Investigation of the Electrical properties of Phosphatidylserine Lipid Bilayer Membranes

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***Abstract*—In this work we investigate the electrical properties of phospholipid bilayer membranes (LBMs) formed from phosphatidylserine by analyzing two experimental setups. The Electrochemical Impedance Spectra (EIS) of phosphatidylserine show that a lipid bilayer membrane formed from this phospholipid has an average specific electrical resistance of 3.466 k Ω.cm^2 and an average capacitance of 0.385 μF/cm^2. Some of the major factors that affect the LBM resistance include electroporation, the method of deposition, and the surface tension in microchannels for supported LBMs. Therefore, wide apertures remain the most accurate method for supporting LBMs.**

Keywords—Lipid Bilayer Membranes, Electrochemical Impedance Spectroscopy, Phosphatidylserine

I. Introduction

Phospholipid Bilayer Membranes (LBMs) play a crucial role in human cells, and constitute the cellular membranes as well as the boundaries that encapsule many cellular organelles such as the Golgi Apparatus, mitochondria, and endoplasmic reticulum. The remarkably high flexibility of LBMs allows them to form very complex conformations that can be seen in the immense tubular network in the Golgi apparatus and extensive endoplasmic reticulum networks, the dynamics of which are the subject of active research [1] [2].

The phosphoglycerolipid phosphatidylserine (PS) comprises about 8.5% of the cellular phospholipid content [3] as well as about 25 to 35% of the inner leaflet of the membrane [4] in mammalian cells, and the major negatively-charged phospholipid in the membranes. In addition to phosphatidylserine, the phospholipid phosphatidylethanolamine (PE), is another major constituent of biological membranes. Both lipids comprise 25% of the plasma membrane of red blood cells and more than 70% of that of the Escherichia coli (E. Coli) bacteria [5]. As in other phosphoglycerides, the three-carbon glycerol constitutes the backbone of PS, with the phosphate group attached to the sn-3 position of the glycerol while the sn-1 and sn-2 carbon atoms are connected through ester bonds to two fatty acid chains.

To reduce the rigidity of the head group of Phosphatidylserine, and thereby control the fluidity of the lipid bilayer membranes in Eukaryotic cell membranes, PE is found abundantly with PS in the inner leaflets. PE also reduces the negative charge concentration due to PS [6]. In laboratory settings, PS is mixed with phosphatidylcholine (PC) to reduce rigidity [3]. The two enzymes responsible for the synthesis of PS are PS Synthase-1, which replaces the choline headgroup with serine in phosphatidylcholine, and the PS Synthase-2 enzyme which works by replacing ethanolamine by serine in phosphatidylethanolamine [7] [8].

Ever since the cellular lipid bilayer membrane (LBM) was first described in 1925 [9] by Gorter and Grendel, it was modeled numerous times, such as the Fluid Mosaic model in 1972 [10], and the Classic Bilayer Mechanics Theory developed in the 1970's [11] [12] [13] (also known as the Canham–Helfrich–Evans model), the Area Difference Elasticity Model in the 1990's [14] [15] [16] which described the energy due to the monolayer curvature, that was simplified in the previous models.

A comparison in the physical properties between phosphatidylserine and phosphatidylethanolamine is shown in Table 1.

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TABLE I. PHYSICAL PROPERTIES OF TWO PHOSPHOLIPIDS

	Phosphoglycerolipids	
	Phosphatidylserine [17]	***Phosphatidylethanolamine [18]***
Chemical Formula	$C_{42}H_{82}NO_{10}P$	$C_9H_{18}NO_8P$
Molecular Weight	792.1	299.21
Polar Surface Area	172 $A^{O\,2}$	134 $A^{O\,2}$
Physiological Charge	-1	Neutral

In this work, we investigate the electrical properties of lipid bilayers formed from phosphatidylseine and compare the results achieved to those of other research groups conducted on phospholipids.

II. EXPERIMENT AND METHOD

A. Formation of the Lipid Bilayer Membrane

Several methods can be found in literature for lipid bilayer membrane deposition, such as lipid vesicle spreading [19], Langmuir- Blodgett and Langmuir- Schaefer techniques [20] [21] [22] [23] [24], which offer the advantage of tight packing of the lipid molecules, spin coating [25] [26], and the Montel-Mueller method [27]. In this work the Montal-Mueller techniques has been used to form the lipid bilayer as demonstrated in our work in reference [28]. The synthetic phospholipid 1,2-diphytanoyl-*sn*-glycero-3-phosphoserine ($C_{46}H_{89}NO_{10}PNa$) was purchased from Avanti Polar Lipids, Inc and was used to form the LBM due to its long fatty acid chain in order to spontaneously favor the formation of bilayer membranes over micelles [29] [30].

Two different apparatuses were constructed for the experiment, one of which was 3D printed from thermoplastic polyster PolyLactic Acid (PLA) and one apparatus was constructed from glass. Each of the two apparatuses consisted of two chambers separated by a wall with an aperture of 1mm diameter for the PLA apparatus and 1.29 mm for the glass apparatus.

A stock solution of 10 mM NaCl was prepared by dissolving 0.35 grams of NaCl in 600 mL of distilled water. Each chamber of the glass apparatus had a surface area of $W \times L$ = 105 mm x 82.5 mm resulting in a total surface area of 17,325 mm^2.

The apparatus was initially filled with the electrolyte solution up to the bottom boundary of the aperture, and then the phospholipid was added to the electrolyte surface, and was left for two hours to stabilize and to form a monolayer on the water surface. The electrolyte was then added slowly via tubes inserted on the far ends of each chamber so that approximately a 2 mm height increase is achieved to cover the top boundary of the aperture as demonstrated in Fig. 2. The EIS spectra were then collected for the system.

The gradual addition of the electrolyte through the two tubes is necessary to avoid turbulence in the water that may cause the lipid bilayer that form to rupture. In addition to that, the electrolyte is added almost simultaneously in both chambers so that the water heights in both chambers increase concurrently with each other to avoid exerting pressure on the delicate lipid bilayer that forms across the aperture.

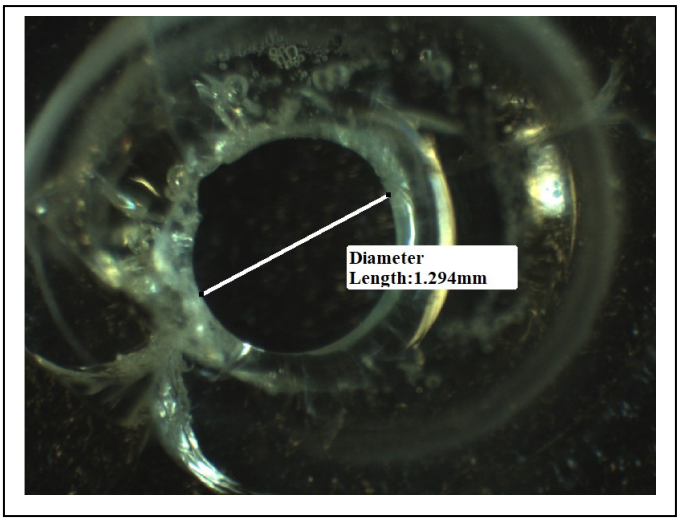


Fig. 1. The aperture that separates the two chambers was laser cut in a 0.5 mm thick glass and with an average diameter of 1.29 mm.

In order to calculate the amount of lipids needed, the total surface area that would be exposed to the lipid includes the surface area of the two chambers, as well as the surface area of the sidewalls (including the aperture) that will have contact with the lipid monolayers after filling in the electrolyte. Therefore the total surface area exposed to the lipid monolayer is calculated as follows:

$$Surface\ Area = 2(W \times L + (W \times h \times 2 + L \times h \times 2)) \quad (1)$$

Resulting in a total surface area exposed to the lipid monolayer of 18,825 mm^2.

With 5 million phospholipid molecules occupying a square of 1 µm x 1 µm [5], and a molar mass of 870.16 g/mole of the phospholipid, it can be shown that the amount needed of the $C_{46}H_{89}NO_{10}PNa$ phospholipid to cover the entire surface area exposed to it is approximately 0.14 mg.

B. Electrochemical Impedance Spectra (EIS) and Electrical Impedance Model

Several methods have been described for measuring the dielectric properties of biological membranes such as dielectrophoresis (DEP) and Traveling Wave dielectrophoresis (TWDEP). However, EIS stands out as a commonly used non-invasive technique that does not require the use of labels for

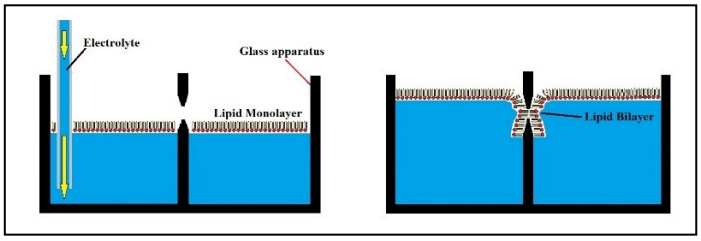


Fig. 2. Formation of the lipid bilayer membrane across the aperture that separates two chambers in a glass apparatus.

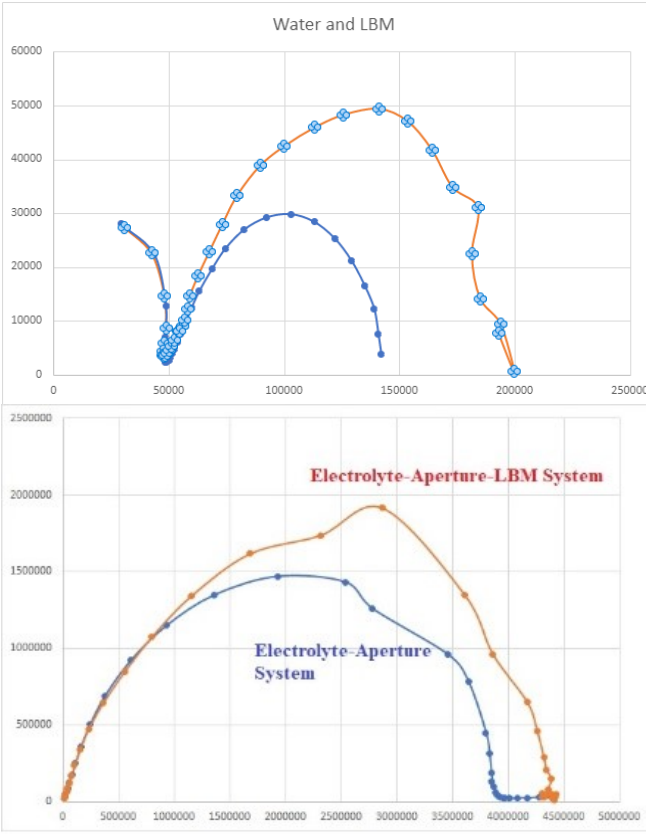
This work is funded by the National Science Foundation Award 2000685

measuring the dielectric properties of both the surface and the internal content of the biological membranes.

The system composed of an electrolyte solution in two chambers with an aperture that connects the two chambers is modeled as a pure resistance $R_{electrolyte}$ in series with a parallel combination of a resistance and a capacitance since the entire current must pass through the electrolyte [31] [32] [23] [33] [34] [35].

Depending on the thickness of the biological membrane, it can be modeled as a capacitor and a negligible resistance [36] for biological membranes that have a thickness of about 5 nm. However, in this work the lipid bilayer has longer fatty acid chains than is normally found in biological membranes for an increased stability as pointed earlier, therefore, it is modeled as a resistance and a capacitor in parallel [34] [23] [32].

By modeling the biological membrane as a capacitor, the membrane will prevent conduction at low frequencies due to the high impedance. However, the experimental results that describe a measurable impedance at low frequencies for artificial bilayer membranes [32] [33] [37] [28] confirm that a more accurate model of the biological membrane is that which includes a resistance and capacitance in parallel. The electrode–electrolyte interface impedance (also called the electrical double layer) is modeled as a capacitor in parallel with a resistance. The ion flow will have to pass through the LBM and the aperture, therefore, the LBM and aperture imedances are in series. The resulting electrical impedance model is shown in fig. 3.



Fig

III. Analysis and Discussion

The Electrohemical Impedance Spectra of the system in each of the two experiments were collected for an input voltage of 10 mV RMS from 100 kHz to 0.1 Hz at 2 second intervals. The EIS spectra of the LBM on the aperture and for the aperture before the deposition of the LBM in both the PLA and the glass apparatus are shown in fig. 4. It can be noticed from the figure that the value of the impedance consistently increases for both cases when the LBM is deposited across an aperture in a PLA apparatus and in a glass apparatus.

The values of the capacitance and resistance of the lipid bilayer were numerically calculated and shown in Table 2. It can be seen in Table 2 that the resistance of phosphatidylserine with the PLA apparatus was measured to be about one order of magnitude larger than in the glass apparatus, and vice versa for the capacitance. This is attributed to phase separation and loss

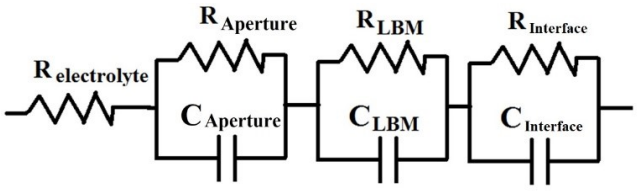


Fig. 3. The Electrical Impedance Model of the system composed of the aperture with a phosphatidylserine LBM across it in a 10 mM NaCl solution.

of continuity in the PLA apparatus due to the high hydrophobicity of the PLA walls which may affect the electrolyte continuity in the aperture. However, both measurements are close to each other when compared to measurements reported by other groups for similar phospholipids. Furthermore, if the LBM thickness of approximately 5 nm is taken into account, then the resistivity of phosphatidylserine is on average 69 x10^6 Ω.m.

One of the major challenges associated with forming lipid bilayer membranes across wide apertures is the limited stability of the lipid bilayers. To overcome this difficulty, researchers relied on supporting structures for the lipid bilayers such as porous silicon and porous alumina [38] [39]. However, the fluid dynamics in pores and microchannels are significantly different than in wide apertures. The surface tension becomes the dominant force, pressure gradients and shear stresses become enormously higher in microchannels. In addition to that, electrokinetic effects along the walls of the microchannels cause a charge distribution in the fluid along the pore walls and channels. Therefore, wide apertures remain the most accurate choice for studying lipid bilayer membranes [40].

The development of pores in the LBMs when an external electric field is applied (known as electroporation) is another factor that can contributes to differences in measurements.

TABLE II. PHYSICAL PROPERTIES OF TWO PHOSPHOLIPIDS

	Phosphatidylserine Electrical Impedance		
	Resistance	***Resistivity***	***Capacitance***
PLA Apparatus	6,280 Ω.cm^2	125.6 x10^6 Ω.m	0.0051 µF/cm^2
Glass Apparatus	653 Ω.cm^2	13 x10^6 Ω.m	0.765 µF/cm^2
Average:	3.466 k Ω.cm^2	69 x10^6 Ω.m	0.385 µF/cm^2

IV. CONCLUSION

In this work we investigated the electrochemical impedance spectra for lipid bilayer membranes of phosphatidylserine formed across an aperture in a 3D printed apparatus made in PLA, and in a glass apparatus. Both apparatuses resulted in a specific resistance and capacitance values about close to each other when compared to results reported for other similar phospholipids.

Recently, wide apertures have regained attention as the preferred choice for studying lipid bilayer membranes due to the difficulties associated with porous support materials and microchannel array-based support materials, primarily, the high surface tensions, pressure gradients in the microchannels, and the electrokinetic effects along the walls of the microchannels which cause a charge distribution in the fluid along the pore walls and channels. Electroporation is one of the major contributors of variations in the reported specific resistance values of phospholipids in literature.

Future perspectives include using an intelligent robot [41] to perform the lipid bilayer formation and deposition to improve accuracy (Fig. 5). Collaborative robots constitute a major element in Smart Manufacturing [42], they are compact in size, light weight, and equipped with vision systems that can process data, and can move payloads with a positioning repeatability in the range of 20 micrometers while carrying payloads and moving them at a Tool Center Point (TCP) speed of up to 1,500 mm/s. Consequently, significant improvements can be achieved in LBM deposition and formation over conventional methods.

Fig. 5. Intelligent robots are compact in size, light weight, and equipped with machine vision systems and can move payloads with high precision and accuracy.

REFERENCES

[1] H. T. Perkins, V. J. Allan and T. A. Waigh, "Network organisation and the dynamics of tubules in the endoplasmic reticulum," *Nature Scientific Reports,* vol. 11, p. 16230, 2021.

[2] H. T. Perkins and V. Allan, "Intertwined and Finely Balanced: Endoplasmic Reticulum Morphology, Dynamics, Function, and Diseases," *Cells,* vol. 10, p. 2341, 2021.

[3] M. Roux and M. Bloom, "Calcium Binding by Phosphatidylserine Headgroups. Deuterium NMR Study.," *Biophys J,* vol. 60, no. 1, pp. 38-44, 1991.

[4] H. Antila, P. Buslaev, F. Favela-Rosales, T. M. Ferreira, I. Gushchin, M. Javanainen, B. Kav, J. J. Madsen, J. Melcr, M. S. Miettinen, J. Määttä, R. Nencini, O. H. S. Ollila and T. J. Piggot, "Headgroup Structure and Cation Binding in Phosphatidylserine Lipid Bilayers," *Journal of Physical Chemistry B,* 2019.

[5] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, Molecular Biology of the Cell, 5th ed., New York: Garland Science, 2008.

[6] W. W. Christie, "Phosphatidylethanolamine and Related Lipids," 2022. [Online]. Available: https://dev.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/complex/pe/index.htm. [Accessed 13 January 2022].

[7] J. Hasegawa, Y. Uchida, K. Mukai, S. Lee, T. Matsudaira and T. Taguchi, "A Role of Phosphatidylserine in the Function of Recycling Endosomes," *Frontiers in Cell and Developmental Biology,* vol. 24, 2021.

[8] J. E. Vance, "Historical Perspective: Phosphatidylserine and Phosphatidylethanolamine from the 1800s to the Present," *Journal of Lipid Research,* vol. 59, pp. 923-944, 2018.

[9] E. Gorter and F. Grendel, "On bimolecular layers of lipoids on the chromocytes of the blood," *Journal of Experimental Medicine,* vol. 41, pp. 439-443, 1925.

[10] S. Singer and G. Nicolson, "The fluid mosaic model of the structure of cell membranes," *Science,* vol. 175, pp. 720-731, 1972.

[11] P. Canham, "The minimum energy of bending as a possible explanation of the biconcave shape of the red blood cell," *Journal of Theoretical Biology,* vol. 26, no. 1, pp. 61-81, 1970.

[12] W. Helfrich, "Elastic properties of lipid bilayers: theory and possible experiments," *Z Naturforsch C,* vol. 28, no. 11, pp. 693-703, 1973.

[13] E. Evans, "Bending resistance and chemically induced moments in membrane bilayers," *Biophysics Journal,* vol. 14, no. 12, pp. 923-931, 1974.

[14] L. Miao, U. Seifert, M. Wortis and H. Döbereiner, "Budding transitions of fluid-bilayer vesicles: the effect of area difference elasticity," *Physical Review E,* vol. 49, no. 6, pp. 5389-5407, 1994.

[15] B. Bozic, S. Svetina, B. Zeks and R. Waugh, "Role of lamellar membrane structure in tether formation from bilayer vesicles," *Biophysical Journal,* vol. 61, no. 4, pp. 963-973, 1992.

[16] U. Seifert, "Shape transformation and free, toroidal and bound vesicles," *Le Journal de Physique Colloques,* vol. 51, pp. C7339-344, 1990.

[17] National Library of Medicine, "National Center for Biotechnology Information," 2022. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/9547096.

[18] National Library of Medicine, "National Center for Biotechnology Information," NIH, 2022. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/Phosphatidylethanolamine.

[19] A. Brian and H. M. McConnell, "Allogeneic stimulation of cytotoxic T cells by supported planar membranes," *Proceeding of the National Academy of Science USA,* vol. 81, pp. 6159-6163, 1984.

[20] K. Blodgett and I. Langmuir, "Built-up films of barium stearate and their optical properties," *Phys. Rev.,* vol. 51, pp. 964-982, 1937.

[21] I. Langmuir and V. Schaefer, "Activities of Urease and Pepsin Monolayers," *J. Am. Chem. Soc.,* vol. 60, pp. 1351-1360, 1938.

[22] I. Langmuir, V. Schaefer and H. Sobotka, "Multilayers of sterols and adsorption of digitonin by deposited monolayers," *J. Am. Chem. Soc.,* vol. 59, pp. 1751-1759, 1937.

[23] K. Tantawi, R. Cerro, B. Berdiev and J. Williams, "Investigations on transmembrane ion channels suspended over porous silicon membranes," *Nanotechnology,* vol. 3, pp. 198-201, 2013.

[24] K. H. Tantawi, R. Cerro, B. Berdiev, M. E. Diaz, F. Montez and J. D. Williams, "Lipid Bilayer Membrane with Incorporated Protein on Porous Silicon," in *22nd NSF EPSCoR National Conference*, Coeur d'Alene, Idaho, 2011.

[25] L. Krapf, M. Dezi, W. Reichstein, J. Kohler and S. Oellerich, "AFM characterization of spin-coated multilayered dry lipid films prepared from aqueous vesicle suspensions," *Colloids and Surfaces B: Biointerfaces,* vol. 82, pp. 25-32, 2011.

[26] A. C. Simonsen and L. A. Bagatolli, "Structure of Spin-Coated Lipid Films and Domain Formation in Supported Membranes Formed by Hydration," *Langmuir,* vol. 20, pp. 9720-9728, 2004.

[27] M. Montal and P. Mueller, "Formation of Bimolecular Membranes from Lipid Monolayers and a Study of Their Electrical Properties," *Proc. Nat. Acad. Sci.,* vol. 69, pp. 3561-3566, 1972.

[28] K. Tantawi and H. Hunnicut, "Electrophysiology of Phosphatidylserine Bilayer Membranes Using Electrochemical Impedance Spectroscopy," in *International Mechanical Engineering Congress and Exposition (IMECE)*, 2021.

[29] B. Berdiev and D. Benos, "Epithelial Sodium Channel in Planar Lipid BiLayers," *Methods in Molecular Biology,* vol. 337, pp. 89-99, 2008.

[30] K. H. Tantawi, R. Cerro, B. Berdiev and J. D. Williams, "Lipid Bilayer Membrane Supported on Porous Silicon Substrate for Protein Investigation," in *Alabama Composites Conference*, Birmingham, AL, 2010.

[31] A. J. Bard and L. R. Faulkner, Electrochemical Methods Fundamentals and Applications, Hoboken, NJ: John Wiley and Sons, Inc, 2001.

[32] K. Tantawi, B. Berdiev, R. Cerro and J. Williams, "Porous silicon membrane for investigation of transmembrane proteins," *Superlattices and Microstructures,* vol. 58, pp. 72-80, 2013.

[33] M. S. Khan, N. S. Dosoky, G. Mustafa, D. Patel, B. Berdiev and J. D. Williams, "Electrophysiology of Epithelial Sodium Channel (ENaC) Embedded in Supported Lipid Bilayer Using a Single Nanopore Chip," *Langmuir,* vol. 33, no. 47, pp. 13680-13688, 2017.

[34] K. H. Tantawi, R. Cerro, B. Berdiev, M. E. D. Martin, F. J. Montes, D. Patel and J. D. Williams, "Investigation of transmembrane protein fused in lipid bilayer membranes supported on porous silicon," *Journal of medical engineering & technology,* vol. 37, no. 1, pp. 28-34, 2013.

[35] A. J. Bard and L. R. Faulkner, Electrochemical Methods Fundamentals and Applications, Hoboken, NJ: John Wiley and Sons, Inc, 2001, pp. 368-416.

[36] T. Sun, N. Green and H. Morgan, "ANALYTICAL AND NUMERICAL MODELING METHODS FOR IMPEDANCE ANALYSIS OF SINGLE CELLS ON-CHIP," *Nano,* vol. 3, no. 1, pp. 55-63, 2008.

[37] M. S. Khan, N. S. Dosoky, D. Patel, J. Weimer and J. Williams, "Lipid bilayer membrane in a silicon based micron sized cavity accessed by atomic force microscopy and electrochemical impedance spectroscopy," *Biosensors,* vol. 7, no. 3, p. 26, 2017.

[38] V. Bedekar and K. Tantawi, "MEMS Sensors and Actuators," in *Advanced Mechatronics and MEMS Devices II*, Springer, 2017, pp. 195-216.

[39] K. H. M. Tantawi, Porous Silicon Platform for Investigation of Transmembrane Proteins, Lambert Academic Publishing, 2013.

[40] K. V. Sharp, R. J. Adrian, J. G. Santiago and J. I. Molho, "Liquid Flows in Microchannels," 2005, pp. 10-1 to 10-48.

[41] K. Tantawi, A. Sokolov and O. Tantawi, "Advances in Industrial Robotics: From Industry 3.0 Automation to Industry 4.0 Collaboration," in *4th Technology Innovation Management and Engineering Science International Conference (TIMES-iCON)*, Bangkok, Thailand, 2019.

[42] K. Tantawi, I. Fidan and A. Tantawy, "Status of Smart Manufacturing in the United States," in *2019 IEEE SoutheastCon*, Huntsville, AL, 2019.