substrate. The ubiquitous nature of phospholipids in biological systems provides a wide repertoire of functional materials that can be purified or synthesized, and now integrated on the appropriate micro and nanoscopic length scales.

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