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Bilayer membrane interactions with nanofabricated scaffolds☆

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ABSTRACT

Membrane function is facilitated by lateral organization within the lipid bilayer, includingphase-separation of lipids into more ordered domains (lipid rafts) and anchoring of the membrane to a cytoskeleton. These features have proven difficult to reproduce in model membrane systems such as black lipid membranes, unilamellar vesicles and supported bilayers. However, advances in micro/nanofabrication have resulted in more realistic synthetic models of membrane-cytoskeleton interactions that can help uncover the design rules responsible for biological membrane formation and organization. This review will focus on describing micro-/nanostructured scaffolds that can emulate the connections of a cellular membrane to an underlying "cytoskeleton". Examples include molecular-based scaffolds anchored to a solid substrate through surface chemistry, solid-state supports modified by material deposition, lithography and etching, the creation of micro/nanoporous arrays, integration with microfluidics, and droplet-based bilayers at interfaces.Model systems such as theseare increasing our understanding of structure and organization in cell membranes, and how they result in the emergence of functionality at the nanoscale.

1. Introduction

Cells and their internal compartments are enveloped by membranes based on lipid bilayers. The bilayer serves as host to hundreds of embedded and associated proteins that impart diverse functions, including selective transport, signal transduction, cellular recognition and locomotion (Luna and Hitt, 1992). Most membrane lipids and proteins diffuse freely in the plane of the membrane, described classically as a fluid mosaic (Singer and Nicolson, 1972). The membrane is not just a simple permeability barrier, but a highly complex, dynamic and multifunctional structure. The spatial organization of lipids and proteins in biological membranes is regulated not only by specific molecular interactions with neighboring biomolecules, but also by the structural and mechanical properties of the membrane, such as bilayer thickness, intrinsic curvature, elastic moduli, and lipid composition (Lundbaek et al., 2010). Understanding the lateral structure of membranes and how it relates to function is critical to advancing biological science and technology. It has become increasingly apparent that nanoscale structure and organization in cell membranes play significant roles in cell signaling and membrane physiology (Cho and Stahelin, 2005; Smith et al., 2015; Best, 2014; Sheng et al., 2014; Rozovsky et al., 2012; Best et al., 2011).

Separation of lipids into distinct domains of greater order (lipid rafts) and anchoring to the cytoskeleton are two main mechanisms for organizing the membrane in living cells, features which have proven difficult to reproduce in traditional model membrane systems (e.g., unilamellar vesicles, black lipid membranes, and supported bilayers). Certain mixtures of synthetic lipids have resulted in easily observed, stable microscopic domains (Chen and Santore, 2013). Synthetic lipid mixtures that give rise to nanoscopic domains have also been discovered (Feigenson, 2009). However, these domains are not visible in optical microscopy and are believed to have a fleeting existence, making them difficult to study. The fact that the measured diffusion coefficients of many membrane proteins are far smaller than expected implicates interactions with either the cytoskeleton directly or through intervening proteins (Horton et al., 2010). Both lipid rafts and membrane–cytoskeletal interactions involve the formation of nanoscale structures. In many cases, these nanostructures are transient, further frustrating efforts to study them.

Advances in micro/nanofabrication, however, have resulted in the development of increasingly realistic synthetic models of membrane–cytoskeleton interactions, enabling the discovery of design principles governing biological membrane formation and

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organization, the creation of increasingly complex hybrid micro-nanostructures comprised of both biological and non-biological components, and the emergence of functionality at the nanoscale (Kam, 2009).

This review will describe how established model bilayer systems, such as liposomes, supported lipid bilayers, and suspended bilayers, can be configured to adopt features reminiscent of real membrane–cytoskeleton interactions. Similarly to a real cytoskeleton, a micro/nano-fabricated scaffold should be able to nucleate domains in the membrane and impart order at defined locations, while allowing free diffusion in the bulk of the bilayer. A realistic membrane model should thus have well-defined lateral structure and distributed attachment points to an underlying substrate, functionalized as needed to tailor the surface chemistry. Diffusion coefficients in such a model will decrease with increasing observation times, corresponding to free-space diffusion of molecules within domains at early times, followed by obstruction of diffusion by the barriers between domains at later times (Kam, 2009).

A number of comprehensive reviews have been published on various aspects of lipid bilayers, including their formation mechanisms, characterization techniques, their regulation of membrane proteins, and their integration in biosensors and other devices (Lundbaek et al., 2010; Feigenson, 2009; Kam, 2009; Zagnoni, 2012; Danelon et al., 2008; White, 1986; Chan and Boxer, 2007; Demarche et al., 2011; Czolkos et al., 2011; Mashaghi et al., 2013a; Kelly and Craighead, 2011; Castellana and Cremer, 2006). Owing to space limitations, this review will focus exclusively on micro/nanostructured scaffolds that emulate in some way the connections of a cellular membrane to an underlying "cytoskeleton" (Fig. 1). These structured scaffolds could be fabricated from solid-state inorganic materials like metals, (Reimhult et al., 2006; Li et al., 2008; Brzozowska et al., 2009) semiconductors (Choi et al., 2010) or oxides (Mager et al., 2008a; Lobovkina et al., 2010a), "soft" polymeric biomolecules (Wong et al., 1999; Tanaka and Sackmann, 2005; Sterling et al., 2015) such as PEG, collagen, or actin, or combinations of these. Recent work on micro/nanoporous scaffolds that can also be classified as model systems for understanding membrane–cytoskeleton interactions will be described.

2. Molecular-based "scaffolds"

Supported lipid bilayer membranes self-assemble on hydrophilic substrates in aqueous solution on a thin (1–2 nm thick) lubricating layer of water, which imparts lateral fluidity to the membrane and helps to stabilize the bilayer, but also impedes the incorporation of transmembrane proteins due to steric repulsion with the solid substrate (Fig. 2) (Sackmann, 1996). Supported bilayers can be modified however to include spacer molecules that can decouple the bilayer from the substrate, such as organosilanes on silica, alkylthiols on gold, tethered lipid monolayers, or cushioning polymers (PEG-lipids), hydrogels, or polyelectrolyte films (Demarche et al., 2011). Spacer molecules typically consist of a hydrophobic group that binds to the bilayer, a hydrophilic backbone, and a coupling moiety for tethering the spacer to the solid substrate.

The surface chemistry of molecular scaffolds can be precisely engineered to control the coupling of the bilayer to the surface, the spacer molecular length and shape, and the chemical functionality

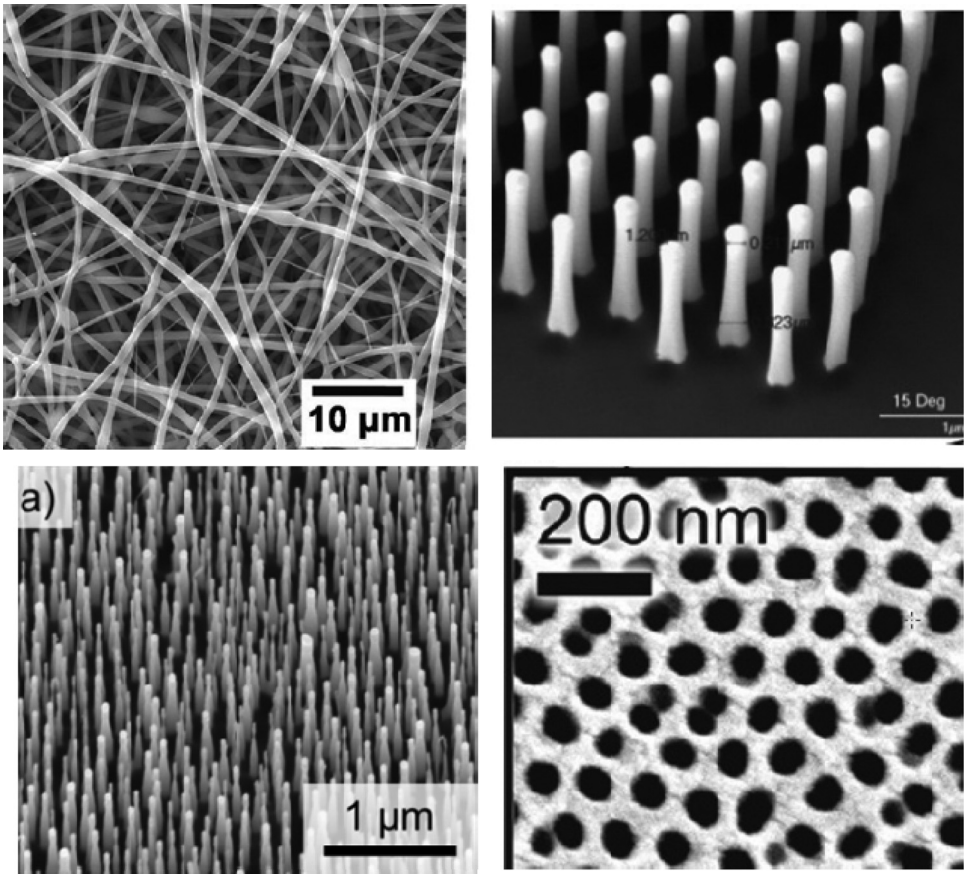

Fig. 1. Examples of nanostructured scaffolds that can mimic the cytoskeleton, including nanofibrous structures (Zha et al., 2011), deterministically patterned nanopillars with EBL (Choi et al., 2010) and stochastically patterned nanopillars (Boreyko et al., 2014) and nanopores (Lazzara et al., 2011).