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Low-cost equipment for electroformation of Giant Unilamellar Vesicles

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ABSTRACT

Giant Unilamellar Vesicles (GUVs) constitute a very useful model for biomembranes. They are usually generated by electroformation using sinusoidal potential waves applied between two platinum electrodes or electrodes composed of glasses covered by indium tin oxide (ITO).

Here we present the design of a programmable, compact and low cost wave generator that allows applying different waveforms, even with initial non-periodical shapes. Thus, it allows to optimize the potential waveform for the particular GUV' composition.

We also describe the design of a low cost, reusable chamber with stainless steel electrodes that allows using very small volumes of solution (0.3 mL). With this chamber, a larger number of GUVs were generated in less electroformation times compared to ITO electrodes. The general characteristics of the obtained GUVs are comparable to those electroformed in platinum or ITO surfaces.

The chamber, combined with the wave generator comprises a simple, reusable, programmable and low cost system for the formation of GUVs.

Specifications table

Hardware name	GUVs generator
Subject area	• Chemistry and Biochemistry • Medical (e.g. Pharmaceutical Science) • Biological Sciences (e.g. Microbiology and Biochemistry) • Educational Tools and Open Source Alternatives to Existing Infrastructure
Hardware type	• Biological sample handling and preparation
Cost of Hardware	75 dollars

1. Hardware in context

Giant Unilamellar Vesicles (GUVs) are micrometer-sized lipid vesicles (liposomes) widely used in membrane biophysics studies. Their main advantage over other model membrane systems is that, having sizes comparable to that of cells, they can be observed with optical microscopy.

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GUVs can be generated by lipid emulsification, swelling, microfluidic-based methods or electroformation [1,2]. This last technique was pioneered by Angelova and Dimitrov in 1986 [3], it involves modulating the spontaneous swelling of lipids within an aqueous solution using an externally applied electric field. Electroformation is the most used method for producing GUVs, as it enables modulation of the lipid hydration process to form relatively monodisperse, defect-free vesicles [4]. For example, from a set of 274 papers randomly selected that deals with topics related to membrane biophysics, and that name "GUV", 131 (∼50%) also name "electroformation".

For electroformation, lipids are dissolved in an organic solvent, and deposited on two electrodes usually composed of indium tin oxide (ITO)-coated glasses or platinum. For example, from the 131 papers in which the words "GUV" and "electroformation" appear, 54 (∼40%) also named "ITO" whilst 25 (∼20%) also named "Platinum".

Lipids self-assemble into a stack of bilayers on a substrate when the organic solvent is removed by evaporation [2]. Following solvent evaporation, the electrodes are placed in contact with an aqueous solution. On hydration, the bilayer stacks separate out very slowly. If the bilayer edges are allowed to merge at a faster rate, multi-lamellar vesicles (MLVs) are formed. Increasing the rate of bilayer separation by application of electric fields, one can obtain uni-lameller vesicles [2]. Therefore, an alternating potential difference is applied across the electrodes, stimulating the swelling process of the hydrated lipid layer. For GUV's electroformation, a wave generator is thus required. The optimal potential field for a high yield of large vesicles has been an active research field, and several cell geometries, wave amplitudes, frequencies and modulations have been tested. For instance, Bi et al studied GUV generation using interdigitated ITO electrodes and different lipid mixtures [5]. They found that smaller widths generated bigger GUVs under the same conditions, and that a solution height above 600 mm has no influence on the GUV formation. Furthermore, the diameters of the GUVs decreased with increasing frequency at a constant amplitude, and increased with increasing amplitude.

Li et al. used two plasma cleaned ITO electrodes in a face-to-face layout in order to successfully generate GUVs from zwitterionic lipids as well as charged lipids in high concentration saline solutions [6]. Drabik et al. used platinum electrodes in a polytetrafluoroethylene (PTFE) cell and analyzed the effect of duration of electroformation, usage of electrodes and frequency of applied AC field and its voltage on the properties of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine GUVs. According to their results, long times does not influence outcome whilst voltage and frequency did significantly influence GUV's properties [7].

All these studies indicated that the optimal waveform depends on the lipid composition and solution conditions. Thus, in order to use different vesicle compositions and conditions, a wave generator with modifiable wave characteristics is necessary.

For GUV formation, a fluid lipid membrane is required, meaning that the temperature has to be higher than the membrane melting temperature. Therefore, depending on the lipid composition and experimental conditions, electroformation has to be performed in a stove at temperatures in the range 30–60 °C. Thus, a compact wave generator is preferred.

Regarding the electrode materials, platinum and ITO-coated glasses are expensive. Furthermore, ITO-coated glasses easily break. ITO-coverages prepared in the laboratory requires the sputtering equipment and are instable (the surface coverage eventually dissolve with consecutive cleanings). Additionally, the conductivity of the surface is not always optimal and is not reproducible. Therefore, alternatives to platinum and ITO-coated glasses have been reported. Cao et al. proposed the usage of glyoxylic acid modified aluminum electrodes for GUV generation. Using eggPC they obtained high yield of vesicles with diameters >0.1 mm, which were not produced on platinum and indium tin oxide at the same experimental conditions [8].

Carbon fiber electrodes have also been tested, and the authors found that fast GUV formation can be achieved due to the small size of the (and thus, fast diffusion toward) electrode surface [9]. On this electrode material, hydration of the lipid layer due to the application of a negative potential has previously been reported [10].

The usage of stainless steel as electrode material has also been reported. GUVs of 1-palmitoyl-2-oleoyl-sn-glycero-3-phos phocholine with similar properties to those formed with platinum were successfully generated [4]. Stainless steel is reusable, unlike ITO-coated glasses, that eventually loses de covering or aging occurs during their repeated use, which is reflected in their surface topography on the nanoscale [11]. Compared to platinum, stainless steel electrodes have the advantage of being rigid, reducing the risk of bending and improving control over the electrode-separation distance, thus leading to a uniform electric field. Furthermore, stainless steel is cheaper than platinum and ITO-coated glasses.

When ITO-coated glasses are used, the chamber is usually prepared between the two glasses in each experiment, thus the volume is not always the same and furthermore, sometimes the lipid suspension leaks from the chamber.

Regarding the chamber material, the use of 3-D printed laboratory tools has boosted in the last years, and become an important source of accessible tools [12]. Here we propose using a 3D printed chamber with stainless steel electrodes with the main advantage of low manufacturing costs and fast production. Different cell geometries can be tested with new chambers, which are easily and economically prepared with a 3D printer.

This chamber, combined with the home-made wave generator described here have the following advantages:

- Compact and programmable wave generator
- Reusable chamber
- Low cost and easily set up