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High Speed Switching for Plasma Based Electroporation

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High Speed Switching for Plasma Based Electroporation

by

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Dedication

I would like to dedicate my work to my professor, Dr. Andrew Hoff and to my family.

Acknowledgments

I would like to sincerely thank, my professor, Dr. Hoff, who helped me through the thesis as smoothly as possible. I thank him for believing in me for getting work completed, for having faith in me, and for supporting me through this. I thank him for being the constant source of inspiration and guiding me through this, as and when the help was required. I cannot imagine any other professor being my thesis advisor, other than him.

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Abstract

Ever since mankind has started identifying the diseases, extensive work has been done, and pathologies are written identifying the cure for them. As technology progressed, the development in the field of pathologies also did. Thus, finding innovative and efficient ways to get the 'cure'. With the advancement in drugs, the delivery methods for these drugs also have advanced. One such delivery method is gene therapy. This method is beneficial when genes are to be used to prevent diseases. Gene therapy has been proven effective for treating a wide range of conditions, such as cancer, heart diseases, and diabetes.

Gene therapy is used in various environments using different types of delivery methods. The environment can be in-vivo, in-vitro, or in-situ. Various delivery methods can be carried out, mainly classified into two groups, viral and non-viral vectors. When the viruses are used to carry the protein in the body, this type of drug delivery is termed as viral vectors. Non-viral vectors are a drug delivery method when naked DNA or physical processes transport the protein to the body.

In this study, we will be discussing a type of non-viral drug delivery known as electroporation. Electroporation is a method that uses a pulse of electric field to open the pores in the cell membrane to let the drug or protein inside the cell for delivery. The device would create an electric field resulting in corona discharge (ultimately forming a plasma), which would help open the cell membrane's pores.

The device is divided into two parts, one that controls the input of high voltage on the required area, and the second controls the electric field through pulses on the surface. The device can take up to 10kV and has been tested under various circumstances.

This study includes the impact of various parameters on the electric field, and how by controlling it, we can perform the delivery successfully. Varying the distance, with varying voltages and then testing has been observed and tabulated. The experiment has been committed to prove its supposed practical implementation in a biological environment to carry out electric field mediated gene delivery.

Chapter 1: Introduction

In 1972, Dr. Friedmann suggested that there might be a way to modify mammalian genes to cure gene-related diseases [34]. This pathology was further noted down as gene therapy. Following this discovery, many types of research were carried out to test its validity and to discover various methods to carry the procedure out. One of which is discussed here and is electroporation.

In the late ’70s, a new theory was coined that could carry out gene therapy by using the electrical aspects of the cell. To use the electric field to open pores in the cell wall, let the gene or drug pass through the wall and into the cell. Although the exact mechanisms of interactions between the electric fields and cells are unknown, various combinations such as weak low-voltage, low-current electric fields applied for long durations and high-voltage short electric fields used should durations, are some of the protocols most known [1,2,3,7,9,14,19,25,33]. With the procedure and type of delivery taken into consideration, the design constraints for the device are set to get the most efficient output.

Electric pulses play two vital roles: permeabilization target cells and the second ensuring the delivery of DNA towards and across the permeabilized membrane by electrophoresis [3,7,9,10,11,13,14,19,23,33]. Electrophoresis is a method that involves the motion of dispersed particles in a fluid under the influence of a uniformly distributed electric field. So, the main parameter of the protocol is the time duration of the electric field on the object under test; either less or more time can result in the outcome, which will not be favorable to the work.

The protocol also depends on which type of procedure is to be conducted, reversible or irreversible electroporation. As the name suggests, reversible electroporation would be when the cell could repair itself after the process has been performed. While irreversible would simply mean that there would be cell ablation [6,14,25]. Determining the most appropriate protocol with the control of the other parameters (such as electric field intensity, the polarity of the electric field, the diameter of the tip of the needle used to control the electric field) helps get the most efficient outcome. Protocol, here, is a set of time-defined operations to be executed while the experiment is being performed by the device used.

Selecting the environment also plays a vital role in deciding which type of delivery will be followed, as do the vectors involved. The background of electroporation and gene therapy is highly recommended so that while designing the device, all parameters are taken into consideration.

Chapter 2: Gene Therapy

The European Medicines Agency (EMA) defines that a gene therapy product as a therapeutic product that can be characterized through following points, (a.) it contains an active substance that consists of a recombinant nucleic acid dispensed to human beings by regulating, repairing, replacing, adding, or deleting a genetic sequence; (b.) its diagnostic effect relates directly to the recombinant nucleic acid or the sequence it contains [4]. It has been a couple of decades when this theory was coined, and since then, this therapeutic approach has been proven beneficial for various diseases. With all the advances that have been made in this domain, this novel form of molecular medicine will have a significant impact on human health in the coming period [6].

Gene therapy is classified into two cell types, (a.) somatic cell, where the therapeutic genes will not be transferred to the offspring; (b.) germline, where there is a certain probability that the therapeutic genes may be transferred to the offspring [4,8,20,22,32]. Somatic cells have been proven a safe option to test and study the effects of gene therapy over the option of the germline. Currently, there have been certain limitations imposed on testing on the germline, as the imminent risk of modified gene replication in the offspring. Hence, each study and observation recorded are mainly acquired from the somatic cells.

The gene delivery has been majorly classified into two categories, namely, viral, and non-viral vectors [20,22,32]. The type of delivery method depends on specific parameters of the procedure that needs to be conducted. Selecting the delivery is the essential part of gene therapy for sustained and efficient results.

2.1 Viral Vectors

The advantage of using a virus as a delivery source is that it can avoid immunosurveillance by an infected host while delivering the protein. Several types of DNA (Adenovirus, Herpes virus) and RNA (Lentivirus, Retro Virus) are being used to carry out the function [20]. Due to this ability, it has a high transduction rate. But with this advantage, it comes with various limitations, one of the important one being the size of specific nucleic acids that cannot be transported using this method [32].

2.2 Non-Viral Vectors

Non-viral systems comprise physical and chemical systems including, chemical methods, such as cationic liposomes and polymers, or physical methods, such as electroporation [7]. In comparison to the viral vectors, they are proven to be less toxic and immunogenic. This method also ensures the protection against the degradation of genes [23]. There are various methods of delivering a protein using non-viral vectors. The natural way of delivery is, injecting naked DNA. Physical methods include electroporation, gene gun, sonoporation, and many more [4,15,21,23,27,33,34]. Chemical methods include the use of oligonucleotides, lipoplexes, polymersomes, polyplexes, inorganic nanoparticles, and cell-penetrating peptides [32].

As time and technology progress, gene therapy and its related delivery methods would also find and discover innovative, sustainable, and efficient ways to get the procedure done to provide the best outcome.

Chapter 3: Electroporation

The concept of electroporation has been known for decades but has recently started receiving attention to manipulate cells and tissues [7]. Because viral methods face several problems, a physical method such as local tissue electroporation can be used as an alternative method [3,5,9,21]. Frankly, our knowledge is phenomenological, as it is based on measurements of electrical currents through planar bilayer membranes (BLM) under the influence of a strong electric field and on delivery of molecules into (or out of) the cells subjected to electric field pulses [8]. The term 'electropermeabilization' is commonly used in explaining the phenomenon behind the experiments conducted using large molecular transport into (or out of) the cell membranes [7,9]. Much of what is observed can be called electropermeabilization of the membrane, i.e., a rapid increase of permeability (or conductivity) and (in some cases) mechanical rupture [7].

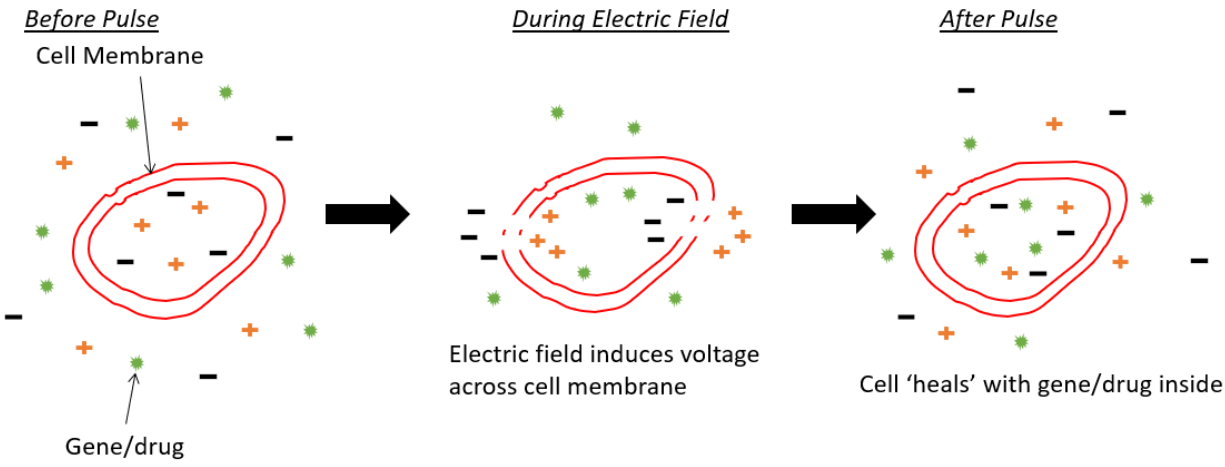

Figure 1: Process of Electroporation.

The essential points for electroporation, as shown in figure 1, include (a.) application of electrical pulses; (b.) charging of BLM; (c.) fast localization of structural rearrangements within the membrane; (d.) transitions to water-filled membrane structures, which creates aqueous pathways; (e.) huge increase in ionic and molecular transport [9]. Hence, understanding the concept of electroporation, mechanism, and its use is essential to carry out the process successfully. A typical experimental protocol involves suspending cells in an aqueous medium containing water-soluble molecules of interest, applying one or pulses, waiting for cell membrane recovery ('resealing'), and then removing the cells for use or study [9]. The design constraints for the experimental procedures (or protocols, explained in chapter 1) are decided to aim to transport foreign molecules or ions into the cells. These design constraints depend on the environment that the electroporation will be performed in, in-vivo, in-vitro.

A typical in-vitro apparatus involves parallel plane electrodes contacting an aqueous electrolyte with suspended cells and laboratory apparatus by introducing DNA [9]. At the same time, in-vivo applications include the local delivery of potent but relatively impermeable anticancer drugs into solid tumors [9].

3.1 Electroporators

There are various electroporators types of electroporators available. They are designed as per the requirement of the procedure to be implemented. These requirements mainly include temperature ratings, voltage ratings, and the type of electrical pulses to be provided. As the requirement changes, the type of the component used to build these devices also changes. In this work, we have mainly focused on building an electroporator that would be voltage rating specific. This means that the voltage provided by the electroporator during the experiment would be particular to a certain range.

In initial days of the experimentation, these electroporators were designed using marx generators, thyratrons, blumelin network, etc. [11-13]. With their excellence in high voltage switching, provided at the time, they had low lifetime, were bulky to use and had to be used with extreme caution and precision. When the semiconductor industry bloomed, these switching components were then substituted by MOSFETs, IGBTs and other transistors which could handle high voltage. With these devices, an assurance for a better lifetime, considerable reduction in power loss and less damage to the device can be considered [11-13].

3.2 Commercially Available Electroporators

Depending on the requirements, these electroporation generators can be constructed by the user, or the user can access the commercially available electroporators. These commercially available electroporators have been designed to work in various biological environments, such as, in-vivo and in-vitro. There are roughly less than ten companies who design these clinical electroporators, and has certain protocols provided with each. These electroporators provide a range of type pulses and pulse widths. One of the most widely used electroporators is designed by BTX. These protocols can be customized also as per the requirements.

These electroporators have been designed with a certain protocol which when implanted should provide expected results. Generally, these electroporators are not used by researchers, as each experiment would require a different set of protocols. These electroporators would be ideal in a scenario where the given protocol can be implanted for experimental purposes.

Chapter 4: Hypothesis and Research Aim

4.1 Hypothesis

In the work explained, corona discharge is used to create an electric field induced environment to carry out electric field mediate gene delivery. The basis of corona discharge related electric field processes for drug or gene delivery are two-fold, 1) the charge density on a substrate (cells or tissue) surface produces the field. And 2) temporal modulation of the E, or pulsing, as described throughout most of the archival literature on the topic [15].

But there are various factors to deal with when relying solely on corona flux for experimentation. Corona is basically flow of charged particles. The problem with using only corona is that, after a certain density is deposited on a substrate, the field due to this charge would repel further charges from approaching and depositing on the same space. This phenomenon effectively limits the potential a surface may be charged to, limiting the electric field. Modulation of this surface charge is the basis of this work.

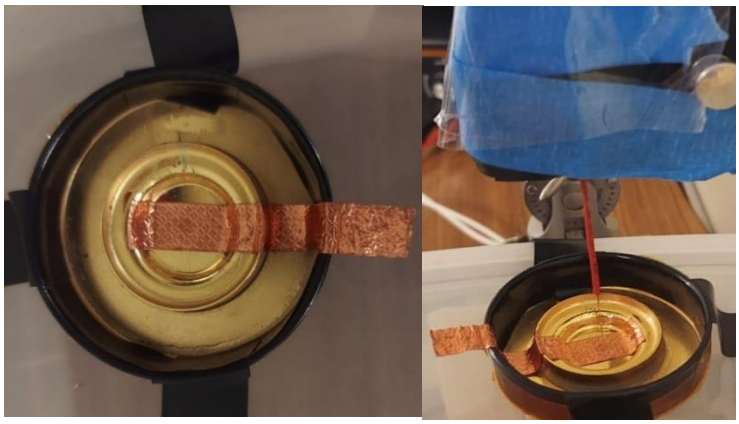

Figure 2: Gold Plate and Needle. The left image shows the gold plate with copper. The image on the right shows the plate with the suspended needle.

A small radius electrode immersed in an electric field of sufficient magnitude will induce the formation of corona ions of polarity opposite to the charge creating the discussed electric field. Alternative would be to apply these corona ions to drop the substrate potential to the required level.

4.2 Research Aim

As time has passed, there has been various discoveries and information collected for electroporation method, but there has been very few research done to get an appropriate pulsed power supply to carry out the procedure [15]. The primary aim of the work is to construct a device that would enable a new approach to modulating charge densities in a time related manner and hence effectively pulse the electric field with a specific time in the absence of high currents.

Implementation of the study involves a plate electrode, mimicking a charged tissue surface, charged to many thousands of volts of potential. A component controlling corona emission is placed in proximity to the plate surface. This component will be responsible to generate ions of opposite polarity to the plate, attracted by the high field theses ions will the charge density on the plate thereby maintaining the plate potential and field [21]. So, electronic controls attached to the plate and corona source will be activated to create pulses of electric field emanating from the plate. The currents involved are small (mainly in the range of micro to nano amperes) and insufficient to cause damage to cell cultures or tissue.

The device is divided into two sections, one connected to the gold plate that would be controlling the input of high voltage on the gold plate (where the said electric field must be created, which is substitute for the electrode carrying cells or tissues), and the other will be responsible for draining out the electric field from the plate using the ‘suspended’ needle. The needle provides compensating charge to the surface and hence changes the net field of the plate.

As we can infer from figure 3, we propose to let the charge stay for certain amount of time and then discharge, but not discharge it instantly. The designing and construction of this device would have to take these requirements into consideration, also keeping in mind that the current generated should be minimal, resulting in reduced power generation and loss.

4.3 Proposed Mechanisms

Proposed design mechanism provided is that there should be a delay of around 500ms. After that for 150ms a pulse should occur which should have an amplitude with respect to the voltage supplied through the high voltage supply. Nearing the end of the 150ms period, needle should engage, which would then help in discharging the plate. Once the needle has discharged the gold plate (maintaining the potential and field), there should again be a delay of 500ms before another pulse occurs. This is explained in figure 3.The point shown, 'needle engagement' is the point when the needle is charge with opposite polarity charged particles to maintain the potential on the plate.

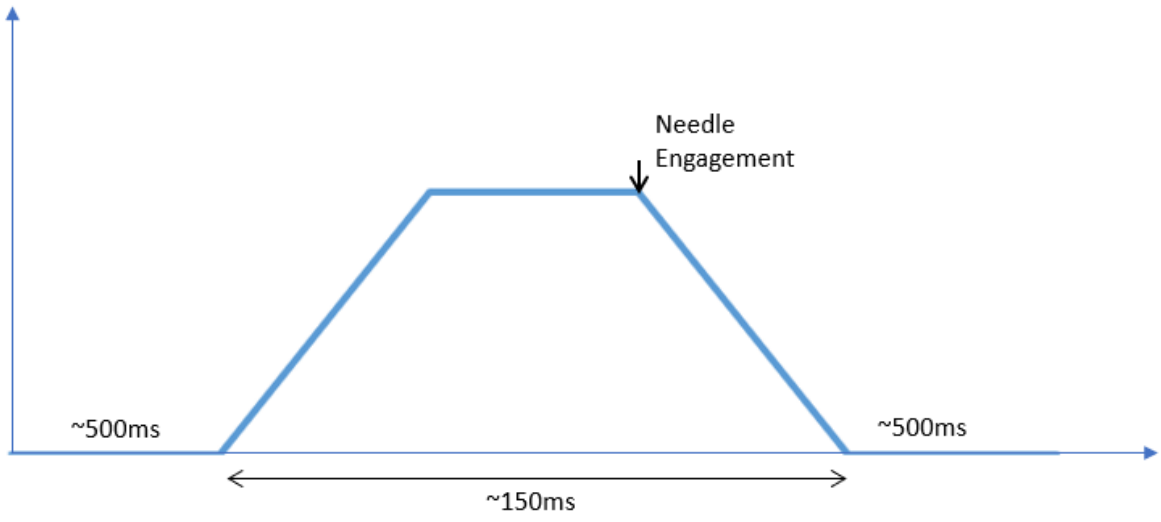

Figure 3: Proposed Design Requirement. The slopes provided to give the switching components required amount of time to switch, so as no device is harmed during the experimentation.

The device has been divided into different parts:

(a.) one to charge the plate with high voltage, and,

(b.) another one to discharge through the non-contacting needle.

The non-contacting needle is responsible for the flow of opposite charge on the surface of plate to counter the ongoing deposition of charges on the plate. Each one of them has been constructed in a way that would take into consideration the design constraints provided to have a functional device.

Chapter 5: Experimental Setup

The device consists of various parts controlling the charging and discharging of the gold plate. The electroporator constructed needs to have a certain pulse width to compensate the design constraints provided.

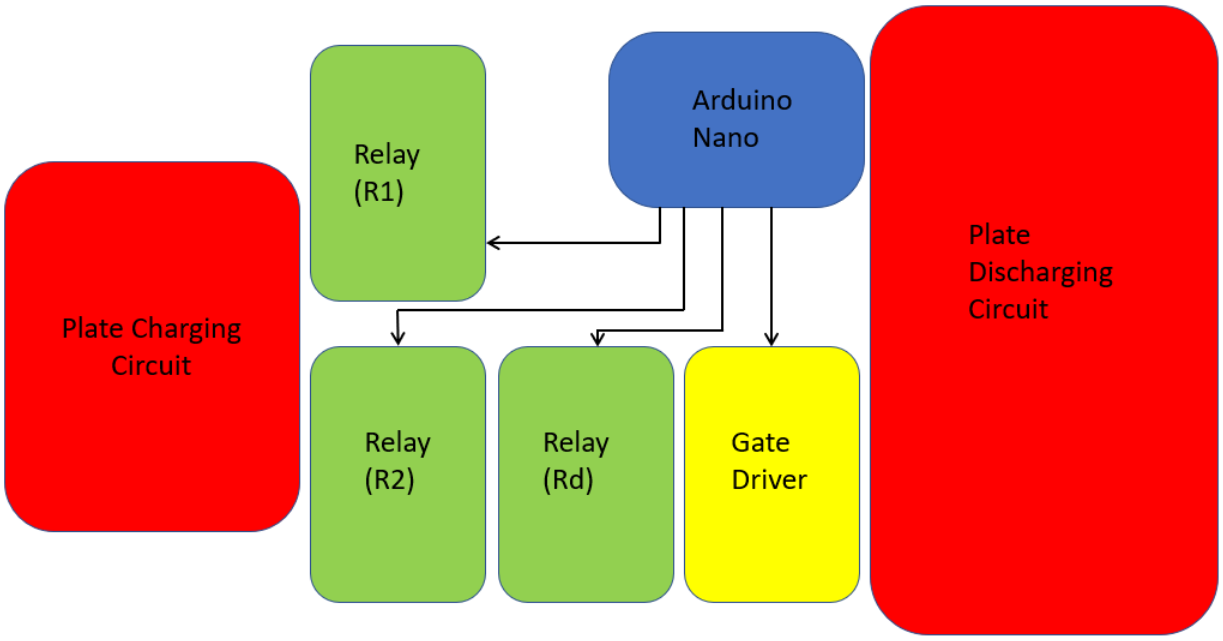

Figure 4: Experimental Layout.

Figure 4 here, gives a layout of the electroporator. The two main parts of the device are, shown in red, controlling the charging and discharging of the electric field induced on the plate. The yellow part of the device is the part that controls the gate driver, which is responsible when turning ON the circuit. The green indicates the individual component, which is a crucial component of the device, which would be controlling the current flow with respect to the time throughout the device. Finally, the blue, Arduino, controls the switching of the various

components. The experimental layout is the basic layout of the construction of the device and the idea behind the working of the device. As shown in figure 5, the setup has been mounted on the board, to provide stability to each constructed part of the device. Just like the plate, all the wires connecting these components are also taped down to avoid any sort of hindrances that can cause problems while taking the data. The white wires shown in the MOSFET circuit, are high voltage cables that are used to make their respective safe connections. The device also has a plate discharging relay. It has not been shown in figure 5. Each circuit constructed has been covered in corona epoxy, for our safety as well as the safety for the connections between the components. These components have also been glued to the wooden plank so that we have a comparatively stable structure.

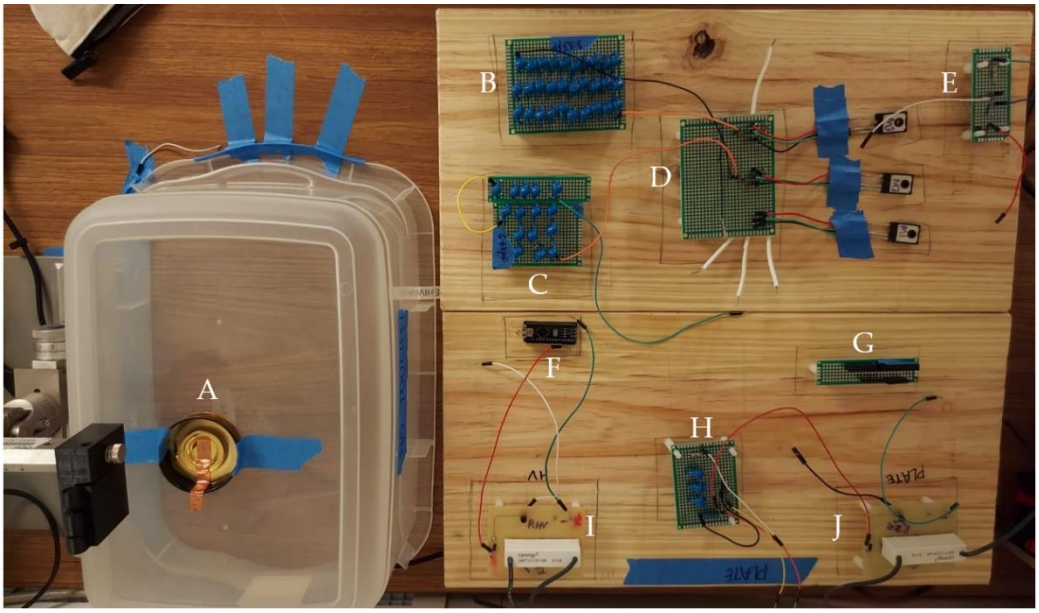

Figure 5: Experimental Setup in Lab. Here, (A) is the gold plate, (B) is 3.33pF capacitor, (C) is the 6.67 pF capacitors, (D) is the MOSFET circuit, whose parts are components (B) and (C), (E) is the gate driver circuit, (F) is the Arduino nano R3, (G) is the common ground and +12V DC connection, (H) is the circuit responsible for charging the plate, and, (I) and (J) are the relays controlling the charging of the plate and charging of the 300pF capacitor, respectively.

The placement of each component has been done to separate the high voltage carrying components, such as the relays and the MOSFETs to low voltage carrying components, such as the Arduino. This would let the system to function in a proper manner. Usually, at high voltage, accumulation of stray charge would pose as an obstacle in proper functioning of these devices, which would lead to incorrect reading collection. A faraday cage is advisable while operating on the device. Faraday cage would help keep these stray charges at bay and assure proper functioning of the device.

5.1 Plate Charging Circuit

The circuit has been devised in a way that it would supply high voltage to the plate in a controlled form.

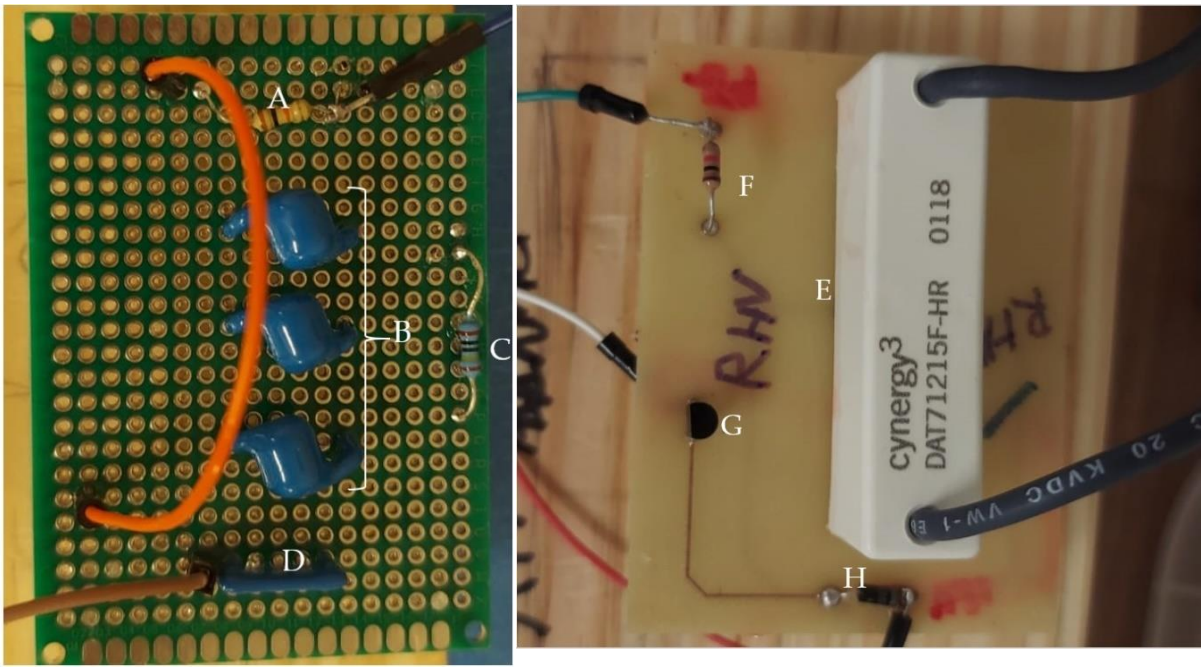

Figure 6: Plate Charging Circuit. Left image shows the circuit responsible for charging, and the image on the right is the relay used to control the charging of the capacitor and the plate. Components used in the construction are, (A) is the 2kΩ resistor, (B) 300pF capacitor, (C) 1 MΩ resistor, (D) 5 GΩ resistor, (E) high voltage switching relay, (F) 1kΩ resistor, (G) npn transistor, (H) diode.

The circuit in figure 6, comprises of four main components, the relays, 300pF capacitor, 5GΩ resistor and 2kΩ resistor. The relays, R1 and R2, are never ON simultaneously. At t=0, R1 closes and lets the 300pF capacitor charge. Once the capacitor is charged, R1 opens and then R2 closes (keeping each component's operating time as specified in their datasheet). R2 then lets the charge on 300pF to flow and charge the gold plate. Till this time the needle has not been engaged.

This construction has been confirmed after various experiments, as it was proven to be more controlled charging of the plate. Note that when the charging of the plate is discussed, it means that the copper strip on the plate is being charged to charge the surface of the gold dish. The circuit analysis has been discussed in section 6.2.

5.2 Plate Discharging Circuit

The circuit being discussed here is connected to the suspended needle. This circuit is responsible for discharging the plate.

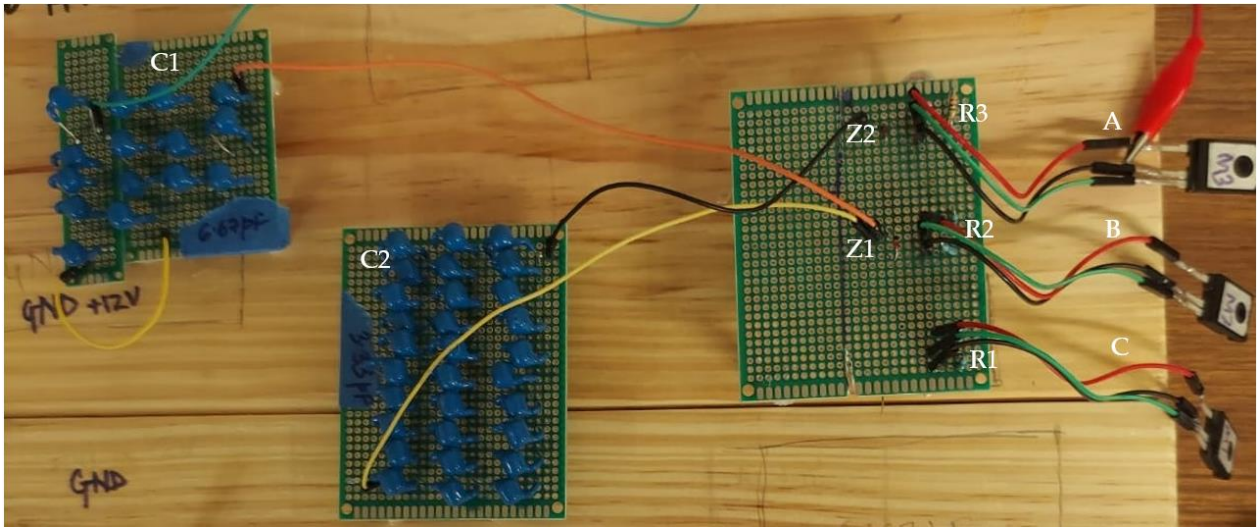

Figure 7: Plate Discharging Circuit. Here, the components shown are, A, B and C are the high voltage MOSFETs; R1, R2, and R3 are the 2GΩ resistors; Z1 and Z2 are the zener diodes; and C1 and C2 are the 6.67pF and 3.33pF capacitors, respectively.

The circuit shown in figure 7, has been taken in use by following the research provided in [16-20]. The schematic shows a connection of three high voltage MOSFETs with three equivalent

resistances of 2GΩ each. The 100MΩ and the 110kΩ resistance is connected to the needle, so that the MOSFETs will not get a large voltage drop immediately. The circuit is designed in a way that it provides opposite polarity ions on the plate through the needle to counter the neutralization of the additional charges being deposited on the surface of the gold plate. This is the entire idea behind construction of the circuit.

The MOSFET circuit will turn on, only when the charges have stayed on the plate long enough as provided in the design constraints. As and when the needle engages, the discharging would start, and since the MOSFETs would provide a low resistance path, it would help in dropping the voltage on the plate to ~3kV. These pulses will be governed by the switching of the gate of MOSFET A, as shown in figure 7. The switching ON of this MOSFET A is being controlled by the gate driver (as shown in figure 8) which is connected to the Arduino. Then the relay, which is normally open in nature, will drain the excess charge remaining on the plate. This relay will switch ON, according to the design constraint, after the corona current goes to ~zero.

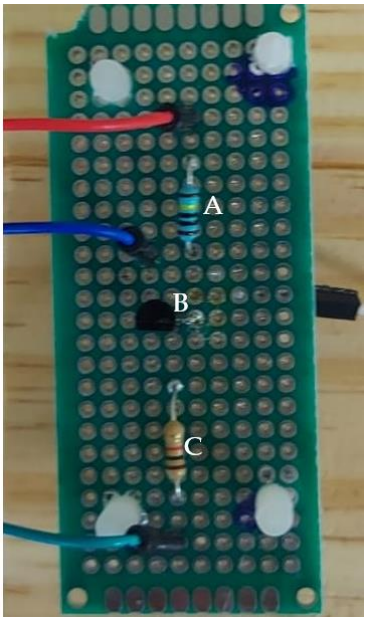

Figure 8: Gate Driver Circuit. Here, the components used are, (A) is 2kΩ resistor connected to the +12V DC power supply, (B) npn transistor, (C) is the 1kΩ resistor connected to the Arduino.

The off - state of the MOSFET's have an equivalent resistance much higher than 1MΩ, which is then compensated by the array of 2GΩ connected in parallel. Because of these, voltage balancing resistors, the voltage is equally distributed [19]. The Zener diodes are connected, primarily, to control the Vgs to not exceed by 20V. The capacitors connected to the gate, are the gate-trigger capacitors [19], which help in setting a certain potential for the MOSFETs, drain potentials of A and B, and source potentials of B and C MOSFETs. The MOSFETs will be OFF constantly, once the relay used for draining extra charge would be switched ON (the construction is showed in figure 6, on the right). If the timing of any of these switches would overlap, it would create a haphazard and uncontrolled flow, which would be harder to control. When working in a biological environment, the scenarios discussed above must be avoided.

Chapter 6: Circuit Analysis

The primary aim while constructing the electroporator, are the provided design constraints (explained in section 4.3). Fulfilling them is the main motive. The crux of this device are the components. Selection of the components used in the construction of the device is important, as it provides a better understanding of the reason behind construction of the device.

6.1 Component Selection

Each component used, has a certain voltage rating that could withstand the high voltage being supplied. The table 1 shown below shows the voltage rating and other parameters taken into consideration while selecting them. The resistors used are not mentioned here as the power dissipated through them is not going to be high, so the normal through-hole resistors were used. Whereas capacitor was selected for its high voltage rating because the charge accumulation in the capacitor will be accordingly high.

Table 1: List of Components.

Components	Part Number	Notes
Capacitor	HVCC153Y6P101MEAX	Voltage rating 15kV
Relays	DAT70515F-HR	Switching Voltage 10kV
Zener diode	1N5250B-T	Vz = 20V
MOSFETs	IXTH02N450HV	Vds = 4500V

For relays, the switching voltage is defined as the maximum value of the voltage the relay can withstand for switching. Use of additional relay has been done, that has the same switching voltage limit as others is used to drain out excess charge remaining on the plate after each pulse. The zener diodes are used mainly to control Vgs up to 20V, and not let it max out. Hence, its Zener voltage is set to 20V. MOSFETs, as we will further analyze in section 6.3, are chosen for a Vds of 4500V as when connected in series, the entire series array can withstand of a potential of 13.5kV, which is high enough to carry out our experimentation. Keeping all these factors into consideration, a schematic is designed that will cooperate these characteristics to give us a more precise output in the required conditions. Initially, we would be discussing the component which would control the switching of these components, hence controlling the pulse shape and duration.

6.1.1 Arduino

As discussed previously (in section 4.3) and displayed in figure 3, the shape and duration of the pulse required as per the design constraints provided. We have used Arduino nano R3, to control the switching of various components. This is provided in the figure 6 below.

The needle engagement, as given in the figure 2 above, is governed by the MOSFET circuit and the 6GΩ resistor stack connected in parallel to it. This in turn is controlled by the first MOSFET, according to figure 7, MOSFET A. According to the datasheet provided by the company for the MOSFETs, they require a minimum of 10V, to trigger the gate of the MOSFET in turn starting the circuit. Figure 6 provides a flow chart, which is used to program the Arduino. Arduino would be the controlling unit, as it will be controlling the component's switching, hence, controlling the electric field pulses to be subjected. The programming has also been done by taking into consideration the charging and discharging of various components (as described in sections, 6.2 and 6.3).

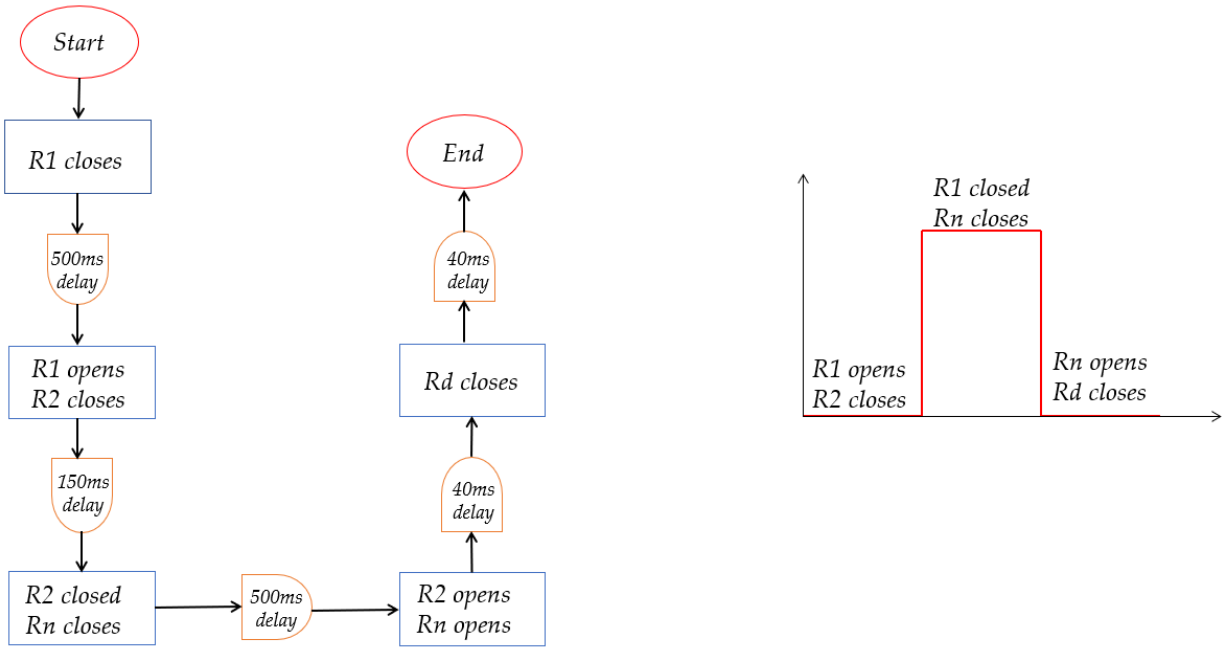

Figure 9: Flow Chart for Arduino. Right figure shows the relation between the pulse requirement with the flow chart explained.

6.2 Plate Charging Circuit

Capacitances used in the circuit is because the plate capacitance was observed to be 160pF, theoretically the capacitor charging the plate needs to be almost the double in value, hence, 300pF. This so that the capacitor can charge up to double charge as per required by the plate and then when needs to be disposed, the plate would charge to the point necessary to the design constraints.

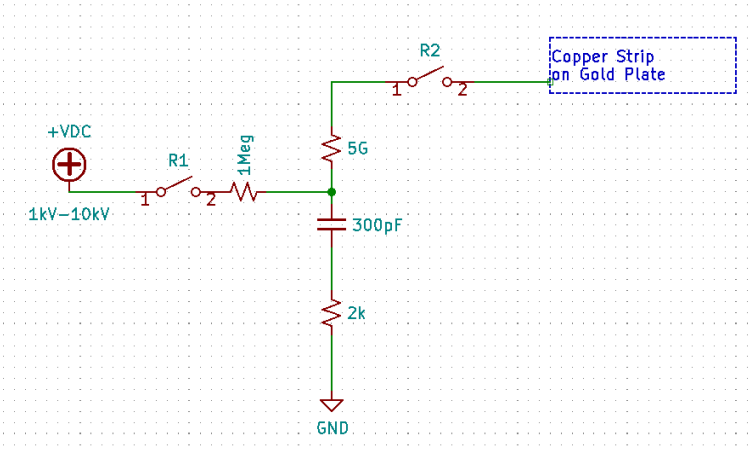

Figure 10: Plate Charging Circuit Schematic.

Controlling the charge and discharge of these two capacitors (300pF and the plate) are the resistors connected. The resistor, 1MΩ connected in series, controls the current flow to the capacitor. Similar work is for the 5 GΩ for the plate. The 2kΩ resistor is primarily used to observe the displacement current of the 300pF capacitor.

At t=0, the relay connected to the high voltage supply would turn ON. As specified in the datasheet, the operating time of the relay is around 3ms. When the said relay turns ON, the 300pF would charge up. Theoretically, according to the formula of time constant,

$$\tau = RC \tag{i}$$

and it takes around, 5τ time to charge the capacitor completely. For 300pF, this value came out to be 1.5ms. Once this capacitor is completely, the relay opens, and the relay charging the plate switches. Since, as calculated prior, the capacitance of the plate is around 160pF and the approximate time of charging the plate and discharging of the needle is around 3.5 seconds. Given the design constraints, Arduino is instructed to keep these calculated values also into consideration.

Since, the relays require a 12V supply to start switching, a circuit was constructed individually to control its efficient working. Three pins of this circuit need to be connected, one to Arduino, one to the 12 V DC supply, and the other to the ground (refer figure 14).

6.3 Discharging Plate Through Needle

Once the plate has been charged and the charges have stayed on the gold plate for the required amount of time, the needle engages to discharge the charges from the plate. The method of discharging through the needle has been discussed in [21], to maintain the potential on the plate, in turn maintaining the field on the plate. Once, the needle has been engaged according to the requirements, the relay will be switched on for 40ms to let the excess charge drain from the plate. This avoids the current to drop below a certain point. Also, these stray charges would add up to

the electric field, which would provide us the result which will be unpredictable. Here, initially, we will be discussing the MOSFET system then we will be discussing the working of this relay.

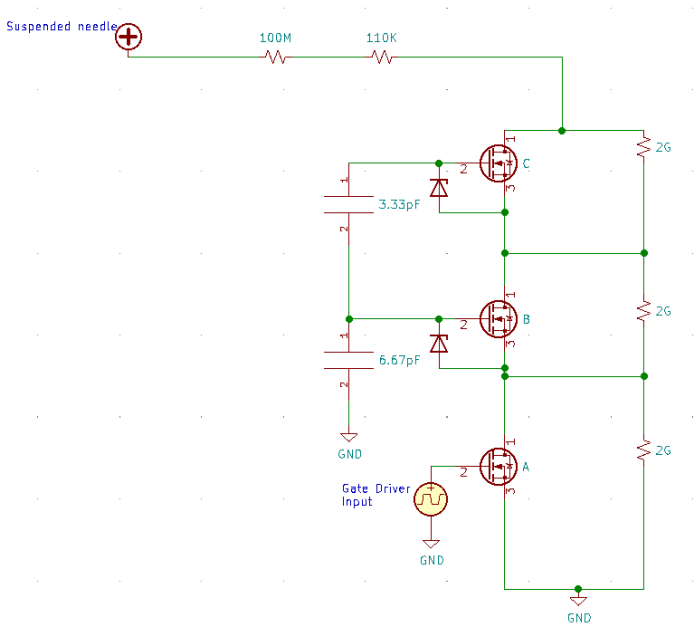

Figure 11: Plate Discharging Circuit Schematic.

The circuit is constructed by series connection of the MOSFETs. The construction, as discussed in [16-20], is in series because this connection lets the individual MOSFET's Vds to be added up. Since, we want a circuit that could handle a high voltage discharge, this type of construction was the most plausible one. Here, each MOSFET has a provided Vds limit of 4500V, which when added up could provide us a circuit that could, theoretically, withstand 13.5kV. The capacitors connected to the gate of the MOSFETs required to have a value that would trigger the gate to turn ON all the subsequent MOSFETs. So, its value needs to be approximately equal to the equivalent capacitance, all the gate and source capacitances of the MOSFETs. As described in the paper [17], the value of the capacitance should be,

$$C_{eq} = C_{iss} + (A_v \,.\, C_{rss}) \qquad \text{(ii)}$$

$$\text{where, } A_v = Vds/\, Vgs \qquad \text{(iii)}$$

A_v , here, is the voltage gain[17]. Once, the equivalent capacitance value is calculated, we can then calculate the value of the capacitance to be connected to every gate. Keeping in mind, that these capacitor values need to be calculated in a way that it controls the source potential of MOSFETs B and C and drain potentials of MOSFETs A and B.

6.3.1 Gate Driver

Gate driver is required to provide the required gate-source voltage, otherwise known as threshold voltage of the MOSFETs to turn them ON. The schematic of the gate driver is shown in figure 12.

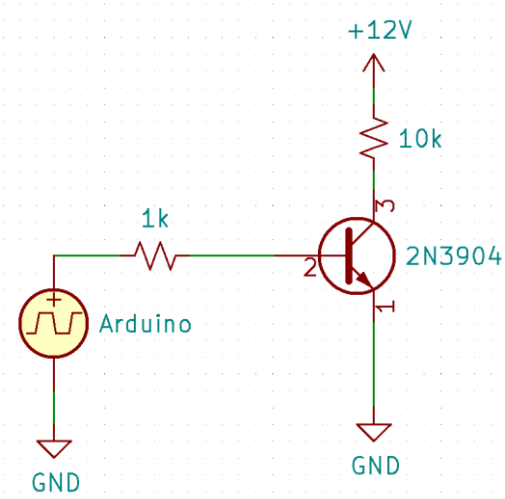

Figure 12: Gate Driver Circuit Schematic.

We have an npn – transistor, which would regulate and amplify the 5V pulses coming from the arduino to 12V to turn ON the MOSFETs. As we know that, the active region of a npn – transistor is when emitter is grounded, and collector is high. The working of the npn transistors is that it inverts the incoming pulse, when high at the gate it will input low and vice versa. This must be taken into consideration while programming the Arduino (refer Appendix II, provide 'HIGH' input when needed 'LOW' and vice versa).

Here, the 5V pulsed signal would be amplified to 12V and then provided to the gate of the first MOSFET. The gate would be connected to the collector of the npn-transistor. We have connected a 12V DC power supply for ease of connections. But connection of any value greater than 10V, which is the threshold voltage of the device, is advisable.

6.3.2 Circuit Analysis of the MOSFET Circuit

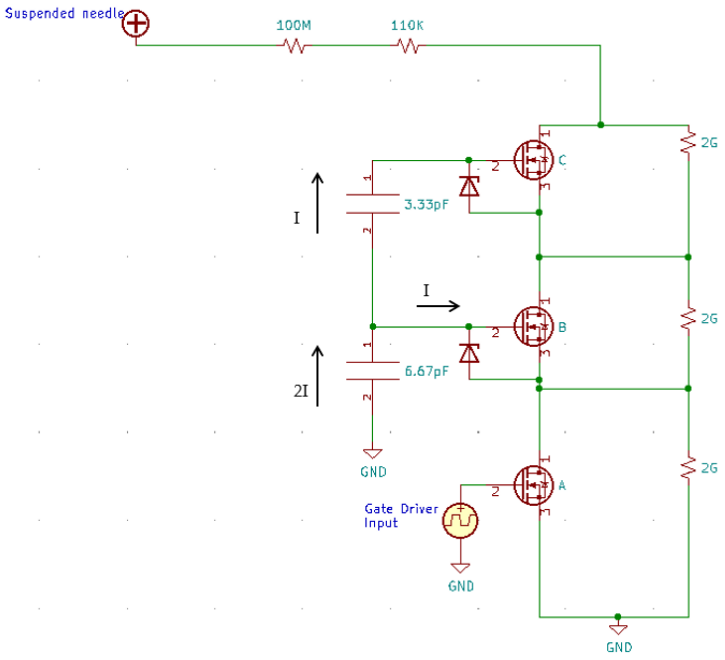

Figure 13: MOSFET Array Current Distribution.

Figure 13 shows the basic current flow through the circuit. Here, the current flowing through the MOSFET gate and the gate capacitor (C1 and C2) would be equivalent, as shown in figure 13, whereas the current flowing through these capacitors would be different [17]. This specification would help maintain the voltage between the gate and the source of MOSFET constant. To be discussed further in chapter 7, the voltage at the needle will not necessarily be equal to the voltage on the plate but will be some factor less, as discussed in section 7.2 and 7.3. This is because of the electric field generated between the tip of the needle and the plate.

Needle is engaged when the gate drives provide the threshold voltage and switches the MOSFET ON. When MOSFET A is turned on, 6.67pF capacitor charges up and triggers the MOSFET B to turn ON and similarly for MOSFET C. The key factor here is that all the switching time of the MOSFETs should be almost coherent. If the switching times differ by a factor of microseconds, there would be a certain charge buildup which would lead to uncontrolled switching of these MOSFETs.

When high pulse is provided by the gate driver, the MOSFET stack will turn ON and the current will flow through it. And when low pulse is provided, the current will flow through the resistor stack. So, when the MOSFET stacks are ON, the voltage reading at the drain of MOSFET C would be, using the concept of the voltage divider,

$$V_{d\_atC} = \frac{V\_needle*(3Ron)}{100M\Omega+110k\Omega+(3Ron)} \quad \text{(iv)}$$

As provided in the datasheet of the MOSFETs, the R_{ds_on} (resistance when MOSFETs are ON) for the MOSFETs is less than or equal to 625Ω. Here, V_{d_atC} is the voltage at the drain of MOSFET C, V_needle is the potential that needle will have after engagement and R_on is the R_{ds_on} of the MOSFETs.

From (iv), we will have an approximate value of V_{d_atC} as,

$$V_{d\_atC} = \frac{V\_needle*(3Ron)}{100M\Omega} \quad \text{(v)}$$

The equivalent resistance of the system will be around 100MΩ. Similarly, the voltage at drain of MOSFET C when all the MOSFETs are OFF will be,

$$V_{d\_atC} = \frac{V\_needle*(6G\Omega)}{100M\Omega+110k\Omega+(6G\Omega)} \quad \text{(vi)}$$

Simplifying equation (vi), we get,

$$V_{d\_atC} = \frac{V\_needle*(6G\Omega)}{6.1G\Omega} \quad \text{(vii)}$$

As we can infer from the equation (vii), the value of V_{d_atC} would be approximately equal to the V_needle. Comparing equations, (v) and (vii), we can see that the value of V_{d_atC} when the MOSFETs are ON would be much lower than when they will be OFF.

6.3.3 Excess Charge Removal from Plate

A relay and three wires connected. One goes to the ground, one goes to the Arduino which controls the switching, and the last one goes to the +12V DC power supply. We will be using a relay that has a switching voltage of 10kV because this relay used to drop the plate voltage when the current goes down a certain limit. The excess charge remaining will not cause a voltage as high as its switching voltage, but just to be prepared for the worst scenarios, selection and placement of the component has been done which can handle high voltage, if it may ever come to that.

Chapter 7: Results and Analysis

The device has been constructed in a way that it will let the electric field stay on the gold plate (or any apparatus to carry out experimentation), according to the design constraints provided. The primary focus is to develop a characteristic behavior of the charge deposition or potential development on the gold plate. This is a tricky task to perform as, since the entire circuit is handling high voltage, we need to devise methods or track test points that would be safer enough to observe data from. Hence, the observation of the characteristic of the charge on the plate has been performed using the method of displacement current.

Displacement current is observed across the gold plate in real-time, using the Arduino. Reffering to figure 10, we will be collecting the data at 2kΩ. Assuming the plate capacitance is 160pF, it can be used to derive the voltage seen on the plate with respect to the displacement current through it. Derivation of the equations used is,

$$V_r = iR \tag{viii}$$

$$Q = CV_c \tag{ix}$$

Equation (viii) shows the voltage drop across the 2kΩ resistor and equation (ix) shows the voltage drop across 300pF capacitor. Here, V_r is the voltage drop across 2kΩ resistor and V_c is the voltage drop across the capacitor. Since the displacement current is equivalent to the current flowing through the resistor, so, combining both the equations

$$i = C\, dV_c \tag{x}$$

From equation (viii), the final derived formula is,

$$V_r/R = C\, dV_c \tag{xi}$$

$$V_c = 1/(RC) \int V_R \quad \text{(xii)}$$

$$\text{or } V_c = 1/(\tau) \int V_R \quad \text{(xiv)}$$

Once we calculate the voltage across 300pF capacitor, according to the relation between the capacitances 300pF and 160pF, there is a 2/3rd factor when observing voltage across the plate (refer to Appendix I). So, following this method, a certain value of charge deposition and voltage drop can be observed which in turn can be used to characterize the potential on plate. But before the behavioral analysis of the plate subjected under high voltage, understanding of charge deposition and the interaction between the plate and the needle (setup shown in figure 2) is important, as analyzing them would make characterizing potential on the plate understandable.

7.1 Experiment to Show Charge Deflection Above Given Potential

Theoretically, there is a charge limit that any surface would sustain that is being consistently being exposed to corona emission before it itself goes into corona itself. Here, since the gold plate is being under the exposure of emission, we need to find the limit of the maximum charge deposition.

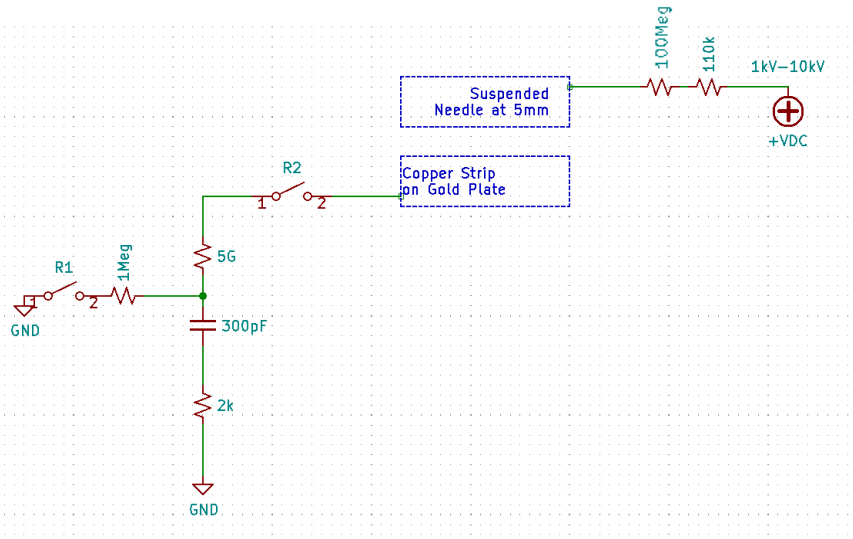

Figure 14: Charge Limitation Experiment Schematic.

Referring to the schematic shown, the gold plate is connected to the relay circuit via the copper tape (as shown in figure 2), where the relay handling the high voltage is being grounded. The high voltage, instead, is connected to the needle.

. The needle would charge up the plate by corona emission and the goal is to observe the potential on the plate, using the displacement current method as described above. The results have been collected for the high voltages, 3kV, 6kV, and 9kV. The shown data is in voltage observed across the plate vs voltage supplied through the high voltage power supply. So, observing the data provided in the figure 15, we can see that for the 9kV supply only 4500V was only reached on the plate.

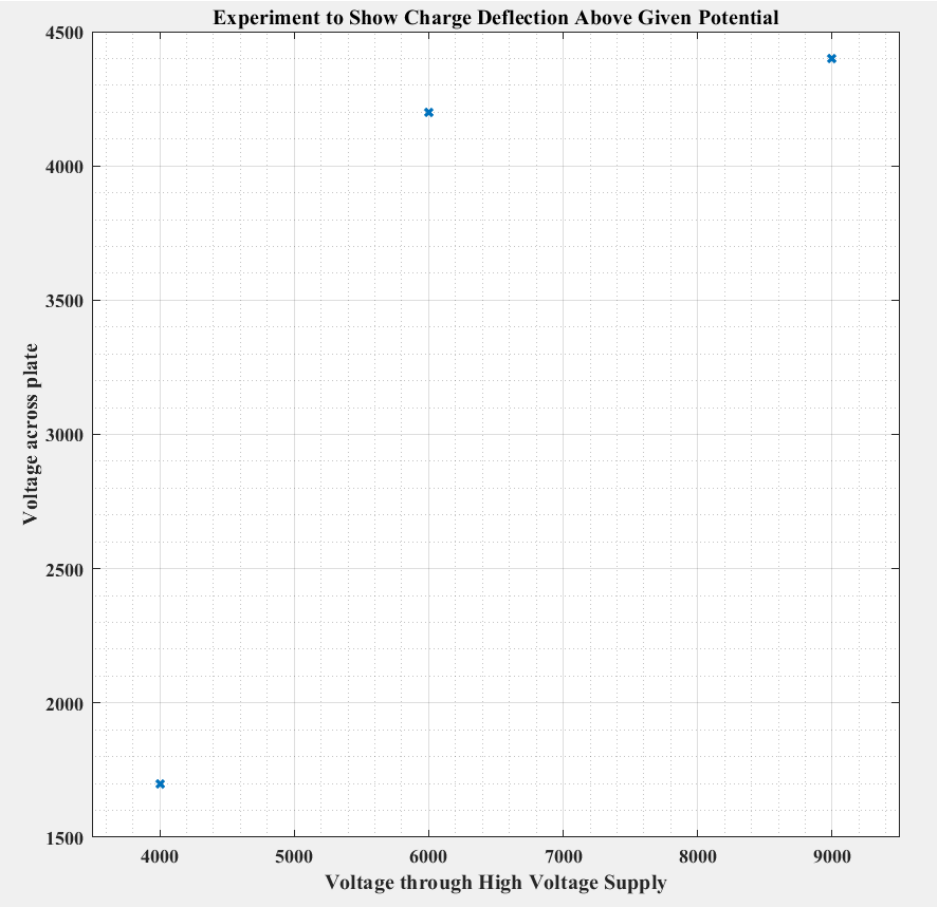

Figure 15: Plot for Charge Deflection Experiment. Charge limitation of the plate with respect to the high voltage supplied through the needle using displacement current method.

The data recorded shows that beyond 6kV, the potential on the plate is somewhat consistent. This proves the theory discussed. Also, this is the very reason that sole corona flux is not used as it would charge up the surface quickly which would limit the deposition of any additional charges. Having an object which would be responsible to provide opposite charges would help us regulate the deposition of these additional charges and even buy more time to perform electric field mediated gene delivery with more precision.

7.2 Behavior Between Plate and Needle

Characterizing the field between the plate and the needle is the key factor to understand the characterization of the potential on the plate with respect to the voltage supplied through high voltage power supply.

The experiment has been conducted in a manner that, the plate would get direct DC supply from the high voltage power supply and the needle would be grounded as shown in the schematic in figure 16.

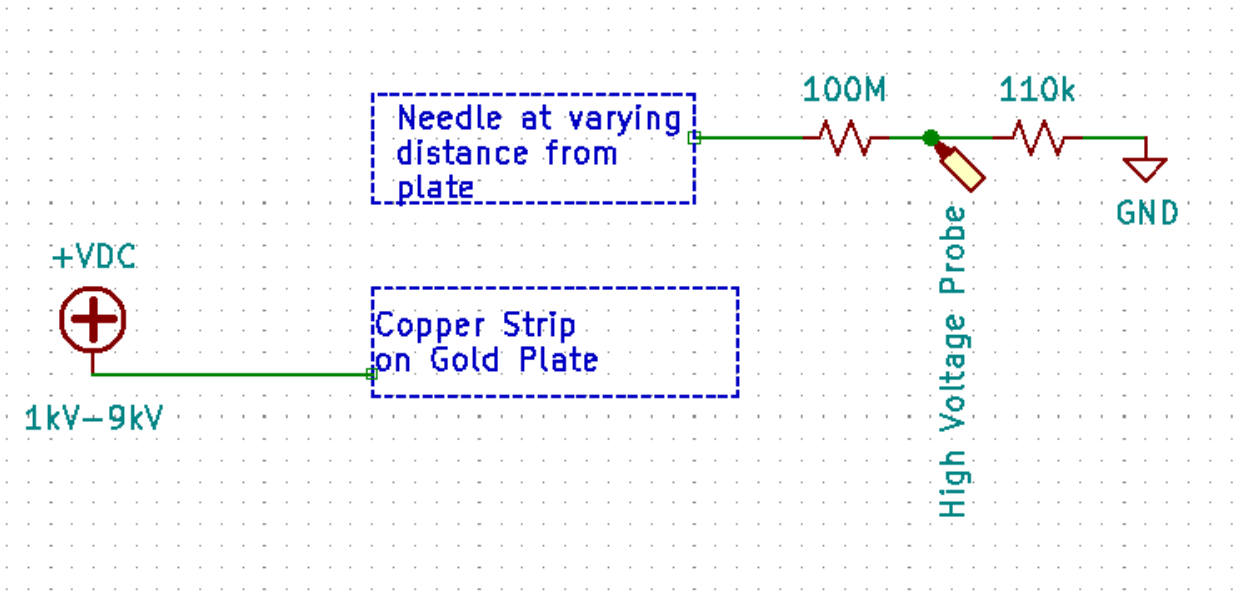

Figure 16: Schematic for Behavioral Characterization Between Plate and Needle.

The high voltage probe is used here and connected between the resistors of the needle for safe connection. The needle was varied at distance of 3mm to 8mm with a difference of 1mm each. Each distance was characterized with application of high voltages, in the range of 3kV to 9kV. Since, the resistance of the high voltage probe at 1000x attenuation is 900M, it will not affect the resistance value of the needle while calculating the potential on the plate or in the calculation of current flowing through the needle. The relation between the voltage acquired at the probe to the voltage at the needle is,

$$I_n = \frac{V_{probe}}{110k\Omega} \qquad \text{(xv)}$$

here, V_{probe} is the voltage reading through the high voltage probe and I_n is current flowing through the needle. The relation provided in (xiii) helps in providing a basic IV characteristic plot.

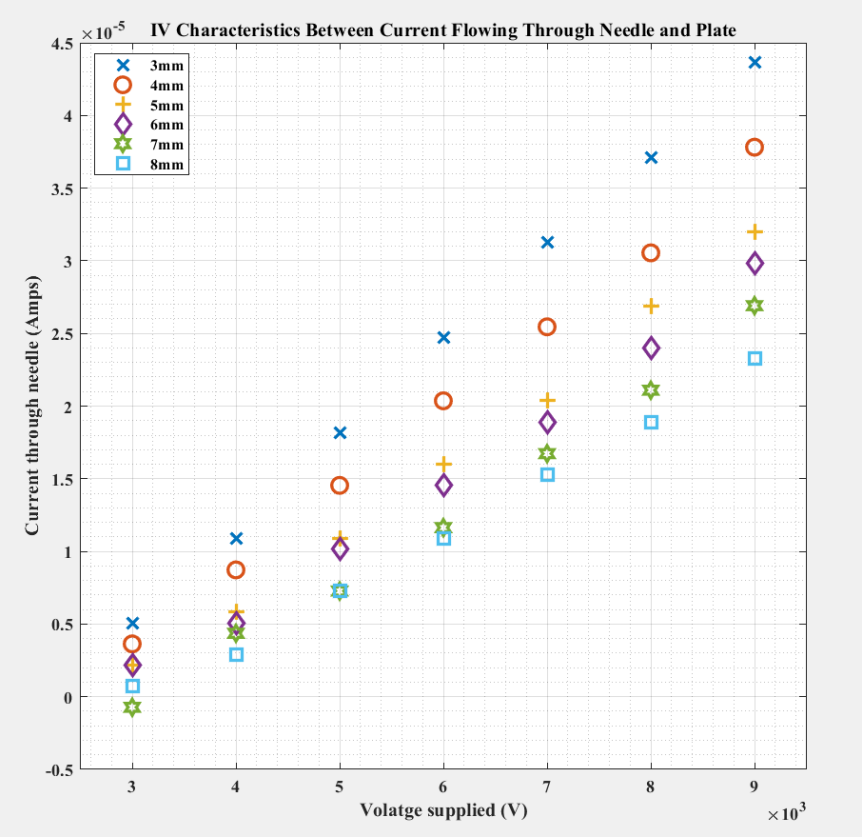

Figure 17: IV Characteristics Between Needle and Plate. Current through needle vs voltage supplied on plate curve, with respect to the varying distance between the plate and the needle.

According to the experiment, with each reading taken, the distance between the needle and the plate was kept constant and the voltage supplied was varied from 3kV to 9kV. So, for every increase in voltage there would be a proportional decrease in the current through needle, which is observed in the plot, as shown in figure 17. From bottom to top, there is a decrease in the distance, as shown in the legend. It can also be observed that, the values are comparatively nearer at 3kV, with respect to the values at 4kV or above.

7.3 Characteristics of Plate

Using the similar experiment, we have observed in the previous section, we will be characterizing the voltage across the gold plate during the implementation of the entire device. The schematic of the implementation is shown in the figure below. Here, the needle is 5mm away from the surface of the gold plate.

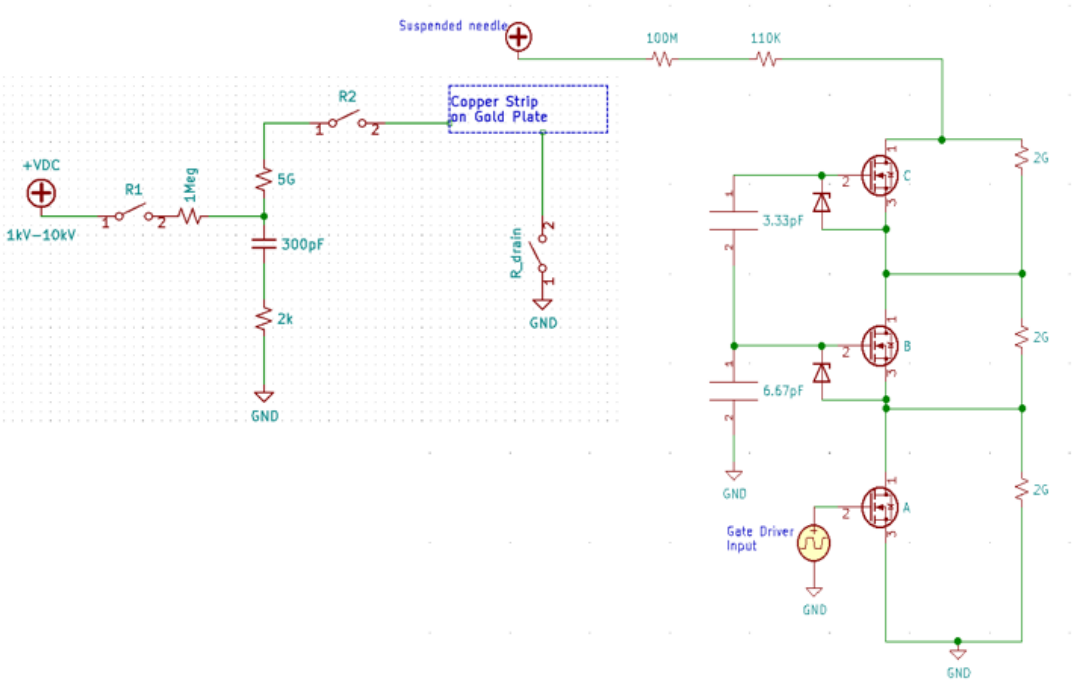

Figure 18: Characterization of Potential on Plate Experiment Schematic. This is the entire schematic of the device under observation. The distance between the plate and the needle is 5mm.

In the schematic, 1MΩ is used to limit the current value at the point above the 300pF capacitor, so that the voltage drop across 2kΩ resistor does not exceed 5V. This is mandatory, as the displacement current has been observed through Arduino, and to avoid any harm to the Arduino, the voltage drop across the resistor should be less than or equal to 5V, preferably less than 5V.

The voltage has been observed across the gold plate at varying voltages between 1kV to 9kV. The data has been shown in the figure below. The plots given are in potential observed on the plate vs voltage supplied. As observed from the figure, the voltage on the plate never actually reaches the voltage as provided by the high voltage supply, but somewhere less than that. The reason behind this observation has been discussed in section 7.2.

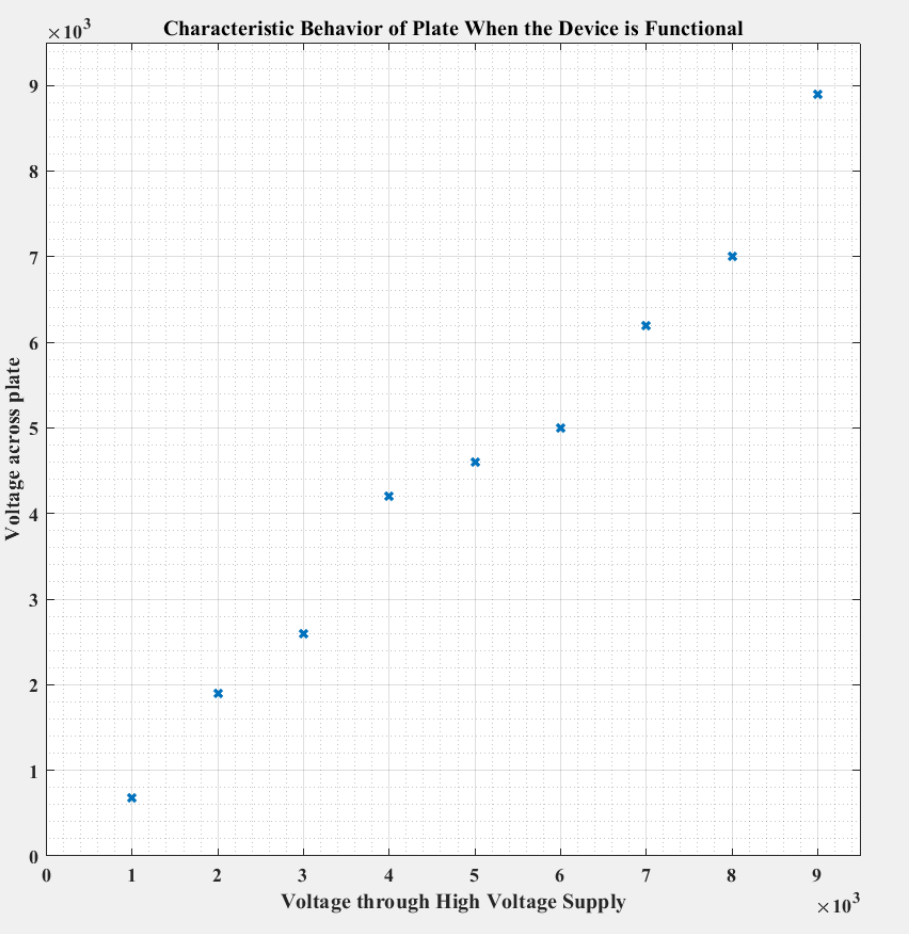

Figure 19: Plot for Characterization of Potential on Plate.

As observed from the plot, there is a spike after 4kV. That is when the air breakdown voltage is reached, and the field becomes strong enough between the needle and the plate that it goes under corona discharge. After the 4kV, almost equivalent voltage is observed as provided by the high voltage power supply. Since, the air breakdown voltage is reached at 4kV, a higher value is observed on the plate, greater than 4kV.

Chapter 8: Conclusion

Gene therapy is an initiative, a rather new concept, but provides a hopeful future to researchers, doctors, and the public. Its flexible and adaptive nature in terms of pathologies and improved efficacy has proven that it can be used as a therapeutic approach for various other treatments as well. Gene therapy is flexible in terms, that it can function in any given environment, in-vivo or in-vitro, using any mode of delivery, such as viral, non-viral vectors, for any biological entity (cells, tissues, etc.).

The device has been designed to be used for electroporation. Provided design constraints stated that a square pulse for a given time needs to be provided and the voltage that it should be able to carry 10kV. Here, the device has been used in a corona-charged environment, which is assumed in the area where the cells are going to be kept.

As suggested in the paper [36], they have used a helium-gas-based plasma pen to create a corona discharge in the requirement, for an in-vivo delivery for the DNA. The results of the paper suggested that the plasma could be used for an effective reversible, non-invasive, and in-vivo electroporation. A similar idea has been used in the work provided by Jaroszeski, et al. [35]. Here, the device constructed will require an apparatus (here, gold plate is used) where the gene delivery would take place. The nature of the interaction between the gold plate and the tip of the needle has been explained by Ravi [20].

As observed, through the results, that the voltage increased rapidly after we increased the voltage supplied from the power supply to be more than 3kV. That is when the voltage reached the air breakdown voltage, and plasma was created. The problem with corona discharge in the air

is that with the required area to be charged, the excess area will also be charged which would be harmful to our device's working and the excess electric field created in the required area. So, different parts of the circuit have been constructed, one for charging, one for discharging and then, one for draining off the excess charge.

From the results, it can be inferred that the device is not completely draining off the charges, but it is functioning in a controlled manner. We were able to get the requirements fulfilled and get the electroporator working. With some modifications, this device should be able to get the necessary experiments done. It can be described as flexible with the pulse duration and width, as changes the Arduino code can be done (provided in Appendix II), keeping the charging and discharge times of various capacitors used in consideration. If this condition is not met, then the output may vary, and the device may not function as discussed.

Chapter 9: Future Work

Since the discovery of gene therapy, advancement in pathologies has been observed in the domain of gene therapy to cure gene-related diseases to curing various other diseases such as, cancer and tumor [2-15]. It has only been a decade since electroporation has been from bench to bedside [25]. As time progresses, innovative ideas have been introduced to carry out the delivery process of electroporation, such as voltage-dependent voltage, time-dependent pulses, type of pulse, etc. [15,27].

As previously discussed, there are only two types of electroporators available. One that is commercially available, and the other that is constructed in the labs and is experiment specific. Any of these electroporators, available, is not meant to bend according to various experiment requirements but is feasible for a set of experiments that has proven to provide a predictable set of outcome data. Here, the device has been constructed for experiments for the design constraints already provided and discussed.

Following research to be done with the device is how to make it safer from the stray charges that will not affect the function of the device. Also, a thing to note here is that to drain the excess charge, the use of a normally open relay has been done, as they are much easily accessible for high voltages as compared to the normally closed relay. But using a normally close relay would be more beneficial as if the device would shut down in the middle of the procedure due to some reason, every other relay would be open, which would let the normally closed relay to ground the plate as soon as possible.

Future research for this model should also entail a much secure or rather compact construction of the device so that while using it during electroporation procedures, the loose wires and the components will not create that much of a hurdle. The construction explained here, is a bit rough and was just to test out theories if it could work in a biological environment.

Discussed here, is just a theoretical model of an idea of using corona discharge for electroporation. Various other constructional modifications are required for it get working in a biological environment. And once the device has been proven clinically viable, this method would be the attainment of goals addressed initially and would be an accomplishment in the field of non-viral gene therapy.

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Appendix I: Relation Between the Potentials of the Capacitors

The following schematic (shown in figure a) shows the circuit which is used to derive the relation of voltage drop between the two capacitors, the 300pF and the gold plate (160pF).

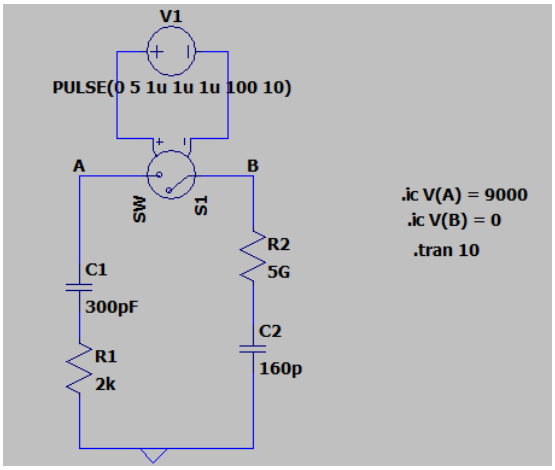

Figure a: Schematic for Relation Between the 300pF Capacitor and Plate.

Referring to figure a, node A has initial voltage of 9kV and 0V for node B. Here, figure b shows the voltage drop across 300pF and 160pF (the gold plate).

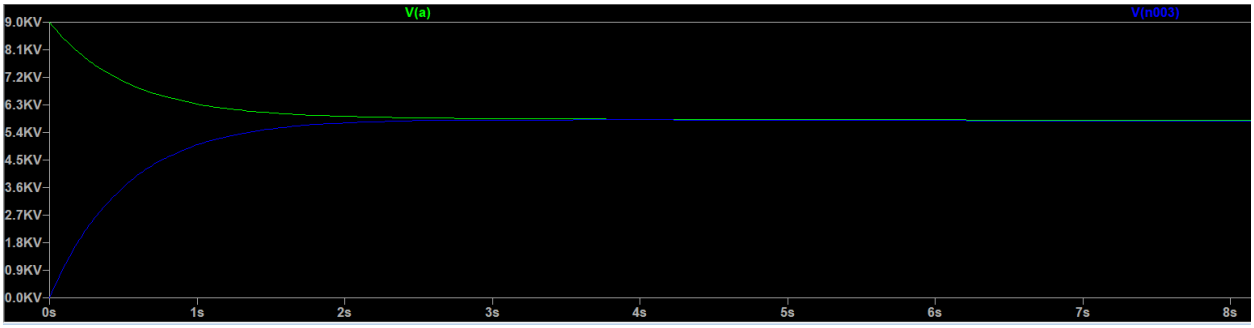

Figure b: Simulated Result for the Relation Between the Capacitor and Plate.

In figure b, V(a) curve (green line) represents the voltage characteristics of the 300pF capacitor. While V(n003) (blue line) represents the voltage characteristic of 160pF capacitor (gold plate). As observed from figure b, the system (figure a) reaches equilibrium at around 6kV, which is $(2/3)^{rd}$ of 9kV. Hence, the relation was applied in chapter 7.

Appendix II: Arduino Code

Following consists of the Arduino code used to provide switching instruction to the components as well as the calculation of displacement current at these switching times.

```
int output_r1 = 11; // arduino pin to enable charging of 100pF capacitor
int output_r2 = 10; //arduino pin to enable charging of the plate
int output_rn = 9;  //arduino pin enable needle
int output_roff = 8; //arduino pin to enable discharge of remaining charges of the plate
const int in_pin = A3;
int sample;
unsigned long last_time_sampled = 0;
unsigned long last_time_printed = 0;
float integral1 = 0;
float integral2=0;
const float conversion = (5.0/1023);
const float tau1 = 2e3*300e-12;
const float tau2 = 5e9*160e-12;


void setup() {
  Serial.begin(9600);

  pinMode(output_r1, OUTPUT);
  pinMode(output_r2, OUTPUT);
  pinMode(output_rn, OUTPUT);
 pinMode(output_roff, OUTPUT);

  digitalWrite(output_r1, LOW);
  digitalWrite(output_r2, LOW);
  digitalWrite(output_rn, HIGH);
  digitalWrite(output_roff, LOW);

}

void pulse_new() {
  unsigned long start = micros();
  float integral1 = 0;
  float integral2 =0;

  digitalWrite(output_r1,HIGH);
  digitalWrite(output_r2,LOW);
  digitalWrite(output_rn,HIGH);
  digitalWrite(output_roff, LOW);

   unsigned long now = micros();
   last_time_sampled=now;
```

```
  while(now-start<501.5e3) {
     int sample = analogRead(in_pin);

  integral1 += sample*(now-last_time_sampled)*conversion;
  integral2 = (3/2)*integral1;
 // Serial.print("input=");
  //Serial.print(sample);
    //Serial.print(',');
  //Serial.print("output");
 Serial.println(integral2/tau2);

  now=micros();

}
   delay(1);


 digitalWrite(output_r1,LOW);
  digitalWrite(output_r2,HIGH);
  digitalWrite(output_rn,HIGH);
  digitalWrite(output_roff, LOW);
  start=micros();
  now=start;
  last_time_sampled=now;
  integral1=0;
  integral2 = 0;
  while(now-start<40e3) {
  sample = analogRead(in_pin);
  integral1 += sample*(now-last_time_sampled)*conversion;
  integral2 = (3/2)*integral1;
 //Serial.print("input = ");
  //Serial.print(sample);
    //Serial.print(',');
  //Serial.print("output");
  Serial.println(integral2/tau2);
  last_time_sampled=now;
   now=micros();

 }
   delay(1);
```

```
 digitalWrite(output_r1,LOW);
  digitalWrite(output_r2,HIGH);
  digitalWrite(output_rn,HIGH);
  digitalWrite(output_roff, LOW);
  start=micros();
  now=start;
  last_time_sampled=now;
  integral1=0;
  integral2 = 0;
  now=micros();
  while(now-start<400e3) {
   sample = analogRead(in_pin);
   integral1 += sample*(now-last_time_sampled)*conversion;
   integral2 = (3/2)*integral1;
  //Serial.print("input");
 //Serial.print(sample);
  //Serial.print(',');
  //Serial.print("output");
  Serial.println(integral2/tau2);
  last_time_sampled=now;
  now=micros();
  }
  delay(1);

  digitalWrite(output_r1,LOW);
  digitalWrite(output_r2,LOW);
  digitalWrite(output_rn,LOW);
 digitalWrite(output_roff, LOW);
  delay(500);

  digitalWrite(output_r1,LOW);
  digitalWrite(output_r2,LOW);
  digitalWrite(output_rn,HIGH);
  digitalWrite(output_roff,HIGH);
  delay(50);


 }

void loop()
{


  delay(1);
    pulse_new();




}
```