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Review

Lipid bilayer technologies in ion channel recordings and their potential in drug screening assay

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ABSTRACT

Ion channels are expressed in every tissue and are important for many physiological processes. Malfunction of ion channels can lead to diseases in many tissues. All these make ion channels ideal targets for drug development. Many techniques are employed to study ion channel functions including the lipid bilayer technique. Lipid bilayer membranes are being synthesized in vitro to mimic natural cell membranes. Ion channels are then incorporated into the bilayer membrane for ion channels activity recordings. The use of Lab-on-a-Chip (LOC) technologies to form lipid bilayer has made lipid bilayer technique efficient for ion channel recordings and has brought great potential for this technique to be scaled up to a high throughput platform for ion channel drug screening. In this review we discuss the use of lipid bilayer for ion channel studies, conventional methods and current LOC technologies on lipid bilayer formations, comparison of lipid bilayer high throughput platform to automated patch clamp system, and finally the potential of lipid bilayer platforms in drug screening assay.

Contents

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1. Introduction

Ion channels are fundamental in many physiological processes, for instance action potential generation, heart and skeletal muscle contractions, transepithelial transports, cell volume regulations, etc. Ion channels are pore structured proteins that allow traverse of specific ions across the lipid bilayer membrane. Ion channels are classified as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chloride (Cl^-), and unspecific cation channels based on the types of ions traversable. The selective property of ion channels, together with active pumps and co-transporters generate electrochemical gradient across lipid bilayer membranes. Ion channels undergo cycles of opening and closing, or 'gating', to permit ions movement across the channel pore. Regulation of channel gating can be classified as voltage gated, ligand gated, and mechanosensitive gated.

Ion channels are ubiquitously expressed in all biological cell membranes. Malfunction of ion channels can lead to diseases in many tissues such as heart, bone, muscle, kidney and neuron [1,2]. These make ion channels ideal as targets for drug development. About 13% of all marketed drugs target ion channels, and that accounts for $375 million [3]. However, the small numbers of drugs that targets ion channels [4] in contrast to the big amount of money being invested in this market indicates that there is room for improvements in this area.

Several techniques have been employed to study ion channel properties including the lipid bilayer technique. The study of lipid bilayer or artificial cell membrane began in the 1880s (for review see [5,6]). The first artificial lipid bilayer membrane was demonstrated by Mueller et al. in 1962 [7]. The lipid bilayer was formed by 'painting' techniques, where lipids in organic solvent were painted over an μm wide hydrophobic aperture, resulting in a 'free standing' bilayer. Under reflected light, lipid bilayer appears black and therefore being called Black Lipid Membrane or BLM. In 1969, Bean et al., observed fluctuated conductance of lipid bilayer membrane when bacterial toxin was introduced into the membrane [8]. The discrete in ionic currents were demonstrated later by Ehrenstein et al. [9], and Hladky and Haydon [10] in 1970. To date, it is well known that the fluctuated membrane conductance and ionic current are the result of ion channels or pore forming proteins situated on the lipid bilayer membrane. Based on this concept, lipid bilayer has been extensively used as a platform to study many ion channels [11–19] and pore forming proteins properties [20]. Secondary to ion channel function study, the ability to insert ion channels into lipid bilayer has led to the development of a new biosensor concept, the nanopore sensor, which utilizes the specificity of ion channel function and the change in ionic currents to identify the present/activity of interest biomolecules. Excellent reviews on nanopore sensor have been discussed elsewhere [21–26] and will not be discussed here. In this review we will focus solely on the use of lipid bilayer for ion channel function studies.

Microfabrication techniques are capable of scaling down complicated laboratory procedures into small chips typically referred to as Lab-on-a-Chip, or 'LOC' technologies [27]. LOC technologies have emerged as an important aspect in electrophysiology field and in drug discovery investigation [27]. The growing trend in applying LOC technologies in lipid bilayer platforms offers alternative methods for ion channel drug screening assay. These technologies have the potential to entirely change the field of ion channels drug screening in terms of cost and throughput.

In this review, we will discuss the use of lipid bilayer for ion channel study, the conventional technique for lipid bilayer formation, the existing LOC lipid bilayer technologies for lipid bilayer formation and for electrophysiology study, and finally the potential of lipid bilayer in ion channel drug screening assay.

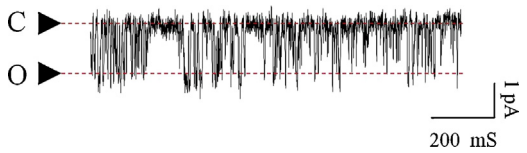

Fig. 1. Example of single channel recording obtained from HEK cells overexpressing small conductance potassium (SK4) channels. Inside out patch clamp configuration was performed under equimolar K^+ concentration on the intra- and extracellular side (140 mM) with 1 μM Ca^{2+} on the intracellular side. The cell membrane patch was clamped at −40 mV. C and O indicate close and open state of the channel respectively.

2. The use of lipid bilayer for ion channel function studies

For the study of ion channel functions, patch clamp is considered the gold-standard technique as it is able to monitor the actions of ion channels in real time, with very high sensitivity and temporal resolution typically in the order of microseconds and sub-picoamperes. Data obtained from patch clamp can be classified as whole cell recordings and single channel recordings. Data obtained from single channel recordings have aided massively in understanding ion channel functions and their role in cell physiology. Single channel recordings in patch clamp are obtained from cell membrane patch. Application of voltage clamp across the membrane causes fluctuations of current which represents the opening and closing state (or gating) of ion channels situated on the cell membrane (Fig. 1). Single channel recordings can also be obtained from a lipid bilayer membrane with ion channels incorporated. The lipid bilayer membrane resembles the cell membrane patch at the tip of the glass capillary in patch clamp. Similar to the patch clamp technique, single channel recordings are achieved by applying voltage clamp across the bilayer membrane in order to monitor changes in ion channel gating.

Lipid bilayer has been used as a model to study the properties of many ion channels for instance acetylcholine receptor [11], sodium channels [12], big conductance potassium channels (BK or Slo1) [19], ryanodine receptors (RyR) [14,16,18], mitochondrial-ATP sensitive potassium channels (mitoK_{ATP}) [17], and purinergic receptors ($P2X_7$) [28]. In many cases lipid bilayer is preferred to patch clamp for example in the study of the lethal ΔF508 Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutation. Normal patch clamping of ΔF508 CFTR is not applicable as this mutated protein fails to traffic to the apical membrane and retains in the endoplasmic reticulum (ER) [29]. Therefore, to study them, the purified ΔF508 CFTR channels are being reconstituted onto the lipid bilayer membranes for single channel recordings [30–32]. Moreover, lipid bilayer is being used intensively for the study of intracellular ca^{2+} regulations [16].

3. Conventional techniques for lipid bilayer formation and incorporation of ion channel proteins into lipid bilayer

Lipid bilayer is a well known technique used to study ion channel functions. In conventional technique, lipid bilayer can be formed by three techniques: the 'painting' [7], 'folded' [33], and 'vesicle rupture' techniques. In the painting technique, an experimental chamber is divided into two partitions where both partitions are connected via a small aperture (50–200 μm in diameter) located on a thin hydrophobic septa (in general made of Teflon) (Fig. 2a). After both compartments are filled with aqueous solutions, a lipids/solvent mixture is painted on both sides of the aperture in the two compartments using thin red-sable brush or glass rod to form a monolayer of lipid on each side. The two lipid monolayers are then pulled naturally by van der Waals forces to a close proximity, in a process known as "thining" [7], forming a lipid bilayer. Unlike natural lipid bilayer, the bilayer formed by