

Measuring Transmembrane Potential of Myelomas Hypridoma cell culture in Radio Frequency Spectrum

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Abstract—This article explores using dielectric spectroscopy in an attempt to measure the trans-membrane potential of synthetic cells in radio frequency spectrum. The purpose of this test is to "mimic" a capacitor with the cell suspension acting as a dielectric. This is a non-invasive way to measure the average membrane potential across your cell suspension and has been proven in [?] for frequencies up to $10^5 Hz$.

I. IMPLICATIONS OF MEMBRANE POTENTIAL

Although there may be many implications of transmembrane potential at radio frequencies, the focus of this example is in disease models. In the late 1930's it was suggested that a relationship lie between cancer and the bioelectric properties of host tissue. Interestingly, membrane voltage (V_{mem}) analysis in many different mammalian cell types reveals that proliferative potential is correlated with unique ranges of V_{mem} : quiescent cells tend to be hyperpolarized, whereas highly plastic cells such as embryonic cells, adult stem cells and tumors cells are depolarized. [?]. During the early stages of tumor formation, V_{mem} is a key regulator of the cell cycle and determines the proliferative state of many different kinds of cells. These statements excite the possibility of non-invasive techniques being used to track bioelectric cell states and detection of tumors, or even mitigation of tumor formation by canonical oncogene.

II. HYPOTHESIS

III. CALCULATING MEMBRANE POTENTIAL

We model the impedance of the cell suspension as a resistor $R = d/\sigma A$ in parallel with a capacitor $C = \epsilon A/d$, where σ and ϵ are the conductivity and dielectric permittivity of the cell suspension and A and d are the surface area of the two disk electrodes and the distance between them.

$$\sigma(\omega) = Re \frac{d_1 - d_2}{A(Z_1^O - Z_2^O)} \quad (1)$$

$$\epsilon(\omega) = Im \frac{d_1 - d_2}{\omega A(Z_1^O - Z_2^O)} \quad (2)$$

Electrode distance variation technique:

$$\begin{aligned} Z_1^S - Z^P &= Z_1^O \\ Z_2^S - Z^P &= Z_2^O \end{aligned} \quad (3)$$

$$Z_1^S - Z_2^S = Z_1^O - Z_2^O \quad (4)$$

Where Z^S is the sample impedance, Z^P is the unknown polarization impedance, and Z^O is the measured impedance.

I have been assured by Dr.Dharmakeerthi Nawarathna that equation (1) and equation (2) are sufficient in finding the potential of the solution.

IV. DISTANCE BETWEEN PLATES:

With regards to near and far field transmission. The most agreed upon definition of near field transmission is less than one wavelength(λ) away [?]. If we consider a sinusoidal wave traveling at a constant speed, we can calculate wavelength with the following formula ...

$$\lambda = \frac{v}{f} \quad (5)$$

Where v is the magnitude of the phase velocity and f is the frequency of the sinusoid. It is difficult to determine the phase velocity of our electromagnetic wave while propagating through our cell suspension, but if we consider water, we can make a prediction of the wavelength. The velocity of EM waves is more than 4 orders faster than acoustic waves according to [?]. Knowing that the speed of sound is $343.2m/s$ we can say ...

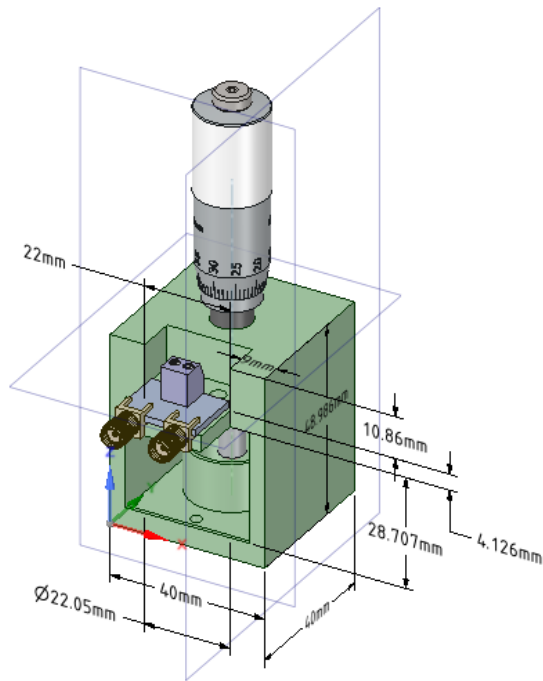
$$\lambda = \frac{343.2 \cdot 4}{10^9} = 1.3728 \times 10^{-6}m \quad (6)$$

Water is a good basis for the phase velocity as the cell suspension is made from deionized water and synthetic cells. I used $10^9 Hz$ as our frequency, which is in the radio spectrum. Our micrometer is sensitive to $10\mu m$, thus our test fixture will be capable of measuring only waves 10 times greater than the fundamental wavelength, meaning far field transmissions will be measured. This is a satisfactory result, as the far field is the "real" radio waves, that propagate through space at just about the speed of light [?].

V. MATERIALS

A. Copper Plates

Two copper plates $3/4 inch$ in diameter will be used. One plate will be fixed to the bottom of the beaker and the other is attached to a micrometer. To create the copper plates a hole puncher will be used.



B. Copper Wire Leads

Two wires will be attached to the copper plates. The wire attached to the fixed bottom plate will be attached to the center and run through the bottom of the test fixture to later be attached to a network analyzer. The second wire will be attached to top plate on one of the edges and run up the side of the beaker to be attached to a network analyzer. These two wires will provide the radio frequency signal and the probes to be attached to a network analyzer.

C. 3-D printed Beaker

A 3-D printed beaker will be made to perfectly fit the test fixture. The beaker will be made of ABS plastic and is used to hold the cell suspension and two copper plates. It will be placed in the center of the Test fixture stand.

D. Micrometer

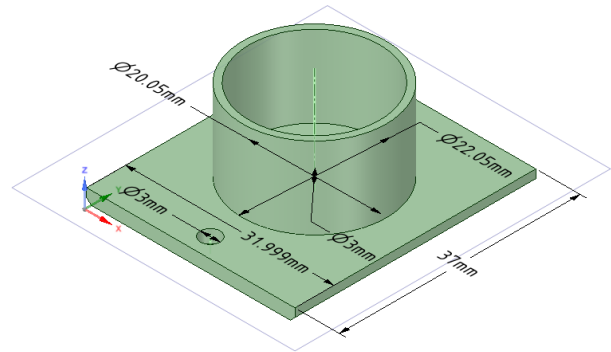
A micrometer will be used to adjust the height of one of the copper plates. The micrometer will be attached to the test fixture stand, and the drive of the micrometer will be attached to one of the copper plates. A micrometer cap will be 3-D printed in ABS plastic to perfectly fit the micrometer and later be adhered to the copper plate.

E. Test Fixture Stand

Provide a base for the beaker as well as an accurate and stable micrometer mount. This item will be 3D printed with ABS plastic.

F. Cells

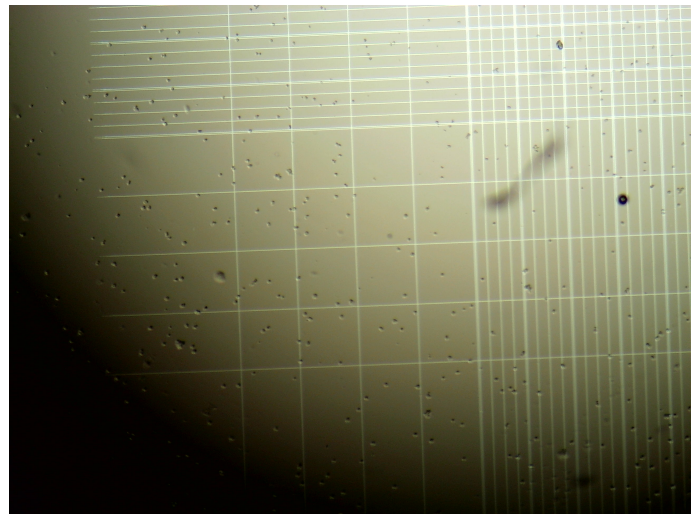
SP 2/0 myelomas hybridoma cell line. These are nonadherent cells, and are essentially cancer cells from mice.[?] They are rated at biosafety level 1. This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Research with these agents may be performed on standard open laboratory benches without the use of special containment equipment and it is not necessary for Biosafety level 1 labs to be isolated from the general building.[?]



VI. METHODS

A. Cell Concentration

The cell concentration will be measured using a hemocytometer. An example of the process of counting the cells can be seen in figure



VII. PROCEDURE FOR SPLITTING CELLS FOR REGROWTH AND TESTING

A. Tools and Equipment

- New cell culture flask
- Centrifuge
- 1ml centrifuge vials
- Pipette with 10ml graduated tips

- Bleach
- Phosphate buffer solution (PBS)
- Cell food (10% FBS PFS solution)
- Vial rack for the centrifuge vials
- 50ml sealable test tube

B. Personal Safety Equipment

For bio safety level 2, use of hood, gloves, and lab coat for person protection. With the hood shield down, protective eye ware was not needed. The hood should be turned on, and the shield lowered so only your hands can fit underneath it, before any work with the cells is done. The cells are not to leave the bio safety level 2 lab room.

C. Notes

- The cell suspension will become more acidic over time, and thus, the more yellow or orange than dark pink (the color of the cell food).
- The cells are being kept under conditions of 37 °C and 4.9% CO₂ in the incubation chamber when not in use.
- The food is being stored in a standard refrigerator in a bio safety lab room.
- The process does not need to leave the room to be completed.

D. Procedure

Centrifuging:

- 1) Remove the flask from the incubation unit and place it in the hood
- 2) Using the pipettes, put 1 ml of cell solution from the cell culture flask into each of the centrifuge vials until no more solution in the flask. 10 vials should be full.
- 3) Seal the vials, and evenly distributed them in a centrifuge which is then run at 1.7krpm for 5 min. Once complete, there will be a pellet of cells in the bottom of those vials. If there is an odd number of vials, do not use the odd one, place it aside on the rack for later disposal.
- 4) Split the vials into two equal groups. One group will be used to create the solution for testing and the other will be used to regrow the cell population.

For cell regrowth:

- 1) Siphon off the solution in the vials and put it in the test tube.
- 2) Using a new pipette, add 1ml of food to the vials. The tip of the pipette will not touch the cells.
- 3) Shake the vials to dissolve the pellet.
- 4) Using a pipette, add the solution of cells and cell food to a new cell culture flask. The flask should be labeled with the type of cells or strain, and what path number they are on.
- 5) The flask is then sealed, and placed horizontally back in the incubation chamber until this process needs to be repeated.

Preparing Cells for testing:

- 1) Repeat step 1 from the Cell regrowth section

- 2) Using the pipette, add 1 ml of a buffer solution, phosphate saline buffer solution is added to the vials
- 3) Repeat step 3 of the Cell Regrowth section
- 4) Using the pipette, the solution can be added as needed to the test fixture. It can also be stored temporarily in test tube or a cell culture flask.

For unused centrifuge vials:

- 1) Repeat step 1 from the Cell regrowth section
- 2) Using the pipette, add 1ml of bleach to the vials
- 3) Shake the vials to dissolve
- 4) Using a pipette, the bleach and cell solution is added to the mixture of old cells already in the disposable test tube.

Clean up:

If the disposable test tube has not had bleach added to it, several milliliters should be added to it. The liquid should turn light blue to clear, and now can safely be disposed. All surfaces in the hood and materials that are not disposable should be wiped down with 70% ether solution. All equipment used that is disposable should be disposed of in bio-safe bins which should be located near the hood.

VIII. CONCLUSION

The reason for the test fixture is to hold a beaker dish and adjust the height between the two parallel copper plates. In order to be as accurate as possible a micrometer will be attached to the top copper plate and then attached to the top of the test fixture. This will allow us to accurately set the parallel plate distance and also change the height as needed.

IX. ACKNOWLEDGEMENT

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REFERENCES

- [1] C.Prodan,F.Mayo,J.R.Claycomb,J.H.Miller,Jr,M.J.Benedik. *Low-frequency, low-field dielectric spectroscopy of living cell suspensions.* Journal of Applied Physics, Volume 95, Number 7, April 2004
- [2] Shan Jiang, Stavros Georgakopoulos. *Electromagnetic Wave Propagation into Fresh Water.* Journal of Electromagnetic Analysis and Applications,2011,3,261-266
- [3] Lou Frenzel. *What is the Difference Between EM Near Field and Far Field.* Electronic Design, June 8, 2012
- [4] Brook T.Chernet and Michael Levin *Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a Xenopus model.* Disease Models Mechanisms 6, 595-607 (2013)
- [5] Center for Disease Control and Prevention *Recognizing the Biosafety Levels.* www.cdc.gov/training/QuickLearns/biosafety/
- [6] ATCC *Mouse Myeloma Hybridoma Strain 6C2-4 (PTA-970).* American Type Culture Collection