Functional Lipid Nanostructures Fabricated by Dip-Pen Nanolithography

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ABSTRACT

Lipids are well known to form the structural basis of biological membranes, and the reconstruction of functional lipid nanostructures is an active field. Parallel and multiplexed dip-pen nanolithography (DPN) using phospholipids is well suited for this purpose, as it is allows multiple lipid materials to be integrated over large surface areas, with submicron lateral resolution[1]. Furthermore, the unique properties of phospholipids permit control of multilayer stacking and encapsulation of materials into the nanostructured lipid multilayers on surfaces[2]. A combination of the advantages of the structural, chemical, and lyotropic properties of membrane lipids with the nanostructuring capabilities of DPN permits creation of semisynthetic functional lipid nanostructures[3-5]. Phospholipid-based DPN's ability to pattern biofunctional lipids has applications in biological studies of cell adhesion[5], as well as biophotonic structures for label-free investigation of liposome function and biosensing[6].

Keywords: phospholipid, nanostructure, dip-pen nanolithography, liposome, photonics

1 INTRODUCTION

Biological systems are composed of numerous functional structures, the critical dimensions of which are on hierarchical levels ranging from the molecular to the entire organism (Figure 1) and even to larger scales such as populations, ecosystems, and the entire biosphere. Lipids are especially crucial to the organization of biological systems, as they form the structural and functional basis of biological membranes and are therefore essential components of all known life.

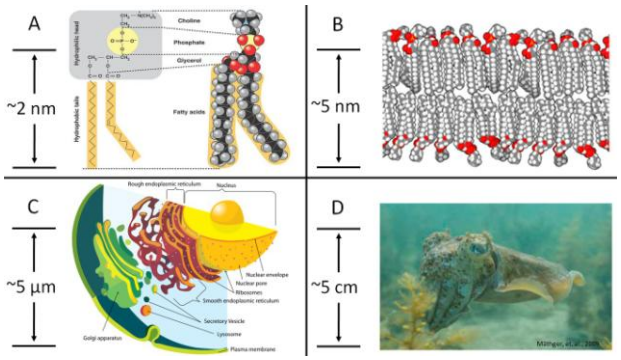

Figure 1: Hierarchical structure of lipids in biological systems. (A) The chemical structure of a phospholipid, showing hydrophobic hydrocarbon tails linked to a polar headgroup[7]. (B) when fully hydrated, e.g. by exposure to water, phospholipids are in a bilayer phase with a bilayer thickness on the order of 5 nm. (C) When viewed on larger, micrometer scales, a variety of functional lipid structures are visible within individual eukaryotic cells. (D) On the organismal level, multiple cells interact and communicate. For example, iridophores in cephalopods are iridescent cells that can rapidly change color for purposes of communication and camouflage.[8]

The amphiphilic chemical structure of biomembrane molecules such as phospholipids (shown in Figure 1A) allows them to self-organize spontaneously under aqueous conditions into lipid bilayer membranes (Figure 1B), essentially providing a two dimensional organic phase that separates two aqueous phases on either side of the membrane. These membranes allow larger cellular compartments to form, as illustrated in Figure 1A–C. Such compartments range from the cell itself to a variety of subcellular organelles such as the nucleus, mitochondria, and endomembrane systems

(Figure 1C). In multicellular organisms, a variety of differentiated cells form larger structures that allow organisms to carry out a variety of functions, many of which have their basis at the nanometer scale.

One striking example of such a nanostructure-function relationship on the organismal level is the iridescent cells (iridophores) present on the surfaces of cephalopods such as squids that allow the organism to change color rapidly in a highly patterned way[8]. These cells function by locally tuning the spacing of nanoscale stacked structures that function as Bragg reflectors. This dynamic photonic structure gives the organism the ability to communicate and camouflage itself within a larger ecosystem.

A promising approach to the synthesis of functional synthetic systems, i.e., one approach to synthetic biology, is to reconstitute purified biological molecules in a controlled environment. It has potential for novel technological applications as well as better understanding of and interfacing with biological systems. Applying this approach to purified or synthetic phospholipids has led to the development of liposomes or vesicles, which are lipid-based systems widely used for drug and gene delivery, as well as model systems for studying simplified cellular systems. A difference between synthetic liposomes and biological systems is that the biological systems are composed of much more diverse molecules interacting on nanoscopic scales in a variety of functional ways. Technologies capable of directing these interactions among multiple materials are therefore promising approaches to improved reconstitution of biological function.

Dip-pen nanolithography (DPN) is one such approach[9]. Use of the tip of an atomic-force microscope to position molecules locally on a surface permits fabrication of structures with ultrahigh resolution from a single material, and when multiple tips are used in parallel, multiple materials can be integrated with resolution below 100 nm. Using phospholipids as the ink for DPN therefore has the potential to create an unprecedented combination of multicomponent, synthetic, lipid nanostructures[2-6]. Because these structures are formed from biological lipids, they are inherently compatible with other biological molecules and able to serve as model systems.

2 RESULTS

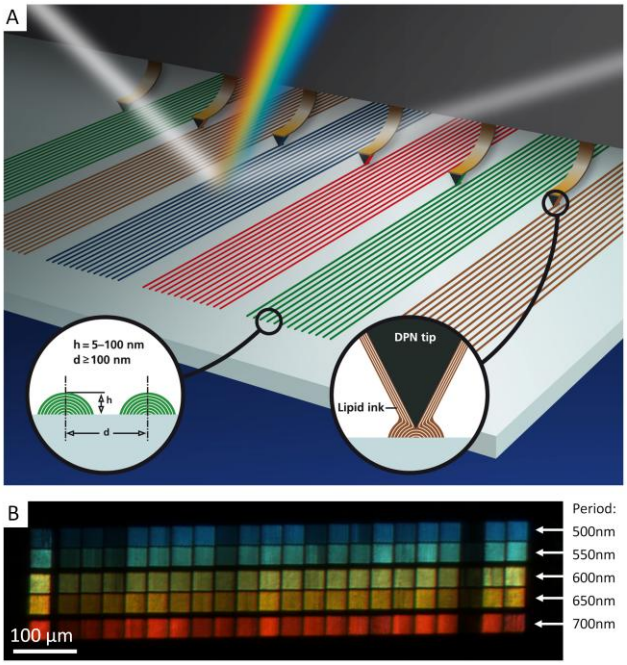

Figure 2: Optical diffraction gratings fabricated by parallel DPN. (A) Schematic illustration of the concept (graphic by Wilfrid Schroeder). Parallel arrays of DPN tips are used for direct printing of phospholipid multilayers of controllable multilayer thickness and line spacing, allowing optical diffraction to be observed. (B) Optical micrograph of light diffracted from gratings of different period from 500 nm to 700 nm. The color observed corresponds to the spacing of the grating lines, whereas the intensity of diffraction is determined by the quality and height of the gratings[6].

Figure 2A shows the concept of using the high resolution and multiplexing capabilities of DPN to fabricate multicomponent diffraction

gratings composed of phospholipids. When the diffraction gratings are illuminated with white light from an angle, the color of the light diffracted from the structures is described by the grating equation:

$$d(\sin\theta_m + \sin\theta_i) = m\lambda \quad (1)$$

where d is the period of the grating, θ_m and θ_i are the angles of diffraction maxima and incidence, respectively, *m* is the diffraction order, and λ is the wavelength of light. The intensity or efficiency of light diffracted from the gratings depends on the quality and especially the height of the grating. Figure 2B shows an example of gratings made from the phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC).

As each tip in a parallel array can be dipped in a different type of phospholipid by means of microfluidic inkwells[2] or inkjet printing[4], multiple phospholipids can be integrated onto the same surface. As this constructive printing process can also be carried out on a prestructured surface, it can also be combined with more traditional top-down microfabricated substrates[6].

Because the lipids are fluid, being essentially nanoscopic oil droplets on a surface[10], their shape is governed by the physics of adhesion of a liquid on a solid surface. The shape of the liquid droplet is therefore best described as a functiton of the interfacial energies of the interfaces involved, as well as the local interfacial geometry and kinetic effects. Monitoring diffraction from these liquid gratings provides a label-free method of observing these properties, as illustrated in Figure 3.

In air, the lipid fluidity depends on the humidity. At low humidity, the lipids are dehydrated and effectively frozen in a gel phase. When they are exposed to humidity above 40% however, they become fluid and, depending on the surface energy of the solid substrate, can begin to spread to form a molecularly thin layer[6, 11]. This spreading can be observed through use of fluorescently labeled lipids and, in the case of diffraction gratings, monitored in a label-free manner by a decrease in the diffraction intensity as the grating height decreases. This property gives the gratings the potential to function as an indicator for the presence of vapors.

Of particular relevance for model biological systems as well as for biosensing applications is the ability of gratings to function under water. Because the lipids are immiscible in water, yet biofunctional, they are uniquely capable of remaining both fluid and stable under water. In fact the insolubility of phospholipids in water has been recently demonstrated to allow lipid-DPN to be carried out in aqueous solution[3]. Although the lipids do tend to spread when immersed in water on the majority of substrates, immersing them while dehydrated, on a hydrophobic surface poly(methyl methacrylate) (PMMA), in the presence of the blocking agent bovine serum albumin (BSA), permits maintenance of stable multilayers under water.

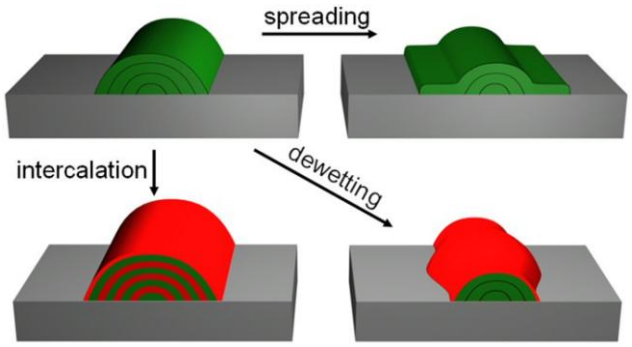

Figure 3: Dynamic shape and size changes of a single lipid multilayer grating element (graphic by Tobias Heiler). Depending on the interfacial energies, local geometry, and kinetic factors, the starting structure (top left) can (1) spread on the surface to form a molecularly thin film, (2) dewet from a line to form droplets, or (3) change size by intercalation of materials into the lipid multilayer[6].

By incorporating biofunctional lipids into the lipid multilayer gratings, one can then investigate how these materials respond to interactions with other biological molecules, as

both a model system and a candidate for a new type of biosensor element. This potential is demonstrated in Figure 4; biotinylated lipid gratings were used to detect the presence of streptavidin protein at concentrations down to 5 nM after 15 minutes of incubation.

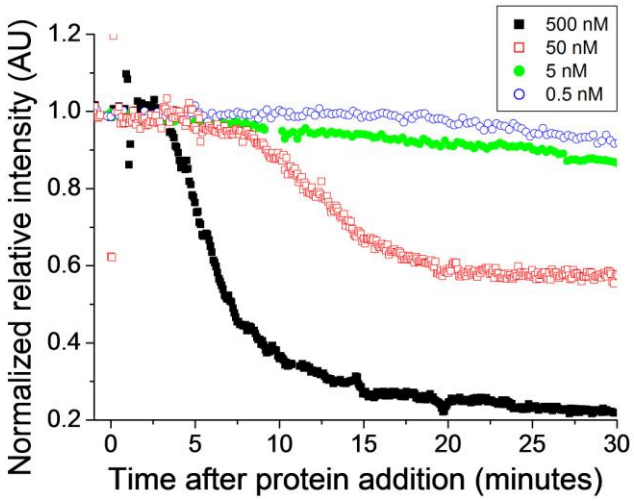

Figure 4: Detection of protein by means of a lipid multilayer grating at a concentration of 5 nM after 15 minutes. The intensity of light diffracted from the gratings rapidly decreases upon protein binding because the protein increases the surface tension of the lipid-water interface, causing it to dewet from the surface[6].

3 CONCLUSIONS

Using phospholipids as an ink for dip-pen nanolithography opens up the possibility of reconstituting biological lipid function on surfaces, with the added capability of controlling nano- to micrometer-scale structure as well as integration of multiple materials. In particular, two critical length scales become amenable:

1. The size of an individual cell, where compartmentalization occurs (~1–100 micrometers).

2. The wavelength of visible light (~380–750 nanometers).

Optical diffraction gratings can be fabricated in this way and, if biofunctional lipids are incorporated into the ink, become responsive to the presence of other biological molecules such as protein. Their response to the environment can be observed in a label-free manner if the optical diffraction is monitored, for example as a model system for studying liposome function, as well as providing a label-free biosensor element. The resulting material can be considered a biometamaterial as it is a naturally occurring biomaterial that has been structured in a way that gives it a unique function.

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