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Characterization of Surface Charges and Compensating Charges for Gene Delivery to Tissue

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Characterization of Surface Charging and Compensating Charges for Gene Delivery to Tissue

by

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of the requirements for the degree of
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DEDICATION

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

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I would like to sincerely thank my professor Dr. Andrew Hoff who believed in me that I could complete this work. With vast knowledge he had, he was always a constant source of inspiration to me and would express my deepest gratitude for his time.in sharing his knowledge. I heartfully thank my professor for letting me complete my thesis with no pressure and for guiding, motivating, supporting and helping me in all ways to progress with the degree. And I would never think of another professor to guide me with my thesis.

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ABSTRACT

Ever since the discovery of DNA, there has been many pathologies identified effecting mankind. With the development in technology, there are many methods to alleviate these pathologies. One such is gene therapy or gene delivery. It is a process of introducing some foreign material into the body to correct the effected cells. In principle, it is a modern method to cure cells or a method to transfer nucleic acid into a cell to treat specific cells in the body. The process of delivering a genetic material is carried out using vectors, namely, viral vectors and non-viral vectors. In viral vectors, viruses are modified to make it efficient for delivery into the host cells. This method has high transduction rate as compared to non-viral method. Non-viral methods include chemical and physical transfection methods, which are used to deliver the gene of interest into the host cell unlike viral methods.

In this study, a physical method using high voltage is used to deliver a genetic material into cells. High voltages are used to permeabilize the cell to allow the foreign material into it and to express it in the host cell. This process is termed as Electroporation. In specific, in this research, studying a process of charging a region that mimics skin and trying to localize the presence of electric fields on the surface where the strongest uptake of genetic material is found. In other words, region where the gene expression is strongest at a specific region if performed on skin is studied by localizing electric fields on the surfaces. My work is to characterize and develop where this effect takes place on the surface based on both positive and negative electric fields. A physical method is useful as it is a non-toxic way to get a DNA/protein into someone's body without making

them sick, unless if not using a virus to deliver. This is all done using high voltages up to 8kV and the electric fields produced due to high voltages are localized, visualized and characterized with both positive and negative polarities of voltages.

In this study, experiments with high voltages are performed and the spread of charges at specific regions are collected using a needle. This needle goes into corona, which may be called as a secondary corona. It might be called a secondary corona because the flat conductor is being charged by a metal finger but not directly by the power supply. Here, the conductor is charged by a metal finger of high input voltage, which ionizes the air molecules above the flat conductor to form a conductive region. As the input voltage is increased further, electrons escape from the needle to air or from molecules to needle forming negative or positive ions respectively. The outputs at needle were measured on the oscilloscope. In this study, repeated sets of experiments are carried out to collect consistent and reliable data. Visualizing/characterizing these fields are important as maximum delivery takes place at high voltage regions, with a condition that permeability of the cells should be known for proper transfection to occur, otherwise cells would die due to high voltages or no transfection takes place due to poor permeability of cell membrane.

CHAPTER 1: INTRODUCTION

With the progress in the development of recombinant DNA technology, there arrived a modern way to treat tumor or cancerous cells that was unthinkable two decades ago. The process of delivering a foreign material, say a new genetic material, into the targeted cells is called Gene Therapy. Since past two decades, enormous research had been done in this field of modern medicine worldwide in the laboratories of pharmacy, medicine, biochemistry, and chemical engineering [1]. The main objective of gene therapy is to develop an efficient, non-toxic gene carrier that can encapsulate and deliver foreign genetic materials into specific cell types [1]. In addition to above, few factors should be taken into consideration in a process like this to hamper the progress [3]. One of such is efficiency. The carrier vectors should be chosen to demonstrate high gene delivery without showing any toxicity. Second is long term duration of expression or their integration into host genome. And finally, safety standards should be met for proper transfection to host cells. Most importantly, efforts should be made to reduce the cytotoxicity and immunogenic nature of the genetic material to be integrated to cells [3].

1.1 Different Gene Delivery Systems

There are various types of gene delivery methods to carry out this process. Also, there are various types of carriers or vehicles to deliver gene to the host cells. These carrier vehicles are referred to as Vectors. These vectors are expected to transfer the gene to specific cell nucleus allowing it to express the genetic material transferred without causing any toxicity and being non-immunogenic and safe to cells they are transferred to. This process of transferring genetic material

into the cell nucleus is called transfection. And this is classified into Viral (Biological Gene Delivery System) and Non-Viral (Non-Biological Gene Delivery System) systems. That is based on the type of vehicle that transfers the genetic material, gene delivery systems are classified as Viral Vectors and Non-Viral Vectors [1] [2].

The process in which the transfer of gene is done via viral vectors is called Transduction, whereas the process in which delivery is carried out using a non-viral vector is called Transfection [2].

1.1.1 Viral Vectors

Delivery of exogenous nucleic acids into specified cells is important in gene therapy. A biological system in which viruses are used as vehicles to deliver gene to the host cells without the viruses showing its toxic nature are termed as viral vectors. Some viruses which are modified to eliminate their pathogenic effect and maintain high gene delivery efficiency are retrovirus, adenovirus, herpes simplex virus (HSV), adeno-associated virus(AAV), poxvirus and lentivirus [1], [3].

1.1.2 Non-Viral Vectors

Non-viral vectors are considered as non-biological vectors and are mostly cationic in nature [1]. Transfection of genes into cells are done either by chemical or physical approaches [4]. Chemical techniques include cationic polymers, peptides and lipids (liposomes). Physical techniques include electroporation, gene-gun, ultrasound and hydrodynamic pressure [5].

CHAPTER 2: VIRAL VECTORS

As the name implies, viral vectors or viral methods uses viruses as a transport medium to transfer genetic material into the targeted cells. The viruses used in this process are first modified to eliminate their toxic nature. These modified viruses are then transduced into targeted cells to transfer their genetic material into host cells for required action. Modification to viruses are made taking into consideration that they do not lose their capacity to transfer genes. These biological carriers evolve naturally to infect cells. For these systems both DNA and RNA are being evaluated as possible gene carriers and so are popular carriers of genes [6] [7].

Some most common and popular viruses are adeno virus (AV), adeno-associated virus (AAV), retrovirus (RV), herpes simplex virus (HSV), lentivirus (LV) vaccinia virus (VV). Each of these viruses have their own characteristics, however, there is no single universal ideal vector [3] [8]. So, before taking a decision of which viral vector to use, features of various viruses and the type of disease had to be considered to move forward with the gene transfer [3]. The payload capacity of the mentioned viruses are adeno virus (AV) 36kbp of dsDNA, adeno-associated virus (AAV) 4.7 kb of ssDNA, lentivirus (LV) 10 kb ssRNA, retrovirus (RV) 7.5 kb of ssRNA, vaccinia virus (VV) 190 kbp of dsDNA, and herpes simplex virus (HSV) 152 kbp dsDNA [Z. Raduly, G. A. Calin and I. Berindan-Neagoe, "Progresses towards safe and efficient gene therapy vectors," Oncotarget, vol. 6, no. 31, pp. 30675-30703, 2015.].

Although having high transduction rate, these viruses are limited in their use for issues in terms of safety, that is immunogenicity, have limited capacity of transgenic materials, that is

limiting payload capacity, which in-turn restricts the size of gene available for delivery [1]. In addition, these procedures are lengthier, costlier and requires stringent quality control procedures.

These limitations encouraged researchers to think of an alternative vector– non-viral vectors for foreign material transfers into body to correct the infected cells [1] [2] [4] [5].

CHAPTER 3: NON-VIRAL VECTORS

Non-viral vectors unlike viral vectors, though not as efficient as viral vectors, have many advantages over viral vectors. Few of them are they do not have safety related issues like viral vectors do, they have high gene encapsulation capability, and they are simple to set up [1] [5]. In these type of vectors, delivery of DNA is mediated by either of two - physical and chemical methods. Physical methods involve delivery of DNA using electroporation, gene gun, ultrasound, hydrodynamics injection, whereas chemical methods involve using chemicals such as polymers and lipids as carriers [4].

3.1 Chemical Methods

Generally, these vectors are both anionic and cationic in nature, but mostly cationic being used [1] [4] [15]. These methods strive to neutralize these cationic charges (negative charges) on the DNA while trying to express the DNA of interest [12] [13]. Polymers, lipids (cationic) and peptides are most commonly used as carriers in this process [4] [14]. Procedures with these are cheap, nonpathogenic and can be easily produced as compared to viral vectors. As it is known that non-viral systems have reduced efficiency than viral systems, these can be conjugated to viral proteins/virally derived peptides to increase DNA transport to cells [15] [16]. However, with the issues of heterogeneity in polymer based methods, there seemed a control over size of DNA resulting in reduced transfection efficiencies. Adding to the above, it also shows cytotoxic behavior with low transfection efficiencies as relative to viral as well as other physical methods [13].

3.2 Physical Methods

Any delivery system is designed to provide safe, efficient and targetable gene carriers to host cells [1]. Also, transfection by such methods takes a very short period of time. From past few years, emphasis is made on physical methods of delivery systems. Some of the techniques include delivery using electric fields [4] [22] [23] [24], magnetic fields [21], lasers [17], ultra sound [4] [18] [19] and high pressures [4]. Some of these technologies are discussed below. Among all the technologies mentioned, those with electric field mediated methods are gaining reputation as the most efficient methods. And the work presented in this paper also focuses on electric fields mediated way of non-viral technology.

3.2.1 Electroporation

This technique uses electric fields to permeabilize cells to enhance the uptake of gene into cells after they are being injected [4] [11]. This technique uses controlled electric fields to open pores of cells for the delivery. This method can be used in various tissues, such as skin, muscles, or liver [4]. Efficiency in this method can be achieved by optimizing few factors such as dose of DNA, shape of electrode, electric field strength and the duration of exposure to electric fields and duration for expression [4]. This method mostly employs local injection of plasmid DNA before application of electric fields.

3.2.2 Gene Gun

In this technique, the DNA is loaded onto the microscopic gold beads and is shot into the cells with a helium gun [4] [15]. This shooting of gold particles coated with DNA into the body allows direct penetration into the cytoplasm and even the nucleus and appear to be innocuous [15]. This technique can be used to treat skin [4] [15], liver [4] to vaccinate against tumor or to deliver genes that can promote wound healing [4] [15].

3.2.3 Ultrasound

Like other methods in this section, first a DNA of interest is injected into the cells where it is to be treated. Then those cells are treated with irradiating ultrasonic waves to increase cell permeability to macromolecules such as plasmid DNA [4] [25] [26]. This technique is safe and flexible to use in gene delivery and can be used to treat vascular cells [27] [28] [29] [30] [31] and muscles [31] [32].

3.2.4 Hydrodynamic

In this method, large volume of naked DNA solution is being injected with high pressure to see a potent gene transfer in internal organs [4]. This high pressure mediated transfection is found suitable for liver and some tissues and showed results of high transfection [33] [34].

CHAPTER 4: GENE DELIVERY BY ELECTRIC FIELDS

As mentioned earlier, gene delivery using electric fields gained reputation these days. Delivering a genetic material by employing physical forcing functions to accomplish gene delivery are considered to be safe having no effects of immunogenic response and toxic effects. One of such method is discussed in the previous section. With the main aim of characterizing the electric fields on the surface of a conductor (to mimic a skin), my work points out to show the spread of electric fields/charges takes place on surface of a conductor to identify the position of maximum uptake of drug into cells in presence of controlled electric fields.

Many terms are in use to refer to processes using electric fields to deliver genes. Few of them are electroporation, gene electro-transfer, gene therapy, electrochemotherapy, electro-pulsation and others. However, all these processes use electric fields to permeabilize cells for gene delivery. The phenomenon underlying all those mentioned processes are almost the same and will be explained below.

The cell is permeabilized using short or intense electric fields to create pores in cells. These pores allow the drug to enter through the cell membrane. When specific electric fields are chosen, the genetic material of interest pass through the membrane of cell to the nucleus of cell to express the DNA that is injected to it [4] [35] [36] [37] [38] [39]. In this process, an electrode used to permeabilize cells is placed in contact to the tissue/cell to be treated. As high electric filed strength of the order of few hundreds of V/cm is formed [40] [41], ions start to accumulate at the inner and the outer leaflets of cell membrane due to high electric fields on the surface [42]. As more ions

accumulate, the membrane potential reaches the breakdown voltage of insulating phospholipid bilayer and current starts flowing through the membrane, thus creating pores in the cells, which is called as permeabilization [43].

For all this to happen, proper voltages should be chosen to create enough electric fields at the surface of the tissue/cell. And these localized electric fields are studied for maximum gene delivery and this serves as the purpose of this work.

CHAPTER 5: HYPOTHESIS AND RESEARCH AIM

5.1 Hypothesis

From the literature, it is evident that many techniques were introduced to permeabilize cell for drug intake. The most commonly used techniques to create pores in cells are physical techniques using voltages.

This research uses a physical technique, that is, it uses high voltage, for example from 0kV to around 9kV to electropermeabilize cells. All the experiments in this research were performed not directly on skin/cells, but on mimics of skin, that is an anti-static clear tape with sheet resistance 9.8X10E9 ohms/sq and thickness 2.4 mils. This electropermeabilization is done to target specific spot on the surface, in other words to localize the gene delivery at a particular region. Localizing drug uptake is done by localizing electric fields at the desired location aiming for maximum uptake in that area. To achieve this, an experiment is set up to characterize electric charge on the surface by exploring the spread of charges on the surface, which is done by generating corona in point to plane system. The goal of this research is to explore the characteristics of novel charging apparatus to be utilized in future electropermeabilization processes. Studying a process of charging a region of surface and localizing where the uptake of exogenous material is strongest is what drives this research.

Recently, an in vivo method to deliver genetic material by using high purity helium plasma was proposed. It uses helium plasma at a rate of 15 l/min to deliver genetic material into the targeted region and was performed for 10 minutes on an animal. This is in reference to the Ph.D

dissertation “Plasma Mediated Molecular Delivery” by Richard J. Connolly, University of South Florida dated October 29, 2010. On exposing tissue to plasma for longer times, charges on surface of tissue pile up into air, that is charge density increases and on further exposure to plasma, the ions will be repelled by strong field lines and tend to fly/move sideways charging the whole animal or will be repelled entirely after reaching certain charge density above the surface. Here repelling causes the ions to float sideways. See Figure 1 below.

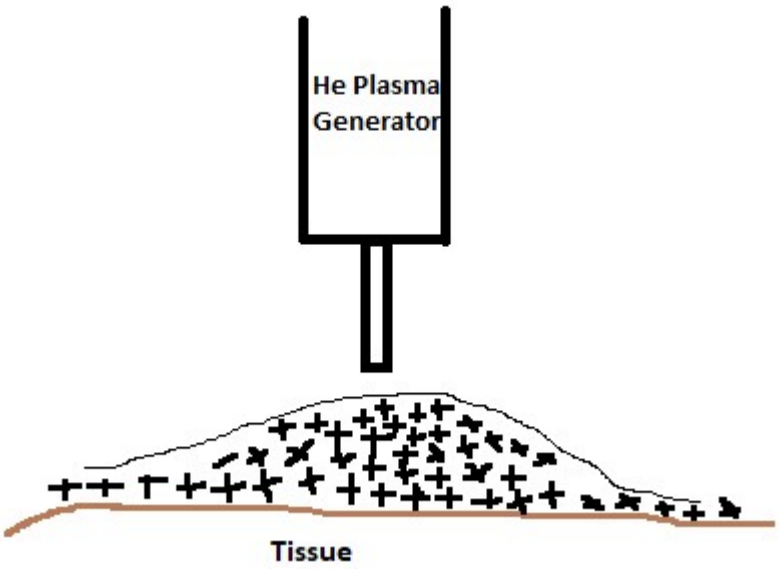

Figure 1 Mechanism of gene delivery using He plasma generator.

A controlled process for charging a surface for maximum uptake is needed. This research aims at controlled localized charging of a specific region.

5.2 Experiments

In order to achieve aforementioned hypothesis, the following experiments were performed.

5.2.1 Compensating Charges

A basic experiment is performed by powering surface electrode, a stainless steel metal piece and a pickup electrode as needle is used to collect the charges in that region. On doing this,

the input currents, voltages and output currents, voltages are being measured and calculated. Experiments were done with both positive and negative polarity voltages.

5.2.2 Ground Reference Dependence for Kelvin Measurements

On moving forward, an experiment was done by placing a Cu tape on the reverse side of glass plate at one end and a clear tape on another end. Voltages were measured from the top side at both ends of glass plate using Kelvin probe and were compared. Such experiments address the possibility that Kelvin probes need a reference potential below the measurement site to accurately provide a value.

5.2.3 Sheet Resistance

Sheet resistance of clear tape for few different lengths were measured. Also, experiment on wiping the clear tape with wet cloth was also performed and the results of both set of experiments were compared. This experiment was performed to characterize charge transport on skin mimic surface. It was predicted that charges move by means of water molecules coating the surface from ambient air. To validate this prediction, this experiment was conducted

5.2.4 Kelvin Probe vs Length from Source

A power supply connected to metallic finger is placed in contact with clear tape on glass plate and Kelvin probe is moved along the length of tape.

5.2.5 Needle vs Length from Source

An experiment that mimics the skin in localizing the strongest uptake is performed. These set of experiments were carried out with positive as well as negative voltages. And the voltage at the needle is collected.

5.3 Corona Discharge

Corona discharge is an electrical discharge which is created by ionization of gas molecules around a conductor subjected to high voltages. The conductor subjected to high voltages create high electric fields around it. As the strength of electric fields go beyond a certain threshold value, a bluish/white glow is seen as a result of formation of a conducting region which is called as corona. These are generally seen in transmission lines or any high voltage applications [44].

5.3.1 Mechanism

The high current carrying conductor creates a strong potential gradient around it. This strong electric field ionizes the air molecules to form both positive ions and free electrons. The strong field strength accelerates both these oppositely charged particles in opposite directions preventing them from recombination. These positive ions and free electrons collide with neutral atoms in air to ionize more creating a corona (https://www.revolvy.com/topic/Corona%20discharge&item_type=topic).

CHAPTER 6: COMPONENTS/EQUIPMENTS AND EXPERIMENTAL MODELS

6.1 Components/Equipment's

The below are the major list of components/ equipment's used in this research.

6.1.1 High Voltage Power Supply

There are two power supplies used in this work. One of the high voltage DC power supply used in this work is from Hipotronics with model number R15B. It operating range is from -15kV to +15kV.

And the other is Spellman power supply (model number CZE30PN10) which is connected to a laptop running NI LabView. This was used to get details of current to load, that is the input current. It can be operated in the range of -30kV to +30kV.

6.1.2 Resistors

Resistors are connected in series and in parallel in the circuit to form voltage divider and parallel combination to get the desired result.

6.1.3 Metal Finger

A metal finger which is connected to the power supply through which it acts as a medium to supply charges to the targeted surface by contact electrification of induced charges.

6.1.4 Stainless Steel Metal Piece

As with beginning of the research, a metal plate was used as a flat electrode which serves as a plane in point to plane corona discharge set up.

6.1.5 Needle

A sharp pointed sterile acupuncture needle with model number NA3040 is used as another electrode to pick up charges from the flat electrode. The high curvature of the needle is responsible for creating corona discharge at a point near the surface. The length and diameter of needle is 100mm and 0.30mm respectively.

6.1.6 Oscilloscope

The digital storage oscilloscope is used to see an output waveform on screen, also stores data on a USB device if connected externally. This is connected to the needle via resistors. It is a four channel digital oscilloscope (200Mhz) from Tektronix, TDS2024C.

6.1.7 Kelvin Probe

Like the sharp needle which is used to pick charges from the flat electrode, kelvin probe is also used to determine the electric potential without contact at any place on the flat conducting surface. This is to be placed at certain distance to avoid direct arcing from the source. The output of this on a voltmeter is limited to only 3kV. This device is from Monroe electronics with model number 244.

6.1.8 Clear Tape

An anti-static clear tape is used as a conducting plane surface which is stuck on a glass plate. Its width is 2.4 mils and its surface resistivity is 9.8X10E9 ohms/sq. This is used as a mimic to skin. This tape is from STATICO with its series as S5100.

6.2 Experimental Models

Based on the research aim, this section describes the working of experimental setups made in this research

6.2.1 Compensating Charges

6.2.1.1 Connections

In this experiment, the high voltage (HV) power supply is used as a source to drive the process. This input is connected to a combination of resistors connected in parallel. Each of these resistors have resistance of 1G Ohm. The equivalent resistance of parallel connected resistors is given by,

$$\frac{1}{R} = \frac{1}{R1} + \frac{1}{R2}$$

Here, R1=R2=1G Ohm;

R=0.5G Ohm=500M Ohm

These parallel resistors act as a current limiter in the circuit which is then connected to a metal finger to charge up the stainless steel plate surface. These constitute one side of the experimental “source” circuit.

On the other hand, a needle is held just above the flat electrode, metal plate. The distance from the needle to the metal piece is chosen to be 1mm, 2mm, or 3mm. This sharp pointed needle is then connected to a series of resistors which acts as a voltage divider at the output. The resistors connected in series as shown in Figure 2 are a high value resistor of 1M Ohm and a low value resistor of 441 Ohm. The output is collected at 441 Ohm resistor on a oscilloscope. This arrangement provided for determination of current in the needle part of the circuit while protecting the oscilloscope from high electric potential.

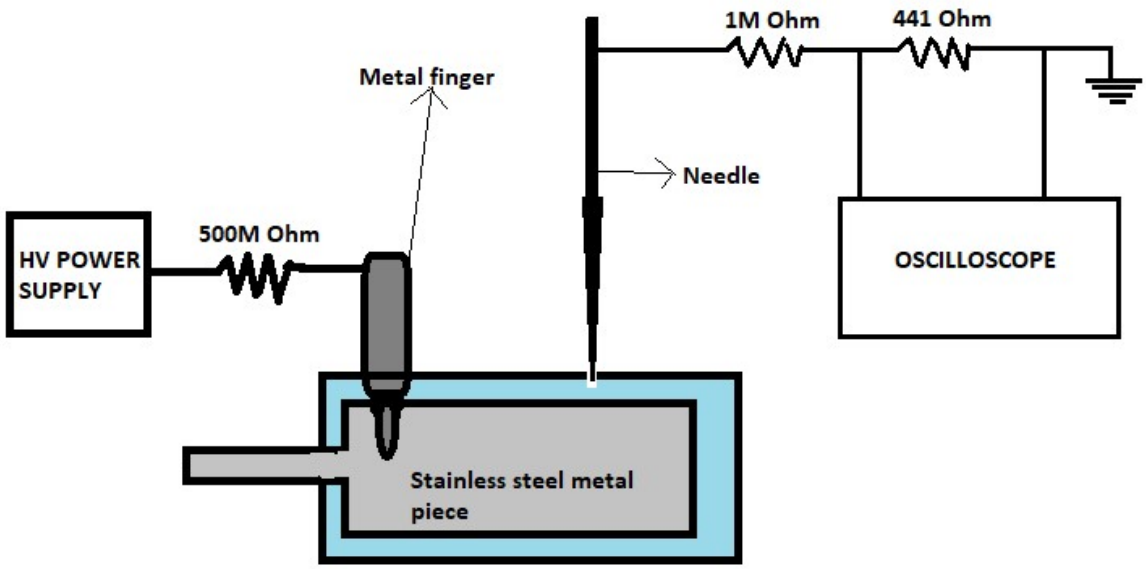

Figure 2 Experimental set up using stainless steel metal piece.

6.2.1.2 Working

On applying input voltage to the circuit, the metal finger charges up the entire surface of metal piece. This experiment is conducted with both positive and negative input voltages ranging from 0kV to 8kV. On applying voltage to the surface of steel piece, its surface gets uniformly charged. When a sharp needle is brought into the vicinity of conducting surface, high electric fields are created at tip of the needle. This is because, the needle has higher radius of curvature, that is the tip of the needle has smaller radius as compared to the flat steel piece.

So, greater the radius of the object, smaller is the surface charge density, indicating low field strength. This shows that at an object with small radius, having larger curvature, there exists strong electric fields.

Analogous to this, here the needle has higher curvature than the steel piece. So, strong electric fields are generated at a point where needle comes in proximity of metal piece. Due to strong electric fields, the molecules in air are ionized to form positive ions and free electrons. These ions and free electrons generated along with primary electrons gain high kinetic energy and

collide with other neutral atoms or molecules in air to multiply the ions in air. On increasing voltage from 0kV to 8kV, these ions/atoms/molecules in air are accelerated to form more ions of which few recombine to form neutral molecules producing a glow, which is called corona discharge, which occurs only if the electric field strength at needle is above the threshold value.

For instance, if positive voltage is applied to the flat surface, electrons tunnel from tip of the needle to air, thus loosing electrons to gas. As electrons are added to the molecules in air below the needle, they tend to form negative ions (like $CO_3^-(H_2O)_n$, here n, degree of hydration = 1, 2, 3,..), (Sakata, Soichiro, and Takao Okada. "Effect of humidity on hydrated cluster-ion formation in a clean room corona discharge neutralizer." Journal of aerosol science 25.5 (1994): 879-893.). These negative ions finally settle on positively charged surface and recombine with those oppositely charged ions lowering the potential below the needle. See the Figure 3 below. "i_e" denotes electron current and the conventional current is opposite to the direction of electron current. The surface is charged with positive input which is indicated as "+" in red color. Note the net potential line, "V" below the needle is lowered as negative and positive ions at the surface recombine, while the potential elsewhere is at higher level than below the needle. The lines drawn from surface to needle are electric field lines and the direction of these lines are indicated in the direction of positive current. Note that these field lines are perpendicular to the needle plane. Electric field lines are not drawn to scale as perpendicular, these are shown only for understanding.

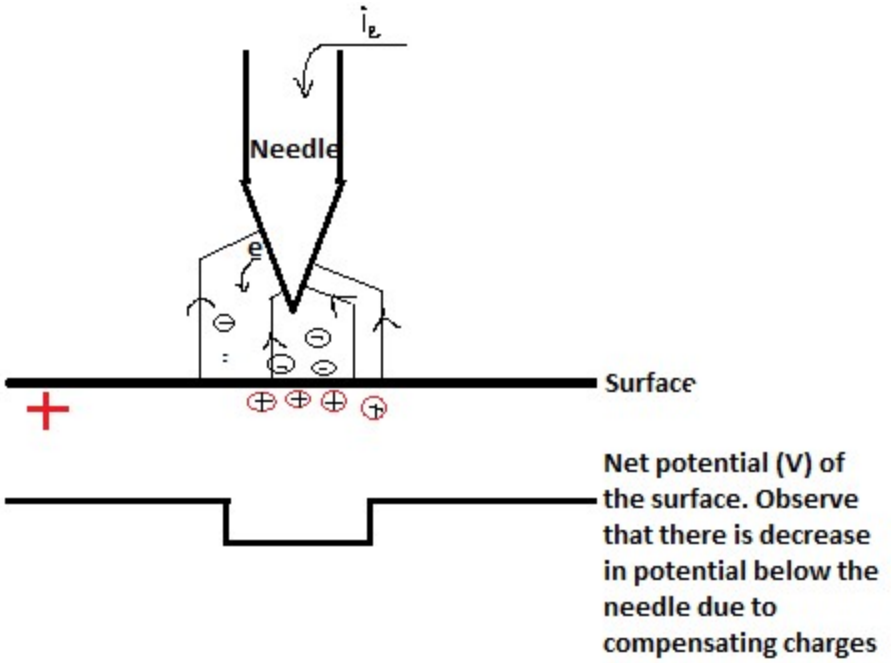

Figure 3 Negative ions compensating positive charges.

Similarly, if negative input voltage is given to surface, electrons tunnel from molecules in air to needle creating positive ions in the atmosphere (like $H^+(H_2O)_n$, where n, degree of hydration = 1, 2, 3,…). (Sakata, Soichiro, and Takao Okada. "Effect of humidity on hydrated cluster-ion formation in a clean room corona discharge neutralizer." Journal of aerosol science 25.5 (1994): 879-893.). These positive ions float to the metal surface where they compensate negative charges on metal surface increasing the potential below the needle. See the below Figure 4. "i_e" denotes electron current and the conventional current is opposite to the direction of electron current. The electric field lines are denoted from needle to flat surface, in the direction of positive current. Note that there is an increase in potential below the tip of needle.

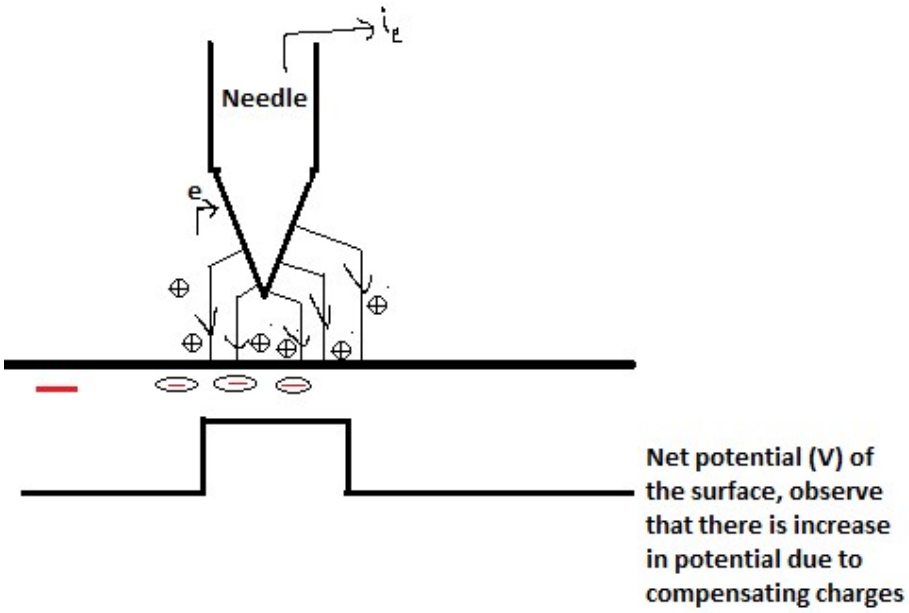

Figure 4 Positive ions compensating negative charges of surface.

And this current is measured across a voltage divider connected at the output. The output voltage collected across 441 Ohm resistor increases positively on increasing positive input voltage. If considered for negative polarity voltages, the output increases negatively, in fact, if seen on oscilloscope it goes down.

See the below Figure 5 representing field lines, flow of electrons and currents for positive and negative voltages. The same is the phenomenon for negative polarity voltages. Note that the direction of electric field lines, direction of currents and electrons are reversed for opposite polarities. Red lines indicate field lines and arrows on these red lines indicate direction of electric field lines.

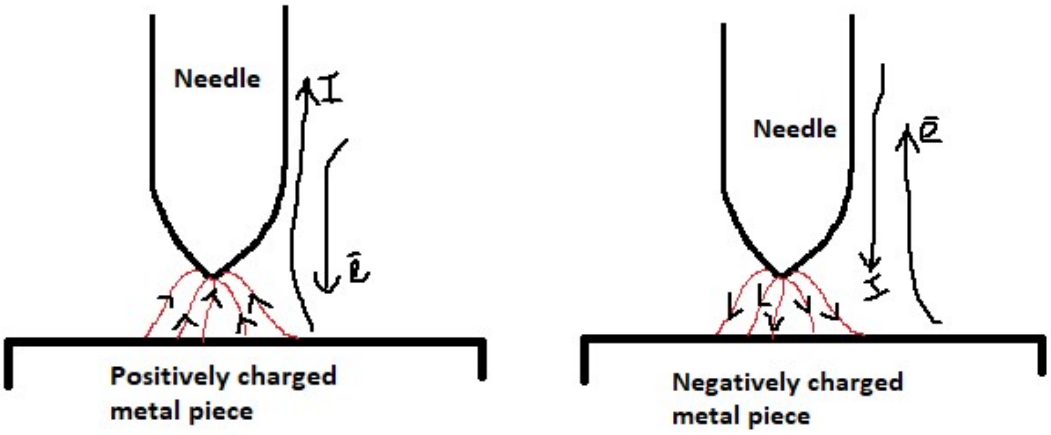

Figure 5 Working mechanism for positive (right) and negative (left) voltages.

6.2.2 Ground Reference Dependence for Kelvin Measurements

6.2.2.1 Connections

In this experiment, power supply is given to resistors connected in parallel having total resistance as 500M Ohm. After some voltage is dropped across resistors, it is then connected to the metal finger to charge the surface. The surface here is a clear tape. The clear antistatic tape is firstly stuck on a glass plate on the top side, while the bottom of the glass plate is stuck with conductive and non-conductive tape at end of both sides beneath the clear tape on top. See Figure 6 below. And a Kelvin probe (KP) is used to determine the electric potential of the surface, which is connected to a voltmeter for output voltages.

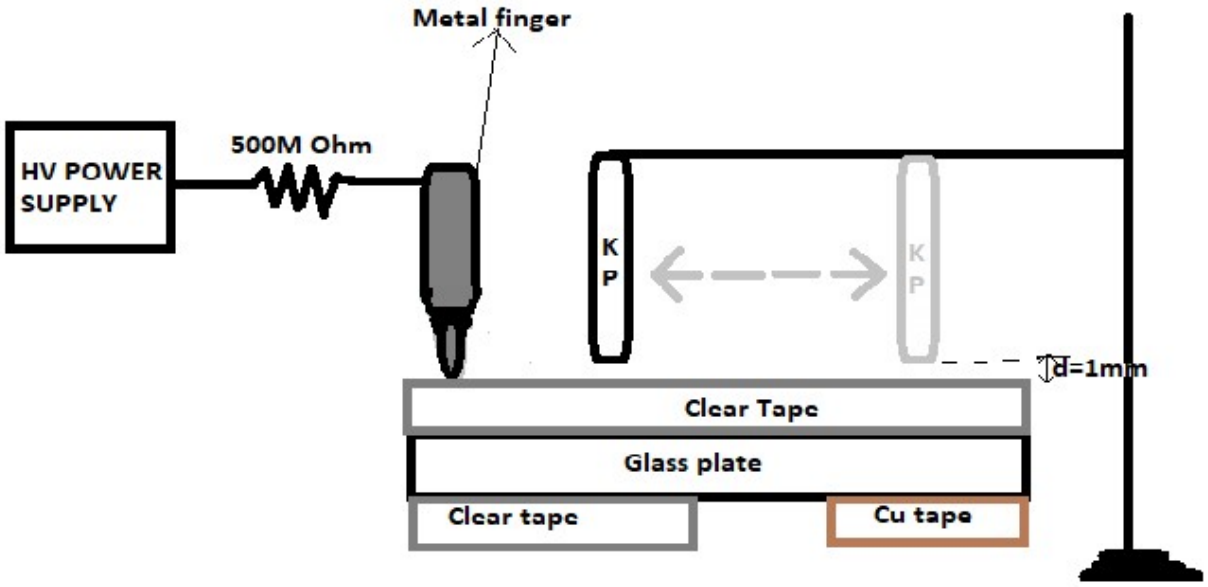

Figure 6 Experiment to measure voltages at two different positions.

6.2.2.2 Working

Usually the final experiment is done on a clear tape placed on a glass plate. The glass plate is normally placed on the wooden bench. To see if there is any change in movement of charges on top of clear tape, if the glass plate is placed on a conducting surface or on a non-conducting surface, this set up is made. Keeping that in mind, this experiment is supplied with voltages from 0kV to 3kV through resistors to metal finger to the targeted surface. The kelvin probe is placed 1mm (=d) above the tape surface. As input voltage is supplied, the clear tape becomes conductive and supports flow of charges through it. The Kelvin probe is then placed once below non-conducting tape (clear tape) (see Figure 6), to measure the deflection in voltage and then is moved carefully to the conducting tape (Cu tape) to note down the deflection. Both the measured regions were compared to see if they exhibit similar voltage values.

6.2.3 Sheet Resistance

6.2.3.1 Connections

Input high voltage power supply here is directly connected to a piece of conductive tape, a Cu tape which is stuck to the clear tape at one end on the top and the output is determined by connecting one end of a pair of resistors connected in series to a Cu tape on the other end of clear tape and the other end to an oscilloscope. A Cu tape is stuck to the bottom of glass plate and is grounded.

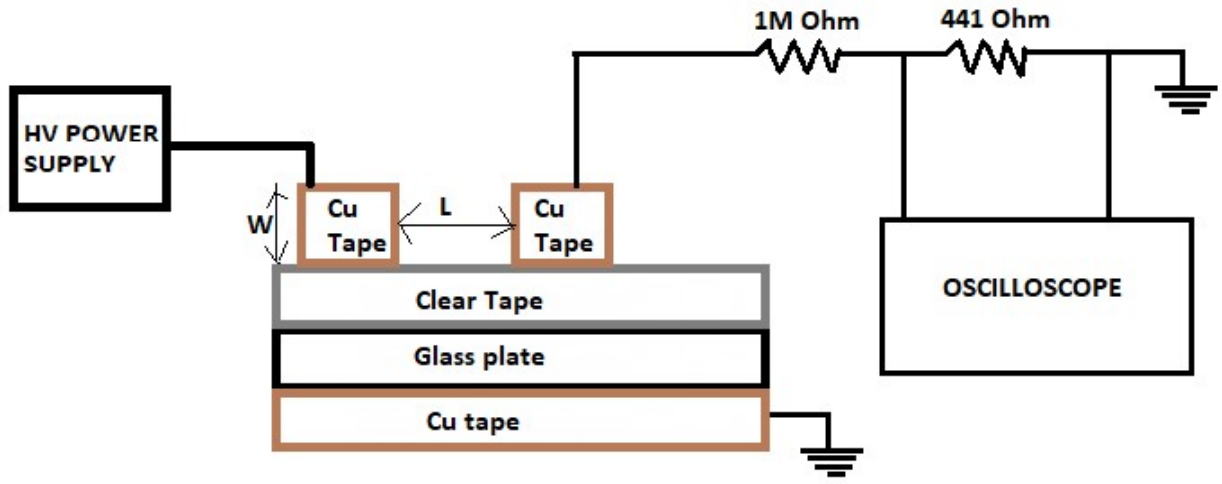

Figure 7 Sheet resistance measurement.

Here, W is width of clear tape as Cu tape is placed on top of Clear tape. Only for understanding, it is shown as such in figure.

6.2.3.2 Working

The aim of this experiment is to see movement of charges, if transport of charges is due to the moisture on surface of clear tape or through the clear tape. This can be explained on measuring the sheet resistance of tape at different lengths. On applying voltage to Cu tape, charges are being transported from one end of Cu tape to another piece of Cu tape on the other end. Having fixed voltage, varying the length L, see Figure 7, the output voltage is measured on the oscilloscope.

The circuit set up in Figure 7 looks like the below figure on measuring the unknown resistance.

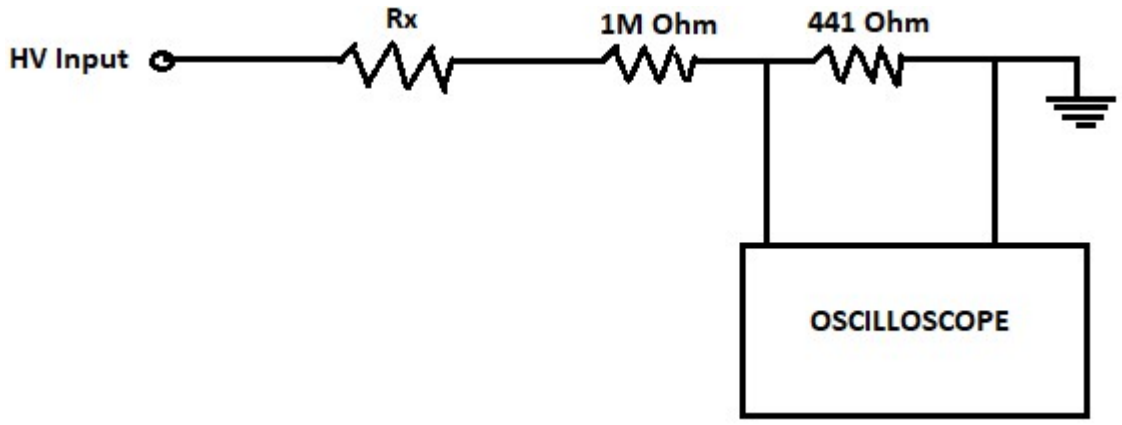

Figure 8 Effective resistance of Figure.7.

The sheet resistance measurements are done as follows. Knowing the input voltage, width of Cu tape, W attached to clear tape and by varying L, output voltage is to be noted. Let us say it as V_o. Now current through the circuit is to be calculated. As all resistors are connected in series, same current flows through the circuit. Knowing the output voltage measured across 441 Ohm resistor, I is calculated as

$$I = Vo/441$$

Applying Kirchoff's law to the above circuit,

$$Vi = IR_x + I * 10^6 + Vo$$

Find R_x

Sheet Resistance is given by

$$R_s = \frac{R_x}{L/W}\,\Omega/sq$$

Look at Figure 7 for L and W indications. Sheet resistance found for different values of L are compared.

6.2.4 Kelvin Probe vs Length from Source

6.2.4.1 Connections

The Spellman power supply connected to laptop running NI LabView is used as power supply for this set up. This system allows the user to set the experimental run time, operating voltage, and maximum current output. The power supply is connected to a parallel pair of current limiting resistors with effective resistance of 500M Ohm, which is then connected to a metal finger. The metal finger is used to power the clear tape for transport of charges. A glass plate with clear tape on top and a Cu tape at bottom grounded is used. At the output, a Kelvin probe is then moved over the tape at height of d=1mm is used to measure charges at different distances, L.

6.2.4.2 Working

At fixed input voltages, the Kelvin probe is placed at different distances, L and their corresponding voltages read on voltmeter are noted. The grounded Cu tape acts as a reference potential.

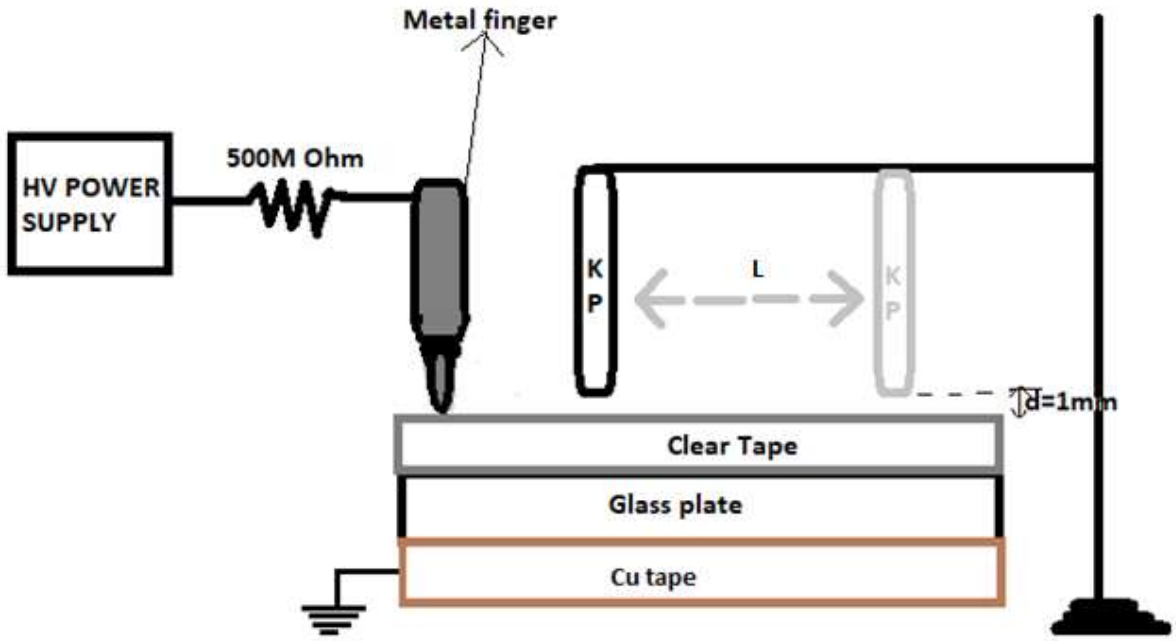

Figure 9 Decay of output voltage with distance, L.

6.2.5 Needle vs Length from Source

6.2.5.1 Connections

A high voltage power supply which is connected to a laptop running LabView is used as the input source. The input is then connected to resistor in parallel who's effective resistance is 500M Ohm. This combination acts as a current limitor to protect the circuit. The voltage after being dropped is then connected to a metal finger. This metal finger is placed in contact to the clear tape which is placed on top of glass plate. The bottom of glass tape is stuck with a Cu tape and is grounded to prevent any charges from the base where the glass plate is placed. This ensures that charges picked on tape is purely from the input voltage at the input side.A sharp pointed needle is held certain distance above the clear tape surface. This needle is then connected to a voltage

divider formed by resistors connected in series. Finally the output is measured from the oscilloscope across 441 Ohm resistor. See the Figure 10 below.

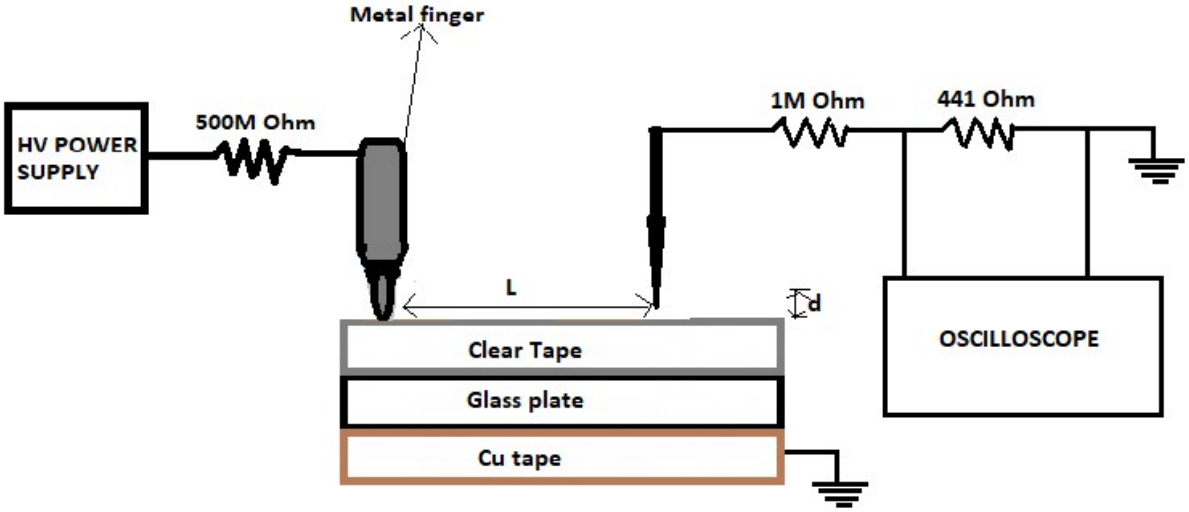

Figure 10 Characterizing electric fields on varying L, d.

6.2.5.2 Working

On applying input voltage from a metal finger which is in contact with clear tape, a conductive path is formed on the clear tape. This surface is uniformly charged. As when a sharp pointed needle is brought near to the surface of the clear tape, but not in contact with the tape, say at distance d=1mm/2mm/3mm, due to its high curvature, the needle creates a strong electric field below it. The field where the needle is pointing the surface electrode, the clear tape, is stronger than anywhere else. Due to the presence of high field strength, ionization of molecules in air takes place giving rise to positive or negative ions. As the field strength increases on increasing voltage, more ions are formed. Also, some recombine to from neutral molecules. When the field strength exceeds certain threshold value, positive and negative ions gain enough energy and tries to recombine giving rise to a bluish/white glow, named as corona discharge. Voltage measurements

are made on applying both positive and negative polarity voltages. Refer to Figure 5 in 6.2.1 for the direction of current, field and electrons.

CHAPTER 7: EXPERIMENTAL RESULTS AND CONCLUSIONS

This chapter presents the results of all experiments mentioned previously in Chapter 6. All experiments in this work are conducted thrice to attain reliable and consistent data. Any data stating V1, V2, V3 and I1, I2, I3 represents voltages for repeat 1, repeat 2, repeat 3 and currents for repeat 1, repeat 2, repeat 3 respectively.

7.1 Compensating Charges

This experimental set up works on charge by induction. Charge by induction is a process of charging a neutral conductor on bringing it into the vicinity of charged conductor. Here, the stainless steel plate is powered and the needle gets charged by the field created by the surface plate to the sharp pointed needle. So, the needle here charges by induction (The Book by John Avison "The World of Physics" published by Nelson Thornes,2014). The charge density on the steel plate creates electric field into air to ionize the water molecules, principal common positive corona ion species, or CO_2 molecules, common negative corona ions in air.

On applying a positive bias to the plate, the charges on the plate create electric field around it. The strongest gradient of electric field lines above the plate occurs at the tip of the needle. The electrons tunnel from the needle and forms negative ions in air which floats on to metal surface. These negative ions compensate positive charges on metal surface. The current measured on using voltage from oscilloscope at output is actually the positive current as electrons are lost in air. So, the direction of current which is opposite to the direction of flow is electrons is away from the steel plate to needle. Refer section 6.2.1. for further understanding this mechanism.

The same is with negative voltage. On applying negative voltage, electrons tunnel to needle creating positive ions at the tip of needle and will move toward the plate and land on the plate, compensating the charge on the plate and rises the potential locally by combining with negative charges.

7.1.1 Results

The data here is measured for d=1mm, where d is the distance between the needle and steel plate. The designation I1, I2, I3 indicates that each of them are from repeat 1, repeat 2 and repeat 3 respectively. The output current measured for positive voltages here indicates positive current, not the electron current, that is, the current measured by losing electrons to gas from the needle. This is measured on an oscilloscope across 441 Ohm resistor.

Table 1 Positive voltage stainless steel output and input currents.

	Current Sourced from PS			Current across 441□ Resistor		
Input V (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
1	3.6	4.5	4.8	1.15	2.09	2.32
2	4.6	5.8	6.1	1.89	2.75	1.64
3	6.9	7.7	7.9	3.39	2.71	4.68
4	8.9	9.6	10.1	5.13	4.99	4.53
5	11.1	11.8	11.9	7.28	8.99	6.14
6	13.1	13.6	14.5	8.41	8.11	8.01
7	15.2	15.7	15.9	8.48	10	10.4
8	17.3	17.7	17.8	13.1.	11.4	9.4

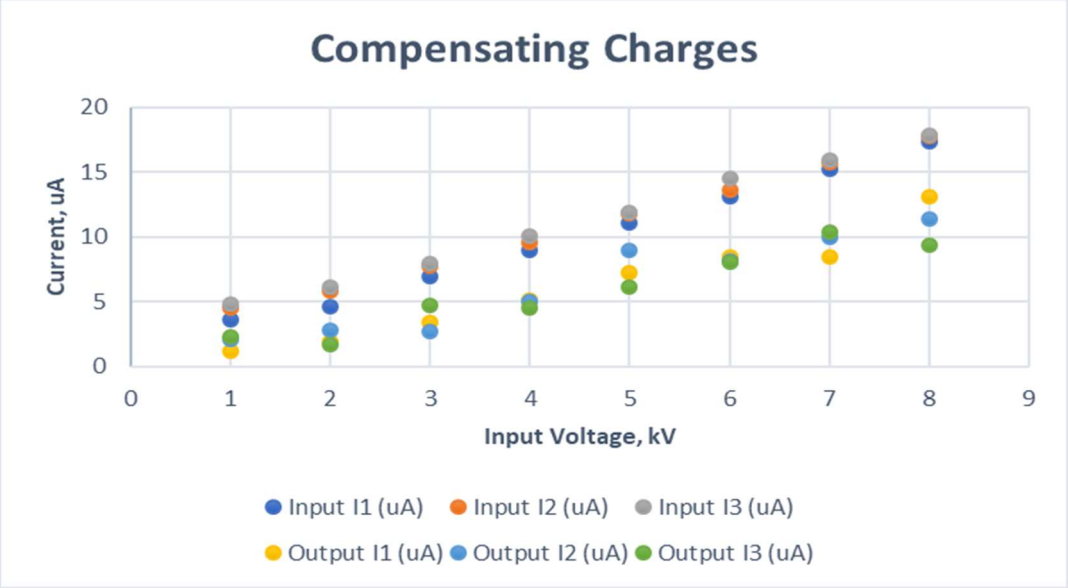

Figure 11 Positive voltage stainless steel output and input currents.

It can be observed from Figure 11 that the output current increases with increase in input voltages and are smaller than input currents.

The data mentioned below is measured for d=1mm negative input voltages. Where d is the distance between the needle and steel plate. The designation I1, I2, I3 indicates that each of them are from repeated experiments namely, repeat 1, repeat 2 and repeat 3 respectively. The power supply used in this experiment is the Spellman power supply running with NI LabView on laptop. It is to be noted that the output current measured for negative voltages indicates the electron current into the needle.

Table 2 Negative voltage stainless steel output and input currents.

	Current Sourced from PS			**Current across Resistor**		
Input V (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
-1	-4.00	-4.50	-4.70	-1.50	-2.58	-1.40
-2	-5.00	-5.60	-5.60	-6.21	-1.64	-6.18
-3	-7.00	-7.80	-7.70	-7.08	-3.84	-3.08
-4	-9.00	-9.50	-9.50	-9.26	-5.45	-5.42
-5	-10	-11	-11	-8.21	-7.34	-7.55
-6	-10	-13	-13	-7.82	-7.00	-7.87
-7	-10	-15	-15	-7.38	-8.78	-9.73
-8	-20.00	-18	-17	-10.8	-1.05	-9.27

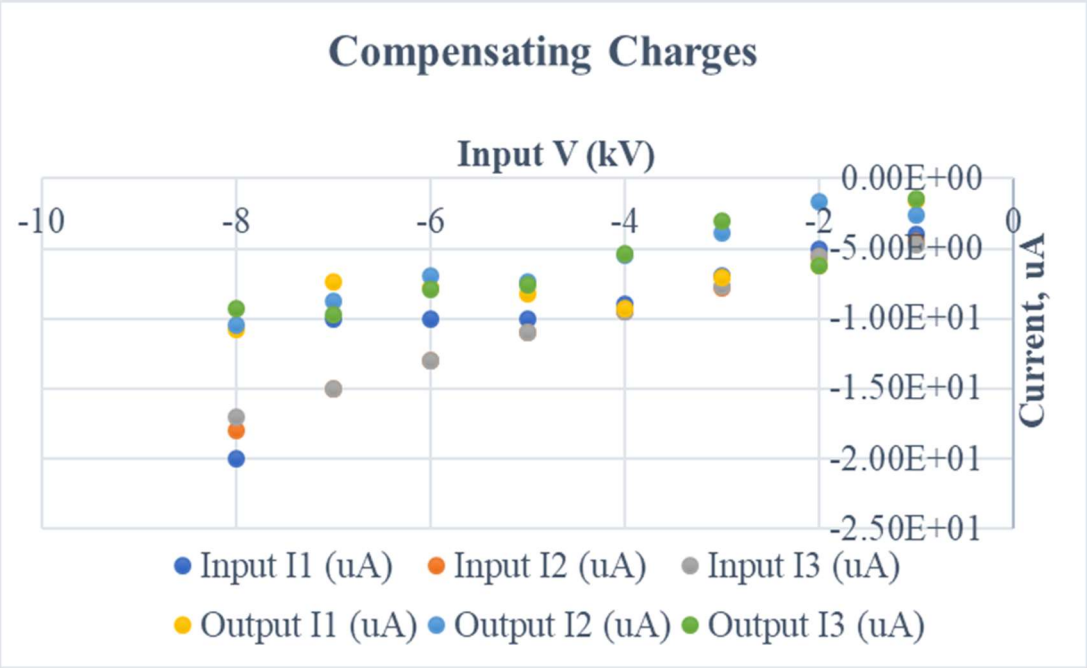

Figure 12 Negative voltage stainless steel output and input currents.

It can be observed from Figure 12 that the output current increases negatively with increase in input voltages and are smaller than negative input currents.

7.1.2 Conclusion

It can be observed from the graphs that the data mostly follows a linear behavior that is on increasing input voltage, the output current correspondingly increases. In this experiment, negative ions compensate positive charges on steel plate, thereby lowering the potential below the needle for positive inputs and positive ions formed from electrons tunneling to needle compensate with negative charges on surface, thereby increasing potential. With this kind of behavior, we can locally modulate the potential at desired regions on the surface.

7.2 Ground Reference Dependence for Kelvin Measurements

This experiment is performed with the metal finger on the Cu side or on the Clear tape side. For consistency, the experiment is given 3 repeats. The experiments are conducted with both positive and negative polarity voltages.

7.2.1 Results

Here, Vo1 represents repeat 1 output voltage, Vo2 represents repeat 2 output voltage, Vo3 represents repeat 3 output voltage These output voltages are collected by Kelvin probe which is held at 1mm above the surface.

Table 3 High voltage power supply at Cu end.

Cu end	**Output voltages measured using Kelvin probe**					
Vin in kV	**Vo1 at Cu end**	**Vo1 at Clear tape end**	**Vo2 at Cu end**	**Vo2 at Clear tape end**	**Vo3 at Cu end**	**Vo3 at Clear tape end**
1	964	959	970	970	965	955
2	1968	1956	1947	1938	1987	1977
3	2887	2854	2864	2838	2839	2824

It can be observed from the above table that output voltage measured at Cu end and Clear tape are almost the same.

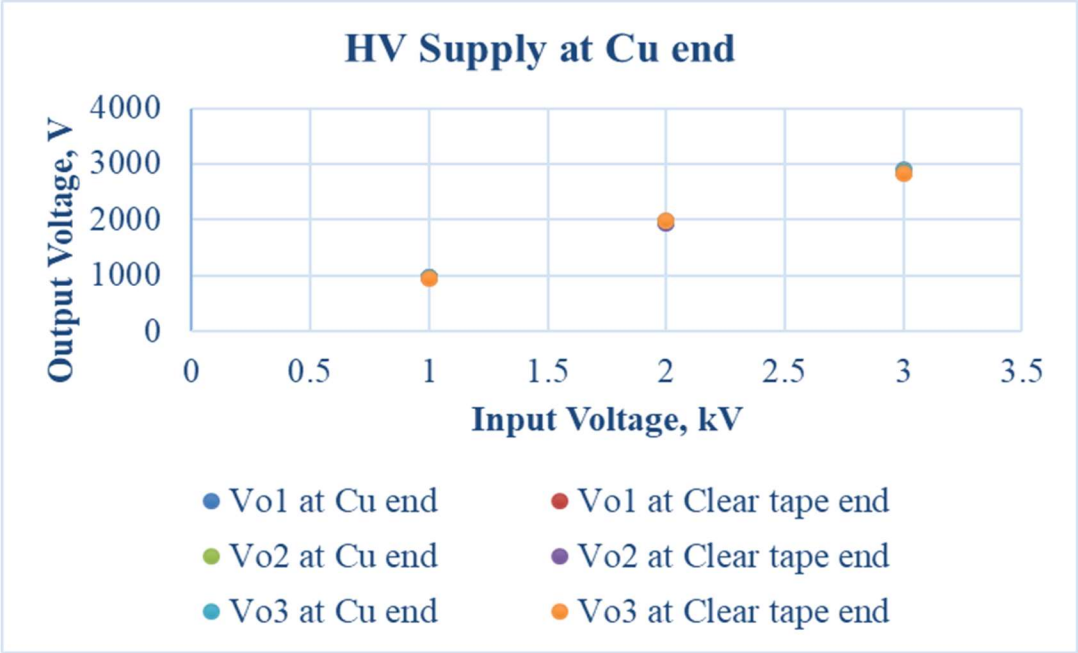

Figure 13 High voltage power supply at Cu end.

The above figure shows that output voltage measured is almost the same as input voltage.

Table 4 High voltage power supply at clear tape end, positive V.

Clear tape end	**Output voltages measured using Kelvin probe**					
Vin in kV	**Vo1 at Cu end**	**Vo1 at Clear tape end**	**Vo2 at Cu end**	**Vo2 at Clear tape end**	**Vo3 at Cu end**	**Vo3 at Clear tape end**
1	962	969	960	963	960	965
2	1888	1908	1894	1918	1888	1905

This table that output voltages measured at Cu end and clear tape are again the same though powering is done at Clear tape side.

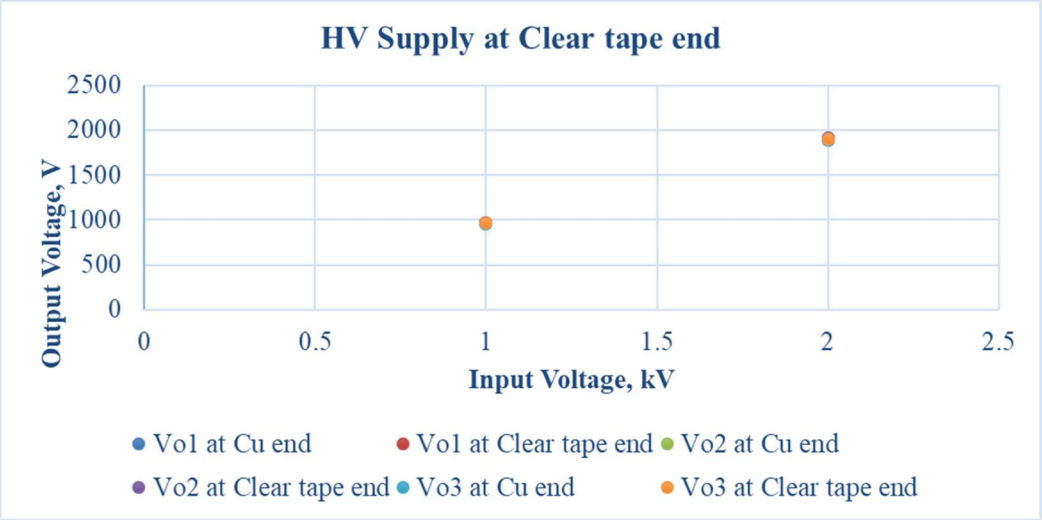

Figure 14 High voltage power supply at clear tape end, positive V.

The below table presents output voltages with corresponding negative input voltages measured using Kelvin probe It can be noted that the voltages measured at both Cu and Clear tape end are the approximately the same.

Table 5 High voltage power supply at clear tape end, negative V.

Clear tape end	**Output voltages measured using Kelvin probe**					
Vin in kV	**Vo1 at Cu end**	**Vo1 at Clear tape end**	**Vo2 at Cu end**	**Vo2 at Clear tape end**	**Vo3 at Cu end**	**Vo3 at Clear tape end**
-1	-953	-960	-948	-957	-953	-961
-2	-1886	-1902	-1886	-1900	-1887	-1902

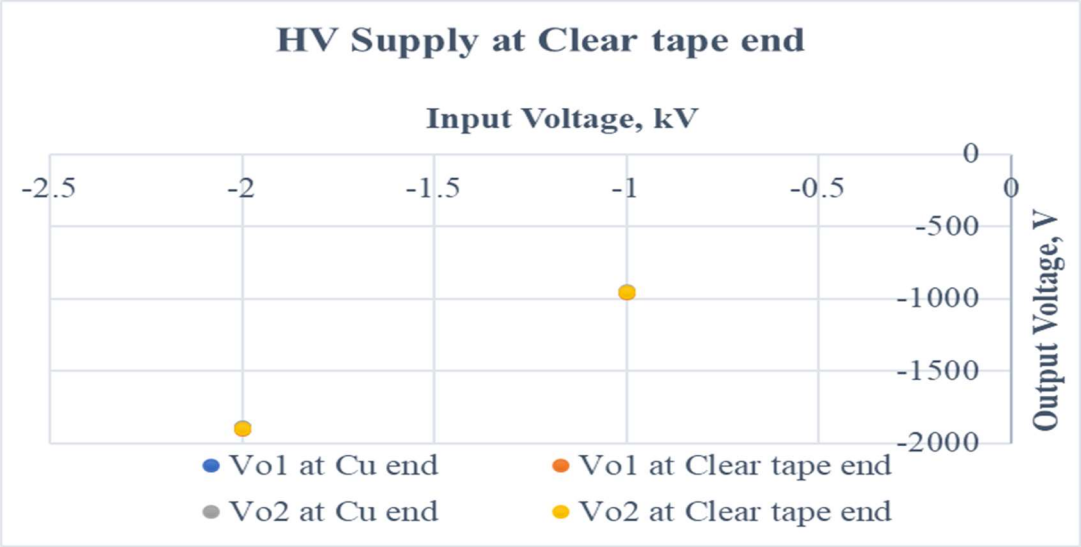

Figure 15 High voltage power supply at clear tape end, negative V.

7.2.2 Conclusions

The output voltages measured at both the ends of Clear tape and Cu tape using Kelvin Probe are nearly the same from tables presented above or it can be easily observed from their respective figures. The experiments performed using this set up indicates that a grounded reference for Kelvin probe measurements are not required for this set up. However, for other experiments not involving Kelvin probe measurements, a Cu tape was used as a reference, was placed at bottom of glass plate and was grounded.

7.3 Sheet Resistance

This experiment was used to measure sheet resistance of clear tape for different lengths. This experiment was also performed to see what drives the charge transport on the surface. With the set up discussed in 6.2.3, experiments were conducted using dry and wet surface (by just wiping the surface with a wet cloth) and results are presented below with sheet resistance calculations. Hipotronics was used to power this experiment.

7.3.1 Results

For first set, L=5cm and by without wetting the surface, for fixed input voltage of 5kV, three repeats of experiments were performed. Here W= 2.4cm. The results and calculations of these repeats were as follows.

For repeat 1, output current is measured as, I = 3.56uA

On referring to section 6.2.3, Figure 8, Resistance, Rx is measured by,

$$R_x = \frac{\left(Vi - I\left(10^6 + 44\ \right)\right)}{I}\ Ohms \quad (1)$$

Rx = 1.4G Ohm

And the sheet resistance is measured by,

$$R_{s=}\frac{R_x}{\left(L/W\right)}\ Ohms/sq \quad (2)$$

Rs = 673M Ohm/sq (i)

Similarly,

For Repeat 2, I=3.43uA

Using Equation (1), Rx = 1.4G Ohm and by using equation (2),

Rs = 699M Ohm/sq (ii)

For Repeat 3, I=3.32uA

Using Equation (1), Rx = 1.5G Ohm and by using equation (2),

Rs = 723M Ohm/sq (iii)

On wetting the surface, that is just by wiping the tape with a wet cloth, for same L=5cm, the following are the results of three repeats.

For Repeat 1, I=95.2uA

Using Equation (1), Rx = 51MOhm and by using equation (2),

Rs = 24M Ohm/sq (iv)

For Repeat 2, I=95.6uA

Using Equation (1), Rx = 51M Ohm and by using equation (2),

Rs = 24M Ohm/sq (v)

For Repeat 3, I=96.4uA

Using Equation (1), Rx 50.9M Ohm and by using equation (2),

Rs = 24M Ohm/sq (vi)

For the second set, L=8cm and by without wetting the surface, for fixed input voltage of 5kV, again three repeats of experiments were performed. Here W= 2.4cm. The results of these repeats were as follows.

For Repeat 1, I=3.00uA

Using Equation (1), Rx = 1.6G Ohm and by using equation (2),

Rs = 500M Ohm/sq (vii)

For Repeat 2, I=3.21uA

Using Equation (1), Rx = 1.5G Ohm and by using equation (2),

Rs = 467M Ohm/sq (viii)

For Repeat 3, I=3.27uA

Using Equation (1), Rx = 1.5G Ohm and by using equation (2),

Rs = 463M Ohm/sq (ix)

On wetting the surface, that is just by wiping the tape with a wet cloth, for same L=8cm, the following are the results of three repeats.

For Repeat 1, I=96.1uA

Using Equation (1), Rx = 51MOhm and by using equation (2),

Rs = 15M Ohm/sq (x)

For Repeat 2, I=96.1uA

Using Equation (1), Rx = 51MOhm and by using equation (2),

Rs = 15M Ohm/sq (xi)

For Repeat 3, I=95.4uA

Using Equation (1), Rx = 51M Ohm and by using equation (2),

Rs = 15M Ohm/sq (xii)

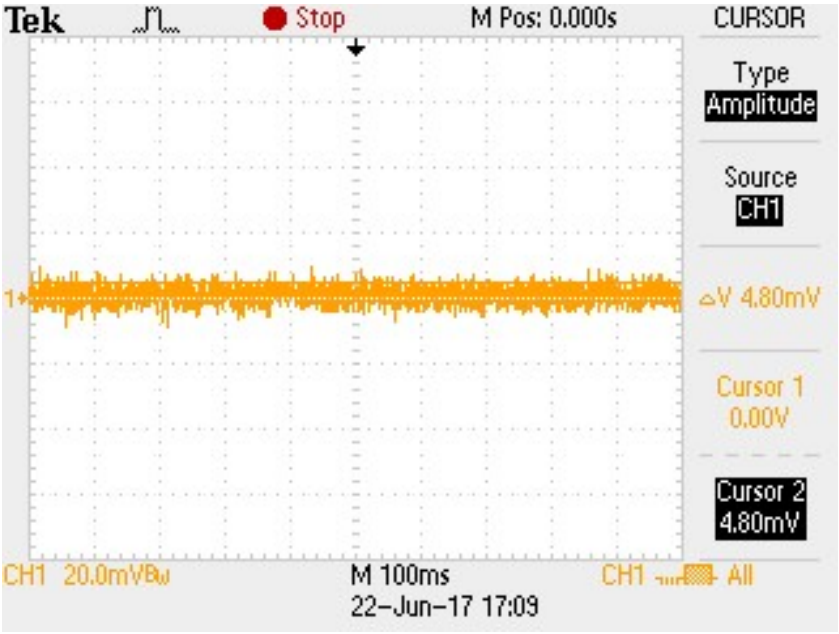

Figure 16 Image from an oscilloscope for dry surface, L = 5cm.

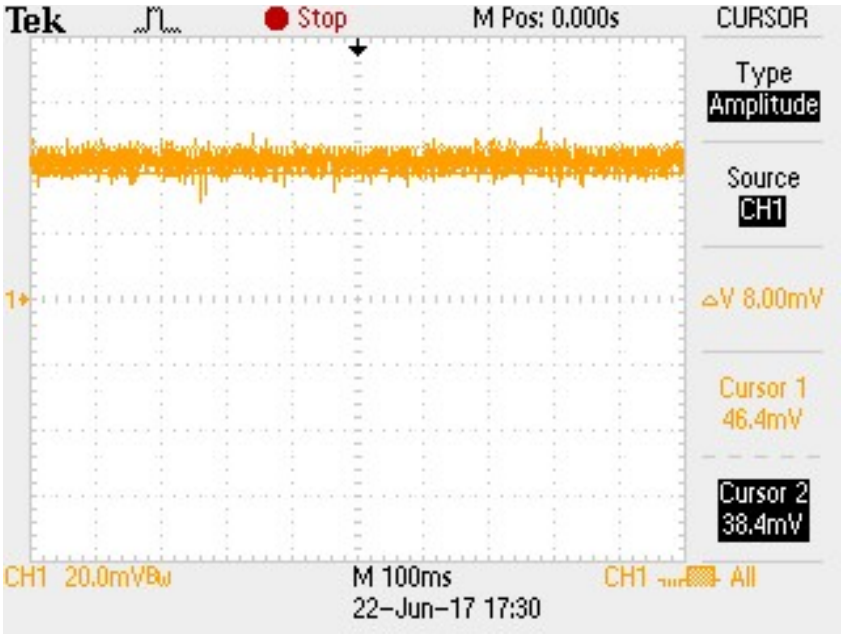

Figure 17 Image from an oscilloscope for wet surface, L = 5cm.

On observing Figure 16 of dry surface and Figure 17 of hydrated surface, it can be observed that Figure 17 shows a significant increase in voltage measured across 441 Ohm resistor on wiping the surface with wet cloth which validates the assumption mentioned in section 5.2.3 that the surface charging is driven by water molecules present in atmosphere. The same behavior was observed for L=8cm.

7.3.2 Conclusions

From (i), (ii), (iii), (vii), (viii), (ix), it can be observed that all these values lie around 500M Ohm for 2.08 squares (no. of squares=L/W=5cm/2.4cm) to 700M Ohm for 3.33 squares (no. of squares=L/W=8cm/2.4cm). But the sheet resistance of clear tape is 9G Ohm/sq. From this it is evident that a portion of current may be carried by surface molecules.

Also, from (iv), (v), (vi), (x), (xi), (xii), it can be noted that on increasing the water molecules on surface, surface becomes more conductive. So, the transport of charges on the clear

tape may be due to the presence of water molecules on the surface. This indicates that water molecules drive this process.

7.4 Kelvin Probe vs Length from Source

This experiment was carried out for different lengths given a fixed input voltage. For this, experiments with input voltages 1kV, 2kV for both positive and negative polarities for lengths, L=5cm, 7cm, 9cm, 11cm, 13cm, 15cm and 17cm are conducted and their outputs are measured using Kelvin probe. These results are shown below.

7.4.1 Results

For V=1kV, d=1mm. Here L is the distance between metal finger and Kelvin probe and d is the height at which Kelvin probe measurements were made from the surface.

Table 6 V=1kV, decaying voltages.

	Output voltages measured on Kelvin probe		
L in cm	**Vo1 in V**	**Vo2 in V**	**Vo3 in V**
5	899	900	902
7	886	882	886
9	868	867	869
11	858	856	858
13	846	844	847
15	832	832	834
17	823	821	824

It can be perceived from the above table that as we move along the clear tape from the metal finger for various lengths, there is a notable drop in potential. See below Figure 18.

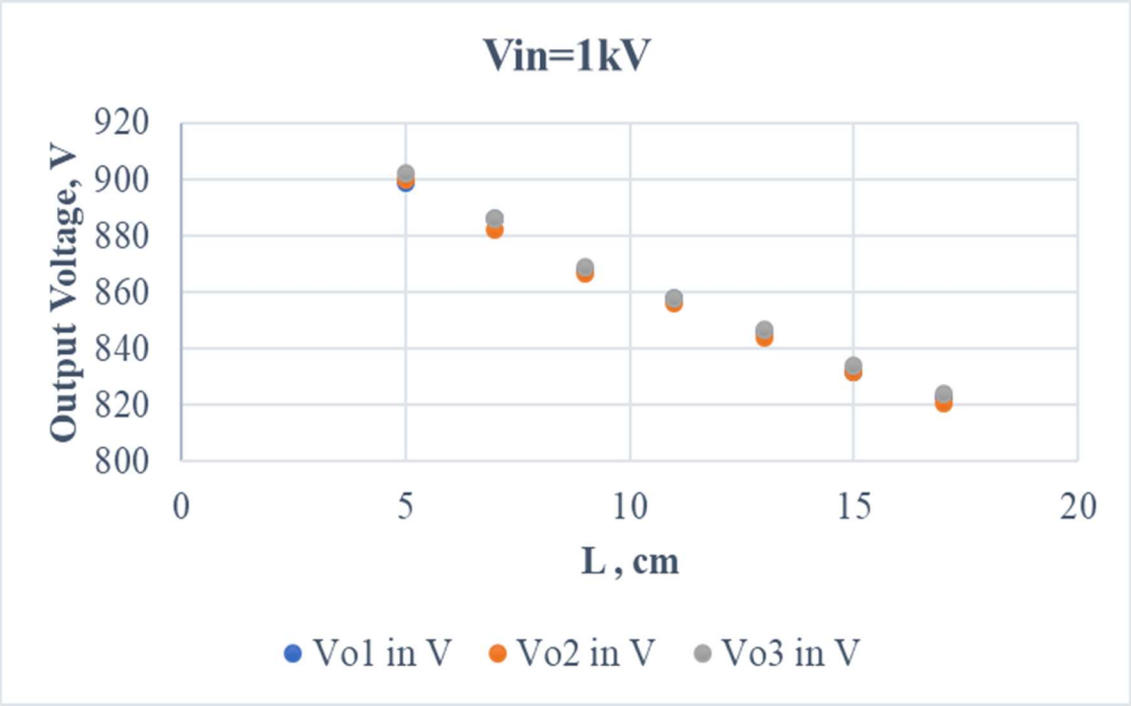

Figure 18 V=1kV, decaying voltages.

For V= - 1kV, d=1mm

Table 7 V= - 1kV, decaying voltages.

	Output voltages measured on Kelvin probe		
L in cm	**Vo1 in V**	**Vo2 in V**	**Vo3 in V**
5	-876	-888	-887
7	-863	-872	-876
9	-844	-858	-862
11	-836	-846	-850
13	-827	-835	-835
15	-813	-823	-827
17	-807	-811	-816

Like for 1kV, the outputs are decreasing negatively on moving away from the metal finger.

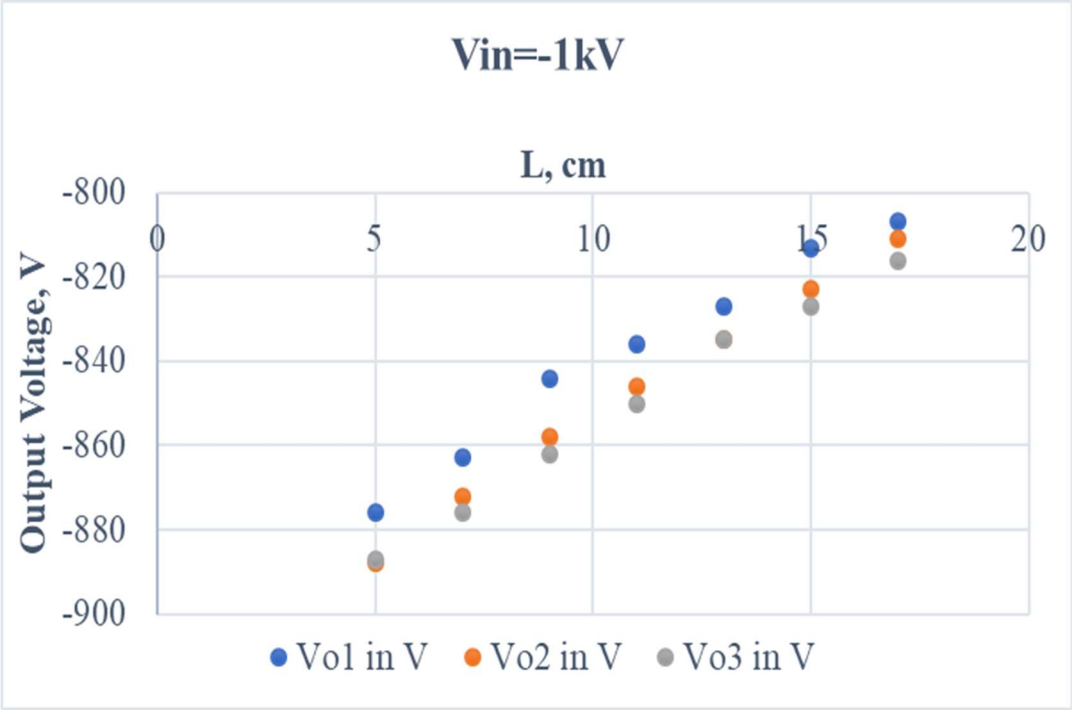

Figure 19 V= - 1kV, decaying voltages.

For V=2kV, d=1mm

Table 8 V=2kV, decaying voltages.

	Output voltages measured on Kelvin probe		
L in cm	**Vo1 in V**	**Vo2 in V**	**Vo3 in V**
5	1777	1783	1770
7	1745	1747	1744
9	1716	1711	1718
11	1688	1685	1683
13	1660	1655	1659
15	1632	1624	1634
17	1609	1605	1611

It is seen that there is some potential drop even on increasing voltage to 2kV.

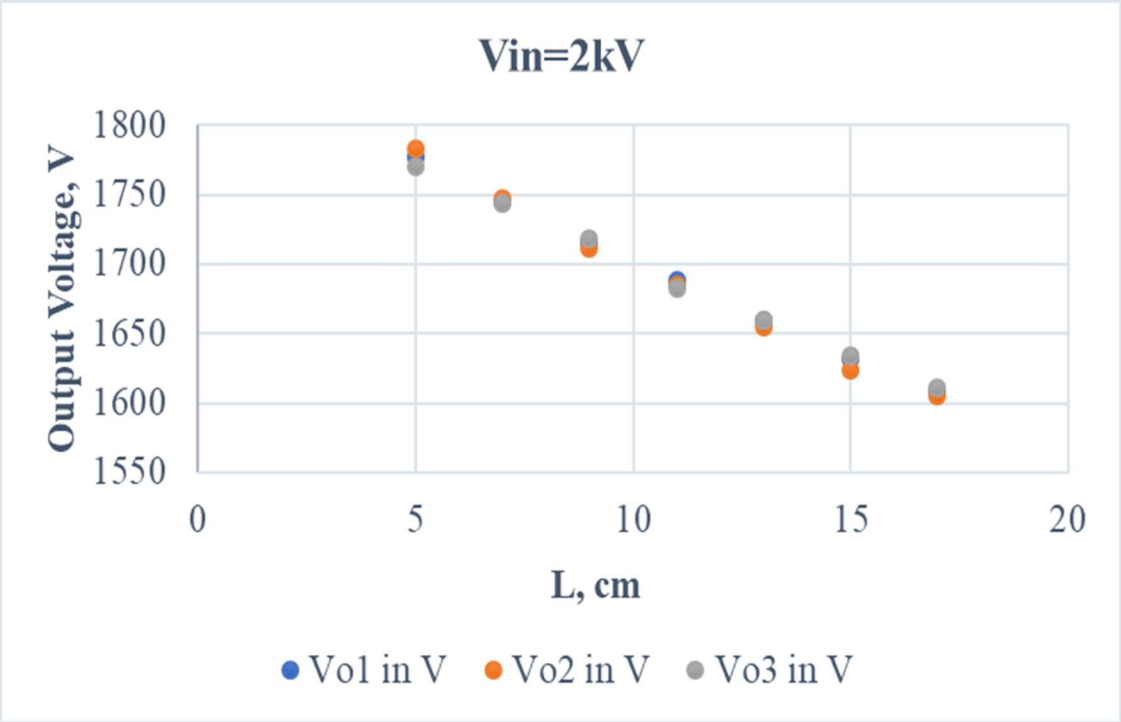

Figure 20 V=2kV, decaying voltages.

The above figure shows exponential decay characteristics for different lengths.

For V= - 2kV, d=1mm

Table 9 V= - 2kV, decaying voltages.

	Output voltages measured on Kelvin probe		
L in cm	**Vo1 in V**	**Vo2 in V**	**Vo3 in V**
5	-1754	-1761	-1766
7	-1718	-1716	-1729
9	-1683	-1694	-1709
11	-1657	-1664	-1681
13	-1629	-1648	-1659
15	-1601	-1621	-1631
17	-1586	-1594	-1608

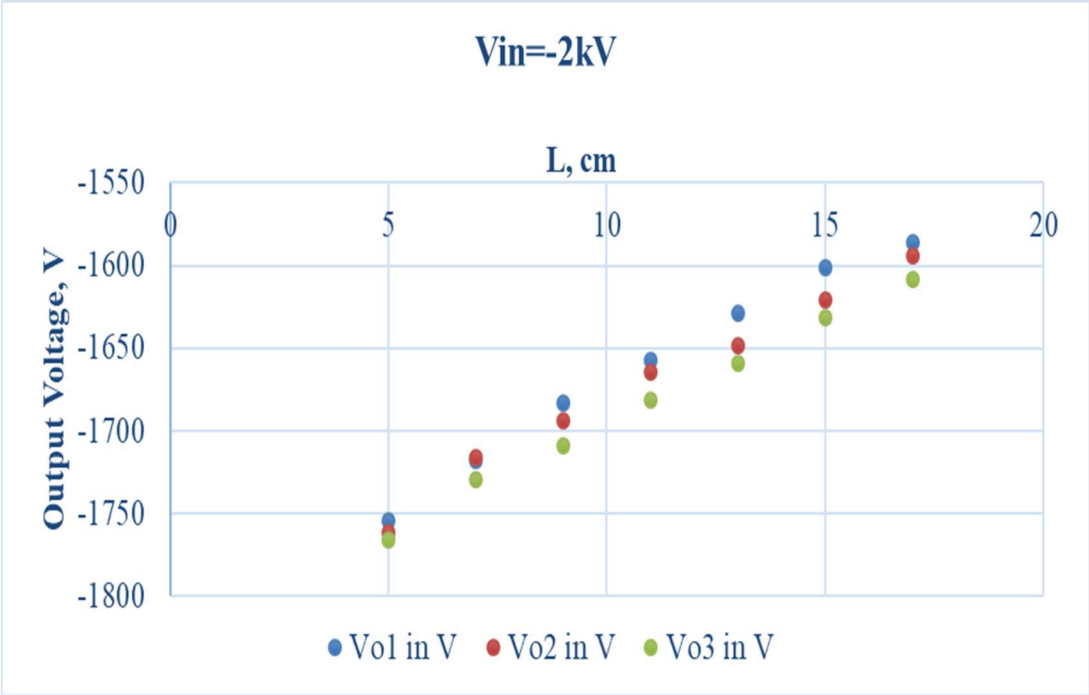

Figure 21 V= - 2kV, decaying voltages.

7.4.2 Conclusions

On observing all tables and figures above in this section, it can be depicted that there is some potential drop as L increases. So, there is potential drop as we move away from the source.

7.5 Needle vs Length from Source

This experiment was performed at various L's, which is the distance between metal finger and the charge pickup needle to observe its characteristics on varying lengths. All the experiments are performed at d=1mm, that is the needle is held 1mm above the surface for both positive and negative voltages ranging from 1kV to 8kV.

For positive inputs, the electrons tunnel from the needle and creates negative ions in air. These negative ions compensate positive charges on the below biased surface by settling on surface. The output current measured on using voltage from oscilloscope is the positive current as

electrons are lost from the tip. This applies to all experiments mentioned below conducted for positive inputs. Refer to section 6.2.1. for further understanding this mechanism.

For negative voltages, electrons tunnel to the needle creating positive ions at the tip of needle which will land on plate, compensating the negative charge on it and rises the potential locally. It is to be noted that for all experiments in this section, the output currents measured for negative voltages indicate the electron current into the needle, measured as negative current.

7.5.1 Results

For L=1cm

Table 10 L=1cm, positive input voltages.

	Current from Spellman			**Current through Resistor, 441 □**		
Vin (+) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
1	0.8	0.6	0.8	0.45	0.43	0.46
2	1	1.1	1	0.39	0.53	0.55
3	1.2	1.3	1.2	0.45	0.65	0.63
4	1.5	1.4	1.4	0.99	0.89	0.93
5	1.8	2	1.9	1.22	1.29	1.27
6	2	2.2	2	1.54	1.67	1.59
7	2.5	2.9	2.7	1.94	2.47	1.99
8	3	3.5	3.3	2.55	3.23	2.87

It can be inferred from the above table that currents measured at output increases with increasing input voltages.

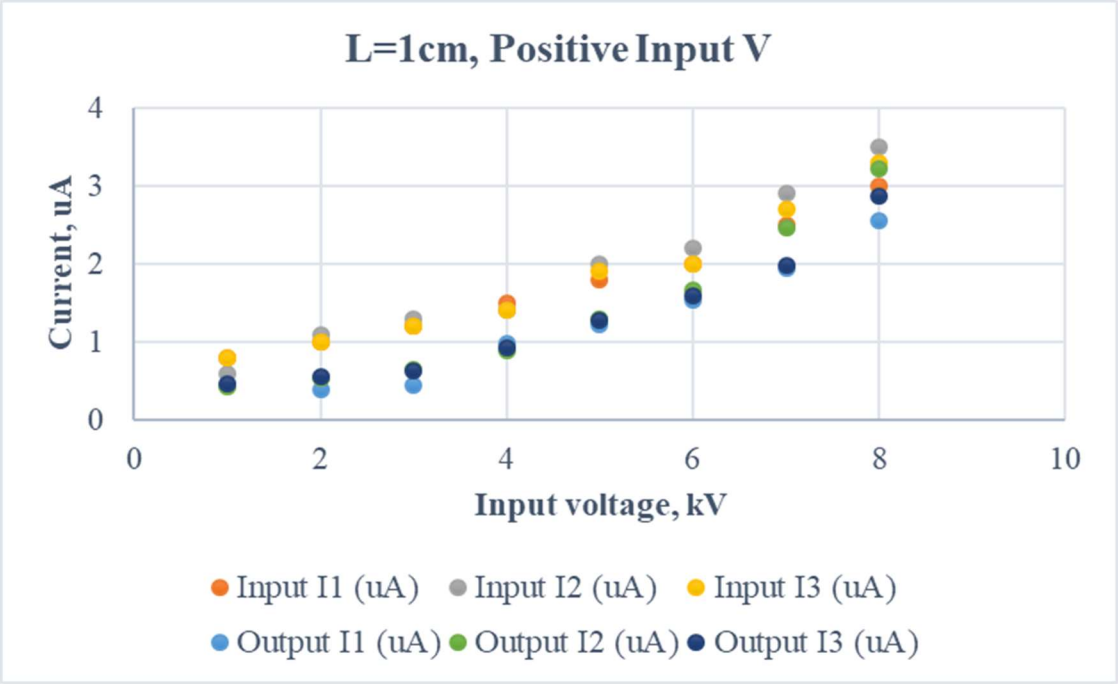

Figure 22 L=1cm, positive input voltages.

For the above figure, set of colored dots above represent they are input currents and the below set of dots represent that of output current indicating input currents are greater than output currents.

On observing Table 11, same behavior like the Table 10, output currents are increasing negatively with increasing negative input voltage.

Table 11 L=1cm, negative input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (-) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
-1	-0.7	-0.3	-0.8	-0.5	-0.26	-0.56
-2	-0.8	-0.7	-1.3	-0.55	-0.45	-0.72
-3	-0.8	-0.9	-1.5	-0.61	-0.66	-0.76
-4	-1.4	-1.6	-1.8	-0.87	-0.89	-0.9
-5	-2.3	-2.3	-2	-1.3	-1.25	-1.13
-6	-2.8	-2.5	-2.6	-1.49	-1.37	-1.42
-7	-3.6	-3.3	-2.9	-1.93	-1.8	-1.45
-8	-4	-3.8	-3.5	-2.98	-2.97	-2.51

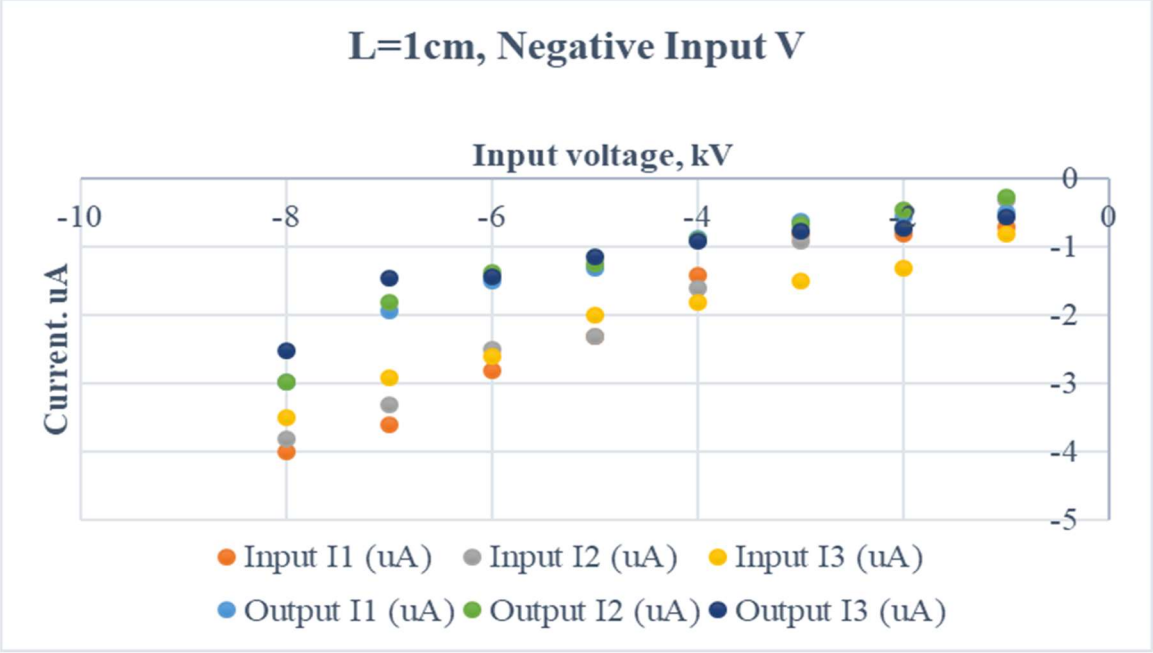

Figure 23 L=1cm, negative input voltages

For L=3cm

Table 12 L=3cm, positive input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (+) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
1	0.8	0.84	0.8	0.43	0.49	0.44
2	1.3	1	1	0.52	0.51	0.51
3	1.8	1.9	1.87	0.53	0.59	0.57
4	2.7	1.2	2.3	0.72	0.62	0.6
5	2.6	1.5	1.71	1.04	0.82	0.98
6	3	3.1	2.9	1.11	1.35	1.21
7	3.5	3.3	3.24	1.24	1.44	1.3
8	4.8	3.7	3.59	1.33	1.71	1.54

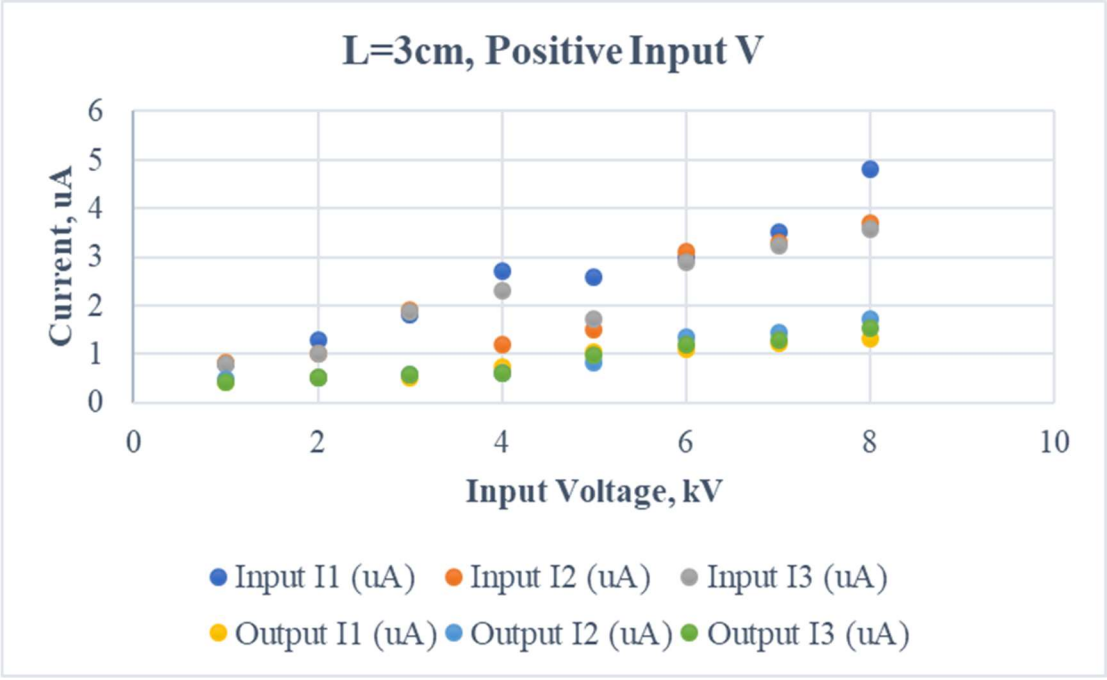

Figure 24 L=3cm, positive input voltages.

From Figure 24, group of colored dots above represent input currents and the group of dots underlying them represent those of output currents. A linear behavior is observed.

Table 13 L=3cm, negative input voltages.

	Current from Spellman			**Current through Resistor, 441 □**		
Vin (-) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
-1	-0.4	-0.7	-0.2	-0.31	-0.4	-0.38
-2	-0.43	-0.71	-0.5	-0.35	-0.47	-0.39
-3	-0.7	-0.8	-0.7	-0.51	-0.57	-0.62
-4	-1.5	-0.89	-0.94	-0.69	-0.66	-0.71
-5	-2.2	-2.27	-2.2	-0.68	-0.83	-0.79
-6	-2.5	-2.58	-2.63	-1.06	-1.19	-1.2
-7	-2.85	-2.91	-2.9	-1.4	-1.48	-1.48
-8	-3.3	-3.5	-3.62	-1.87	-1.95	-1.97

Like for positive input at L=3cm, output negative electron currents increases negatively with input voltages.

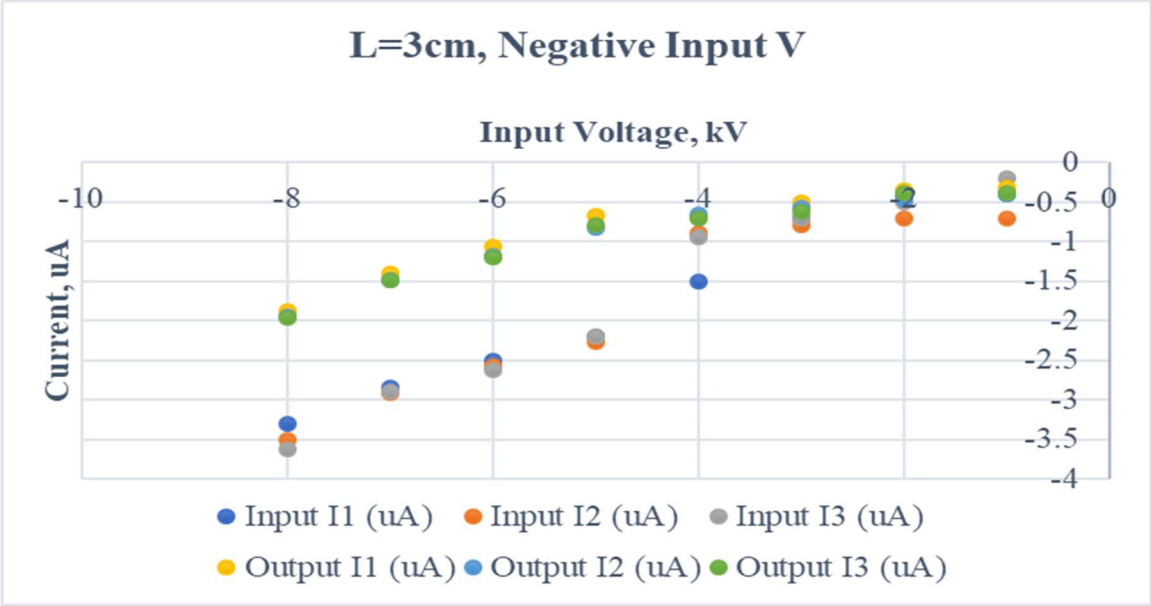

Figure 25 L=3cm, negative input voltages.

For L=7cm

Table 14 L=7cm, positive input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (+) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
1	0.8	0.6	0.8	0.41	0.49	0.47
2	1	1.1	1	0.57	0.48	0.51
3	1.3	1.3	1.2	0.7	0.42	0.55
4	1.5	1.4	1.4	0.67	0.62	0.6
5	1.9	2	1.9	0.82	0.68	0.79
6	2.5	2.2	2	0.8	0.99	0.91
7	3	2.9	2.7	1.05	1.17	1.07
8	3.5	3.5	3.3	1.08	1.09	1.08

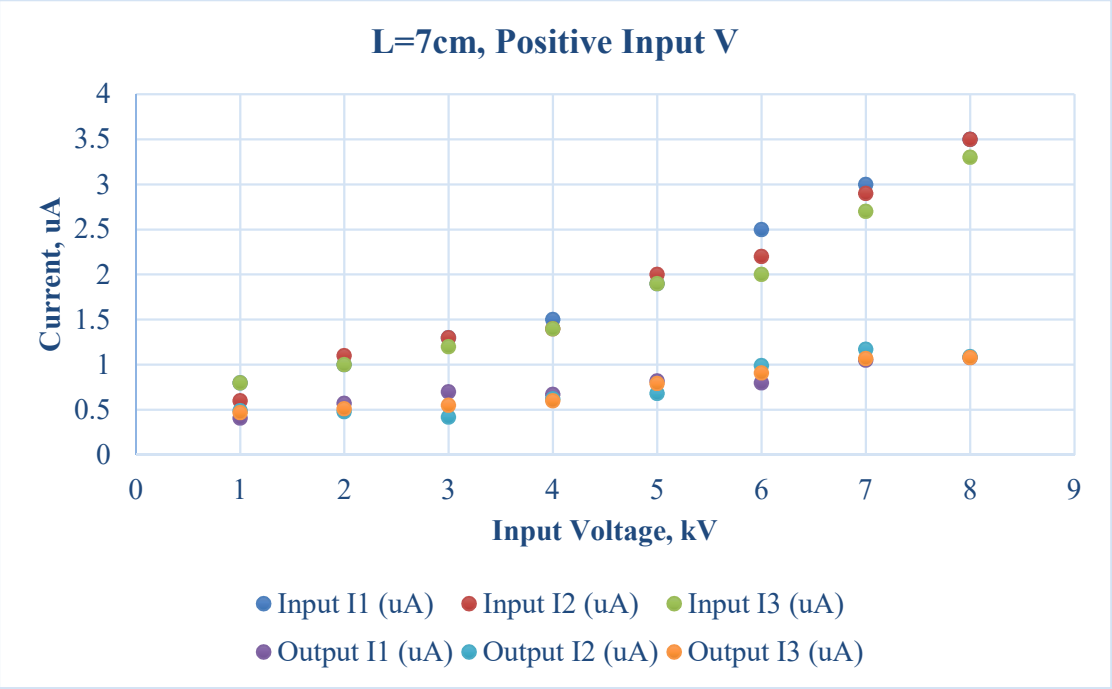

Figure 26 L=7cm, positive input voltages.

Table 15 L=7cm, negative input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (-) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
-1	-0.4	-0.8	-0.8	-0.2	-0.33	-0.47
-2	-1.15	-1.1	-1	-0.47	-0.47	-0.45
-3	-1.2	-1.6	-1.6	-0.47	-0.52	-0.55
-4	-1.35	-1.5	-1.4	-0.65	-0.72	-0.69
-5	-2	-2	-1.9	-0.8	-0.83	-0.76
-6	-2.5	-2.4	-2	-0.99	-0.91	-0.89
-7	-2.6	-3	-2.7	-1.06	-1.07	-1.03
-8	-3	-3.5	-3.7	-1.1	-1.17	-1.12

The output currents for positive and negative inputs follow the same behavior like for L=1cm/3cm but has lower output current values validating the results of section 7.4 that There exists drop in potential as we move along the length of tape.

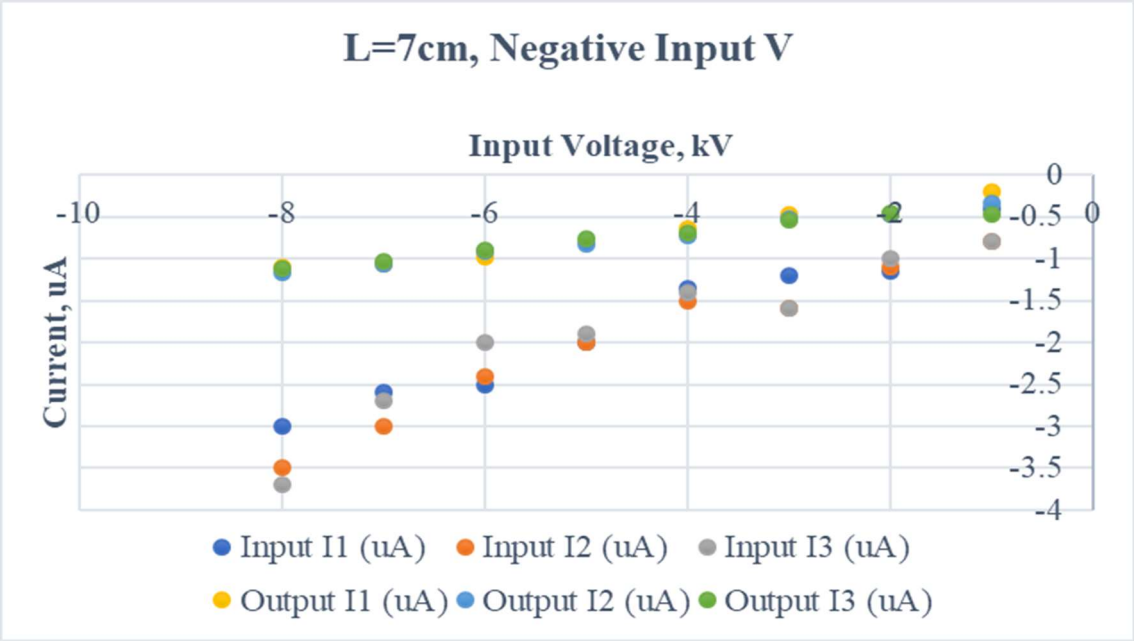

Figure 27 L=7cm, negative input voltages.

For L=10cm

From the below Table 16 and Table 17, output currents are lower than input currents and show a linear behavior on increasing inputs.

Table 16 L=10cm, positive input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (+) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
1	0.8	0.6	0.8	0.53	0.5	0.44
2	1	1.1	1	0.56	0.56	0.49
3	1.3	1.3	1.2	0.57	0.53	0.5
4	1.5	1.4	1.4	0.59	0.55	0.51
5	1.9	2	1.9	0.94	0.65	0.7
6	2.5	2.2	2	0.96	0.7	0.8
7	3	2.9	2.7	1	0.95	0.89
8	3.5	3.5	3.3	1.01	0.995	0.99

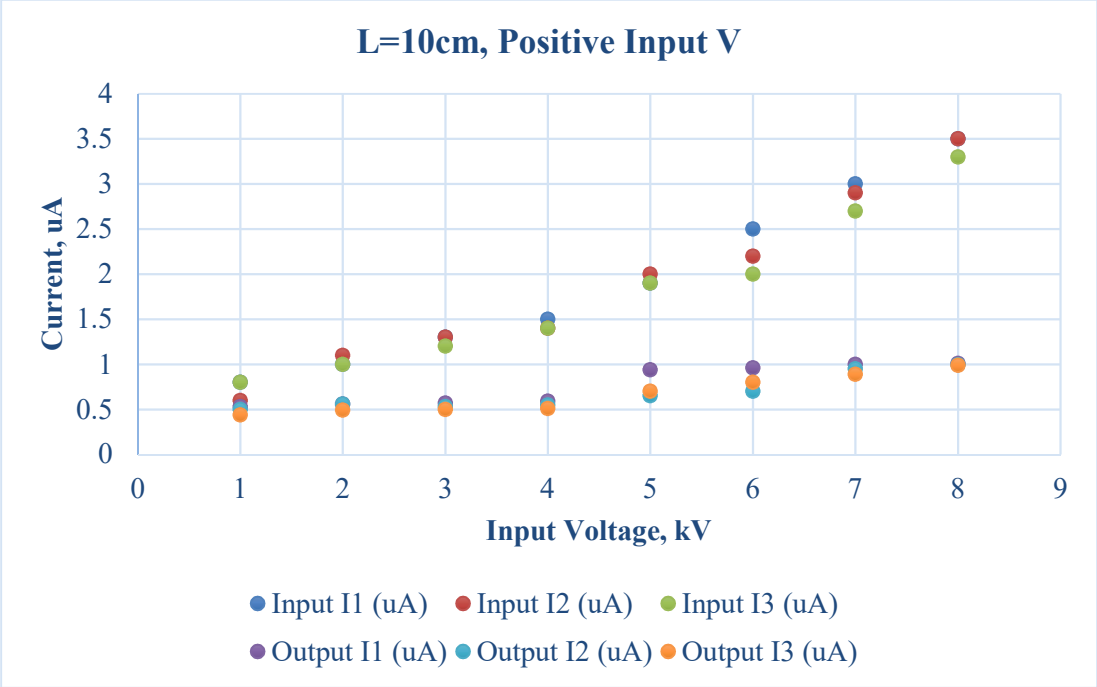

Figure 28 L=10cm, positive input voltages.

Table 17 L=10cm, negative input voltages.

	Current from Spellman			Current through Resistor, 441 □		
Vin (-) (kV)	**Input I1 (uA)**	**Input I2 (uA)**	**Input I3 (uA)**	**Output I1 (uA)**	**Output I2 (uA)**	**Output I3 (uA)**
-1	-0.65	-0.71	-0.77	-0.29	-0.3	-0.45
-2	-0.8	-0.89	-0.9	-0.41	-0.39	-0.46
-3	-1.3	-1.5	-1.4	-0.55	-0.54	-0.55
-4	-1.8	-1.8	-1.76	-0.6	-0.65	-0.59
-5	-2.2	-1.9	-2.1	-0.75	-0.71	-0.71
-6	-2.6	-2.4	-2.41	-0.81	-0.83	-0.8
-7	-3	-2.9	-2.5	-0.87	-0.87	-0.89
-8	-3.6	-3.3	-3.5	-0.9	-1	-1.05

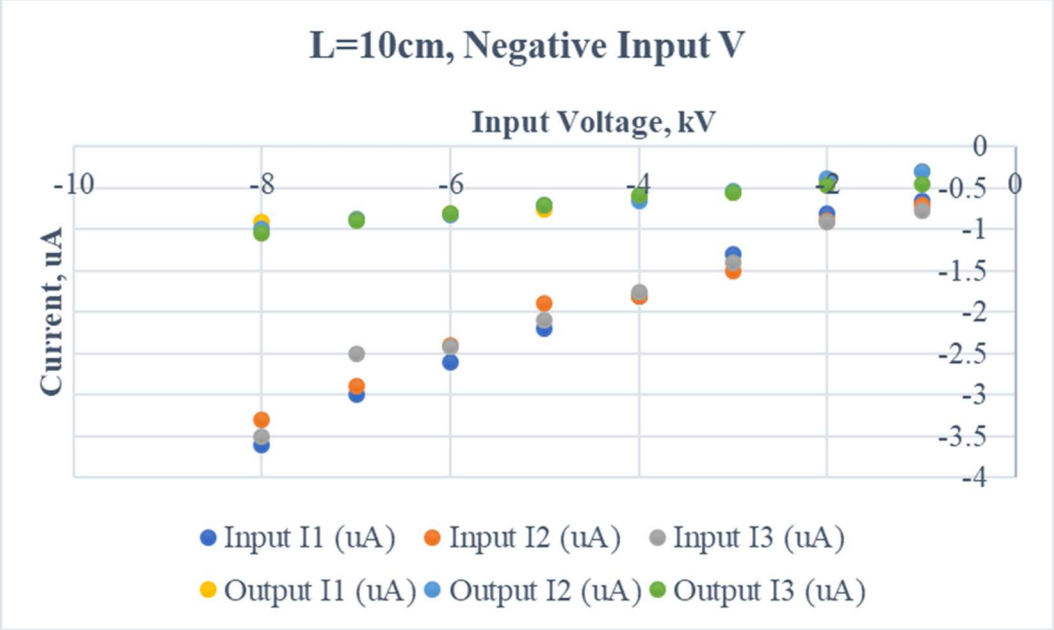

Figure 29 L=10cm, negative input voltages.

7.5.2 Conclusions

The below table summarizes the above graphs. It was done by averaging repeated experiments for their respectively positive inputs and lengths.

Table 18 Summarized table for positive value inputs of section 7.5.1.

	Different lengths on Clear tape			
Vin (+) (kV)	**L=1cm**	**L=3cm**	**L=7cm**	**L=10 cm**
1	0.44	0.43	0.45	0.49
2	0.49	0.51	0.52	0.53
3	0.57	0.56	0.55	0.53
4	0.93	0.64	0.63	0.55
5	1.26	0.94	0.76	0.76
6	1.6	1.22	0.9	0.82
7	2.13	1.32	1.09	0.94
8	2.88	1.52	1.08	0.99

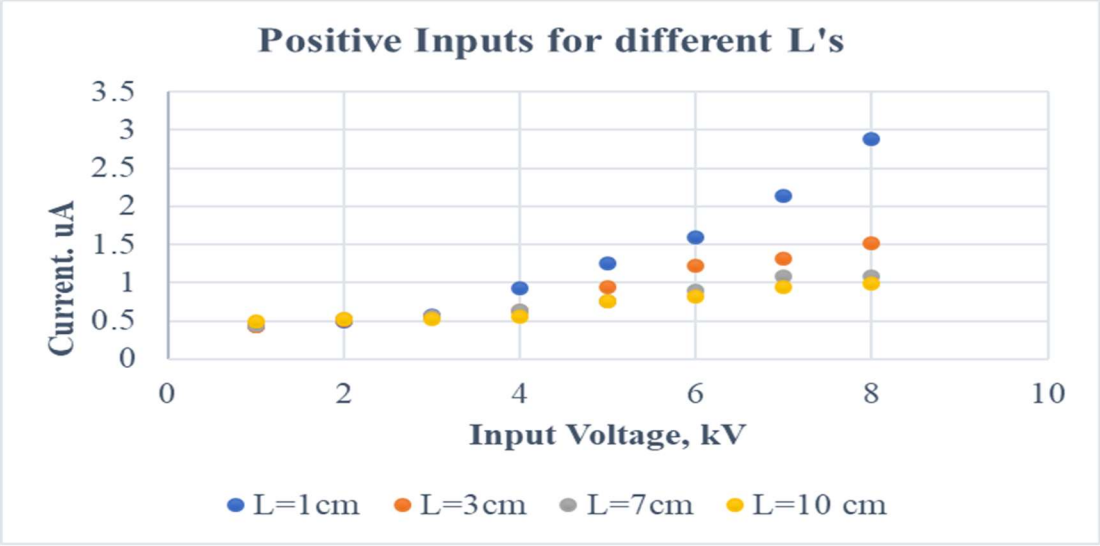

Figure 30 Summarized graph for positive input results of section 7.5.1.

The above figure shows the measure of output currents for various input voltages as function of length. It can be observed that for L=1cm, currents are higher as compared to L = 3cm/7cm/10cm. As we move away from the source, there was more voltage drop which shown a decrease in output current. If observed for L=3cm, output current dropped by half at 8kV input. Moving away from source, shows reducing positive currents. The same can be observed with negative inputs. With this kind of set up, a more modulated process where the strongest gene expression is needed would be achieved.

CHAPTER 8: DISCUSSION

From the past, there were many advancements induction and compensating charges to study surface charging phenomenon is revealed in this study. Physical delivery methods are mostly used in clinical purposes these days. Of these, using electric fields to augment the delivery is the most commonly used delivery technique. There are many ways to deliver a genetic material using electric fields. Injecting the genetic material into the tissue and applying electric fields to create pores in tissue, confining the region of treatment with a conducting material and treating the region inside the conducting ring, current contact dependent techniques, techniques using ionized charges, plasma to deliver a foreign material into the tissue are all seen in recent findings [4], [35], (A Ph.D. dissertation "Plasma Mediated Molecular Delivery" by Richard J. Connolly, University of South Florida dated October 29, 2010).

With the drawbacks of techniques using more than the amount of charge needed to treat a particular area, spreading charges to undesirable regions in process of treating at one place, throwing charges on tissue/skin without modulating the current, this study explores the amount of charges being spread on the surface while localizing and compensating charges at desired location.

This research uses a physical method, specifically, electric fields. The electric fields in this system are localized fields which were developed by setting up a point to plane corona discharge. Also, this system compensates charges to localize current at the region. From the experiments mentioned in previous sections, when a metal surface is charged with positive voltage, the electrons emitted from the tip create negative ions which settle on the metal surface and

compensate positive charges. These compensating charges lowers the potential below the needle providing localized field modulation by reduction of net charge density below needle.

In this research, an anti-static clear tape is used as a mimic for skin. Experiments were performed to show that a clear tape or Cu tape placed at the bottom side of glass plate present approximately same values on measuring voltages at both ends, that is at Cu and clear tape. This was performed to choose a reference. However, Cu tape was used as a reference and is grounded at the bottom side of glass plate, along the length of clear tape on top. A conclusion that water molecules on the surface drive charges in this process is shown. All repeated sheet resistance measurements for different lengths show that the tape is conducting uniformly along the length and some current is carried away by surface molecules. Also, along the length of surface, some voltage drop is observed and were shown as decaying curves. Keeping this property of decaying voltages, the final experiment was done by modulating fields for different lengths. All these experiments explored surface charging on mimics to skin. All the experiments performed in this research would explore the novel characteristics of surface charging apparatus for future electropermeabilization processes.

CHAPTER 9: FUTURE WORK

From the past there were many advancements in the method of treating a deleterious cell/tissue or delivering a gene into tissue. Exploring different delivery approaches and by introducing new developments, using electric fields in gene delivery became the most popular approach in clinical applications. An ultimate goal of any delivery system is to achieve higher levels of gene delivery being minimal toxic and provide persistent successful clinical outcomes. Sharing the same goal, this study provides characteristics of charges on surface.

Referring to Figure 3 and Figure 4, the net potential lines shown were not practically observed as the Kelvin probe is limited to 3kV. So, on using better equipment, that is by using Kelvin probes that operates for higher voltages, for example till 10kV would be a good start to proceed with the idea localizing fields at a specific region.

Also, a ground Cu tape reference for glass plate on performing experiments was cumbersome as wires go below the glass plate letting it not sit properly on table. So, a raised experimental set up above table would be comfortable to work with.

This study shown results as function of input voltages with output currents, lengths from source to sharp needle/Kelvin probe, L and for fixed height of needle/ Kelvin probe held above the surface (d=1mm). Partial experiments were done on increasing the distance of needle/ Kelvin probe above the ground for d=2mm and d=3mm which is not presented in this paper. Doing experiments with different L's and d's would provide more detailed results for later model work.

In this research, a way to modulate electric fields at a particular region and its charging phenomenon are provided. Moving forward, the first step could be testing surface charging phenomenon in vitro with cells to see if it shows the same result. Repeated experiments in vitro should be conducted for reliable and consistent results.

Also, a more localized process can be implemented by using a trigger or switch at the input so that it is turned on and off for some time interval to create pulsed input. On doing so, augmented uptake of exogeneous material can be confined to a particular region. If promising results of gene expression for several repeated trials is observed in in vitro, it can be tested in vivo. If clinical results turn out to be persistent, this method of delivering gene into tissues would be a new achievement in this field of study.

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