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Developing a labview based thermally stimulated current (tsc) controller to measure residual charge in electrospinning

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ABSTRACT

DEVELOPING A LABVIEW BASED THERMALLY STIMULATED CURRENT (TSC) CONTROLLER TO MEASURE RESIDUAL CHARGE IN ELECTROSPINNING

by

Jai Abhishekh Veezhinathan

Electrospinning is an electrohydrodynamic process for the fabrication of nanofibers which are widely used in therapeutical tissue engineering approaches. It utilizes the potential difference of an electrostatic field to overcome the surface tension of the polymer solution to extrude a fine jet of fluid which deposits on the grounded collector as a nanofiber mat. Using this process, nanofibers with diameters less than a micron can be produced.

Previous studies have shown the presence of residual charge in electrospun nanofibers. The presence and decay of residual charge during cell culture media is still unknown. In an attempt to clarify the presence or absence of residual charge during cell culture, a LabVIEW based Thermally Stimulated Current controller is designed. The LabVIEW controller encapsulates the temperature and the electrometer control using RS-485 and GPIB interfaces. The controller outputs a Temperature-Current plot which is the Thermally Stimulated Current spectrum. The current observed at the melting point of the electrospun material is a direct measure of the residual charge trapped within the nanofiber mat. Using this technique electrospun PolyCaproLactone (PCL) mats are analyzed before and after immersion in culture media and Phosphate Buffer Solution (PBS) for 1, 4 and 7 days.

DEVELOPING A LABVIEW BASED THERMALLY STIMULATED CURRENT (TSC) CONTROLLER TO MEASURE RESIDUAL CHARGE IN ELECTROSPINNING

by
Jai Abhishekh Veezhinathan

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DEVELOPING A LABVIEW BASED THERMALLY STIMULATED CURRENT (TSC) CONTROLLER TO MEASURE RESIDUAL CHARGE IN ELECTROSPINNING

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“The scientific method is doing it the best way you can with what you have”

To
Dr. George Collins

I dedicate this work to the man who taught me this and a ton more. You made a champion out of me, for which I am grateful, eternally. I look back upon this journey with the pride of having worked with a champion who makes champions and for all this, thank you, sir.

To
Divya Nithya

For being the light during the dark,
For being the shoulder when legs give away,
For love,
For life.

Thank you, dear.

To my mummy,
Sumathy

For endowing me with a good physical and mental faculty,
For watching over me from above the skies.

To my Parents,
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CHAPTER 1

INTRODUCTION

1.1 Objective

Electrospinning is an electrohydrodynamic process wherein polymer solutions are extruded as a charged jet of fluid into nanofibers. The mechanism behind this process lies in the injection of charges into the polymer fluid. The application of an external field produces an electrostatic force that acts as the driving force to overcome the surface tension of the fluid to extrude a charged jet of polymer into the grounded collector plate [1-2]. This method can be used to fabricate polymer fibers with sub-micron diameters. The fibers produced by this method bear close resemblance in morphology to the protein filaments in the Extra Cellular Matrix (ECM) due to which electrospun nanofiber scaffolds are widely used in tissue engineering.

In electrospinning, charges govern the motion of the polymer solution and after accounting for the loss of charges through electrospraying and corona discharge in the nanofiber mat, there exists a residual charge that is trapped in the bulk volume of the nonwoven fibrous scaffold and on the surface. After the dissipation of the surface charges on deposition at the grounded collector plate, the residual charges remain in the material as they are trapped in the volume of the material.

Although widely used as a substrate for cell culture there has been no explanation in literature on the effect of residual charge in the culture medium. Therefore, it is the objective of this thesis to clarify the effect of residual charge in the electrospun nanofiber mat as this can give vital information on the effect of residual charge in cell culture media and on the culture itself. Since the charges are trapped between the amorphous and crystalline layers of the polymers the only way to measure them is by carrying out thermal analysis of the electrospun samples by Thermally Stimulated Current (TSC) experiments.

In this thesis, a LabVIEW based TSC controller is built to analyze residual charge. The LabVIEW controller automates temperature, valve and electrometer control during the TSC process. Furthermore, it offers easy and efficient measurements while ensuring portability of Control and Monitoring using a Smartphone or Tablet.

1.2 Background

1.2.1 Electrospinning

Electrospinning is the process of fiber fabrication that utilizes the electric potential difference to produce continuous fibers from polymer fluids with less than a micron of diameters. It has been a source of scientific interest ever since the discovery of electrospraying in the 16th Century [3]. Furthermore Zeleny observed the effect of the potential difference on the process and concluded that it was the motion of the fluid due to the influence of an electric field that lead to the electrohydrodynamic process of electrospinning [2]. Although electrospinning has been under investigation since the 16th century, it wasn’t until Formhals patented it in 1934 that it was commercialized [4].

As illustrated in figure 1.1, the positive electrode acts as a source point for charge injection into the polymer solution. The grounded collector plate serves as a target for the collection of the electrohydrodynamically driven fluid jet. The potential difference between the capillary and the collector is usually in the order of 10-30kV. Conventionally, the positive electrode is clipped to the capillary and the negative electrode is grounded to the collector plate. This type of setup can be called as Positive Voltage Electrospinning (PVES). This setup is the most widely used configuration in electrospinning.

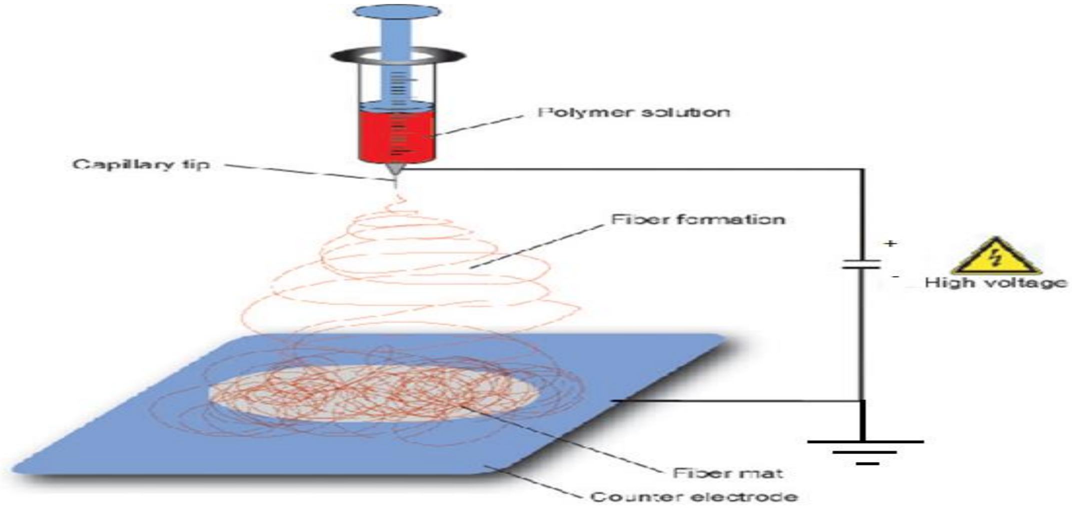


Figure 1.1 Electrospinning setup. [6]

Due to the phenomenon of charge separation (figure 2), a region of excess charge is created. The region of excess charge is created due to an inductive action where a charged electrode is brought into contact with the conducting needle as a result charges with opposite polarity are induced on the other side of the conducting needle to maintain equilibrium. The region of excess charge may be positive or negative depending on the electrode that is attached to it. In PVES, positive charges dominate the region of excess charge and in Negative voltage electrospinning (NVES) negative charges dominate this area. It is ultimately the charges in this region that get attracted to the oppositely charged electrode.

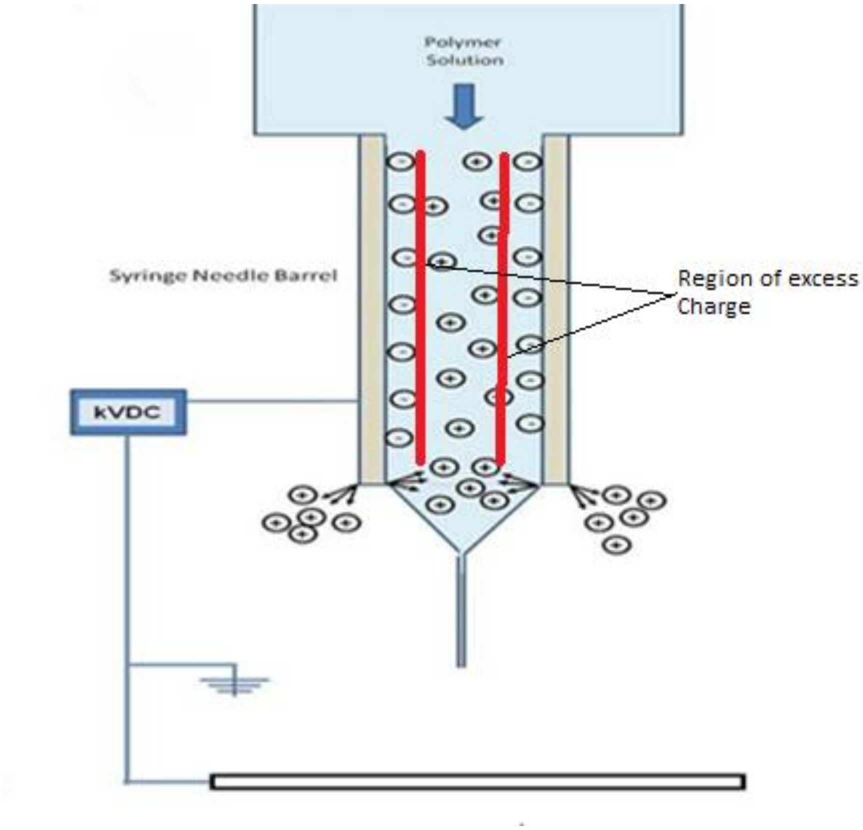


Figure 1.2 Charge separation in electrospinning. [1]

As the fluid containing the charges is pushed out to the capillary at a definite flow rate from the syringe, due to the electrostatic stresses applied on the capillary, it is distorted into a cone like structure known as the Taylor cone (figure 1.3). When the electrostatic stresses or the surface charge density within the cone overcome the surface tension of the fluid, a fine fluid jet of solution is propelled towards the oppositely charged electrode. As soon as the fluid leaves the tip of the capillary via the Taylor Cone, it then undergoes a bending instability known as “whipping” during which the solvent evaporates and the polymer fibers are collected at the grounded collector plate. It is said that whipping plays in important role and may be responsible for the stretching elongation and thinning of the electrostatic fluid jet [5].

Figure 1.3 Taylor cone formation.

From the electrical perspective, it can be said that the process of injection of charges into the polymer solution induces an opposite charge on the collector, similar to that of an electric dipole configuration. The field lines follow a point to plate fashion and indicate the direction of the electric field and by convention it always flows from positive to negative.

Being a process strongly influenced by electrostatics, a reversal of the polarity between the collector and the capillary also results in the formation of these non-woven nanofiberous mats which are being used for different purposes in tissue engineering. This process is known as Negative Voltage Electrospinning (NVES). Although, NVES was not used in this work it is to be noted that NVES leads to the deposition of the negative charges on the nanofiber mat provoking the thought that the presence of negative charges in the electrospun scaffold may have an entirely different effect on tissue engineering [3].

1.2.2 Residual Charge in Electrospinning

During the electrospinning the charges generated in the electrode are transported by the main jet, secondary jet and the corona discharging [6]. After one accounts for charge transportation through these processes, a considerable amount of charge is solidified in the charged filament after the electrospinning process (C. Lien). He observed a very low current during the actual electrospinning process which points to the fact that it is these charges that are the driving force behind the entire process of electrospinning. The presence of low current during electrospinning opens up the plausibility that these charges are carried by the polymer fluid jet as it undergoes the bending instability before being trapped as it is vitrified on the collector.

These charges are trapped within the amorphous and crystalline interfaces of the filament and hence cannot dissipate to ground [7]. Figure 1.4 illustrates the presence of these charges in the vitrified filament after deposition on the collector.

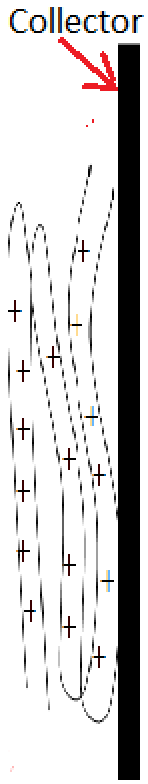


Figure 1.4 Charges trapped in the electrospun mat.

Faraday cup measurements, surface potential measurements and Thermally Stimulated Current measurements are common measurement techniques used to measure residual charge in the electrospun materials. In Faraday Cup measurements, the sample is enclosed within a metal cup and the charges induced by electrostatic induction phenomena was used as a measure of residual charge.

In surface potential measurements as used by Tang et al. [3]. the measurement is done by using a probe tip to induce a local electric potential due to the presence of these charges, the magnitude of the electric potential depends on the size of the probe (large or small). The probe can be moved over the electrospun material to study the variation of surface potential in the electrospun mat. In this work, Thermally Stimulated Current (TSC) technique was used. Catalani et al. first demonstrated the presence of residual charges in electrospinning, they measured a current of 9nA from electrospun PolyButylene Terepthalate (PBT) nanofibers. The measurent was done through Thermally Stimulated Current (TSC) experiments which is an integral part of this work [7].

There has been little or no reference in literature accounting for the presence and the impact of residual charge in electrospun nanofibers during cell culture. It can be argued that the charges may even be present during cell culture as they are trapped within the electrospun material. This work tries to clarify the presence of residual charge in electrospun fibers.

1.2.3 Thermally Stimulated Current (TSC)

The Thermally Stimulated Current (TSC) technique is based on the principle that an increase in temperature releases the “trapped” charges. Using a high sensitive electrometer these charges can be measured in the form of current as these charges dissipate.

Figure 1.5 Previously working TSC.

Figure 1.6 TSC block diagram.

Typically, a TSC measurement would include a plot between the Temperature vs Current. The current measured reflects the magnitude of the stored charge in the material. The observed current may be positive or negative because of the polarity of the charges which can "flow" to the ground or the positive terminal.

The TSC controller communicates with the temperature controller using an RS-485 interface and a GPIB to communicate with the electrometer that makes the sensitive current measurements. The major focus of this work has been the restoration of the functionality of the TSC which was found to be non-functional due to the failure of the controller. The TSC was reconstructed virtually using LabVIEW 2013 to provide functionality and data logging facility to make TSC measurements in a user-friendly manner.

1.2.4 LabVIEW

With the advancement in instrumentation technology, PCB dependent controllers have been increasingly replaced by LabVIEW's versatile data flow programming which has been both intuitive and user friendly. Therefore, we see a lot of the traditional controllers being replaced by virtual instrumentation softwares like LabVIEW and MatLab. In 2004, National Instruments sold 6 million virtual Instrumentation packages to over 90 countries around the world, this shows the rapid growth for virtual instrumentation [9].

Virtual Instrumentation is a combination of user defined software and modular hardware that implements Process control and Automation (P&A). It can be used to control instruments running under GPIB, RS-485, RS-232, USB and PCI interfaces [9]. Furthermore, the virtually designed controller offers a higher reliability and superior control manipulation when compared to the traditional PCBs and it is because of these reasons that there has been an accelerated growth in industries and the academia calling for the implementation of more virtual instruments across the globe. Another dimension of virtually designed controllers is the portability of the controller as they are ".VI" files of 1MB or less, thus the controller can be used universally on systems running LabVIEW with the same hardware setup. In this work, LabVIEW has been used to build a custom program to control the functioning of the temperature controller and the electrometer based on user inputs of temperature and the running time of the TSC experiment.

1.2.5 Serial Communication

Serial communication is the communication protocol that sends one bit at a time over the “serial port”. It is used widely in industrial communication where the use of parallel ports is impractical due to its bulky cabling requirements. In the old days, due to slower processing speed parallel ports were used to transfer bulk of data at once whereas with the advancement in faster processing speeds the data is now sent serially with much higher transmission speeds.

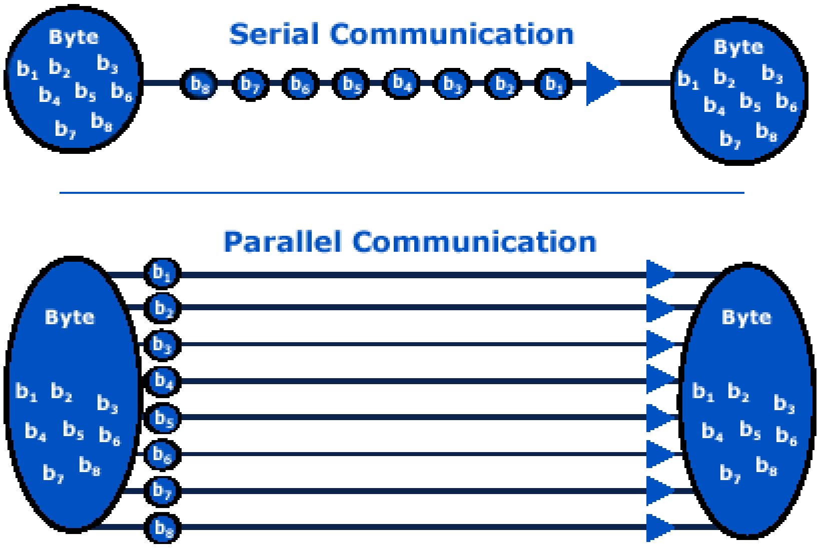


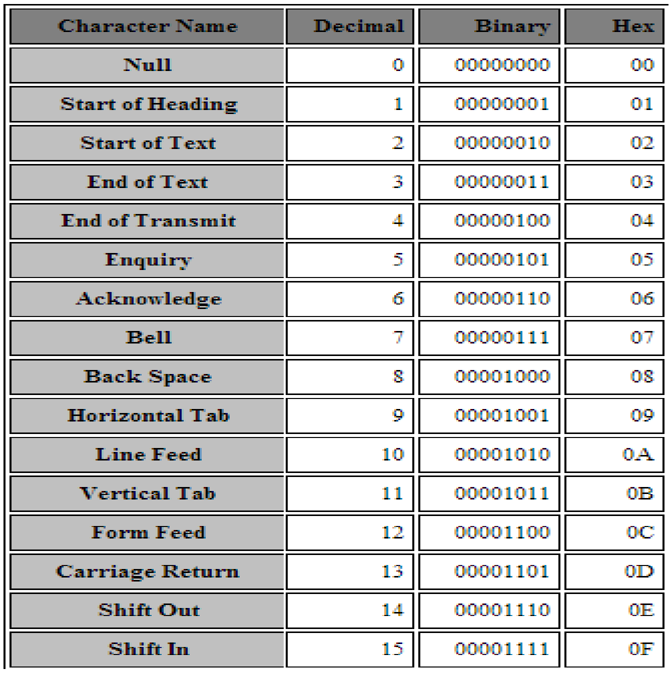
Figure 1.7 Serial vs Parallel.

The data transmission in serial communication is governed by certain parameters such as Baud, data bits, parity and port number. A bit is a packet of information. It can have only two values 0 or 1 (binary). The value is corresponded to a voltage of ~5V for 1 and 2.2 for 0. The variation in the voltage is interpreted as a high (1) or low (0). The variation in the voltage is given by the processor of the host device known as the UART (Universal Asynchronous Reception and Transmission). The processor varies the voltage using the interfaces such as RS-485, RS-232, GPIB (General Purpose Interface Bus) etc.

The data in serial communication in LabVIEW is transmitted as a hexadecimal string encoded in the form of ASCII codes.

1.2.5.1 ASCII. ASCII stands for American Standard Code for Information Interchange. Computers can only recognize numbers, so an ASCII code is the numerical representation of a character such as 'a' or '@' or an action similar to a “space”. ASCII was developed in 1966 by Kenneth Knowlton in Bell Labs. ASCII was actually designed for use with teletypes and so the descriptions are somewhat obscure. Notepad.exe creates ASCII text, or in MS Word you can save a file as 'text only'. Below is the ASCII character table and this includes descriptions of a limited ASCII table. Using Hexadecimal codes, LabVIEW was programmed to control the temperature controller and interrogated every second by the PC to display live temperature

Table 1.1 ASCII table



Character Name	Decimal	Binary	Hex
Null	0	00000000	00
Start of Heading	1	00000001	01
Start of Text	2	00000010	02
End of Text	3	00000011	03
End of Transmit	4	00000100	04
Enquiry	5	00000101	05
Acknowledge	6	00000110	06
Bell	7	00000111	07
Back Space	8	00001000	08
Horizontal Tab	9	00001001	09
Line Feed	10	00001010	0A
Vertical Tab	11	00001011	0B
Form Feed	12	00001100	0C
Carriage Return	13	00001101	0D
Shift Out	14	00001110	0E
Shift In	15	00001111	0F

Typical Serial communication parameters and their values are:

Table 1.2 Serial Parameter

Parameter	**Typical Value**
Baud rate or bits per second	110, 300, 600,1200, 2400, 4800, 9600
No. of data bits	7 or 8
Parity	Odd or even or no parity.
Port number	Port numbers: COM1, COM2, COM3

Using LabVIEW, the host computer and the temperature controller was synchronized to work with defined serial settings. The serial interface used in this work is RS-485.

1.2.5.2 RS- 485. The Recommended Standard (RS) 485 is the communication used in this work to establish communication between the PC and the temperature controller. This protocol is used where implementation of master and slave type structured connection is applicable. The master is usually the device that initiates communication (PC) and the slave is the device that is secondary to the master (temperature controller).

The 485 bus is flexible as it can accommodate upto 32 devices in the same pair of wires with a ground wire as the reference. Due to the use of a pair of wires (referenced to ground) for data transmission, the 485 connection displays superior noise immunity as compared to the 422 bus. Data is transmitted differentially on these two wires twisted together, referred to as a “twisted pair”. The properties of differential signals provide high noise immunity and long distance capabilities. A 485 network can be configured two ways, “two-wire” or “four-wire”. In a “two-wire” network the transmitter and receiver of each device are connected to a twisted pair. “Four-wire” networks have one master port with the transmitter connected to each of the “slave” receivers on one twisted pair. The “slave” transmitters are all connected to the “master” receiver on a second twisted pair. In either configuration, devices are addressable, allowing each node to be communicated to independently [10]. After a character of data is transmitted, the “slave” or the “master” requires some time to “turn-around” to receive or send data. This turn-around delay is an important part of a two-wire network because during that time no other transmissions can occur. An ideal delay is the length of one character at the current baud rate (i.e., 1 ms at 9600 baud). The device manufacturer should be able to supply information on the delay for their products. In this work, a delay of 1ms was used for the Omega Temperature Controller.

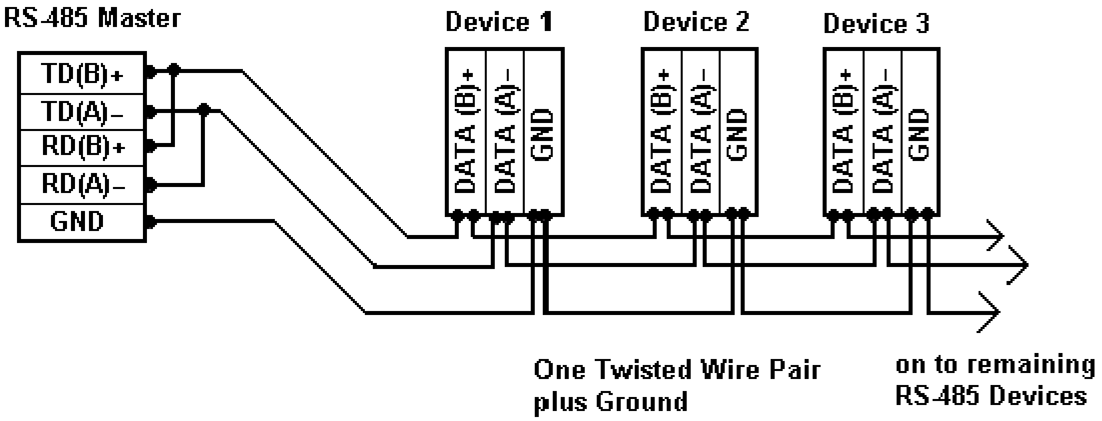


Figure 1.8 485 example.

CHAPTER 2

EXPERIMENTAL

2.1 Materials and Methods

This work encompasses the renovation of the existing TSC apparatus by reconstructing its specific functions of Temperature control and electrometer automation entirely in a LabVIEW based environment. LabVIEW provides a user friendly platform for ease of operation of the entire TSC process. Thus, this section addresses the devices and cabling used to build the instrument in LabVIEW and the subsequent methodology with which the experiments were conducted with electrospun PCL and PCL films.

2.1.1 System Requirements of the Microprocessor-based TSC

The renovation of the microcontroller based TSC was undertaken to replace the failure of the motherboard to reproduce the functional requirements of the TSC. A programmable control board (PCB) approach towards solving the problem seemed complicated as most of the microcontroller's parts were obsolete. Therefore, LabVIEW TSC not only restores the functionality of the TSC but also improved upon its features, as discussed below. The microcontroller TSC did not have direct voltage output to power the heater power supply and the control output was based on the percentage power output. The Omega Temperature controller gives a true PID control signal as voltage output to the power supply giving the temperature controller a more stable temperature control. Although, the microcontroller based TSC had additional functions such as valve control and sensor control, the addition of such features via LabVIEW are listed as future work in this thesis.

Temperature and current readings were made every 1 second by the Thermold TSC, whereas the LabVIEW TSC offers flexibility to record readings up to 100ms thereby offering a versatility in the data collection. Ramp rate of the temperature in the LabVIEW TSC is also user configurable which was not the case in the microcontroller based TSC. The system requirements of the new processor was better temperature control and improved functionality in terms of flexibility in the number of readings per time period. Thus, integration with LabVIEW makes the system more robust and user configurable as compared to the microcontroller based TSC.

2.1.2 LabVIEW Programming Block Diagram

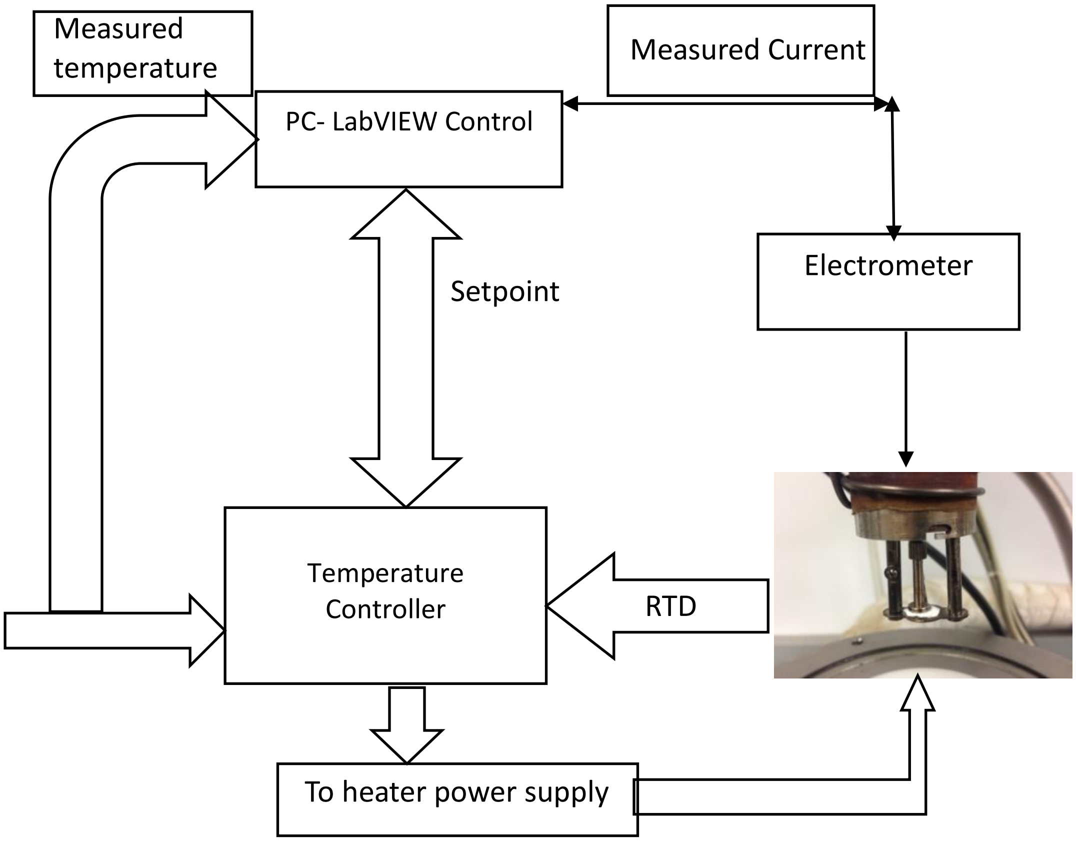


Figure 2.1 LabVIEW TSC block diagram.

The figure 2.1 represents the simplified version of the programming methodology used to program the entire TSC setup. When run, the program prompts the user for temperature setpoint input. Once the user inputs temperature, it is compared with the ambient temperature to establish a constant 7 deg per minute ramp rate to the setpoint. Additionally, users are provided with a “Start” and “Stop” button to exit from the process at any point of time without the instruments being in the “ON” state. The above described block diagram runs every second to give a live display of temperature and current before plotting the TSC graph on completion of the process. Furthermore, users are also required to input the run time of the process for which the TSC spectrum is required. The programming was split block by block using LabVIEW VISA communications to communicate with the serial port.

2.1.3 Temperature Controller (Omega CNi33254-C24) programming

The temperature controller was programmed by using a USB-485 converter as cabling. The “Transmit” and “Receive” pins were shorted in the RS-485end in order to make up a “2 wire” 485 connection to communicate with the temperature controller.

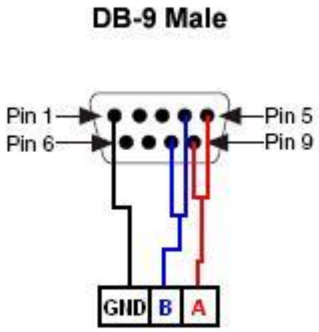


Figure 2.2 RS-485 wiring scheme.

The above wiring scheme shows the conversion of the NI-USB485 for a “2 wire connection” to communicate with the temperature controller. The table below shows the serial parameters used to enable the devices to enter into communication over the serial port.

Table 2.1 Serial Parameters for Temperature Controller

Parameter	**Typical Value**
Baud rate or bits per second	9600
No. of data bits	7
Parity	Odd
Port number	COM1, COM2, COM3 (PC dependent)

Data was sent in the form of ASCII hex codes. Using the appropriate syntaxes the temperature controller was asked to calculate ramp rate of 7 degrees per minute based on setpoint (user input of temperature). Furthermore, temperature measurements were recorded every second for live display as well as data logging of temperature. Resistance Thermometer (RTD-3 wire pt100) was used as temperature sensor (part of the original TSC setup).

The address of the temperature controller was defaulted to “01” as there is only one device in the 485 bus. Thus, every hex code has to be preceded by the asterisk and the address before entry of the hexcode that governs a certain operation. The following table summarizes the hex codes and their application in controlling the temperature. R-read W- write (only read commands specified) “\r indicates a carriage return which the instrument interprets to be the end of a hex code.

Table 2.2 Hex Codes

Hex Code	**Description**
*01X01\r	Read process variable
*01R01\r	Read Setpoint
*01R0E\r	Read Ramptime
*01R11\r	Read display color
*01R17\r	Read Proportional band
*01R18\r	Read reset time
*01R19\r	Read Cycle time
*01Z02\r	Hardware reset

These same codes were manipulated using the write command follow by the parameter hex code and the change of value, all in the hexadecimal ASCII format [11]. Using these codes, the temperature controller was automated to take user inputs and increase temperature by outputting a voltage value between 0-10V. The output voltage of the temperature controller was sent to the programmable power supply, which uses current to heat a resistance (heating coil) by Joule’s heating law. As shown below a feedback loop is established between the measurement of temperature from the RTD and the output of voltage according to the difference between set point and temperature at the heating coil. This type of control of the process variable (in this case temperature) is known as closed loop or feedback control. The controller closely monitors the temperature at every step and takes corrective action. Controller action is PID (Proportional, Integral and derivative of the error signal i.e difference between measured variable and set point). In this work, PID parameters of 4.0, 44 and 13 were used respectively, to obtain the temperature profiles (shown below). A detailed discussion of the temperature profiles for different temperature set points is done in the results section of this work.

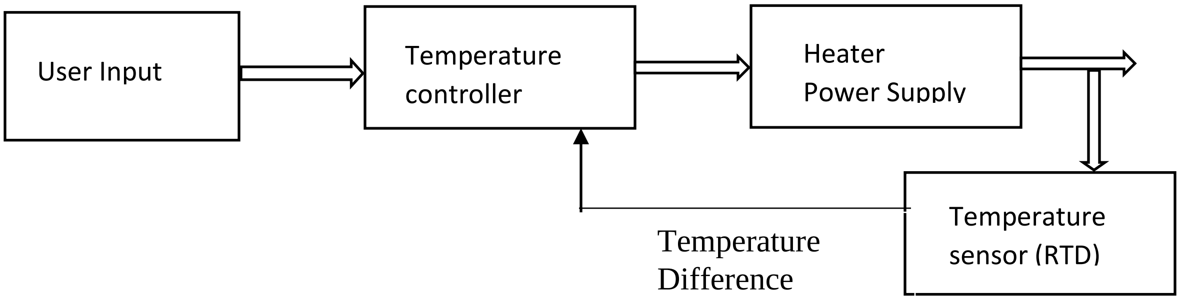


Figure 2.3 Closed loop control system.

2.1.4 Electrometer Automation (Keithley 6517a)

The General Purpose Interface Bus (GPIB) was used as cabling for the electrometer to the PC. Standard Commands for Programmable Instruments (SCPI) protocol was used to measure and transmit current measurements upto the femto ampere range to the PC. The electrometer was programmatically configured to measure and transmit current every second in synchronization with the temperature loop. Communication was by ASCII textual strings.

Figure 2.4 Electrometer (Keithley 6517a).

SCPI protocol is a standard communication protocol for GPIB (IEEE 488.1) instruments due to its simplicity and ease of programming.

2.1.5 Loop Synchronization in LabVIEW

The temperature controller and electrometer automation should run simultaneously to facilitate TSC data logging once the measurement period ends. The measurement period is another user input (in seconds) which determines the running time of the TSC process. Additionally, the user is given options to start, stop or terminate the loop at any time without the instruments in the loop being jeopardized. On terminating the loop the user is given the TSC graph upto the point of termination. User interface is described in the results section.

2.1.6 Electrospinning PolyCaproLactone (PCL)

PCL from Sigma Aldrich (Mn 70,000-90,000) was used to form polymer solutions with Methylene Chloride (CH_2Cl_2) as solvent. Polymer solutions with 10% and 15% by weight were formulated. These solutions were then electrospun to nanofiberous mats. The parameters were as shown in the table below.

Table 2.3 Electrospinning Parameters

Parameter	**Value**
Voltage	25kV
Distance to collector	25cm
Infusion rate	12 ml/hr
Temperature	25 deg C (Ambient)
Humidity	30%

A portion of the electrospun mat of about 6mm was punched out to be used in the TSC experiments. TSC setup includes the enclosure of the sample in between a heating coil within a doubly insulated steel chamber as shown. The sample is held in place by the electrodes of the electrometer.

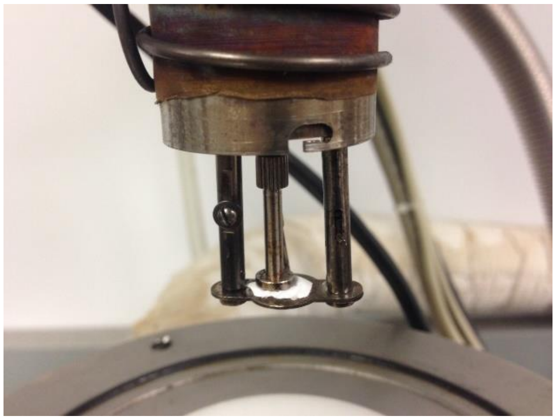


Figure 2.5 TSC setup.

PCL films were cast from the same solution used for electrospinning. The solvent was evaporated by using a vacuum oven maintained at 40 degree Celsius leaving behind a film of PCL which was also used in the TSC experiments by punching out 6mm of the film.

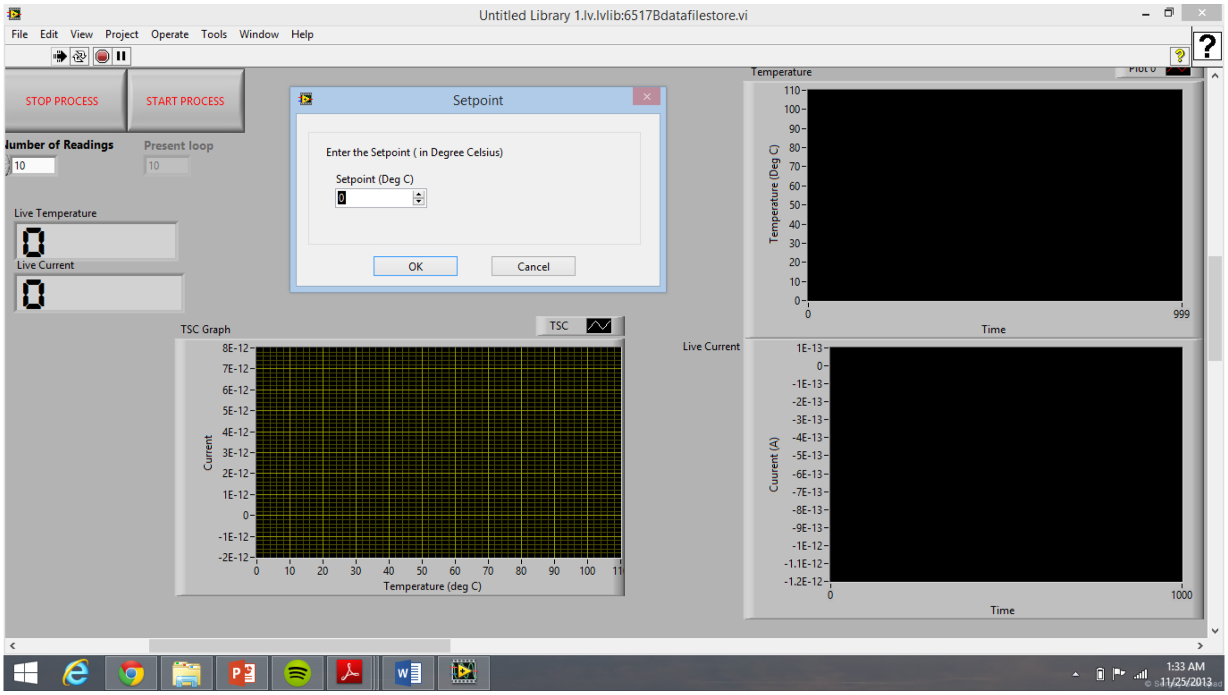
2.1.7 Immersion in Cell Culture media

As one of the objectives of this thesis is focused towards probing for the presence of residual charge during cell culture from electrospun scaffolds, no cells were seeded in the growth media. Two kinds of media were used: DMEM (Dulbecco’s Modified Eagle Medium-InVitrogen) and Phosphate Buffered Saline (PBS). The electrospun PCL scaffolds were then sterilized with 100% ethanol for 20 minutes and dried overnight before immersion in culture media. The DMEM contains 1% antibiotic-antimyotic to prevent bacterial and fungal contamination (Invitrogen), and 10% HyClone FBS-fetal bovine serum (Fisher Scientific). TSC analysis was done on the scaffolds after electrospinning, after sterilization, Day 1, 4 and 7 in 2 types of media (DMEM and PBS) and compared to TSC experiments on PCL films which underwent the same procedure.

CHAPTER 3

3.1 RESULTS AND DISCUSSION

3.1.1 User Interface of the TSC Instrument



umber of Readings: 10

Present loop: 10

Live Temperature: 0

Live Current: 0

Enter the Setpoint (in Degree Celsius)

Setpoint (Deg C): 0

Figure 3.1 LabVIEW Front Panel.

The final user interface of the TSC instrument is shown above. The user inputs the timing of the electrometer-temperature loop in the “Number of Readings” pane. The time of the loop can be modified at any time during the process. The next input is the temperature setpoint which is entered into the “Setpoint” window. When the “OK” button is depressed the setpoint values are waiting to be written into the temperature controller. The setpoint value gets written to the controller only when the user depresses the “Start Process” button. The electrometer also begins to read current only when the “Start Process” button is pressed. The reason for this is to give the user the flexibility to change the input parameters when using the instrument without causing any unexpected triggering of temperature or current measurement. The function of the “Stop Process” button is to terminate the measurement loop. It has to be noted that for user flexibility the “Stop Process” button has been designed in such a way that depressing it at any point during the process should not lead to loss of measured data, therefore stopping the process during measurement will also result in the TSC spectrum.

The charts on the right hand side of the user interface display live temperature and current measurements while the process is running. The TSC spectrum is plotted on the graph on the left handside once the measurement loop ends. The “Present Loop” pane displays the time (in seconds) the process has been running. This can be compared with the user input of “No. of readings” to know the status of the measurement loop. Digital readouts of live temperature and current are also included on the user interface panel.

3.1.1.1 Validation of the LabVIEW-based TSC Instrument. Validation was done by testing the repeatability of the instrument. Repeatability is a measure of the accuracy with which the instrument reproduces any measurement made previously. Therefore, to perform a repeatability test PVDF-TrFE (provided by Sita D.) polymer was used to repeat an experiment done by Yeshuan Lee et al. [12]. As shown below, a peak is observed in the TSC experiment conducted by Lee et al. at about a 110 deg C (see left side), similarly a peak at the same range was observed from the LabVIEW based TSC. The intrinsic presence of noise is seen from the LabVIEW TSC plot, the reason for this is due to the absence of cooling agent like Liquid Nitrogen.

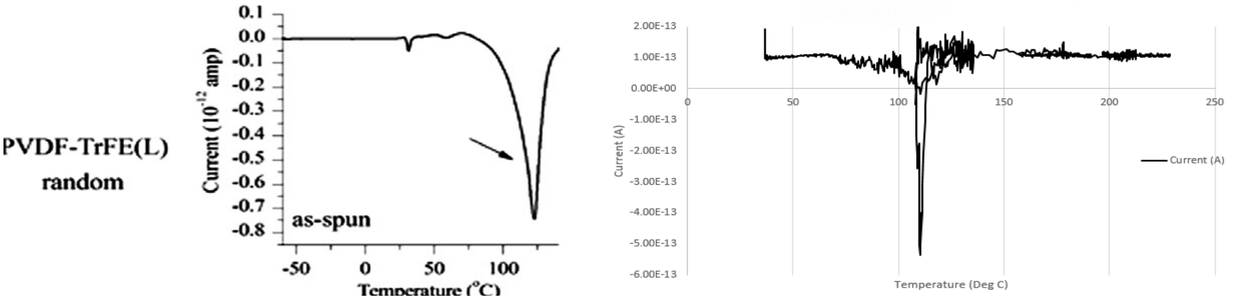


Figure 3.2 Validation curve.

The temperature profile of the LabVIEW TSC is shown below. With the utilization of cooling it is expected that a linear temperature profile can be seen. However, it is important to notice that the loss in the linearity does not impact the fidelity of the current measurements.

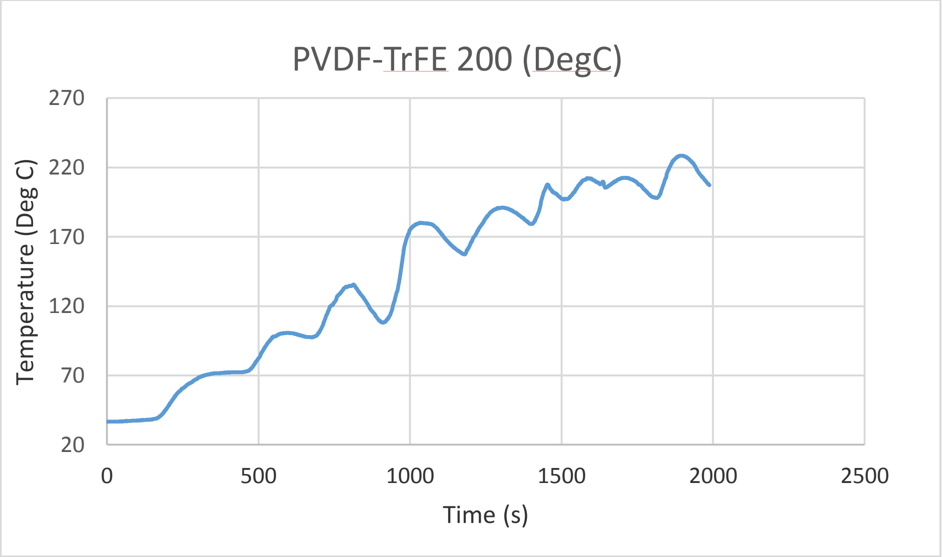


Figure 3.3 Linearization curve (PVDF-TrFE).

Additionally, the 7 degree per minute rate is maintained albeit non-linearly. A better linearity is seen for lower temperatures.

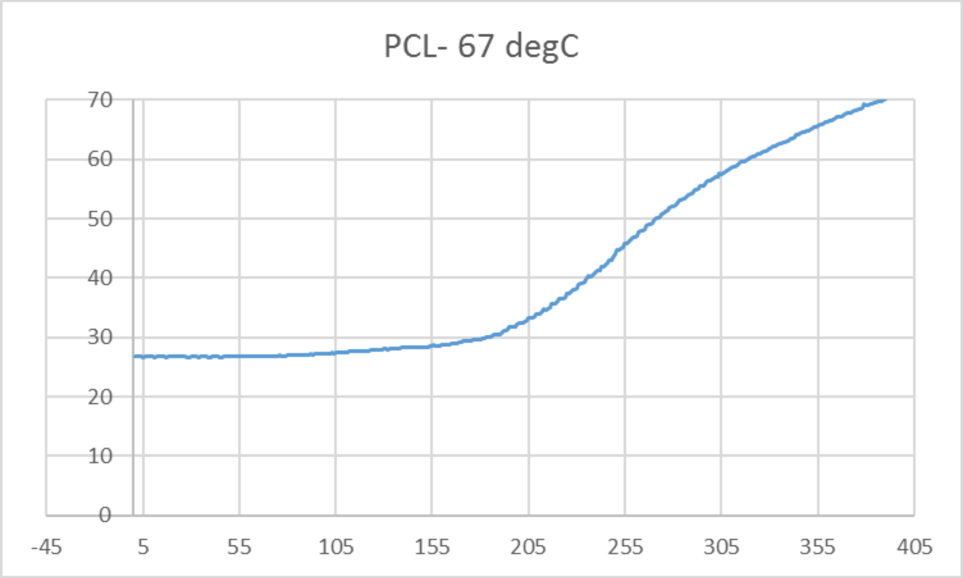


Figure 3.4 Linearization curve (PCL).

All PCL samples in this work were run with 67 degC as setpoint with the temperature profile as shown above. Thus, with the utilization of cooling agents a better temperature profile can be obtained.

3.1.2 TSC of Electrospun PCL vs PCL Film

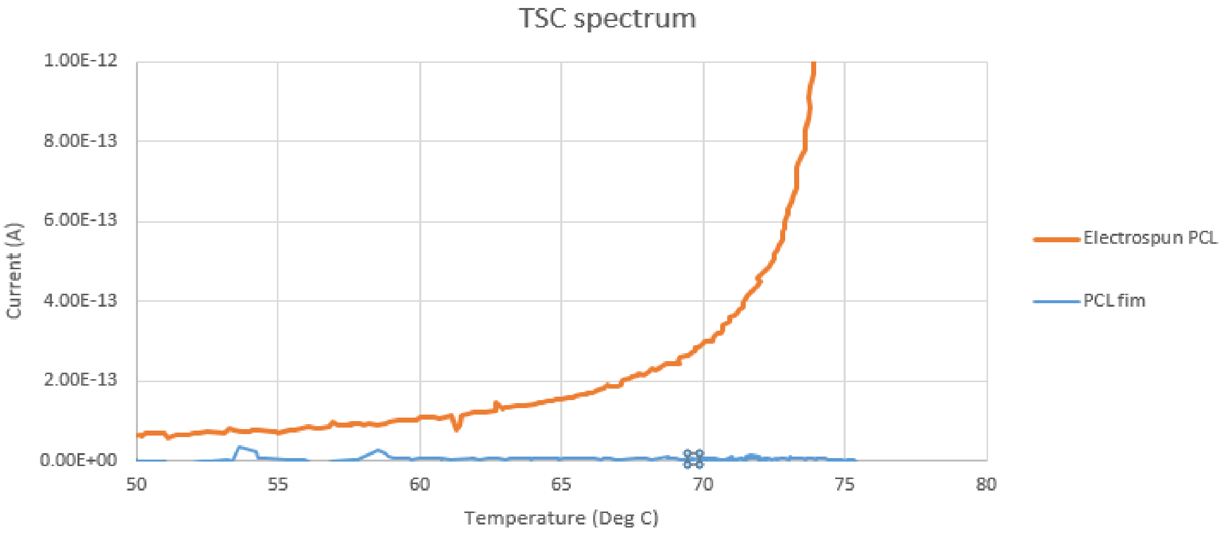


Figure 3.5 PCL (as spun) vs PCL film.

TSC of electrospun PCL (as spun) was first performed and compared to that of PCL film which has not undergone any electrostatic interaction. The observed current is much larger than the current observed in a PCL film which is consistent with our assumption that the low current seen during the electrospinning processmagnitude is present in electrospun scaffolds. The magnitude of residual charge was not seen as a peak due to the enormous amount of charge release seen at higher temperatures (>67 degree Celsius) thus when the temperature decreases due to cooling (PID controller) the magnitude of charges follow the PID oscillations also. Since, the charges follow the PID oscillations it can only be concluded that the electrospinning process Thus, it becomes difficult to determine a peak from the TSC curve of the freshly electrospun PCL scaffolds due to the large amount of charges present in it.

3.1.3 Electrospun PCL (as spun) vs Sterilized PCL

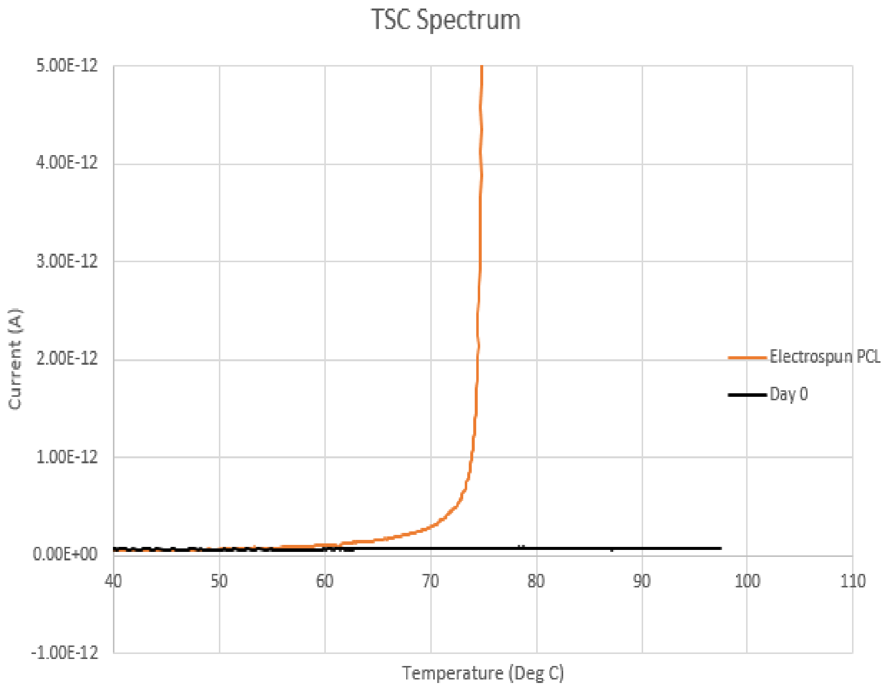


Figure 3.6 PCL (as spun) vs Sterilized PCL (day0).

Sterilization in ethanol (100%) for 20 minutes is the procedure followed before immersion in media. Thus, electrospun PCL was probed for current after sterilization in ethanol. It was seen that residual charge originating from the electrospinning process is dissipated on immersion in ethanol for PolyCaproLactone (PCL) mats. The reason is likely to be the solvation of residual charge which has an opposite polarity when compared to the hydroxyl (OH^-) groups in ethanol. Ethanol is one of the cheaper sterilizing agents used in laboratories for sterilization, it is a possibility that given the nature of charges in the mat that ethanol's hydroxyl groups may be responsible for the solvation of the charges, resulting in the neutralization of the charges originating from electrospinning.

3.1.4 Electrospun PCL (as spun) Vs PCL in PBS (1, 4 & 7 days)

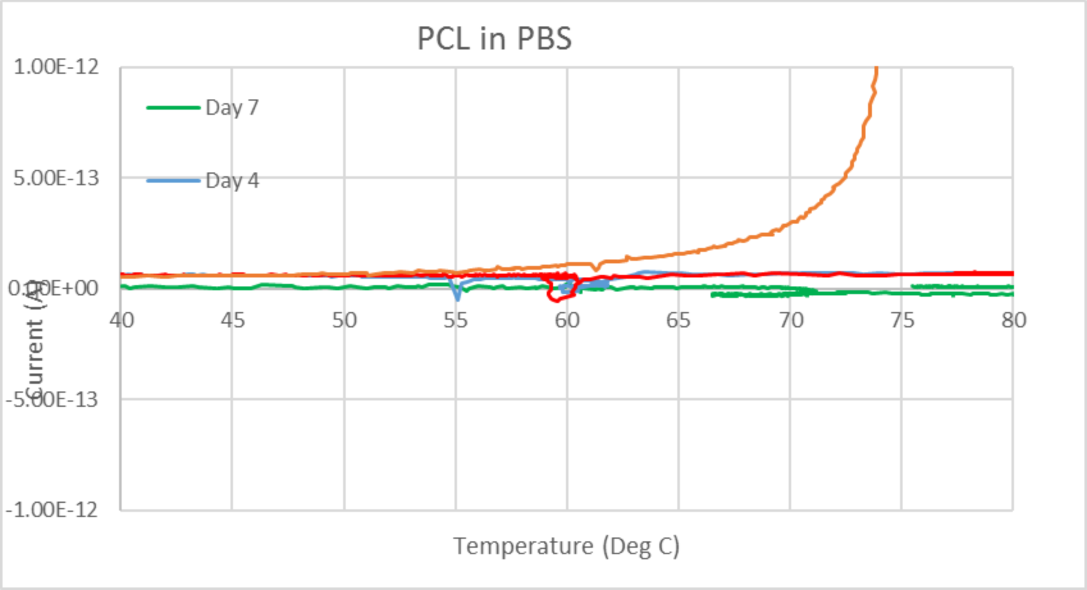


Figure 3.7 PCL (as spun) Vs PCL mat in PBS (1, 4 & 7 days).

The above figure is consistent with the belief that charges emanating from the process of electrospinning are dissipated on sterilization with 100% ethanol. The samples immersed in PBS in all 3 time period show negligible current which is similar to control samples (PCL films). This further asserts the possibility that the charges dissipate in ethanol.

3.1.5 Electrospun PCL (as spun) Vs PCL in Media (Days 1, 4 & 7)

It was observed that the PCL mats immersed in media resulted in an anomalous current behavior during the TSC. The abnormality was seen in the PCL films immersed in Culture media DMEM. The Dulbecco's Modified Eagle Medium (DMEM) contains proteins and salts, it is a likely possibility that the abnormality in the current behavior may be a result of interaction of the scaffold with the contents of the basal growth media i.e FBS, proteins and salts.

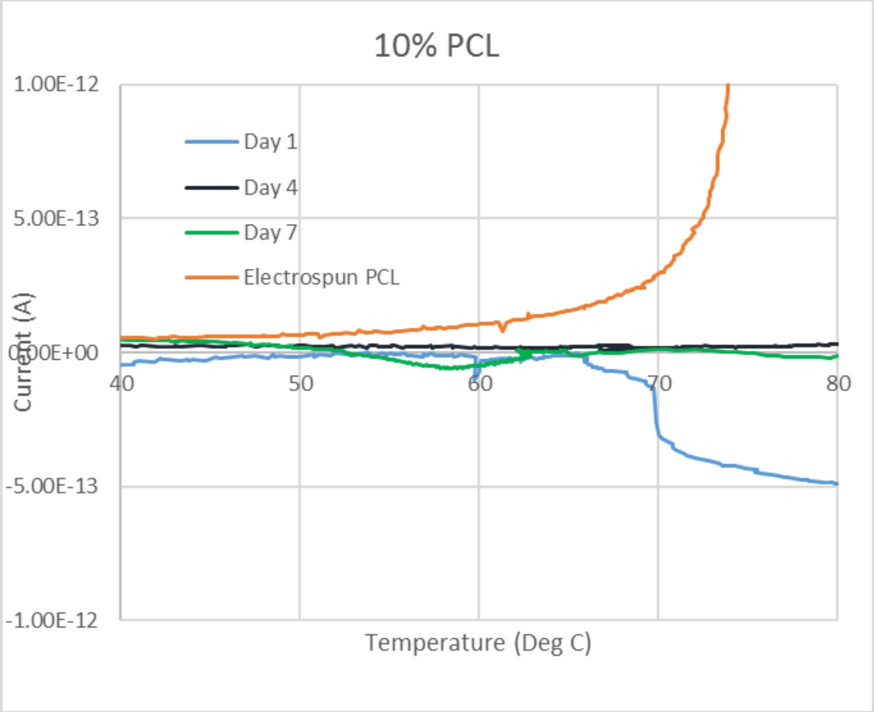


Figure 3.8 PCL mat (as spun) Vs PCL mat in media (days 1, 4 & 7).

A graph of the anomalous current behavior in PCL films is shown plotted with the freshly electrospun PCL.

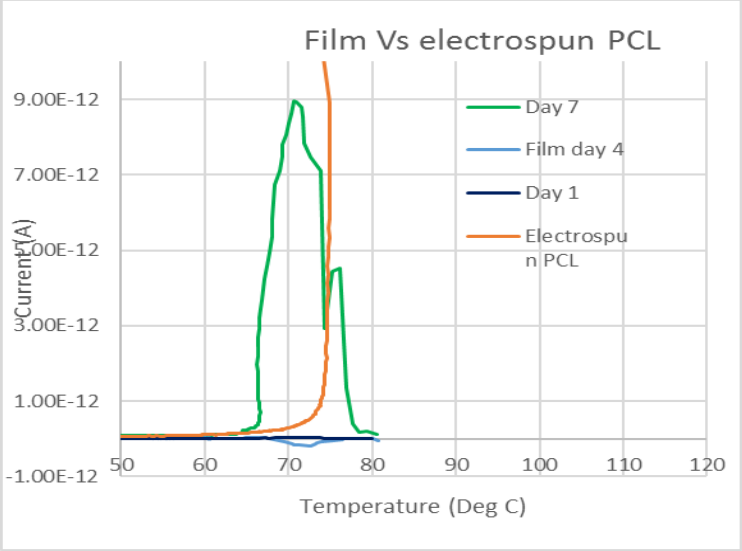


Figure 3.9 PCL film (days 1, 4 & 7) vs electrospun PCL.

3.2 Summary of Results

A LabVIEW based Thermally Stimulated Current (TSC) controller was developed to govern the operation of the previously non-functional TSC. The LabVIEW based TSC encapsulates the electrometer and temperature control while logging the TSC data in an excel file for the user to manipulate it. The non-linearity in the temperature control of the LabVIEW based TSC stems from the absence of appropriate coolants like Liquid Nitrogen.

The charges that are responsible for the governing of the process of electrospinning are deposited on the collector with the charges trapped in the filaments. The trapped charges were previously hypothesized to be present in cell culture [6], but it was found in the course of the experiments conducted in this thesis that the trapped charges in the nanofiber mat are dissipated on immersion into 100% ethanol for sterilization. The results were derived from TSC curves from the LabVIEW based TSC.

Furthermore, anomalous currents were detected from samples immersed in DMEM. It is difficult to even speculate the reason behind these current anomalies. A possible reason could be the interactions between the proteins and the scaffolds. Further experimental evidence is required to better understand the cycle of events leading to the currents observed from the TSC spectrum.

CHAPTER 4

CONCLUSION

The objective of this thesis is to renovate the TSC with a LabVIEW based environment which was done successfully. The LabVIEW based TSC incorporates a greater reliability with upgradation to the latest techniques of industrial control. The user friendly interface provides greater maneuverability of the TSC controller and offers more room for programmable features benefitting the user.

Residual charge from electrospun mats can be measured using the TSC technique. The LabVIEW based TSC was used to measure a maximum current of 50pA. However, due to the oscillations of the PID controller an absolute peak of the current could not be seen in the TSC plot.

One of the major objectives of this thesis is to clarify the presence or absence of residual charge during cell culture. It was observed that during the sterilization process undergone by the electrospun mats, the charges that originated from electrospinning are dissipated in 100% ethanol. TSC curves indicated that there is almost no current on day 0 (after sterilization) indicating that electrospun PCL dissipates charges from electrospinning in ethanol (100%). Charge dissipation in ethanol is likely to be due to the solvation of charge from the nanofiber mat due to the hydroxyl group in ethanol. It is possible that the hydroxyl group solvates the charges which have opposite polarity.

The anomalous current behavior on samples immersed in DMEM requires a deeper understanding of the role of FBS, proteins and salts from the DMEM in charge generation.

CHAPTER 5

FUTURE WORK

Effective cooling mechanism is required to linearize the ramp rate of the temperature control in the TSC. This can be implemented by use of liquid nitrogen as coolant for the process. As inferred from the results the PCL electrospun mats dissipate charge in 100% ethanol. This may also provoke the question if the charge dissipation in ethanol may be material specific. It would be fascinating to know the effect of ethanol on other electrospun materials like PLLA, gelatin etc.

Furthermore, since conventional electrospinning leaves positive charges on the electrospun fiber, it would be interesting to see the effect of reversal of polarities during the spinning process to determine if it leaves negative charges on the electrospun fiber. There has been a dearth of description in literature about the effect of negative voltage electrospinning and the characterization of electrospun substrates that carry negative charges and the ways with which they might affect tissue engineering applications.

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