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Review

Quantitative optical microscopy and micromanipulation studies on the lipid bilayer membranes of giant unilamellar vesicles

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ABSTRACT

This manuscript discusses basic methodological aspects of optical microscopy and micromanipulation methods to study membranes and reviews methods to generate giant unilamellar vesicles (GUVs). In particular, we focus on the use of fluorescence microscopy and micropipet manipulation techniques to study composition–structure–property materials relationships of free-standing lipid bilayer membranes. Because their size (~5–100 μm diameter) that is well above the resolution limit of regular light microscopes, GUVs are suitable membrane models for optical microscopy and micromanipulation experimentation. For instance, using different fluorescent reporters, fluorescence microscopy allows strategies to study membrane lateral structure/dynamics at the level of single vesicles of diverse compositions. The micropipet manipulation technique on the other hand, uses Hoffman modulation contrast microscopy and allows studies on the mechanical, thermal, molecular exchange and adhesive-interactive properties of compositionally different membranes under controlled environmental conditions. The goal of this review is to (i) provide a historical perspective for both techniques; (ii) present and discuss some of their most important contributions to our understanding of lipid bilayer membranes; and (iii) outline studies that would utilize both techniques simultaneously on the same vesicle thus bringing the ability to characterize structure and strain responses together with the direct application of well-defined stresses to a single membrane or observe the effects of adhesive spreading. Knowledge gained by these studies has informed several applications of lipid membranes including their use as lung surfactants and drug delivery systems for cancer.

Contents

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1. Historical perspective: light microscopy and the capability to perform single vesicle (and single cell) experiments

In the last decades, there has been extensive research to elucidate the different physical aspects of biologically relevant membrane systems (mainly liposomes but also cell membranes) using an array of experimental techniques, e.g. fluorescence spectroscopy, differential scanning calorimetry, infrared spectroscopy, EPR, NMR to mention a few (Almeida et al., 1992; Arnold et al., 1981; Bagatolli et al., 1997; Blume et al., 1982; Bultmann et al., 1991; Caffrey and Hing, 1987; Chapman, 1968; Lee, 1975; Lentz et al., 1976a; Mabrey and Sturtevant, 1976; Maggio et al., 1986; Parasassi et al., 1993; Schram et al., 1996; Shimshick and McConnell, 1973; van Dijck et al., 1977; Vaz et al., 1989, 1990). While these techniques produce relevant mean parameters for lipid membranes on the basis of data collected from bulk solution of many (small) liposomes, they lack mechanical and spatially resolved information at the level of *single* vesicles. With the ability to prepare giant unilamellar vesicles (GUVs, see Section 2), a series of microscopic studies over the past 30 years have focused on the characterization of membranes as single vesicle materials. These experiments have largely been directed at the materials behavior of lipid bilayers where their complete characterization requires detailed understanding of the relationships between membrane composition, structure, and properties. Thus, to complement the mean parameters obtained from bulk suspension techniques, structural studies on single vesicle – mainly from fluorescence microscope investigations of phase behavior, have correlated lipid compositions with in-plane bilayer structures and phase separation (Bagatolli and Gratton, 1999, 2000a, b; Bagatolli et al., 2000a; Dietrich et al., 2001; Feigenson and Buboltz, 2001; Korlach et al., 1999). Independently, micromechanical experiments utilizing direct application of well-defined stresses to single GUVs and micro-bubble monolayers have linked similar lipid composition including cholesterol content to elastic expansion and failure (Evans and Kwok, 1982; Kwok and Evans, 1981; Needham et al., 1988; Needham and Nunn, 1990), yield shear and shear viscosity (Kim et al., 2003), molecular exchange with lysolipids (Needham et al., 1997), main acyl as well as solid–solid phase transitions (Needham and Evans, 1988; Needham et al., 1988), and adhesive and repulsive interactions involving colloidal long range forces (Evans and Needham, 1987, 1988) as well as receptor-mediated adhesion (Kim et al., 2000; Noppl-Simson and Needham, 1996). However, despite their individual contributions, the two techniques have not so far been exploited in one system; except for few exceptions (see Section 5). The goal of this review is three fold: to provide a historical perspective for both techniques; to present and discuss some of their most important contributions to our understanding of the biophysics, material science and materials engineering of lipid bilayer membranes; and to outline studies that would utilize both techniques simultaneously on the same vesicle thus bringing the ability to characterize structure and strain responses together with the direct application of well-defined stresses to a single membrane or observe the effects of adhesive spreading.

1.1. Micropipet manipulation of cells and giant unilamellar vesicles

Micromechanical experiments on GUVs were preceded by experimental developments in micropipet manipulation, measurements, analyses and modeling of biological cells, principally the red and white blood cells. Motivated by their new conception of the mechanism of cell division – "the expanding membrane theory", what seem to be the first attempts to use micropipet manipulation to interrogate the properties of cells and their membranes was in a series of three papers by Mitchison and Swann in 1954 (Mitchison and Swann, 1954a, b, c). They developed a method of measuring the properties of the cell membrane with an instrument they called the 'cell elastimeter' (Mitchison and Swann, 1954a). As shown in Fig. 1, and described in their paper, "*It consists of a glass micropipette filled with water and connected by rubber tubing to a small movable reservoir of water. Using a microscope and a micromanipulator the pipette is brought up to a cell (eggs of various sea urchins in the present experiments) and the reservoir is then lowered slightly with a micrometer screw. This creates a small suction, which draws the egg on to the end of the pipette, where it makes a seal, with the surface of the egg bulging slightly up the tube. If the reservoir is then lowered again, the cell surface bulges progressively further up the pipette. The deformation or degree of bulging is measured directly under the microscope; the negative hydrostatic pressure or suction is measured on the micrometer screw*". The second paper (Mitchison and Swann, 1954b) described the application of this method to the unfertilized sea-urchin egg, and showed that the cell membrane behaved as a relatively rigid structure of appreciable thickness. In the third paper (Mitchison and Swann, 1954c) they described measurements of the stiffness