A Versatile DIY 3D Printed Device for the Preparation of Gian Lipid Vesicles as Model Membranes by Electroformation: Design and Fabrication

Claudia Valero-Luna[1], Athziri Herrera-Saucedo[1], Cristina Flores-Cadengo[3], Pablo Luis Hernandez-Adame[4], Francisco Bañuelos-Ruedas[1], Lazaro Canizalez-Davalos[2], Leo Alvarado-Perea[1,2], Alfonso Talavera-Lopez[2], and Alan Bañuelos-Frías[1]*

1 Universidad Autónoma de Zacatecas, Unidad Académica de Ingeniería Eléctrica, Campus UAZ Siglo XXI, Carr. Guadalajara Km. 6, Ejido La Escondida, Zacatecas, Zacatecas, 98160 MEXICO.
2. Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Químicas, Campus UAZ Siglo XXI, Carr. Guadalajara Km. 6, Ejido La Escondida, Zacatecas, Zacatecas, 98160 MEXICO.
3 BC Cancer Research Centre, Department of Molecular Oncology. Vancouver, BC V5Z1L3, CANADA.
4 Laboratorio de Física de Membranas Biológicas, Instituto de Ciencias Físicas (ICF), Universidad Nacional Autónoma de México (UNAM). Avenida Universidad S/N, Chamilpa, Cuernavaca, Morelos, 62210 MEXICO.
**Corresponding author: abanuelos@uaz.edu.mx.*

Recibido 01 de Abril de 2024, Aceptado 28 de Junio de 2024

Abstract
A simple way to study cell membrane properties and their interaction with other molecules is by using model systems with the same basic structure. These use a few basic components that simulate the conditions of the plasma membrane and have been used extensively to study biological membrane properties. Including these model systems, we can find Langmuir monolayers, supported bilayers, and lipid vesicles. Lipid vesicles are particularly interesting because their spherical structure mimics cell membranes. Among the different procedures for preparing GUVs, electroformation stands out because the vesicles produced by this method are mostly larger than 100 nm and unilamellar, making them easily visible through conventional optical microscopy. Although there are commercially available devices, custom handmade setups are usually fabricated. This work describes the design and fabrication of a simple and versatile 3D printed devise to prepare giant lipid vesicles using the electroformation method. The device has been designed to be fixed onto the microscope stage.

Keywords: 3D Printing, Giant Vesicles, Phospholipids, CAD-CAM, Model Membranes.

Resumen
Una forma sencilla de estudiar las propiedades de la membrana celular y su interacción con otras moléculas es mediante el uso de sistemas modelo con la misma estructura básica. Estos utilizan unos pocos componentes básicos que simulan las condiciones de la membrana plasmática y se han utilizado ampliamente para estudiar las propiedades de las membranas biológicas. Entre estos sistemas modelo, podemos encontrar monocapas de Langmuir, bicapas soportadas y vesículas lipídicas. Las vesículas lipídicas son particularmente interesantes debido a que su estructura esférica imita a las membranas celulares. Entre los diferentes procedimientos para preparar GUV's (vesículas unilamelares gigantes), la electroformación destaca porque las vesículas producidas por este método son en su mayoría más grandes que 100 nm y unilamelares, lo que las hace fácilmente visibles mediante microscopía óptica convencional. Aunque existen dispositivos comerciales disponibles, generalmente se fabrican equipos personalizados de manera artesanal. Este trabajo describe el diseño y la fabricación de un dispositivo sencillo y versátil impreso en 3D para preparar vesículas lipídicas gigantes utilizando el método de electroformación. El dispositivo ha sido diseñado para fijarse en la platina del microscopio.

1. Introduction

Cell membranes are highly complex auto-organized structures that contain hundreds of components, among cholesterol, proteins, and a wide variety of lipids [1]. A preferred strategy to study cell membrane mechanical properties and the interaction between cells and molecules is by using model systems composed of the same basic structure [2,3] Therefore, model systems conformed by a few components have been used extensively to study biological membrane properties [4–9].

One of these model systems is Langmuir monolayers, which are used as 2D models to study the interactions present in biological membranes. Langmuir monolayers produce pressure-area isotherms (π-A), allowing us to

perform a physicochemical and mechanical characterization of the monolayers at the air/water interface in a simple way under controlled temperature and pH [6,10]. Furthermore, by using additional characterization techniques such as X-ray and neutron scattering, fluorescence microscopy, Brewster's angle microscopy (BAM), and atomic force microscopy (AFM), the morphologies and textures present in the monolayers can be analyzed [10,11].

On the other hand, GUVs have been extensively used to study cell membrane properties and their interactions with other molecules as they have the same basic structure composed of a single phospholipid bilayer [12]. Different procedures have been used to prepare giant lipid vesicles, among gentle hydration of lipid films, evaporation [13], and electroformation. The electroformation technique for GUV generation was introduced by Angelova y Dimitrov in 1988 [14]; it consists of the hydration of a lipid film in a sinusoidal electric field, obtaining a homogeneous population of vesicles between 30 and 60 µm in diameter. The main advantage of this method is that the vesicles produced are mostly giant (> 100 nm) and unilamellar, which makes them easily visible through a conventional optical microscope [12]. To produce giant vesicles via electroformation, some commercial devices with prices of thousands of dollars are available. Nevertheless, custom handmade setups are usually fabricated, depending on the available resources in the laboratory. Platinum wires inside plastic cuvettes or Indium Tin Oxide (ITO)-coated glass slides connected to a sinusoidal voltage source can be used to promote the electroswelling process [12,14–17].

Additive manufacturing or 3D printing technology has become a valuable and successful tool for many scientific areas [18,19]. It provides a short prototyping time and the capability to create and even test the models before fabrication [20]. By helping to maximize the manufacturing process and reduce the manufacturing cost, 3D Printing has allowed researchers to find new alternatives for the design and manufacture of devices that meet specific requirements for the area of application. This work presents the design and fabrication of a low-cost 3D printed GUV electroformation device that can be fixed to a standard optical microscope platform for direct observation and analysis of the vesicle preparation procedure. The fabricated apparatus can be used for educational or research purposes.

2. Experimental

2.1 Electroformation chamber design and fabrication

The sample holder and base of the electroformation device were designed using Blender open-source design software [21] The selected model was then processed with Ultimaker Cura software [22] and finally fabricated using a ROBO R2 FDM 3D printer with PLA filament with a printing resolution of 20 a 300 mm at approximately 250 mm/s. After the fabrication of the prototype, the fitment on the microscope platform (Velab VE-146YT) was checked to ensure its correct fixation.

2.2 Giant vesicle production

First, two 2.5 cm x 2.5 cm ITO-coated glass slides were sonicated and cleaned with 70 % Ethanol. The conductive sides were identified using a multimeter. 10 ml of a PC:14-DMPC at 1 mg/ml (Avanti Polar Lipids, USA) were carefully spread on the conductive surface of one of the ITO Glass slides (Sigma-Aldrich, Alabaster, USA) using a Hamilton microsyringe, as can be seen in Fig. 1. The sample was then incubated under vacuum for 10 min at room temperature to allow solvent evaporation. Next, 900 µl of deionized water was deposited to fill the chamber. The chamber was then sealed using another ITO glass slide with the conductive surface facing the water. The sample holder was then mounted onto the base fixed on the microscope stage. Then, the holder was connected to a signal generator for two hours with an amplitude of 2V peak to peak and 1Hz of frequency using a BNC connector. At the same time, brightfield images and videos were acquired to analyze the electroformation procedure with an AMSCOPE USB MU-310-BK-Cl microscope camera. Finally, the resulting liposome solution was extracted and stored.

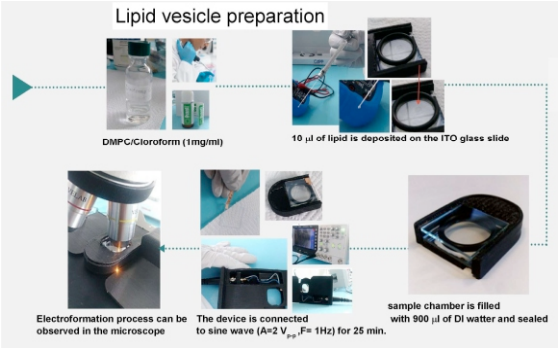

Figure 1. Preparation procedure of Giant vesicles preparation using the designed and fabricated device.

3. Results and discussion

3.1 Device design and fabrication

The designed electroformation device comprises a base and a sample holder, as is shown in Fig. 2 and Fig. S1: CAD model. The base can be fixed to the microscope stage using two bolts, allowing it to move the device freely and focus the microscope into the chamber to observe the electroformation process. The sample holder that forms the electroformation chamber can be attached to the base once the lipid has been deposited and filled with deionized water. The ITO glass slides are then fixed and connected by contact to a copper conductive tape, which in turn is connected by wires to a female BNC connector.

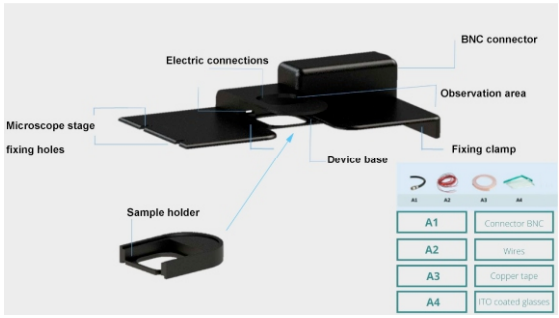

Figure 2. 3D printed GUV electroformation device components. The electroformation chamber is formed.

3.2 Liposome electroformation

The giant liposome preparation was successfully carried out using the designed electroformation chamber. The procedure was observed and analyzed using an optical microscope, as is shown in Fig. 3C, D, and F, where translucent bubble-like structures adhered to the lipid film on the upper inner surface of the chamber are visible. As the sinusoidal electric field polarizes the lipid molecules, fragments of the lipid film detach into the water. Through a self-assembly process, the fragments close on themselves to form thermodynamically favorable structures, in this case, spheres [12,14,23,24]. As a result, the prepared samples produced liposomes suitable for being used as membrane models in further analytical techniques, such as measuring its mechanical properties with electrodeformation, optical tweezers, Etc. [24,25]. In addition, by adding micro-particles, proteins, or molecules of interest to the lipid mixture, its interaction within the lipid bilayer can be studied [16,17,26–28]. On the other hand, by the use of different lipid mixtures, the lipid raft formation phenomena have been extensively studied [8,15,29,30].

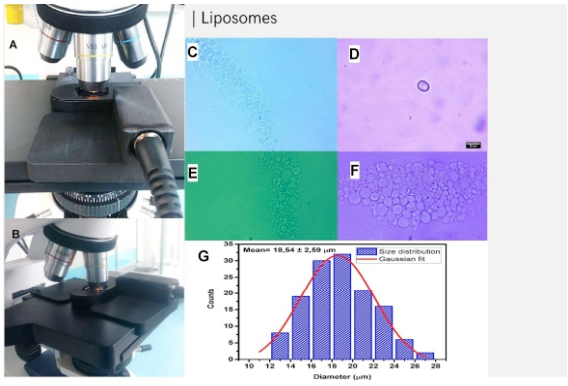

Figure 3. A and B custom-made 3D printed device mounted on the microscope stage. C, E, and F vesicle formation on the edge of the lipid film. D, free-floating vesicle after lipid film detachment under sinusoidal waveform. G, liposome sample size distribution analysis. Scale bar 20 µm

The study of cell membrane phenomena and the analysis of membrane interaction with external molecules or particles is of extensive use nowadays. Moreover, by the use of model membranes such as the giant unilamellar vesicles, these kinds of studies can be done without the implications of having highly specialized cell culture equipment in almost any basic science laboratory. Despite high-end commercially available devices for the preparation of giant vesicles, those are relatively expensive (around USD 4,000) and are not accessible to not specialized laboratories. Therefore, it is convenient to look for alternatives such as the one presented in this work because just is necessary to have inexpensive equipment, such as a signal generator and voltmeter, a regular brightfield microscope with a camera, and a pair of ITO glass slides for the preparation and observation of the GUVs. Therefore, the designed and fabricated device is simple but functional and can be used to prepare giant liposomes for a diversity of further analyses.

On the other hand, 3D Printing, as part of the CAD and CAM technology, is here to stay in scientific research. It can be employed to manufacture inexpensive devices with specific characteristics for the intended applications, not limited to laboratory but everyday life needs. In addition, with the use of other 3D printing techniques, such as high-resolution resin digital light processing (DLP), the device presented in this work can be re-engineered to be smaller and with the integration of microfluidic channels and adaptable for different microscope brands, making it more useful and versatile.

4. Conclusions

In this work, we successfully designed, fabricated, and tested a device to prepare giant lipid vesicles by electroformation. The produced vesicles can be used as model membrane systems given their size of 18.54 ± 2.59 µm (analyzed sample N=135), which allows them to be observed and analyzed using a conventional optical microscope. The designed device was fabricated by Fused deposition modeling 3D Printing with a PLA filament and other low-cost elements, making it cheap and accessible for any science laboratory with access to an FDM 3D printer. Finally, by using 3D Printing technology, we can design and fabricate devices for specific applications straightforwardly and inexpensively.

Author Contributions

Conceptualization, A.B.F.; methodology, A.B.F., and A.H.S.; software, A.H.S.; validation, C.F.C, and P.L.H.A.; formal analysis, C.F.C, and C.V.L.; investigation, A.H.S., C.V.L; resources, L.A.P, A.T.L.; data curation, P.L.H.A; writing—original draft preparation, A.B.F., C.F.C, and A.H.S.; writing—review and editing, A.B.F., C.F.C, F.B.R., P.L.H.A., L.A.P, L.C.D., A.T.L., and A.H.S.; visualization, C.V.L.; supervision, F.B.R.; project administration, A.B.F, and C.V.L.; funding acquisition, A.B.F. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by PRODEP SEP, grant number UAZ-PTC 215.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

CAD models are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to acknowledge the financial support of Consejo Zacatecano de Ciencia y Tecnología (COZCyT) to assist the International Material Research Congress (IMRC). PRODEP SEP, grant number UAZ-PTC-215, funded this research.

References

[1] Kurniawan J, Yin NN, Liu GY, Kuhl TL. Interaction forces between ternary lipid bilayers containing cholesterol. Langmuir 2014;30:4997–5004. https://doi.org/10.1021/la500341c.

[2] Dimova R, Aranda S, Bezlyepkina N, Nikolov V, Riske KA, Lipowsky R. A practical guide to giant vesicles. Probing the membrane nanoregime via optical microscopy. J Phys Condens Matter 2006;18. https://doi.org/10.1088/0953-8984/18/28/S04.

[3] Subczynski WK, Wisniewska A. Physical properties of lipid bilayer membranes: Relevance to membrane biological functions. Acta Biochim Pol 2000;47:613–25. https://doi.org/10.18388/abp.2000_3983.

[4] Veatch SL, Keller SL. Separation of Liquid Phases in Giant Vesicles of Ternary Mixtures of Phospholipids and Cholesterol. Biophys J 2003;85. https://doi.org/10.1016/S0006-3495(03)74726-2.

[5] Van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are. Nat Rev Mol Cell Biol 2009;101:1–4. https://doi.org/10.1038/nrm2330.Membrane.

[6] Stefaniu C, Brezesinski G, Möhwald H. Langmuir monolayers as models to study processes at membrane surfaces. Adv Colloid Interface Sci 2014;208:197–213. https://doi.org/10.1016/j.cis.2014.02.013.

[7] Veatch SL, Cicuta P, Sengupta P, Honerkamp-Smith A, Holowka D, Baird B. Critical fluctuations in plasma membrane vesicles. ACS Chem Biol 2008;3. https://doi.org/10.1021/cb800012x.

[8] Karslake J, Stone M, Veatch SL. Probing Sub-Micron Critical Composition Fluctuations using Super-Resolution Techniques in Giant Plasma Membrane Vesicles. Biophys J 2014;106. https://doi.org/10.1016/j.bpj.2013.11.583.

[9] Veatch SL, Keller SL. Seeing spots: Complex phase behavior in simple membranes. Biochim Biophys Acta - Mol Cell Res 2005;1746. https://doi.org/10.1016/j.bbamcr.2005.06.010.

[10] Bañuelos-Frias A, Castañeda-Montiel VM, Alvizo-Paez ER, Vazquez-Martinez EA, Gomez E, Ruiz-Garcia J. Thermodynamic and Mechanical Properties of DMPC/Cholesterol Mixed Monolayers at Physiological Conditions. Front Phys 2021;9:1–10. https://doi.org/10.3389/fphy.2021.636149.

[11] Brezesinski G, Möhwald H. Langmuir monolayers as models to study processes at membrane surfaces. Adv Colloid Interface Sci 2003;208:197–213. https://doi.org/10.1016/j.cis.2014.02.013.

[12] Méléard P, Bagatolli LA, Pott T. Giant Unilamellar Vesicle Electroformation. From Lipid Mixtures to Native Membranes Under Physiological Conditions. Methods Enzymol 2009;465:161–76. https://doi.org/10.1016/S0076-6879(09)65009-6.

[13] Moscho A, Orwar OWE, Chiu DT, Modi BP, Zare RN. Rapid preparation of GUVs. Proc Natl Acad Sci USA 1996;93:11443–7.

[14] Angelova M, Dimitrov DS. A mechanism of liposome electroformation. Trends Colloid Interface Sci II 2007:59–67. https://doi.org/10.1007/bfb0114171.

[15] Brownell WE, Jacob S, Hakizimana P, Ulfendahl M, Fridberger A, Jørgensen IL, et al. Ternary phase diagram of dipalmitoyl-PC/dilauroyl-PC/cholesterol: Nanoscopic domain formation driven by cholesterol. Biophys J 2013;8:234–67. https://doi.org/10.1007/s00249-016-1155-9.

[16] Estes DJ, Mayer M. Giant liposomes in physiological buffer using electroformation in a flow chamber. Biochim Biophys Acta - Biomembr 2005;1712:152–60. https://doi.org/10.1016/j.bbamem.2005.03.012.

[17] Kuribayashi K, Tresset G, Coquet P, Fujita H, Takeuchi S. Electroformation of giant liposomes in microfluidic channels. Meas Sci Technol 2006;17:3121–6. https://doi.org/10.1088/0957-0233/17/12/S01.

[18] Diegel O, Nordin A, Motte D. Additive Manufacturing Technologies. 2019. https://doi.org/10.1007/978-981-13-8281-9_2.

[19] Ngo TD, Kashani A, Imbalzano G, Nguyen KTQ, Hui D. Additive manufacturing (3D printing): A review of materials, methods, applications and challenges. Compos Part B Eng 2018;143:172–96. https://doi.org/10.1016/j.compositesb.2018.02.012.

[20] Yazdi AA, Popma A, Wong W, Nguyen T, Pan Y, Xu J. 3D printing: an emerging tool for novel microfluidics and lab-on-a-chip applications. Microfluid Nanofluidics 2016;20:1–18. https://doi.org/10.1007/s10404-016-1715-4.

[21] blender.org. blender.org - Home of the Blender project - Free and Open 3D Creation Software. BlenderOrg 2015.

[22] Ultimaker. Cura 3D Printing Slicing Software. Ultimaker 2017.

[23] Simons K, Vaz WLC. Model systems, lipid rafts, and cell membranes. Annu Rev Biophys Biomol Struct

2004;33:269–95. https://doi.org/10.1146/annurev.biophys.32.110601.141803.

[24] Shitamichi Y, Ichikawa M, Kimura Y. Mechanical properties of a giant liposome studied using optical tweezers. Chem Phys Lett 2009;479:274–8. https://doi.org/10.1016/j.cplett.2009.08.018.

[25] Wu H, Yu M, Miao Y, He S, Dai Z, Song W, et al. Cholesterol-tuned liposomal membrane rigidity directs tumor penetration and anti-tumor effect. Acta Pharm Sin B 2019;9:858–70. https://doi.org/10.1016/j.apsb.2019.02.010.

[26] Bertrand B, Munusamy S, Espinosa-Romero JF, Corzo G, Arenas Sosa I, Galván-Hernández A, et al. Biophysical characterization of the insertion of two potent antimicrobial peptides-Pin2 and its variant Pin2[GVG] in biological model membranes. Biochim Biophys Acta - Biomembr 2020;1862. https://doi.org/10.1016/j.bbamem.2019.183105.

[27] Witkowska A, Heinz LP, Grubmüller H, Jahn R. Tight docking of membranes before fusion represents a metastable state with unique properties. Nat Commun 2021;12. https://doi.org/10.1038/s41467-021-23722-8.

[28] Jørgensen IL, Kemmer GC, Pomorski TG. Membrane protein reconstitution into giant unilamellar vesicles: a review on current techniques. Eur Biophys J 2017;46. https://doi.org/10.1007/s00249-016-1155-9.

[29] Hernández-Adame PL, Meza U, Rodríguez-Menchaca AA, Sánchez-Armass S, Ruiz-García J, Gomez E. Determination of the size of lipid rafts studied through single-molecule FRET simulations. Biophys J 2021;120. https://doi.org/10.1016/j.bpj.2021.04.003.

[30] Wesołowska O, Michalak K, Maniewska J, Hendrich AB. Giant unilamellar vesicles - a perfect tool to visualize phase separation and lipid rafts in model systems. Acta Biochim Pol 2009;56:33–9. https://doi.org/20091772 [pii].