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Authors: Dominik Drabik, Joanna Doskocz, Magda Przybyło

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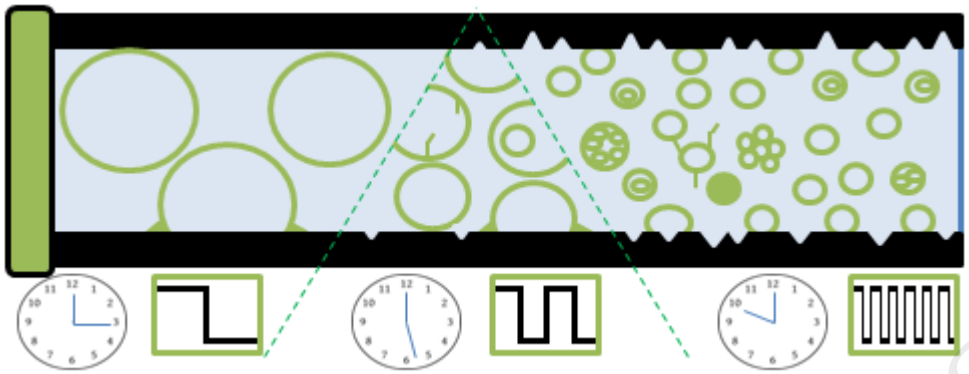
Effects of electroformation protocol parameters on quality of homogeneous GUV populations

Dominik Drabik,[a] Joanna Doskocz,[a] and Magda Przybyło[a,b]

[a]Department of Biomedical Engineering, Faculty of Fundamental Technical Problems, Wrocław University of Science and Technology, 50-377 Wrocław, Pl. Grunwaldzki 13, Poland.
[b]Lipid Systems sp z o.o., 54-613 Wrocław, ul Krzemieniecka 48,C, Poland

Graphical abstract



Highlights

- Electoformation protocol used to form GUVs is extensively varied.
- Assessment of GUV population is done by characterizing vesicles' diameters, structure and amount of lipid.
- Prolonged duration of electoformation did not influence the quality of GUVs populations.
- Frequency and voltage of applied field did influence the quality of GUVs populations.
- State and defects in electrodes did influence the quality of GUVs populations.

ABSTRACT: Giant unilamellar vesicles (GUVs) have become one of extensively studied biological bilayer models especially when investigating topological and mechanical properties of cell membranes. They are also used to visualize membrane-related phenomena. However, the method of preparation and the effects of parameters of preparation on the vesicular structure are extensively varied. Therefore, it is important to understand how the process of formation of GUVs influences the outcome population, as it can influence the outcome of the experiment that is planned. Therefore, in this study, we investigated the effects of protocol parameters of electroformation on properties of homogeneous population of POPC GUVs. The parameters investigated in this study are duration of electroformation, usage of electrodes and frequency of applied AC field and its voltage. The properties investigated, which can be used to describe GUV populations are average diameter of vesicle, the amount of lipid molecules in population, and structure of vesicles. According to our results, prolonged time (greater than 4 h) does not influence outcome; however, parameters of applied electrical field (voltage and frequency) did significantly influence the properties of obtained POPC GUV populations.

KEYWORDS : GUV; electroformation; cytometry; quenching; lipid vesicles.

1. Introduction

Giant unilamellar vesicles (GUVs), lipid vesicles with diameter ranging from 5 to 50 µm, are the extensively studied membrane-related model system. Their size is similar to eukaryotic cells, which allows visualization of topological and mechanical properties of cell membranes by using light and fluorescence microscopy [1]. Thus, they serve as a model system to mimic a freestanding lipid bilayer. Furthermore, due to their size, the curvature of lipid bilayer on molecular level is negligible [2, 3]. GUVs have been widely used to study different aspects of lipid membranes such as fundamental membrane thermodynamics [4], membrane curvature [5], membrane domains [6], fusion [7], lateral structure [8], and bending rigidity [9]. One of the primary advantages of using GUVs as model systems is the ability to precisely control molecular composition of the membranes as well as their environmental conditions. Furthermore, they allow to obtain parameters for each individual vesicles instead of averaged values obtained from large number of liposomes, as in case of large and small unilamellar vesicles.

There are several techniques used for the preparation of GUVs; however, the most common one, due to its convenience, is electroformation [10]. In this method, lipid molecules dissolved in organic solvents, such as chloroform or methanol, were deposited on electrodes' surface. This is followed by drying of electrodes to obtain dry lipid film, which is then immersed in aqueous buffer and then, AC field is applied. As the principle behind the formation of GUVs is not well understood, several different protocols exist. Furthermore, to the best of our knowledge, there are no studies focusing on finding the most optimal protocol for GUV electroformation. The first and