# EFFECTS OF ALTERNATING CURRENT ELECTRICAL STIMULATION ON THE

# CELLULAR CHEMISTRY AND PROLIFERATION OF C2C12 MUSCLE CELLS

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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**ABSTRACT**

The objective of this study was to investigate the effects of electrical stimulation on the cellular chemistry and proliferation of C2C12 muscle cell line. The cells were cultured under the condition of AC electrical stimulation using interdigitated electrode arrays. This research was conducted by applying electrical signals for up to 24 hours to C2C12 muscle cells. After 24 hours, the electrical impedance, cell morphology, proliferation and viability were recorded. The results demonstrated that electrical stimulation have a positive impact on the cell growth of C2C12. These results obtained would aid in future study on improving the techniques of culturing C2C12 muscle cells and also in the field of electrical conductivity in cell proliferation. Lastly, the results obtained also would provide essential information to the studies related to gene and protein expression of cellular and subcellular components after being exposed to electrical stimulation.

## ACKNOWLEDGEMENTS

## DEDICATION

To my beloved parents, Albert and Cath for their endless support.

To my loving brothers, Kar Chun and Kar Hou for their continuous encouragement.

To my dearest Aunt Mary for all the inspiring conversations.

To my one and only, Lenny King for his unconditional love.

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# LIST OF ABBREVIATIONS

3D ………………...…… Three-dimensional

(w/v) ……………...…… Weight/Volume Percent

AC ……………......…… Alternating Current

AM ………………….… Acetoxymethyl

ATCC ……………….…… American Type Culture Collection

$CO_2$ …………...……..… Carbon Dioxide

CRET ……………….…. Capacitive-Resistive Electric Transfer

DC ………………...….. Direct Current

DMEM ………………...… Dulbecco's Modified Eagle's Medium

DMSO……………………. Dimethyl sulfoxide

ES ………………...……… Electrical Stimulation

FBS ………………….……… Fetal Bovine Serum

MEA ……………...……… Microeletrode Array

PBS ………………………… Phosphate Buffered Saline

PDMS ……………………… Polydimethylsiloxane

$V_{pp}$ ………………...……… Peak-to-Peak Voltage

# 1. INTRODUCTION

Currently, there is an increased need to understand the biology of muscle cell and to construct muscle tissues *in vitro*. The main goal of muscle (cardiac or skeletal) engineering is to fabricate *in vitro* functional muscle tissues that can be used to replace animal studies as a biological model for studies of muscle tissue development, drug-screening, gene-screening and even as an implant to replace severely injured muscle tissues [1]. In this respect, the engineering design of muscle tissues has been proposed by a multitude of researchers as a promising approach to reform, replace or recover damaged muscle tissues [2].

Contractility is a vital electrophysiological concept in muscle cells. At the cellular level, muscle cell contraction is regulated through the excitation-contraction coupling process and it all begins with the first action potential [3]. The most important step in the excitation-contraction coupling process is calcium ion balance throughout the cell membrane. The action potentials can be sensed by voltage gated L-type calcium ion channels of sarcolemma (dihydropyridine receptors). Dihydropyridine receptors interact with calcium release channels (ryanodine receptors) localized on sarcoplasmic reticulum to release calcium ions from the lumen into the cytoplasm through ryanodine receptors. The chemical gradient of calcium ions within the cell membrane is important for the propagation of action potential. While they spontaneously propagate throughout cardiac cells, a nervous stimulus is required to activate skeletal muscle cells. Electric field stimulation aims to recreate such electrical signals for muscle cells *in vitro* as to generate action potentials [4].

There are various ways to stimulate the cell samples electrically, mainly divided into two categories: macroscopic and microscopic. In both categories, careful planning and multiple factors have to be taken into account when it comes to experimental setup involving electric field

stimulation. A research group has been studying and created different sets of classical experimental procedures for different types of cells and sets of conditions. According to the researchers, when planning cell culture experiments involving ES, parameters to consider include the duration, type and shape of stimulus waveform, the type and size of the biological specimen, the duration of the experiment and the configuration and properties of electrodes required for the respective experimental setup [5].

On top of that, there are many research studies involving different types of electrical stimulus, such as that of using DC or AC electric field. For example, according to Fredericks et al., there was still a slight disconnection with the understanding behind the biology of the treatment of applying DC signals in spinal surgeries, even with previous research done and results proved that there is an increased fusion rates as compared to rates when only autograft technique alone was applied [6]. Thus, their research was designed to study the response of gene expression related to bone formation relative to expression of autograft alone by direct current stimulation. The goal being to better the understanding the physiological and genetic components of the effects.

According to Salvini et al., they studied the main mechanisms involved in the reduction and increase of myoproteins synthesis, generally linked to muscle atrophy and hypertrophy, respectively. The researchers analyzed the effects of ES and stretching exercise on the molecular pathways in muscle atrophy and hypertrophy. Also, in the paper was described the main effects and limits of these factors in the skeletal muscle, particularly on the denervated muscle. It was found that ES applied in a same manner as performed in clinical practice is able to modify the increase of genes expression involved in muscle atrophy. However, ES was ineffective to prevent the loss of muscle mass caused by denervation. As a conclusion, in clinical practice,

despite the use of stretching exercise and ES to minimize the atrophy of denervated muscle, there was still a need to obtain more scientific evidence to justify the effectiveness of these resources to prevent muscle atrophy in denervated muscle [7].

Next, the research done by Park et al. was focused on the effects of different AC signals on the deposition of type I collagen in 3D cultured C2C12 muscle cells in terms of metabolic activity and cell morphology [3]. Their results indicated that a specific frequency from the AC signals may regulate the accumulation of type I collagen and a specific voltage may alter the differentiation of excitable muscle sarcomere cells.

Following that, this research team discussed the effects of ES on the gene expression of cells and only focused on cardiomyocytes. In this study conducted by Kujala et al., neonatal rat cardiomyocytes were exposed to both long-term and short-term stimulation in order to find out whether it is possible to achieve cell orientation and the maturation of cardiomyocytes with a MEA compatible electrical stimulation platform. Cells were viable after electrical stimulation, but no orientation or other morphological changes were observed [8]. In summary, they found out that the micro architecture of the biomaterials should be carefully designed for cell applications due to the change of cell orientation caused by the MEA. However, as ES and its stimulation duration affected gene expression of some main cardiac proteins, the system may prove useful to improve the cardiac differentiation of stem cells.

Also according to the study done by Ahadian et al., ES substantially improves the process of differentiation of muscle cells to myofibers. They designed a contactless platform using an interdigitated array of electrodes and a coverslip isolator to stimulate C2C12 muscle tissues electrically. Their device could be a great potential for many biological applications as a tool to create safe and noninvasive electric fields as their results shown increased contractile activity

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and greater maturation of muscle tissues in comparison to the tissues that were not electrically stimulated[9].

Next, the gene and protein expression changes of ionic channels in early phase of cultured rat atrial cardiomyocytes induced by rapid pacing are studied by Ji et al. Recent studies have demonstrated that atrial electrical remodeling was an important factor for the occurrence, persistence and maintenance of atrial fibrillation. This study observed the change in ionic channels expression in by applying ES to primary cultured atrial myocytes. As a result, the expressions of L-type calcium channel and potassium channel were both reduced at different levels in early phase of ES [10].

Then, Tandon et al. conducted a series of studies using ES to improve cardiac tissue engineering. They electrically stimulated different types of cells in their research, such as that of cardiac cells from neonatal rat heart ventricles and human adipose tissue-derived stem cells [5]. They conducted their studies in both classical and microscale settings with different experimental parameters including duration of stimulation, types of electrodes used. At the end of their research using the microscale setting to develop a surface-patterned electrode bioreactor for ES, they concluded that the use of interdigitated electrodes allowed them to integrate the use of ES for directing cellular organization and microfluidics that enables the miniaturization of the system of cell culture [11].

McCullen et al. conducted a research to investigate the effects of sinusoidal low-frequency AC electric fields through interdigitated electrodes on cell growth and osteogenic differentiation of human adipose-derived stem cells. They found out that the stem cells were highly responsive to all the AC electric fields at 1, 3, 5, 10, 100 and 1000V/cm at 1Hz and all of the cells were viable up to at least 100V/cm [12]. Similar to McCullen et al., Hernandez-Bule et

al. studied the viability, growth and differentiation of adipose-derived stem cells after a being exposed to CRET therapy. CRET is a noninvasive electrothermal process which applies currents within the frequency range of 400kHz to 450kHz to the treatment of musculoskeletal lesions. They applied the signal of $50\mu A/mm^2$ at 448kHz to the cells and this promoted proliferation of the stem cells. The results also suggested that CRET therapy could promote the regeneration of tissue by activating proliferation of cells in the injured area [13].

In a nutshell, the classical method of ES generally would be placing normal sized electrodes directly onto the cell culturing dish or flask and allow electric field to freely run across the entire layer of cells. On the other hand, the newer method may require the knowledge of lab-on-a-chip, microfluidics and how to correctly utilize different types of interdigitated electrodes because they are all in the micro- or even nanoscale. This method would be considered as a better alternative when resources, such as cell samples, reagents and funding are limited as it does not require a lot of material. According to Halldorsson et al., utilizing various microfluidic devices to culture cells is becoming more common in the cell research field [14]. Ultimately, the use of a microfluidic chamber will be determined by its size to allow new insights into cellular function, especially those that deemed to be difficult or impossible to obtain with macroscopic methods in traditional polystyrene Petri dishes, culturing flasks and multi-well plates.

Many years of heuristic optimization have gone into creating the perfect classical, also known as conventional cell culture devices and protocols [15]–[17]. In comparison, even for the most frequently utilized microfluidic devices, such as those made of PDMS, the bridge to the understanding of the differences in cellular behavior between microscopic and macroscopic method is still establishing [2], [18], [19]. Moving *in vitro* culture from macroscopic culture to microscopic chambers comes with many unforeseen difficult trials. Changes in chamber

material, surface coating, cell number per unit surface area or per unit medium volume all would affect and alter the results of otherwise standard protocols.

After an extensive literature review, it is clear that many studies have used ES to regulate muscle cell functions and behaviors as these studies also showed the different types of methods utilizing the principle of electrical field stimulation and how ES can affect cells at genetic level [1], [20]–[23]. Thus, it is safe to say that there is really no "one-size-fits-all" way of conducting research using ES. Both the classical and newer methods are highly feasible and only the researchers themselves are able to tell which method suits their respective research better.

In order to better understand the microscopic method and outcome of ES, the main purpose of this research is to investigate the effects of AC electric field on the cellular chemistry and proliferation of C2C12 mouse muscle cells cultured on surface interdigitated electrode arrays. The C2C12 cells were cultured on surface interdigitated electrode arrays and exposed to stimulation of 6V/cm at 1Hz and 1kHz for a total period of 24 hours. The results obtained from this research would aid in future studies on improving the techniques of cell culture and the field of electrical conductivity in cell proliferation. This study would also help in providing essential information to the work related to gene and protein expression of cellular and subcellular components after being exposed to ES.

## 2. METHODS AND MATERIALS

### 2.1. Cell Handling

The type of cell line used in this study was C2C12 mouse muscle myoblast purchased from the ATCC. Upon arrival to the laboratory, the vial of cells was thawed by gentle agitation in a water bath at 37°C. After two minutes, the vial was removed from the water bath and 70% ethanol was sprayed onto it for decontamination. It was placed in a laminar flow tissue culture hood and all aseptic conditions were strictly followed. The top of the vial was unscrewed and the contents were transferred to a sterile centrifuge tube containing 9mL of ATCC recommended cell culture medium (DMEM supplemented with 10% FBS). The cryoprotectant agent, DMSO was removed by 125x g for 10 minutes of centrifugation. After that, the supernatant was discarded and the cells are resuspended in 10mL of culture medium in a T-75 cell culture flask for 3 days in the incubator conditioned at 37°C with a concentration of 5% of $CO_2$.

The cell confluency was examined microscopically and when it reached about 90% confluency, the cells were prepared for subculturing. The cell culture medium was removed and discarded from the flask. 3mL of PBS was added to the flask to rinse and remove residual culture medium, then the fluid is discarded. 5mL of trypsin-EDTA solution was added into the flask and incubated at 37°C for 5 minutes to speed up the process of cell detachment. Once the cells appear to be disassociated, 5mL of cell culture medium was added into the flask to inactivate the trypsinization process.

The cell suspension was prepared for cell viability test by mixing 1:10 dilution with 50µL of cell suspension, 400µL of PBS and 50µL of 0.4% trypan blue solution. After mixing, the cells are loaded onto a clean and dry hemocytometer for cell counting. The cells were determined to be at the density of 6 x $10^6$ viable cells/mL and 90% of the cells were prepared for

cryopreservation in freezing medium (cell culture medium supplemented with 5% DMSO) and stored in a liquid nitrogen vapor phase freezer for future use.

### 2.2. Cell Culture on Gelatin-Coated Surface Interdigitated Electrode Arrays

The remaining 10% of cells (~600,000 cells/mL) was prepared to be seeded onto the surface interdigitated electrode arrays for ES. The cell suspension was centrifuged at 400 x g for 4 minutes and the supernatant was discarded. 1mL of fresh culture medium was added to the pellet for resuspension.

Before loading the cells onto the surface interdigitated electrode arrays, the glass surface of the electrodes had to be coated with a layer of gelatin to allow cell attachment. In this research, two sets of electrodes were used and they were purchased from NanoSPR. On each glass slide, there were two interdigitated electrode arrays as seen in Figure 1(B), thus there were a total of four surface interdigitated electrode arrays used in this research. Within each array, there were 20 pairs of interdigitated electrodes shown in Figure 1(A), each of them with the width of 1mm, finger length of 20µm and gap length of 50µm. The total area of each interdigitated electrode array was 2.8mm$^2$ as seen in Figure 1(C).

A 2% (w/v) gelatin solution was prepared by dissolving the gelatin powder in tissue culture grade water. Then, each glass slide surface was coated with 10µL of gelatin solution and allowed to dry for 30 minutes. Then, 10µL of the cell suspension was loaded onto each array surface and placed under the microscope for cell attachment observation. All results were recorded.
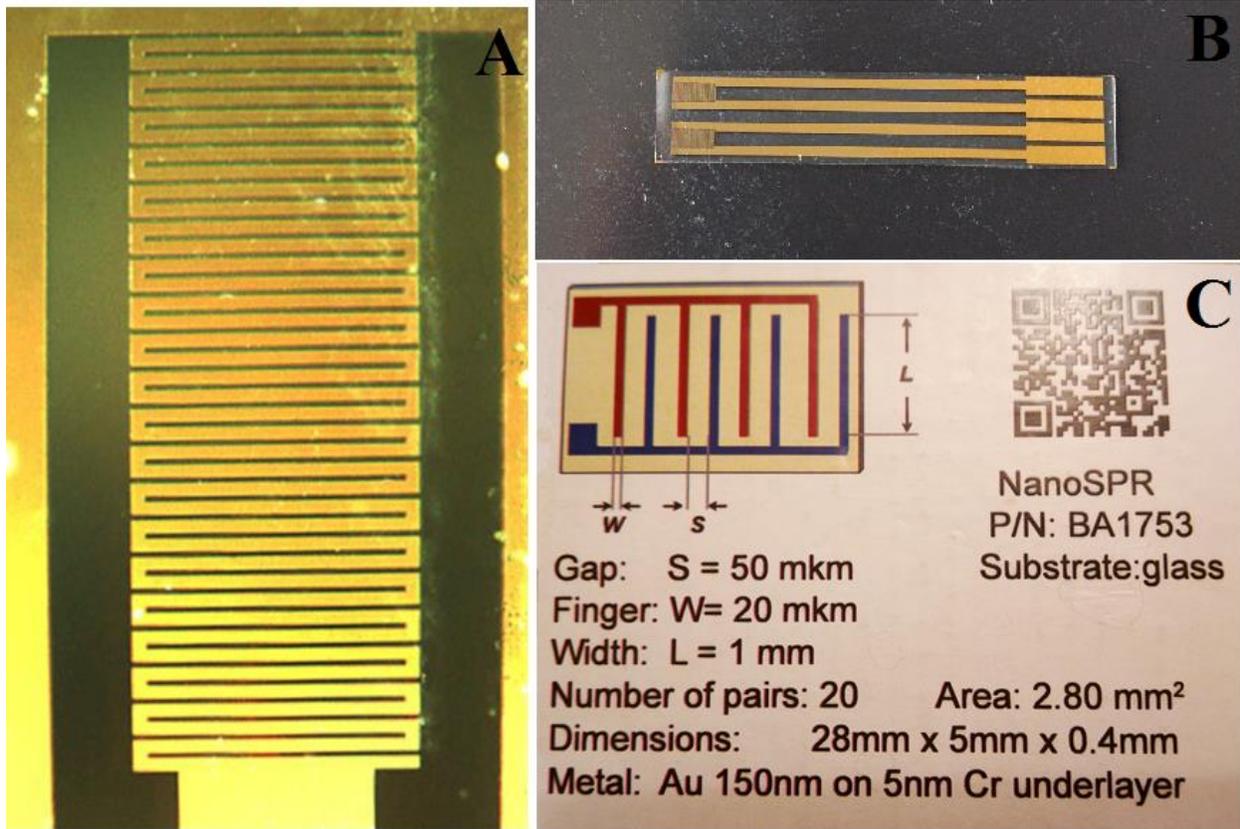
Figure 1. (A) An array of interdigitated electrodes observed under the microscope. (B) A pair of interdigitated electrode arrays (on the left) on a glass slide. (C) Dimensions of the interdigitated electrode arrays.

## 2.3. Alternating Current Electric Field Stimulation

ES of the C2C12 cells was performed by fabricating a custom setup as seen in Figure 2. After mounting the electrodes onto the 50mm opening made on the side of each the 90mm cell culture dishes where the opening is, as shown in Figure 3, a hot glue gun was used to seal the opening to prevent culture medium leakages. After that, due to their fragile nature, the other ends of the electrodes were gently placed into their respective electrode holders to be connected to the waveform generators. After the electrodes were set up firmly, 10mL of culture medium was added into each of the cell culture dish, just enough to cover the surface of the electrodes to maintain cell proliferation.

Before transferring the entire setup from the laminar flow hood into the incubator, each setup is labelled as A or B to avoid confusion. In cell culture dish A, one set of the electrode array was connected to the waveform generator A supplying a sinusoidal 30mV$_{pp}$, 1Hz signal whereas the other set which was not connected to the waveform generator was set as control. As for cell culture dish B, one set of the electrode array was connected to the waveform generator B supplying a sinusoidal 30mV$_{pp}$, 1kHz signal while the other set which was not connected to the waveform generator was set as control. The cells were then transferred and placed in the incubator set at optimal cell growth condition of 37°C with 5% of $CO_2$ for 24 hours as shown in Figure 4.
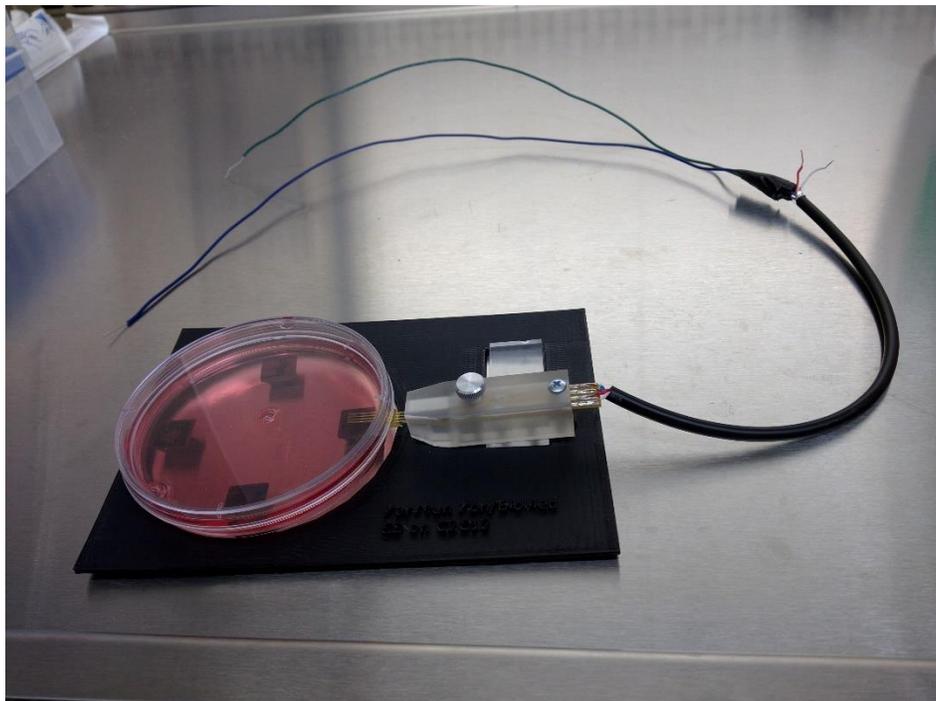


Figure 2. The complete experimental setup of the cell culture dish in the laminar flow hood.

Figure 3. The 50mm opening made on the side of the cell culture dish for electrode mount.
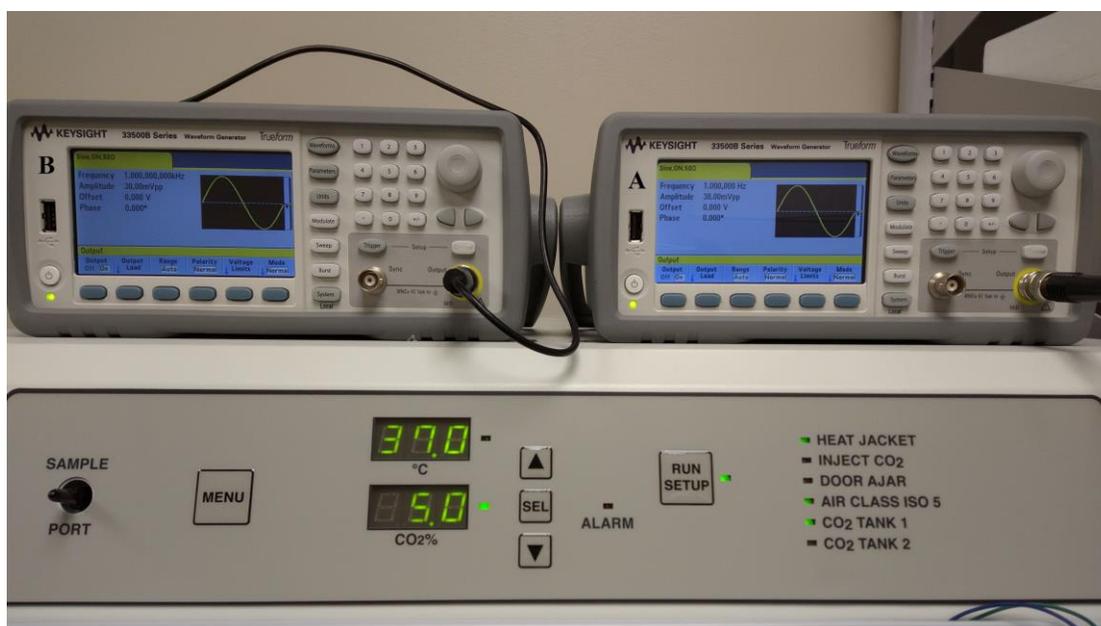


Figure 4. Waveform generator A (on the right) supplied sinusoidal 30mV$_{pp}$, 1Hz signal. Waveform generator B (on the left) supplied sinusoidal 30mV$_{pp}$, 1kHz signal to the cells. Incubator condition was set at 37°C with 5% of $CO_2$.

## 2.4. Impedance Spectroscopy

After 24 hours, electrical signals were stopped and both the culture dishes were removed from the incubator. The culture medium in the dishes were removed and they were rinsed with PBS for impedance measurement. As shown in Figure 5, Gamry Reference 3000 Potentiostat was used to measure the impedance of the cells. The impedance of the cell culture system was measured over the range of frequency of 1Hz to 1MHz at the voltage of $30mV_{pp}$. The frequency responses in magnitude ($\Omega$) were recorded. All numeral data were recorded in an Excel spreadsheet for further analysis.
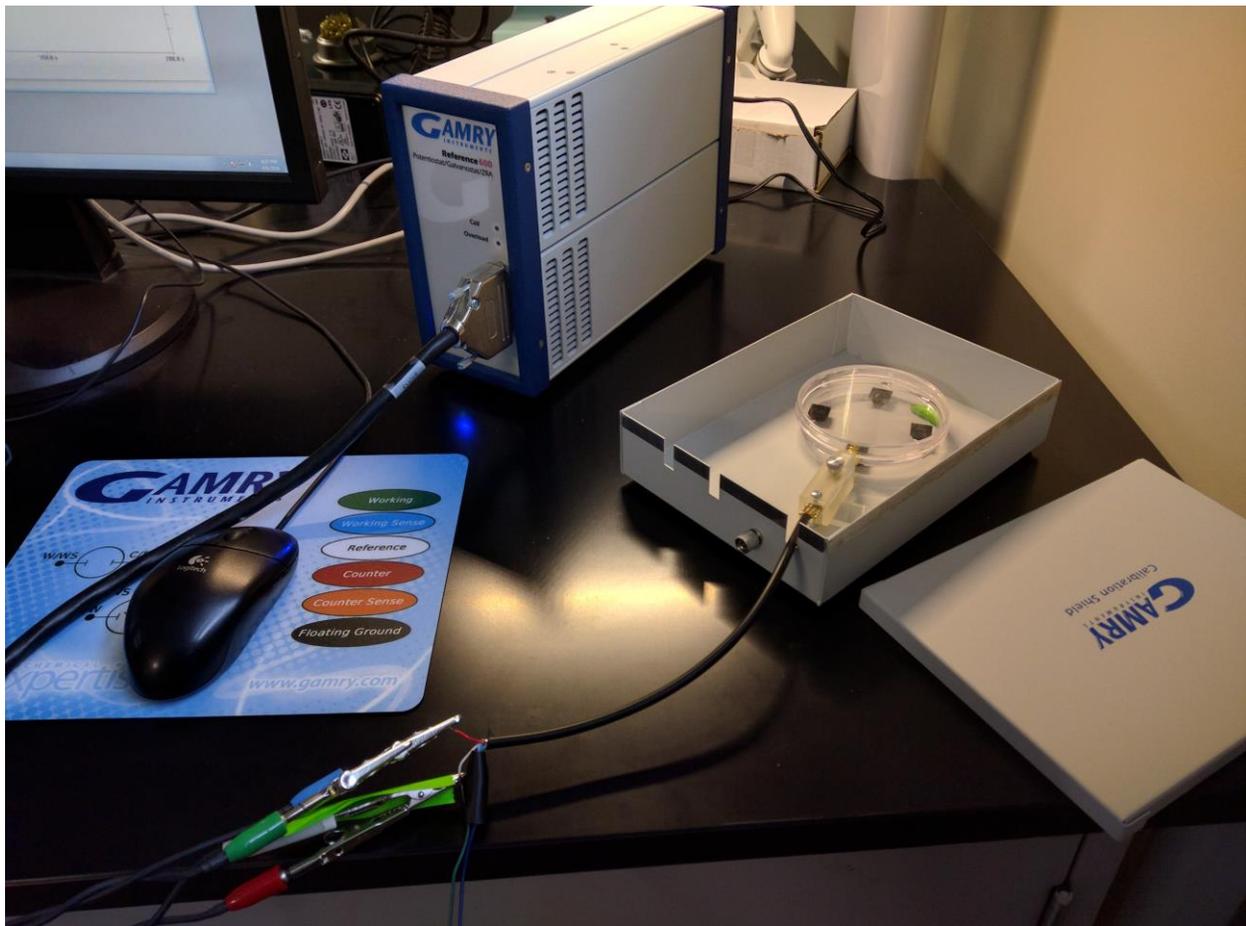


Figure 5. Impedance measurement setup using Gamry Reference 3000 Potentiostat.

## 2.5. Cell Proliferation

The electrode arrays were placed under the microscope for cell attachment and proliferation observation. All results were recorded.

## 2.6. Calcein AM Cell Viability Assay

The calcein AM dye solution was prepared by initially adding 50µL of DMSO into the frozen vial containing solid calcein AM, then 10µL of the dye solution was added to 1mL of PBS for dilution. Then, 10µL of the diluted calcein AM dye solution was added to each array surface of cell culture and they were transferred into the incubator for 30 minutes to make sure the dye permeates the cell membranes. After 30 minutes, the electrode arrays were loaded under the microscope and blue light was used to excite the fluorescent material in calcein AM dye. The results for the cell viability test were observed and recorded.

# 3. RESULTS

## 3.1. Impedance Spectroscopy
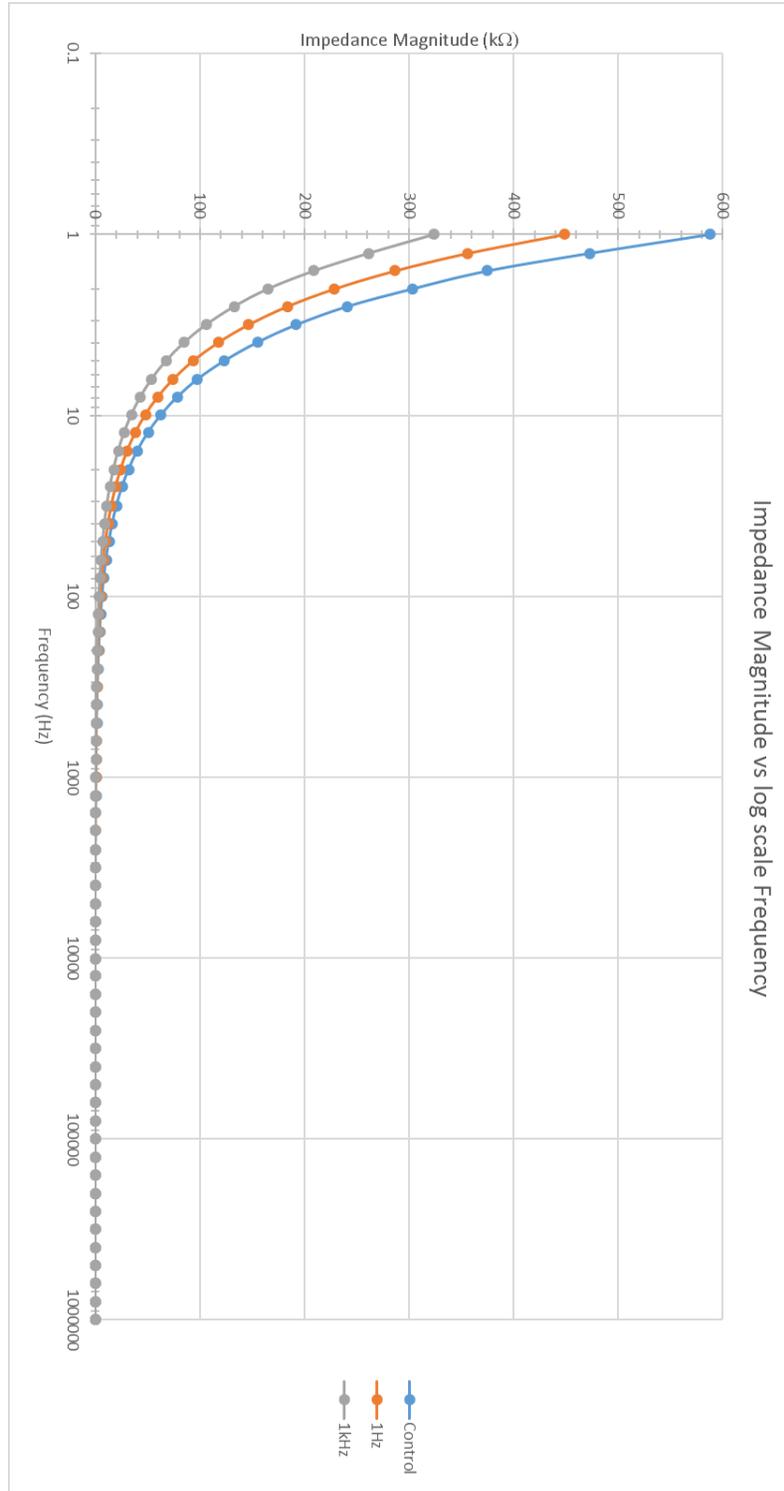


Figure 6. Plot of Impedance Magnitudes vs. log scale Frequency for all data sets.

## 3.2. Cell Proliferation
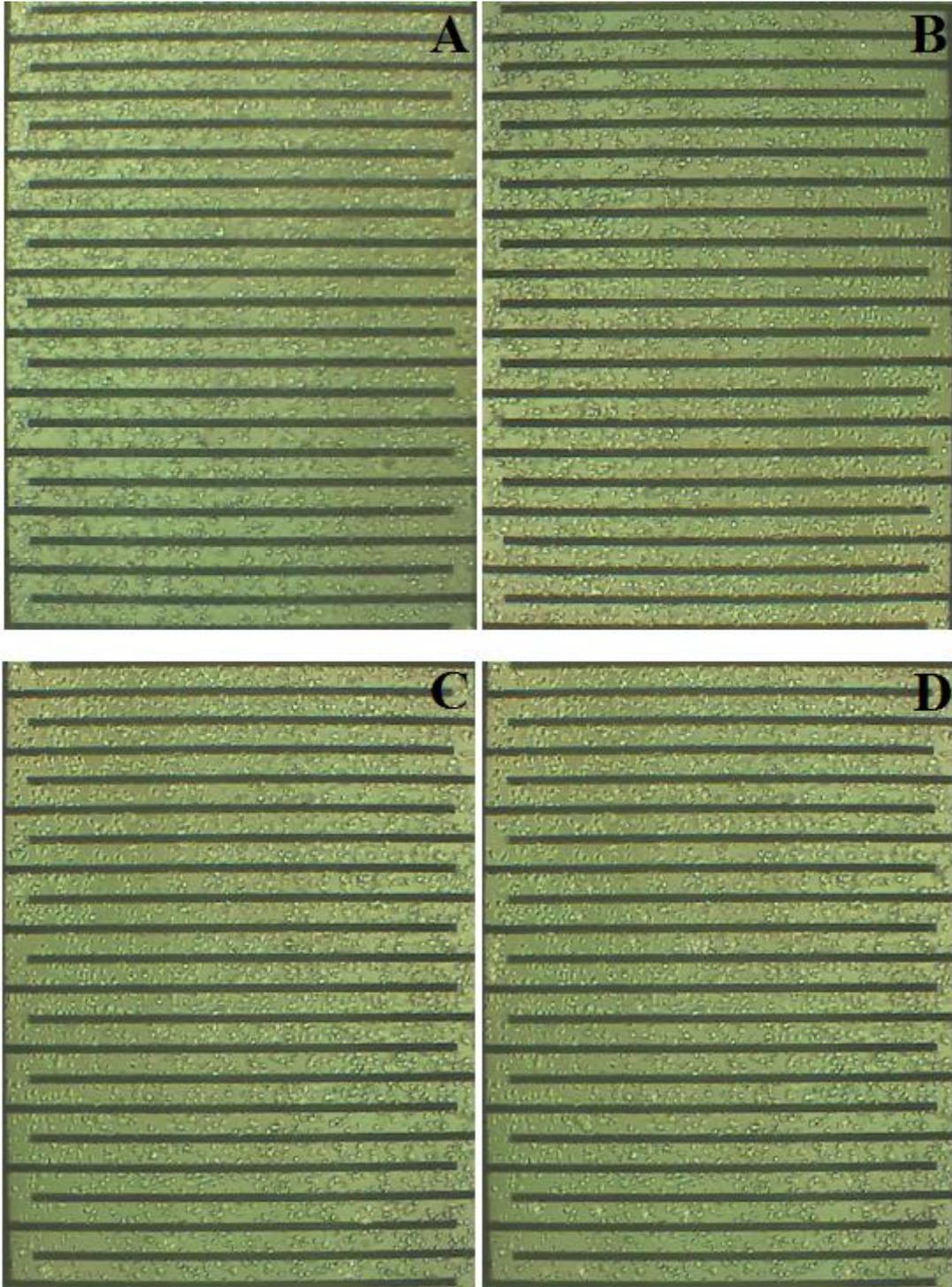


Figure 7. Cell culture on interdigitated electrode arrays before 24 hours of experiment. (A) Control from set A (B) Control from set B (C) Set A: 30mVpp, 1Hz (D) Set B: 30mVpp, 1kHz.
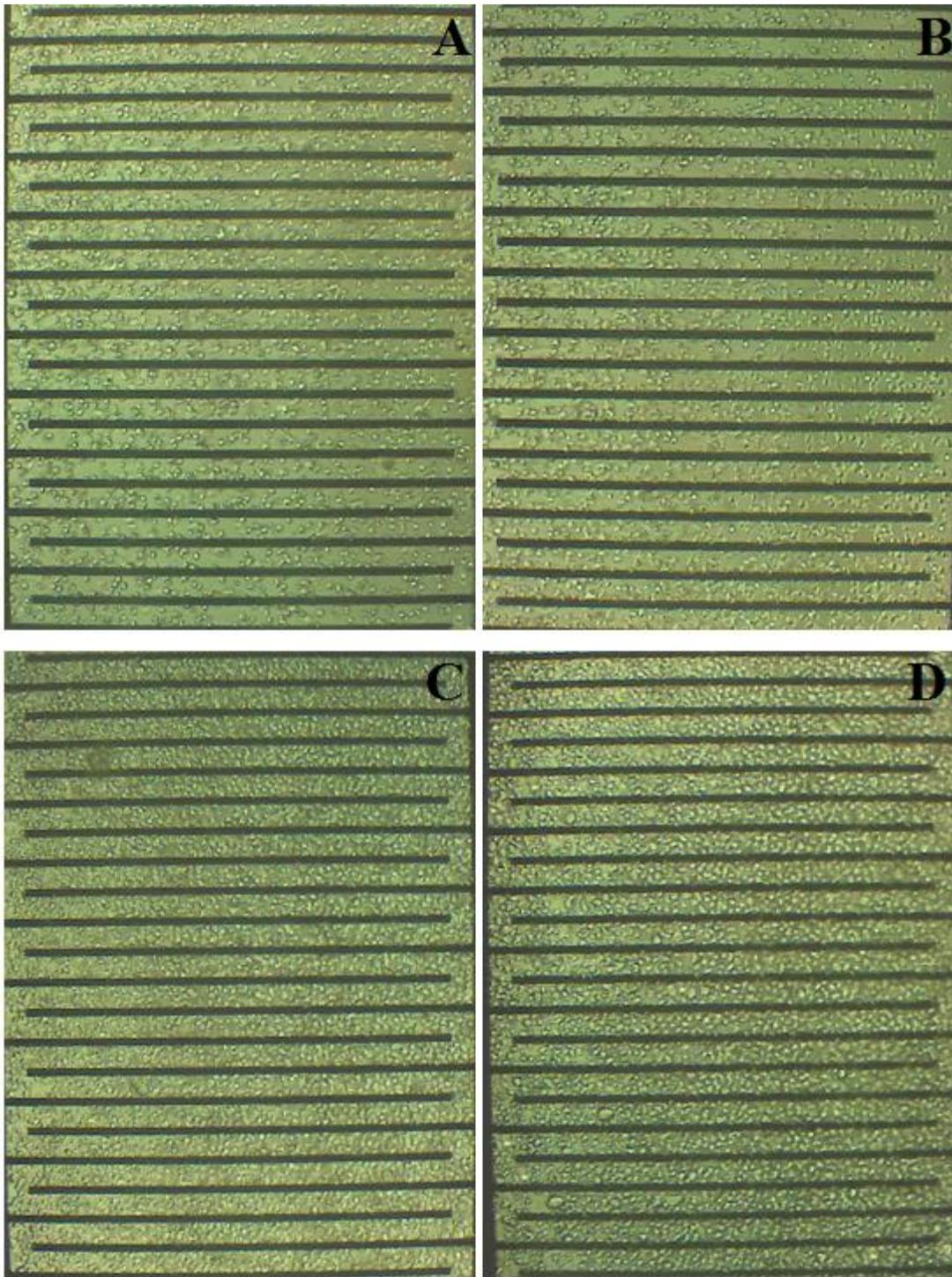
Figure 8. Cell culture on interdigitated electrode arrays after 24 hours of experiment. (A) Control from set A (B) Control from set B (C) Set A: 30mVpp, 1Hz (D) Set B: 30mVpp, 1kHz.

## 3.3. Cell Viability
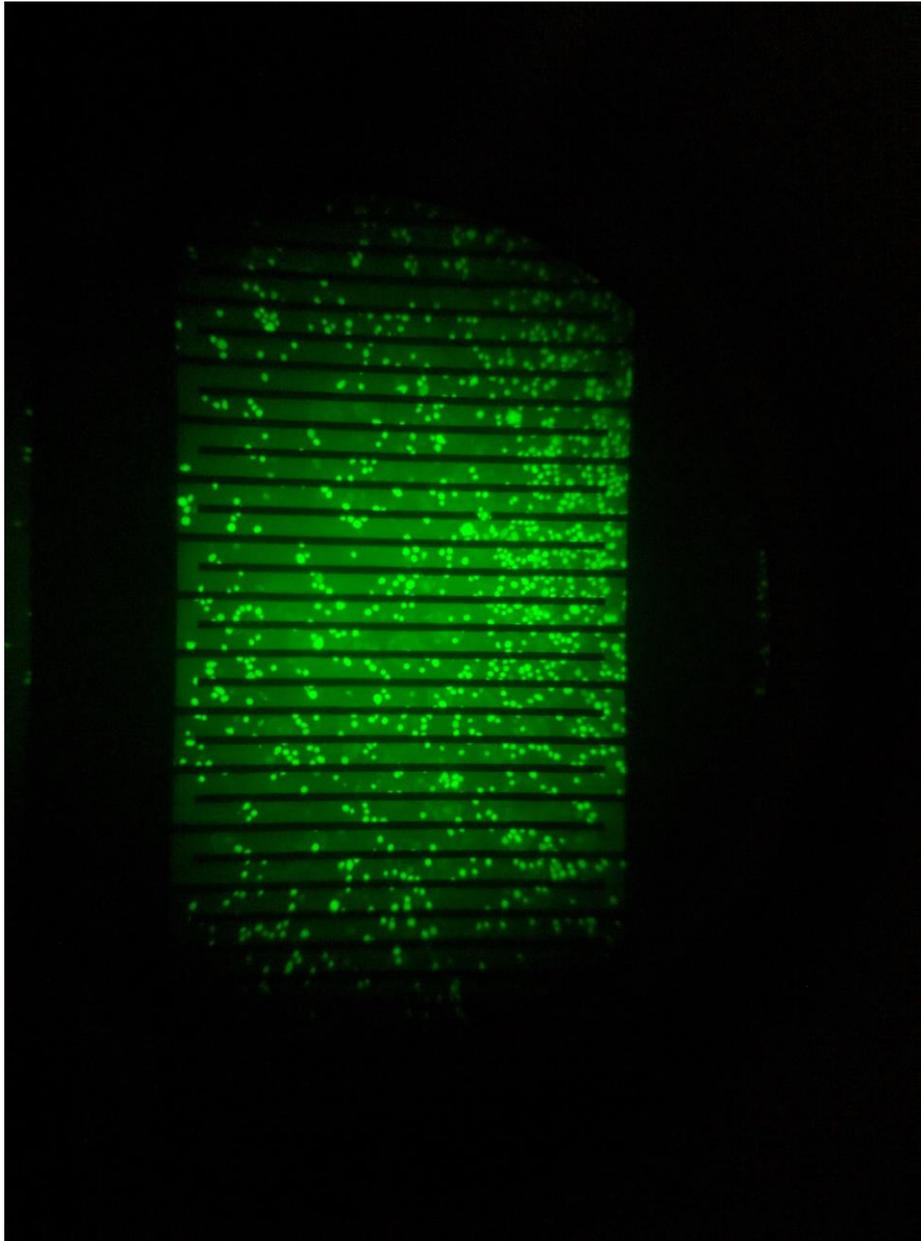


Figure 9. Calcein AM cell viability test after 24 hours of incubation (control from set A).
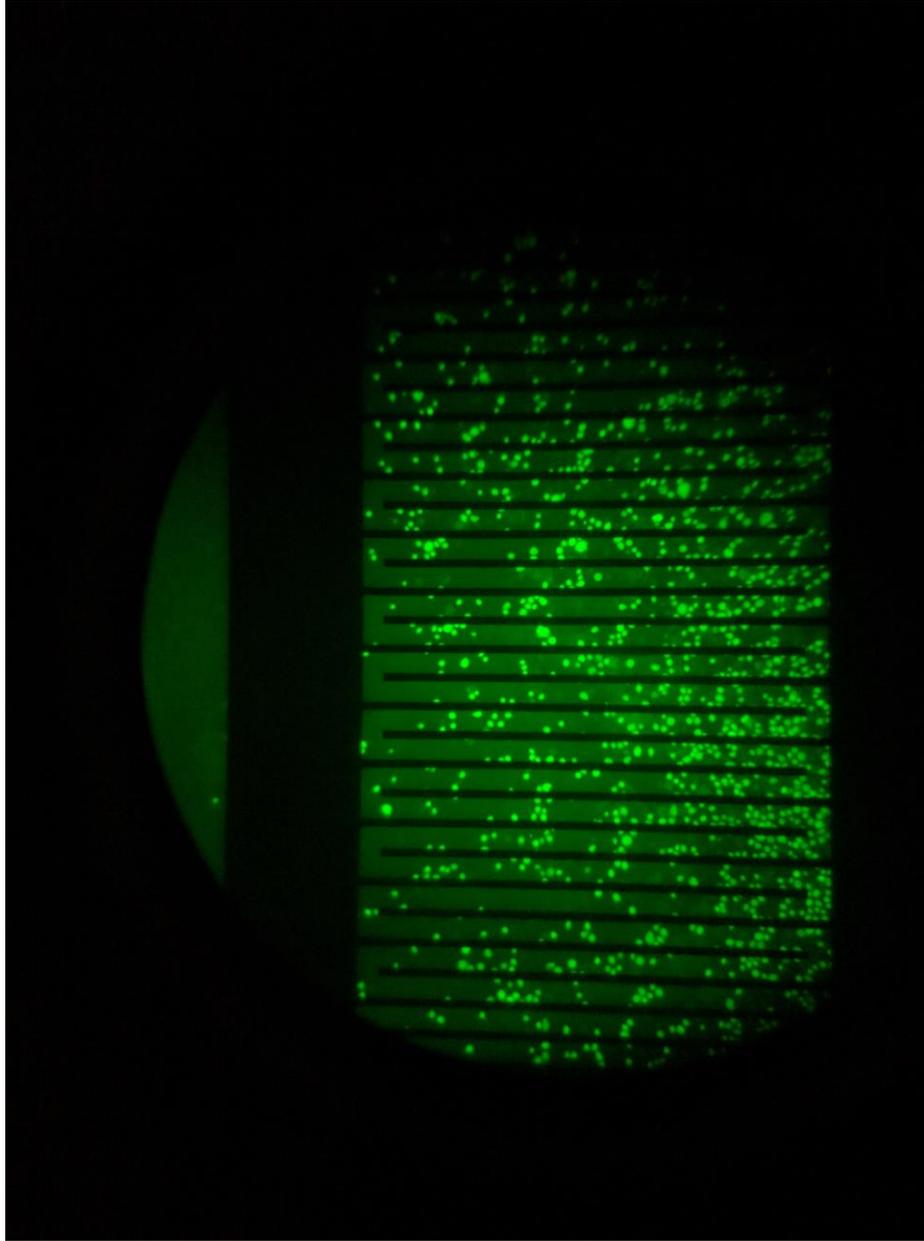
Figure 10. Calcein AM cell viability test after 24 hours of ES (set A: 30mV$_{pp}$, 1Hz).
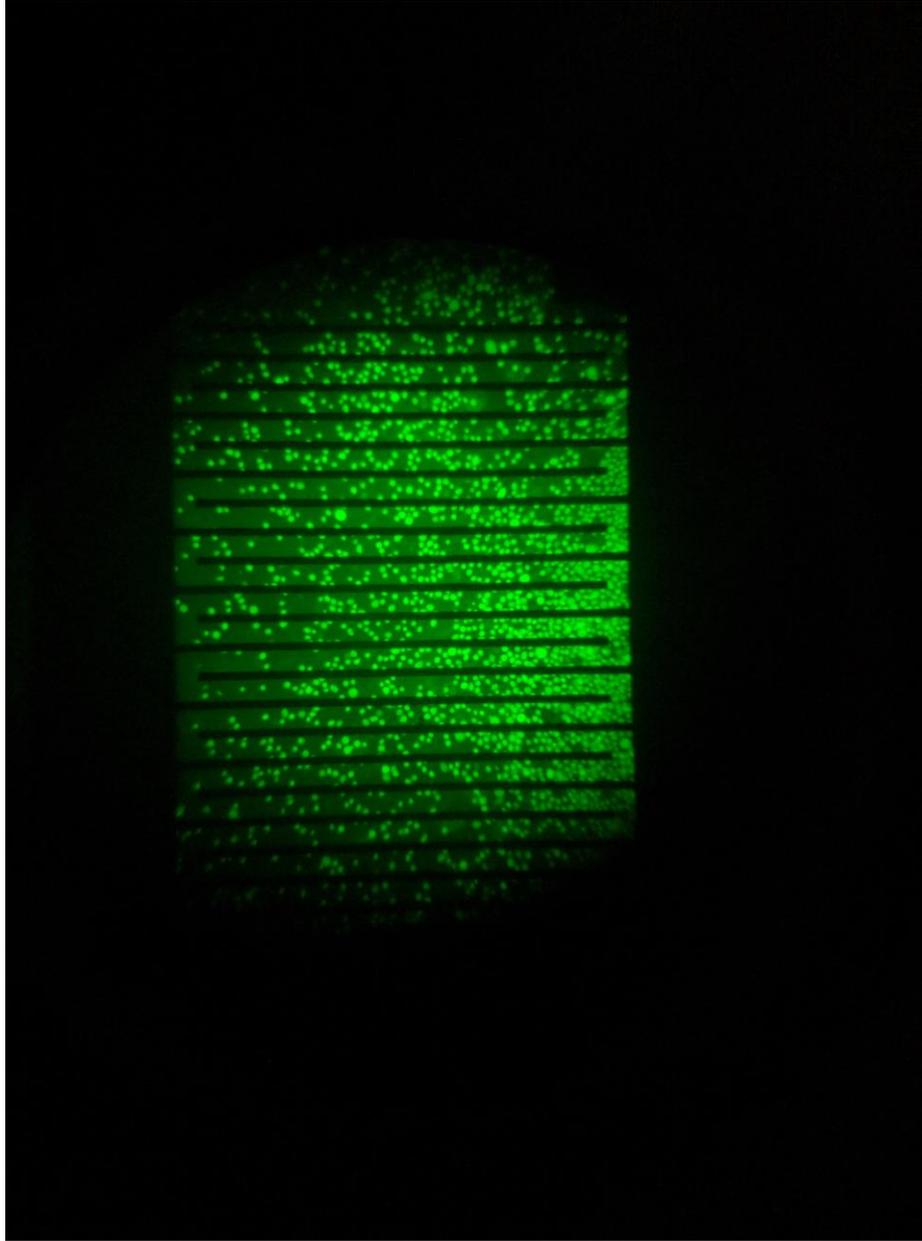
Figure 11. Calcein AM cell viability test after 24 hours ES (set B: 30mV$_{pp}$, 1kHz).

## 4. DISCUSSION

### 4.1. Impedance Spectroscopy

The impedance spectroscopy is a method to measure and analyze the impedance of adherent cells in culture and the key focus of the impedance measurement in this study was on the study of cell proliferation. The numeral data were fully recorded as shown in Appendix Table 1. The two control sets were added and averaged in order to simplify the analysis process because their measured readings were highly similar to each other.

All these plots in Figure 6 looked similar to each other in terms of shape of the curve. From the plots, it was clearly shown that at low frequency, the impedance values were at the maximum thus indicating high capacitive impedance, then the slope rapidly falls and remained constant after.

According to Heiskanen et al., cells will form an integral part on an impedance interface and modulate the capacitive properties until a complete monolayer was obtained [38]; based on the plots obtained, this possibly indicated a rapidly formed cell monolayer due to the rapid change in the curve. Every type of cell has its characteristic attachment and proliferation curve that can be manipulated by varying cell density, coating with extracellular matrix protein layers or other stimuli. One of the major complications to study those processes is to identify between adhesion, spreading and growth [24]. Wegener et al. gave an informative description for the use of a combination of resistance and capacitance to differentiate between those parameters and it was obvious how resistance and capacitance can complement each other [37]. It is vital for the study of attachment and spreading to better understand the effect of the measurement frequency, because the electrical field causes polarization of the membrane of the cells in suspension, which then forces the current to flow around the cells. When the cells adhere, they start to limit the

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current flow by covering all over the electrode surface and capacitance decreases in a linear fashion with the ratio of open area on the electrode array. Szulcek et al. introduced two other parameters to quantify adhesion and electrode coverage: $t_{1/2}$ (half maximum capacitance), which is the time until half of the electrode is covered with cells and the slope of the capacitance curve as the rate of cell spreading and proliferation [24].

## 4.2. Cell Proliferation

Observations were made under the microscope before the cells were transferred into the incubator and the C2C12 cells were able to attach and spread on the surface of the interdigitated electrodes as seen in Figure 7(A), (B), (C) and (D). The size of the cells was normal and approximately 20µm in diameter.

After 24 hours, as seen in Figure 8(A), the cell culture from set A control which was not exposed to any electric field stimulation have almost the same amount of cells as seen in Figure 7(A) which was taken before the 24 hours of incubation period. Similarly, the cell culture from set B control as shown in Figure 8(B) has similar cell morphology and growth rate as cell culture from set A. From observation, these control sets were considered to have a very low rate of proliferation.

Next, Figure 8(C) showed set A of cell culture which was stimulated by a sinusoidal 30mV$_{pp}$, 1Hz signal. It was clearly observed that the cells have increased in great number when compared to Figure 7(C) which was taken before the 24 hours of ES. This set of culture is considered to have a high rate of proliferation but the morphology and size of the cells remained consistent as before.

Then, Figure 8(D) showed set B of cell culture which was stimulated by a sinusoidal 30mVpp, 1kHz signal. Again, similar to set A, the cells have increase in great number when

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compared to Figure 7(D) which was taken before the 24 hours of ES. This set of culture is also considered to have a high rate of proliferation. However, it was noted that there were a few large sized cells in this set of culture and the logical explanation for this was the cells were in the process of separating into two or more cells. This occurrence is normal and it is called cell division.

After all the observation on cell size, morphology and number prior to and after the 24-hour period, there is an obvious difference in the rate of cell proliferation between the control sets and the electrically stimulated sets. The electrically stimulated sets (A and B) have a very high rate of cell proliferation as after ES, the cells covered almost the entire surface of the electrode arrays. In contrast, the control sets have a very low proliferation rate as the cells remained consistent in size and number after the 24-hour incubation period. This directly indicated that the ES promotes cell proliferation in C2C12 cell culture. Many past research studies [25]–[28] have similar positive results on cell proliferation after electric field stimulation, however the rate of proliferation differs according to the type of cells and electrical stimulus used in the studies.

### 4.3. Cell Viability

The calcein AM cell viability test was carried out after the 24-hour period to understand if ES affects the aspect of cell viability. Calcein AM is a cell-permeant dye which is commonly used to determine viability of cells in most eukaryotic cells. The nonfluorescent calcein AM would be converted to green-fluorescent calcein in live cells due to the acetoxymethyl ester hydrolysis by intracellular esterases. In short, viable cells would appear bright green fluorescent under the microscope.

Figure 9 showed the cell viability of cells from set A control, while Figure 10 showed the cell viability from set A and lastly Figure 11 showed the cell viability from set B. As shown in the figures above, both cell cultures from control set A and ES set B have high cell viability. However, for the cell culture from ES set A which the cells have increased in great number due to high rate of cell proliferation did not have that high of a cell viability. The amount of bright green fluorescent dots shown in Figure 10 was almost 50% of the number of proliferated cells in Figure 8(C). A possible explanation for this observation would be due to human error, such as that of the calcein AM dye solution was not spread evenly on the cell monolayer, causing an uneven rate of absorption of the solution by the viable cells.

# 5. CONCLUSION

This research showed the main advantage of utilizing interdigitated electrodes is that cells can be imaged easily during short-term stimulation. With the miniaturization of cell culture system integrated with ES, this research proved to save a lot of resources; namely costs, reagents, cells and time. On top of that, this research provides an efficient method and its results should shed new lights on ES related research, especially in the field of muscle tissue regenerative therapy as it was shown that cell proliferation of C2C12 muscle cells increased significantly in a short period of time after being exposed to 6V/cm at 1Hz and 1kHz sinusoidal signals.

Lastly, ES is very important in order to improve and maintain physiological functions, such as the contractility of engineered muscle tissues for the field of regenerative medicine [29]–[32]. Other interesting applications to manipulate the engineered muscle tissues may be used as drug delivery pumps in muscle tissues for long-term and controlled drugs loaded within muscle tissues [33]–[36]. Finally, more collaborative actions among the biology, clinical and engineering disciplines may further improve potential applications of ES and enhance the use of this area of study for muscle cell and tissue engineering applications. The exchange of knowledge and experience from different specialized disciplines could lead to advances in using electric field stimulation in regulating muscle cell behavior.

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# APPENDIX. COMPLETE NUMERAL DATA FOR IMPEDANCE MESUREMENTS

| Frequency (Hz) | Z_magnitude (control set A) (Ω) | Z_magnitude (control set B) (Ω) | Z_magnitude (control average) (Ω) | Z_magnitude (set A: 1Hz) (Ω) | Z_magnitude (set B: 1kHz) (Ω) |
|---|---|---|---|---|---|
| 9.99E-01 | 5.87E+05 | 5.89E+05 | 5.88E+05 | 4.49E+05 | 323950 |
| 1.27E+00 | 4.75E+05 | 4.71E+05 | 4.73E+05 | 3.57E+05 | 261450 |
| 1.59E+00 | 3.76E+05 | 3.74E+05 | 3.75E+05 | 2.87E+05 | 208900 |
| 2.00E+00 | 3.01E+05 | 3.05E+05 | 3.03E+05 | 2.29E+05 | 165050 |
| 2.50E+00 | 2.41E+05 | 2.41E+05 | 2.41E+05 | 1.84E+05 | 132900 |
| 3.16E+00 | 1.92E+05 | 1.92E+05 | 1.92E+05 | 1.47E+05 | 105800 |
| 3.95E+00 | 1.55E+05 | 1.55E+05 | 1.55E+05 | 1.18E+05 | 85190 |
| 5.01E+00 | 1.23E+05 | 1.23E+05 | 1.23E+05 | 9.34E+04 | 67475 |
| 6.32E+00 | 9.77E+04 | 9.77E+04 | 9.77E+04 | 7.44E+04 | 53785 |
| 7.95E+00 | 7.81E+04 | 7.82E+04 | 7.82E+04 | 5.95E+04 | 43020 |
| 9.93E+00 | 6.29E+04 | 6.29E+04 | 6.29E+04 | 4.79E+04 | 34605 |
| 1.24E+01 | 5.07E+04 | 5.06E+04 | 5.07E+04 | 3.85E+04 | 27855 |
| 1.58E+01 | 3.99E+04 | 3.99E+04 | 3.99E+04 | 3.04E+04 | 21925 |
| 1.99E+01 | 3.20E+04 | 3.20E+04 | 3.20E+04 | 2.44E+04 | 17575 |
| 2.49E+01 | 2.56E+04 | 2.56E+04 | 2.56E+04 | 1.95E+04 | 14075 |
| 3.17E+01 | 2.03E+04 | 2.03E+04 | 2.03E+04 | 1.55E+04 | 11145 |
| 3.97E+01 | 1.63E+04 | 1.63E+04 | 1.63E+04 | 1.24E+04 | 8928.5 |
| 4.99E+01 | 1.31E+04 | 1.31E+04 | 1.31E+04 | 9.94E+03 | 7158 |
| 6.33E+01 | 1.04E+04 | 1.04E+04 | 1.04E+04 | 7.88E+03 | 5672.5 |
| 7.90E+01 | 8.36E+03 | 8.36E+03 | 8.36E+03 | 6.36E+03 | 4575 |
| 1.00E+02 | 6.63E+03 | 6.63E+03 | 6.63E+03 | 5.04E+03 | 3623.5 |
| 1.26E+02 | 5.34E+03 | 5.34E+03 | 5.34E+03 | 4.06E+03 | 2919.5 |
| 1.58E+02 | 4.27E+03 | 4.27E+03 | 4.27E+03 | 3.25E+03 | 2332 |
| 1.99E+02 | 3.43E+03 | 3.43E+03 | 3.43E+03 | 2.61E+03 | 1875 |
| 2.52E+02 | 2.73E+03 | 2.73E+03 | 2.73E+03 | 2.07E+03 | 1489 |
| 3.16E+02 | 2.20E+03 | 2.20E+03 | 2.20E+03 | 1.68E+03 | 1202.5 |
| 3.98E+02 | 1.77E+03 | 1.76E+03 | 1.77E+03 | 1.34E+03 | 964 |
| 5.06E+02 | 1.41E+03 | 1.41E+03 | 1.41E+03 | 1.07E+03 | 767.35 |
| 6.28E+02 | 1.15E+03 | 1.14E+03 | 1.15E+03 | 8.73E+02 | 626.05 |
| 7.97E+02 | 9.12E+02 | 9.12E+02 | 9.12E+02 | 7.00E+02 | 503.4 |
| 9.98E+02 | 7.41E+02 | 7.39E+02 | 7.40E+02 | 5.69E+02 | 410.25 |
| 1.27E+03 | 5.94E+02 | 5.93E+02 | 5.94E+02 | 4.60E+02 | 332.4 |
| 1.58E+03 | 4.83E+02 | 4.83E+02 | 4.83E+02 | 3.80E+02 | 275.15 |
| 1.98E+03 | 3.95E+02 | 3.95E+02 | 3.95E+02 | 3.15E+02 | 228.95 |
| 2.53E+03 | 3.18E+02 | 3.18E+02 | 3.18E+02 | 2.60E+02 | 189.9 |

| Frequency (Hz) | Z_magnitude (control set A) (Ω) | Z_magnitude (control set B) (Ω) | Z_magnitude (control average) (Ω) | Z_magnitude (set A: 1Hz) (Ω) | Z_magnitude (set B: 1kHz) (Ω) |
|---|---|---|---|---|---|
| 3.17E+03 | 2.63E+02 | 2.63E+02 | 2.63E+02 | 2.21E+02 | 162.45 |
| 3.98E+03 | 2.19E+02 | 2.19E+02 | 2.19E+02 | 1.91E+02 | 141.3 |
| 5.02E+03 | 1.84E+02 | 1.84E+02 | 1.84E+02 | 1.67E+02 | 125.35 |
| 6.33E+03 | 1.58E+02 | 1.58E+02 | 1.58E+02 | 1.49E+02 | 113.4 |
| 8.02E+03 | 1.37E+02 | 1.37E+02 | 1.37E+02 | 1.36E+02 | 104.45 |
| 1.01E+04 | 1.22E+02 | 1.23E+02 | 1.23E+02 | 1.26E+02 | 98.035 |
| 1.26E+04 | 1.11E+02 | 1.12E+02 | 1.12E+02 | 1.18E+02 | 93.165 |
| 1.59E+04 | 1.03E+02 | 1.03E+02 | 1.03E+02 | 1.12E+02 | 89 |
| 2.00E+04 | 9.65E+01 | 9.69E+01 | 9.67E+01 | 1.06E+02 | 85.23 |
| 2.52E+04 | 9.13E+01 | 9.17E+01 | 9.15E+01 | 1.02E+02 | 81.575 |
| 3.16E+04 | 8.69E+01 | 8.73E+01 | 8.71E+01 | 9.67E+01 | 77.89 |
| 3.99E+04 | 8.29E+01 | 8.32E+01 | 8.31E+01 | 9.17E+01 | 73.7 |
| 5.02E+04 | 7.90E+01 | 7.93E+01 | 7.92E+01 | 8.65E+01 | 69.26 |
| 6.31E+04 | 7.51E+01 | 7.54E+01 | 7.53E+01 | 8.10E+01 | 64.52 |
| 7.95E+04 | 7.08E+01 | 7.11E+01 | 7.10E+01 | 7.53E+01 | 59.77 |
| 1.00E+05 | 6.66E+01 | 6.66E+01 | 6.66E+01 | 6.93E+01 | 55.14 |
| 1.26E+05 | 6.21E+01 | 6.23E+01 | 6.22E+01 | 6.38E+01 | 51.145 |
| 1.59E+05 | 5.76E+01 | 5.78E+01 | 5.77E+01 | 5.85E+01 | 47.58 |
| 2.00E+05 | 5.34E+01 | 5.36E+01 | 5.35E+01 | 5.39E+01 | 44.76 |
| 2.51E+05 | 4.97E+01 | 4.98E+01 | 4.98E+01 | 5.00E+01 | 42.56 |
| 3.16E+05 | 4.65E+01 | 4.66E+01 | 4.66E+01 | 4.69E+01 | 40.935 |
| 3.98E+05 | 4.40E+01 | 4.41E+01 | 4.41E+01 | 4.45E+01 | 39.815 |
| 5.01E+05 | 4.20E+01 | 4.21E+01 | 4.21E+01 | 4.28E+01 | 39.05 |
| 6.31E+05 | 4.06E+01 | 4.06E+01 | 4.06E+01 | 4.15E+01 | 38.52 |
| 7.94E+05 | 3.95E+01 | 3.95E+01 | 3.95E+01 | 4.06E+01 | 38.17 |
| 1.00E+06 | 3.87E+01 | 3.88E+01 | 3.88E+01 | 4.00E+01 | 37.985 |