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A Microfluidics Based Cell Culture Device with Controlled Temperature Gradient

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A Microfluidics Based Cell Culture Device with Controlled Temperature Gradient

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B.Tech., J.N.T University, 2013

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APPROVAL PAGE

Masters of Science Thesis

A Microfluidics Based Cell Culture Device with Controlled Temperature Gradient

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TABLE OF CONTENTS

Title page	i
Approval page.....	ii
Acknowledgements.....	iii
Table of contents.....	iv
List of figures.....	vii
Abstract.....	ix
Chapter 1 : INTRODUCTION.....	1
1.1 Cell Culture	1
1.2 Hyperthermia – therapy procedure for cancer.....	2
1.3 Temperature variation studies	2
1.4 Microfluidic cell culture devices.....	3
1.5 Advantages of microfluidic cell culture devices	4
1.6 Objective	6
Chapter 2 : DESIGN AND FABRICATION	7
2.1 Temperature sensors.....	7
2.1.1 Principle of Resistance Temperature Detector	7

2.1.2 Fabrication of temperature sensors.....	8
2.1.3 Substrate pretreatment	9
2.1.4 Metal deposition	9
2.1.5 Process flow of UV Lithography.....	10
2.1.6 Wet Etching	14
2.2 Optimizing the design for temperature sensor	16
2.3 Wheatstone Bridge.....	18
2.4 Thin PDMS layer on the substrate	19
2.5 Fabrication of Microchannel.....	20
2.5.1 Negative master design	20
2.5.2 Casting and Curing	22
2.5.3 Releasing	23
2.6 Bonding Microchannel to the substrate	23
2.7 Design of hotplate.....	25
Chapter 3 : EXPERIMENTS AND RESULTS	28
3.1 Calibration of temperature sensors	28
3.2 Experimental procedure	30
3.2.1 PI Control	32
3.2.2 Testing	36

3.3 Experiment – monitoring temperature gradient across the microchannel	37
3.4 Cell Experiment	40
3.5 Integration of the fabricated device with an imaging setup	41
Chapter 4 : CONCLUSION	43

LIST OF FIGURES

- **Figure 1:** Illustration of the microfluidic cell culture device
- **Figure 2:** Thin Chromium metal layer deposited on a glass substrate
- **Figure 3:** Process flow of UV Lithography
- **Figure 4:** Mechanism of positive photoresist
- **Figure 5:** (A) shows the exposure of substrate of UV radiation, (B) shows development of positive photoresist
- **Figure 6:** (A) shows Photomask with resistive patterns, (B) shows magnified image of one of the sensors
- **Figure 7:** Illustration of resistive pattern after development
- **Figure 8:** Microscopic image of substrate with metal layer and photoresist pattern
- **Figure 9:** Illustration of substrate with metal pattern
- **Figure 10:** Microscopic image of metal pattern on the substrate
- **Figure 11:** (A), (B),(C) and (D) show resistive patterns of varying dimensions
- **Figure 12:** Temperature sensor as a part of Wheatstone Bridge
- **Figure 13:** Substrate coated with thin layer of PDMS
- **Figure 14:** Design of Microchannel
- **Figure 15:** (a) Silhouette printer (b) Blade that cuts thin films fed into the printer (c) Negative pattern of the microchannel placed in a petri dish.
- **Figure 16:** PDMS poured on the negative master design
- **Figure 17:** Microchannel
- **Figure 18:** (a) Microchannel, (b) Thin PDMS layer coated on the substrate (c) Substrate with temperature sensors

- **Figure 19:** Microfluidic cell culture device
- **Figure 20:** Peltier Cooler
- **Figure 21:** TMP 36 sensor
- **Figure 22:** Custom-built hot plate with microfluidic device placed on its surface
- **Figure 23:** Block diagram showing the process flow for sensor calibration
- **Figure 24:** Calibration of sensor S_A
- **Figure 25:** Calibration of sensor S_B
- **Figure 26:** Schematic of experimental setup
- **Figure 27:** PI Control loop
- **Figure 28:** Experimental setup
- **Figure 29:** Experimental process flow
- **Figure 30:** Sensor output in case 1 ($K_i = 0.001$ [V/ (°C.s)] and $K_p = 3$ [V/ °C], $\Delta t = 200$ milliseconds)
- **Figure 31:** Sensor output in case 2 ($K_i = 0.001$ [V/ (°C.s)] and $K_p = 3$ [V/ °C], $\Delta t = 200$ milliseconds)
- **Figure 32:** Sensor output (hotside 40°C and coldside 37°C) at $K_i = 0.001$ [V/ (°C.s)] and $K_p = 1$ [V/ °C], $\Delta t = 100$ milliseconds)
- **Figure 33:** Sensor output (hotside 40°C and coldside 37°C) at $K_i = 0.001$ [V/ (°C.s)] and $K_p = 1$ [V/ °C], $\Delta t = 100$ milliseconds)
- **Figure 34:** (a) Microchannel with cells (b) Microscopic image of the cells in the microchannel
- **Figure 35:** Illustration showing the integration of fabricated device with an imaging setup.

Abstract:

Cell culture is an extensively used technique for studying the behavior and growth of cells in response to different conditions. Examining the effect of temperature variation on cells helps in understanding the characteristics of cancer cells and may help in developing therapeutic procedures. To study the effect of this variation, we propose to use a microenvironment with a programmable temperature gradient. Microfluidics based cell culture devices offer advantage of providing a controlled environment, effectively.

The study focuses on creating a microfluidic cell culture device with a controlled temperature gradient. The device consists of two components— a microchannel (18mm×6mm) that holds the cell suspended in culture media and integrated multiple temperature sensors that detect the temperature in the microchannel. Soft lithography of Polydimethylsiloxane was used to fabricate the microchannel. The chromium temperature sensor, which is essentially a resistive pattern, was designed and fabricated using UV lithography technique. To measure the local temperature in a controlled gradient, integrated sensors are placed across the channel. These sensors give a measurement of the temperature inside the channel on the cold and hot regions. In order to provide this gradient, a custom built hotplate was devised which creates a difference of about 3 °C across the width (6mm) of the channel.

First, the designed sensors were calibrated and a linear relation between temperature and voltage was found. The device was evaluated by monitoring the temperature gradient across the channel for 6 hours. Cell compatibility of MCF-7 breast cancer cells was tested in the fabricated device with the set conditions and it can be used for in vitro experiments that last for hours.

Chapter 1

Introduction

1.1 Cell culture

Cell culture is a major tool that is being extensively used in biological sciences ^[1]. Cell culture allows to explore the characteristics of cells *in vitro*. One of the major advantages of cell culture lies in its simplicity to study a single type of cell in a controlled microenvironment as compared to studying a whole organ or body which comprises of different cell types ^[2]. Further, it allows to manipulate the physical, chemical and physiological conditions in which the cells survive ^[2]. This helps researchers study the effect of these conditions on cells. Cell culture enables to better understand the behavior of cells in response to different conditions ^[2].

Cell culture has many applications and it has had an enormous impact on human health. The ability to grow cells outside of their natural microenvironment primarily helps in understanding various cell functions such as proliferation, viability, adhesion and effect of drugs ^[2]. In addition, cell culture studies help in drug discovery and pharmacological applications, cancer research, genetics and gene therapy ^[3]. It also augments studies related to nutrition and aging ^[4]. Although, cell behavior and characteristics can be studied *in vivo*, it is difficult to quantitate and control these experiments ^[4]. Therefore experimental systems that can offer reproducibility and allows to quantitate the effects are required.

One of the areas where cell culture techniques have had a major impact is cancer research. Examining the effect of temperature on cancer cells helps in understanding the characteristics of cancer cells and may help in developing therapeutic procedures. An example of a therapy procedure which can be used to treat cancer is hyperthermia.

1.2 Hyperthermia – therapy procedure for cancer

Based on cell culture studies, hyperthermia – a therapy procedure that can kill cancer cells, has been developed ^[5]. In hyperthermia, the body tissue is exposed to temperature as high as 45°C to destroy the cancer cells ^[5]. More often, this procedure is used in combination with other therapies like radiation and chemotherapy ^[5]. Hyperthermia causes the cancer cells to become more sensitive to these forms of treatment. It is also known to increase the effect of anticancer drugs on cancer cells ^[5]. Takahashi et.al, studied the effect of hyperthermia in combination with radiotherapy and/or chemotherapy and stated in their research that anticancer drugs together with hyperthermia had an enhanced effect in treatment of cancer ^[6]. Hyperthermia is known to destroy cancer cells with least possible effect on normal cells ^[7]. Hyperthermia has been found to increase the drug diffusivity into the cancer cells by modifying local tumor environment ^[6] ^[8]. It can be used as a modality for drugs to target the tumor ^[8]. Hyperthermia increases tumor blood flow and allows better tumor microvascular permeability ^[9]. However, there are a few issues with respect to equipment and procedure that need to be overcome before hyperthermia can be considered a standard technique for cancer treatment ^[9].

1.3 Temperature variation studies and their significance:

Small variations in temperature can cause differences in cell behavior and individual protein activity, as in the study by Das et.al. In general, thermal effects are common occurrences in diseases and are also used in many therapy procedures ^[10]. It is also well known that thermal gradients exist within the human body as these are important for certain cellular functions ^[10]. The temperature of a cancerous tissue is slightly elevated as compared to a surrounding non-cancerous tissue ^[10]. Das et.al, proved in their study that cell viability is low in temperatures higher than the normal body temperature. In temperatures lower than the normal body temperature, the cell density

was low, but no significant effect of the thermal gradient was found ^[10]. In order to explore further the effects of temperature gradient on cancer cells, there exists a need to develop microsystems that efficiently provide a natural microenvironment for the cells and provide variations in temperature.

Apart from cancer cell analysis, temperature gradient studies are also important in other areas. For instance, Lucchetta et.al, studied the effect of temperature gradient on *Drosophila* embryo development. It was shown in their work that the embryo on the warmer side of the designed PDMS microfluidic device developed rapidly relative to the cooler side ^[11].

Conventional cell culture techniques using polystyrene dishes, flasks or well plates involved large fluid volumes that prevent rapid changes in the conditions, manually involved cell manipulation and low accuracy of cell analysis ^[12]. Other limitations of the conventional cell culture techniques are that the device architecture is fixed, the culture surface is rigid and perfusion gradients are difficult to achieve ^[13]. Also, it is difficult to carry out studies that involve temperature gradients using the conventional cell culture techniques ^[15].

1.4 Microfluidic cell culture devices

Microfluidic cell culture provides techniques to culture and maintain cells within a controlled environment in micro-scale volume of reagents ^[12]. Additionally, microfluidic devices offer flexible device architecture, experimental flexibility and control and the ability to perform perfusion culture ^{[12][13]}. Understanding the network between the requirements for cell culture and the conditions provided by microfluidic devices will further the technology of microfluidic cell culture ^[14].

Microfluidic technology has emerged as a potential tool to create and manipulate cellular microenvironment, which can provide greater insight to cellular physiology leading to new discoveries. One of the contributors to the field of microfluidics is microelectronics [20]. The original technique of microfluidics was Photolithography on a Silicon substrate which was successful both in microelectronics and microelectromechanical systems (MEMS) [20]. However, Silicon is expensive and is not optically transparent. Therefore, it has been documented that fabrication of microsystems is easier when elastomeric materials are used [20].

1.5 Advantages of microfluidic cell culture devices:

Microfluidics enables the precise application of experimental conditions to study the behavior of cells. The advantages of microfluidic cell culture over the macro-scale methods include efficient process control, design versatility and the ability to closely replicate the cell's natural microenvironment [13]. Faster analysis is possible considering the augmented transfer of heat and mass over smaller lengths as compared to macrodevices [13]. Reduced reagent consumption, accurate experimentation and reduced risk of contamination add to the advantages of the use of microfluidic cell culture devices [13].

A few advantages of microfluidic cell culture devices are:

1. Flexibility of design^{[12][13]}
2. Fast response of the system^{[12][13]}
3. Precise control over experimental conditions^[13]
4. Low reagent consumption^[16]
5. Perfusion culture can be performed^[17]
6. Ability to integrate biosensors into the cell culture device^{[12][13]}

7. Studies on single cells can be carried which is simple compared to studying the bulk of an organ ^{[13] [18]}
8. Low number of cells suffice the experimental requirements^[13]
9. Sensors can be integrated into the devices allowing analysis of the conditions provided, on the cells ^[13]

Considering the advantages offered by microfluidic cell culture devices, the recent trend has been the development of integrated microfluidic devices with electronic and mechanical components or chemical processes onto the substrate that help in analyzing the system ^[21].

Currently, the most popular method of fabricating microfluidic devices for cell biological applications is the Soft-lithography of Polydimethylsiloxane (PDMS) ^[22]. PDMS based microfluidic devices are easy to fabricate and offer flexibility in design ^{[23] [24]}. PDMS is an elastomer, which is optically transparent, biocompatible and gas permeable ^[22]. In addition, PDMS is inert and non-toxic ^{[21] [22] [23] [24]}. These properties make it a better choice for researchers to use PDMS for cell biological applications ^[25]. Soft-lithography of PDMS helps to fabricate the channels at the dimensions of the cell ^{[23] [24] [25]}.

Important parameters that need to be controlled during cell culture include Oxygen, Carbondioxide, pH and temperature. In the current study, we focus on the effects of temperature gradient on cancer cells. It is known that cell morphology, metabolism, proliferation and adhesion are all temperature dependent ^[22]. In order to study the effect of these variations on cells, it is essential to develop a microsystem that can efficiently provide a temperature gradient ^[23].

1.6 Objective

In the current study, we propose to develop a microfluidics based cancer cell culture device that can provide a programmable temperature gradient. The goal is to create thermal gradient of 3° C across the width (6mm) of the microchannel. Multiple integrated sensors are placed inside the device to measure local temperature in the microchannel. These sensors are resistive patterns of Chromium metal, fabricated using Standard UV lithography techniques. The microchannel, fabricated by Soft Lithography of Polydimethylsiloxane (PDMS), holds the cells. A custom built hot plate has been devised to provide the mentioned thermal gradient. A major effort has also been devoted to monitoring the temperature gradient over a period of time in order to study the effect of this thermal gradient on MCF-7 breast cancer cells.

Chapter 2

Design and Fabrication

The primary requirement to design and fabricate the microfluidic cell culture device (shown in Fig 1) is to provide a controlled temperature gradient and study its effect on the cancer cells. In order to achieve this goal, three essential components are required.

1. A microchannel that can hold cells suspended in the culture medium
2. Integrated multiple temperature sensors that can give a localized measurement of temperature from within the channel
3. A hot plate to provide the temperature gradient

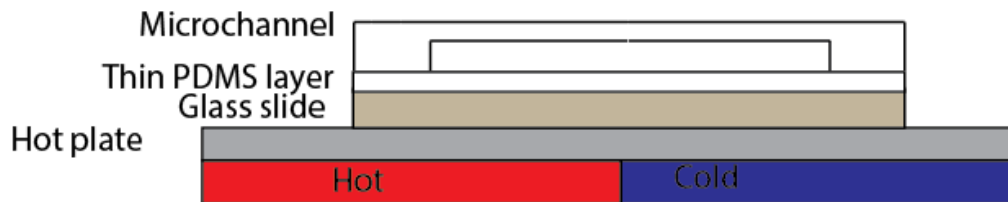


Fig 1: Illustration of the microfluidic cell culture device

The following sections present a detailed procedure on the fabrication methods of each of the component in sequential order in which the device was designed.

2.1 Temperature sensors

2.1.1 Principle of Resistance Temperature Detector:

The integrated temperature sensors were designed based on the principle of a Resistance Temperature Detector (RTD). RTDs are designed using metals whose resistance changes linearly

with temperature i.e. resistance increases with increase in temperature. In other words, RTDs have positive temperature coefficients (PTC) [26].

The equation below gives a relation between resistance and temperature.

$$R_t = R_0(1 + \alpha(t - t_0)) \text{ ----- (1)}$$

where

R_t – resistance of the metal at temperature t

R_0 – resistance at room temperature t_0

α - temperature coefficient of resistance ($^{\circ}\text{C}^{-1}$)

Resistance of any conductor of length L and area of cross-section A can be calculated using the equation

$$R = (\rho * L)/A \text{ ----- (2)}$$

where

ρ – Resistivity of the metal

2.1.2 Fabrication of temperature sensors:

The temperature sensors integrated into the microfluidic device were fabricated using standard UV Lithography techniques. UV lithography is a process used to transfer a pattern onto another layer on a substrate [21]. In order to integrate temperature sensors into a microfluidic device, a thin layer of metal has to be deposited inside the device and a substrate is needed for the same [11].

A glass slide was used as a substrate since it is compatible with all the cleanroom techniques [11].

A microscopic half glass slide of dimensions 37.5mm×12.5mm×1mm was used to fabricate the temperature sensors which are resistive patterns of the metal.

The metal used to design the sensors was Chromium because of the following advantages:

- a) High resistivity
- b) Easy to fabricate
- c) Inexpensive and easily available.

2.1.3 Substrate pretreatment

The substrate must be cleaned before metal deposition. Pretreatment of substrate is an important step in Thermal evaporation, which is a method used for thin-film deposition ^[23].

First, the substrate was cleaned with Acetone in order to wash away any dust particles present on the surface, and dried using Nitrogen. Next, the substrate was subjected to Oxygen plasma (PDC-32G plasma cleaner, Harrick Plasma) for 45 seconds. Plasma treatment is one of the ways to modify the surface of the substrate in order to improve adhesion while preserving the desired properties of the substrate material ^[30]. It chemically activates the surface and promotes the adhesion of metal on the substrate ^[24].

2.1.4 Metal deposition

Chromium metal (Kurt. J. Lesker Company) was deposited on the substrate by Thermal evaporation (Denton Vacuum 502A). The Thermal evaporator is integrated with a Quartz Crystal Microbalance (QCM) to monitor the rate of deposition. The thickness of the metal deposited was found using Dektak Stylus Profilometer. The measured thickness was in the range of 60 to 80 nm. Fig 2 shows a metal layer deposited on the glass substrate.

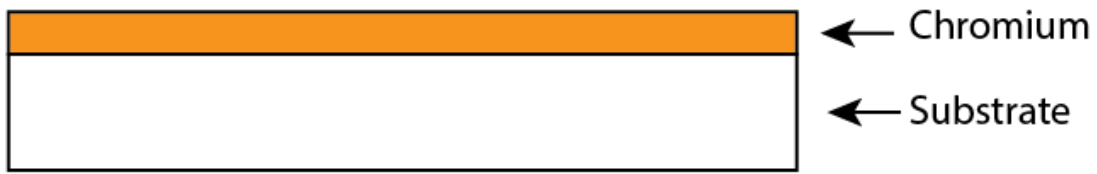


Fig 2: Thin Chromium metal layer deposited on a glass substrate

The desired resistive pattern that constitutes the temperature sensor was transferred onto this substrate by Standard UV lithography technique. The following details the procedure of UV Lithography.

2.1.5 Process flow of UV lithography

UV lithography makes use of a photosensitive material (Photoresist), a photo mask and UV light to aid the process ^[21]. Fig 3 shows the process flow of UV Lithography

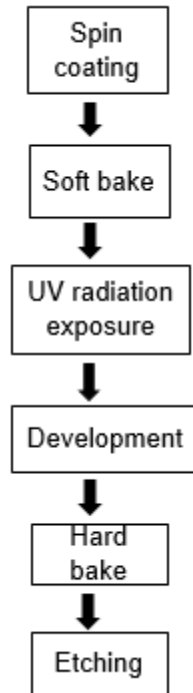


Fig 3 Process flow of UV Lithography

In the following sections, each of the above mentioned steps is described.

Step-1: Spin coating

The surface of the metal film is coated with a thin layer of positive photoresist using a spin coater (Laurell Technologies Corporation, WS-650SZ-6NPP/LITE). A photoresist is a light sensitive material that is used to form a pattern on the surface of a substrate ^[27]. A positive photoresist when exposed to UV radiation becomes more soluble in the photoresist developer. The unexposed portion remains insoluble in the developer ^[28]. Fig 4 shows the mechanism of positive photoresist. This step is followed by soft baking.

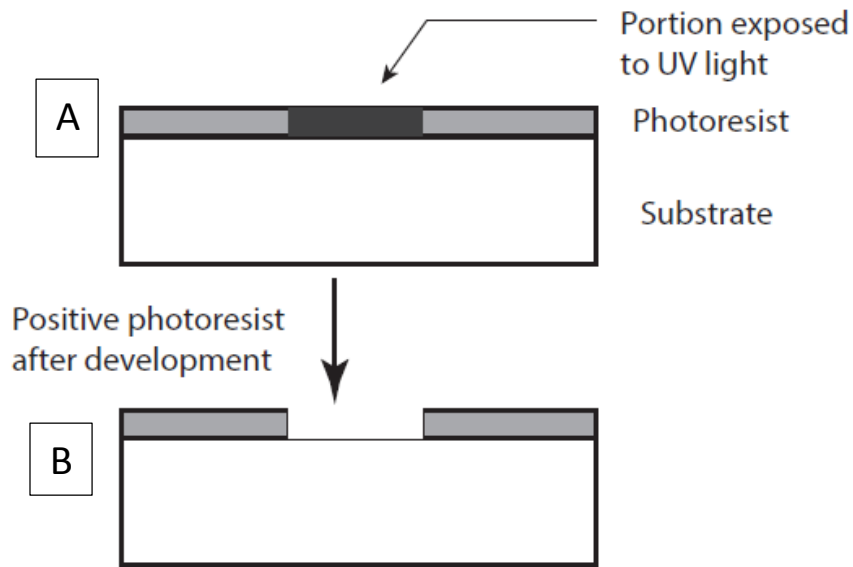


Fig 4: Mechanism of positive photoresist

Step-2: Soft bake

Soft bake is done on a hotplate to remove excess solvent present in the photoresist deposited on the substrate. The temperature at which the soft baking is done is about 113⁰ Centigrade for about 90 seconds.

Step-3: UV Exposure

The substrate with the layer of photoresist on it is exposed to UV radiation through a photomask. The photomask was designed using L-Edit (v11.2). Fig 6 shows the photomask that was used for UV exposure. Time of exposure of UV radiation was 3 seconds. The length of the pattern shown in the figure is 6550 μ m and the width is 30 μ m. Fig 5(A) shows the UV exposure of photoresist on the substrate.

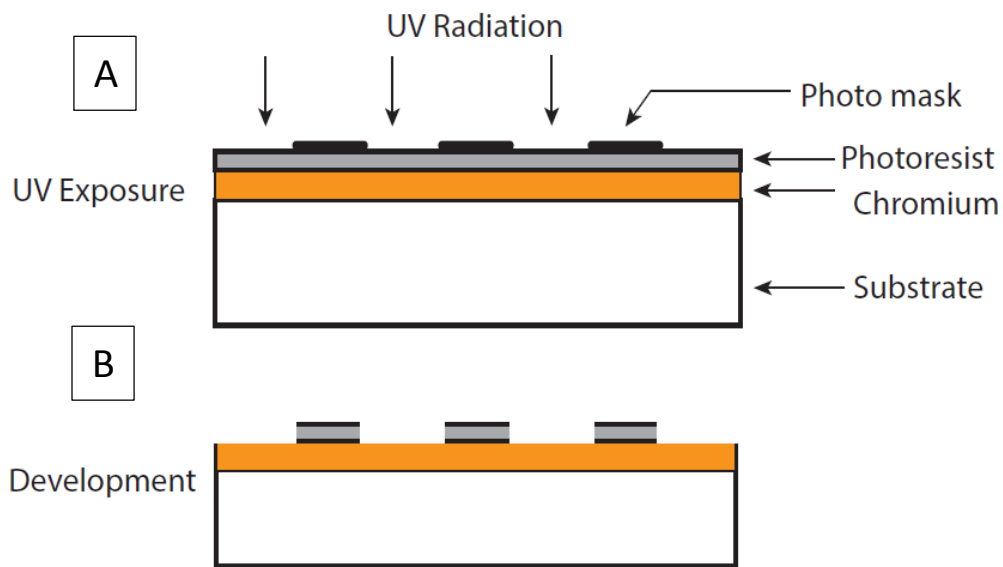


Fig 5:(A) shows the exposure of the substrate to UV radiation, (B) shows the development step.

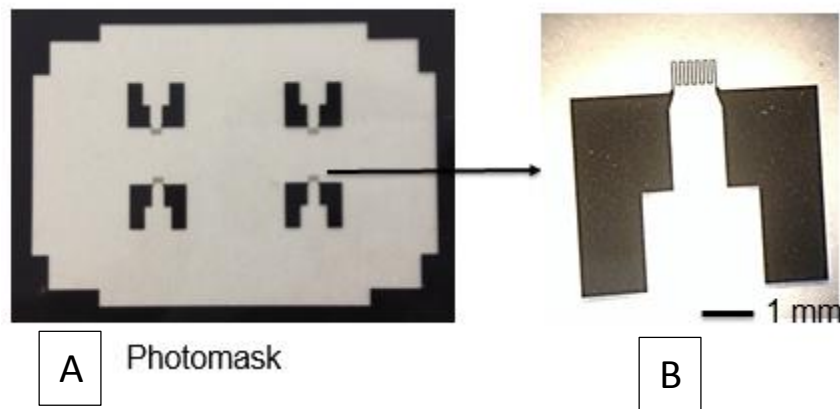


Fig 6: (A) shows Photomask with resistive patterns (B) shows a magnified version of one sensor.

Step-4: Development

In this step, the substrate is placed in a positive photoresist developer (AZ 300 MIF, AZ electronic materials) for 10 to 12 seconds to remove the portion of photoresist that has been exposed to UV radiation and the resistive pattern is protected by the photoresist. Then the substrate is rinsed with

distilled water and dried. Fig 5(B) shows the substrate after development. Fig 7 shows the pattern of photoresist on the metal, after development.

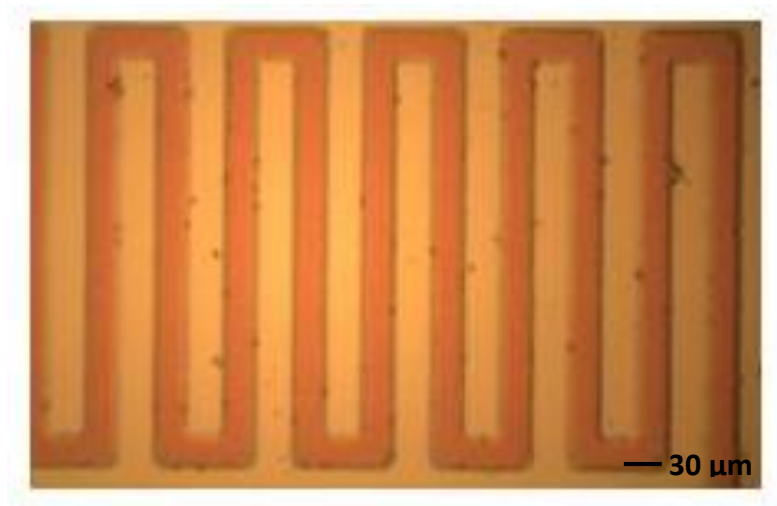


Fig 7: Resistive pattern after development

Step-5: Hard bake

After development, hard baking is performed to improve the adhesion of photoresist structure to the surface of the substrate. Hard baking is usually done at 120⁰ Centigrade for about 10 minutes [14].

2.1.6 Wet etching:

Wet etching is a process that makes use of a chemical solution (etchant) to etch the metals. Etching is usually done to transfer a pattern onto a structural layer beneath the photoresist layer [27]. Wet chemical etching is performed as detailed below.

The metal on the substrate is etched out using Chromium etchant (Transene Company, Inc.), from the areas exposed to UV radiation. On the unexposed areas, there is still a thin photoresist layer

that protects the metal below it. When the metal is etched, the substrate is washed using distilled water. Fig 8 shows the substrate after etching.

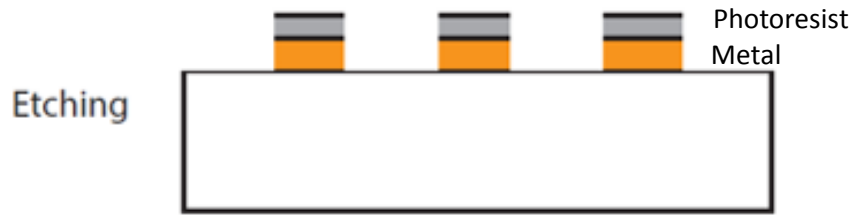


Fig 8: Substrate with metal and photoresist layer (after etching)

Now, the substrate has metal and photoresist layer on the unexposed areas. It was then immersed in a container with Acetone and placed in a Sonicator for about 2 minutes. This removes the photoresist. Finally, the substrate was washed with Isopropanol and dried using Nitrogen. Fig 9 shows the substrate with metal pattern after Acetone sonic wash.



Fig 9: Substrate with metal pattern

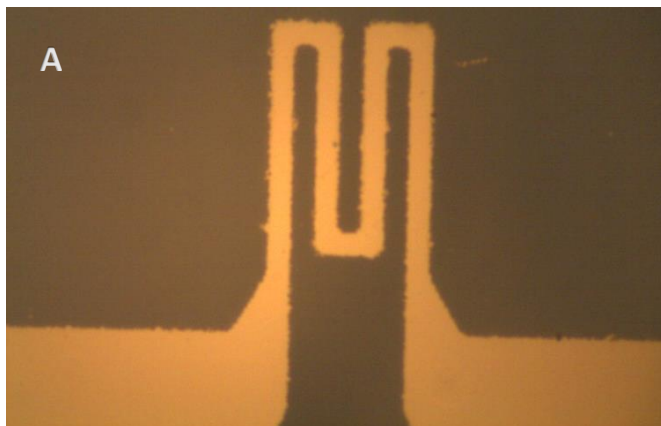
The metal pattern on the glass slide created as detailed above, offered a resistance and this acts as a temperature sensor. Fig 10 shows a microscopic image of the metal pattern on the substrate.



Fig 10: Metal pattern on the substrate

2.2: Optimizing the design for temperature sensor:

Using the procedure detailed in Section 2.1, four resistive patterns of varying lengths and widths were designed. The following provides the dimensions of each of the pattern and the resistance they offered.



Dimensions of the pattern A:

Length = 1275 μm

Width = 25 μm

Resistance offered = 2 - 3 KΩ

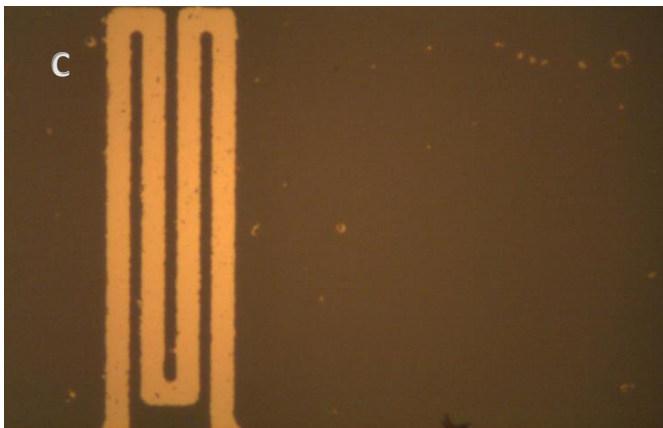


Dimensions of the pattern B:

Length = 6550 μm

Width = 30 μm

Resistance offered = 3.8 - 4.5 K Ω



Dimensions of the pattern C:

Length = 2075 μm

Width = 20 μm

Resistance offered = 0.8 - 2 K Ω



Dimensions of the pattern D:

Length = 1950 μm

Width = 30 μm

Resistance offered = 1.4 - 2 K Ω

Fig 11 (A) , (B), (C), (D) shows resistive patterns of varying dimensions and the resistances offered.

Of the four patterns, pattern B was chosen. The reason was, easy to fabricate, resistance offered was in a medium range when compared to the other patterns and, stability.

2.3 Wheatstone bridge

In order to pick the temperature signals from the sensor, very thin wires were glued to the metal pattern using Silver epoxy glue. It is electrically conductive and can be used on almost any surface.

Thin conducting wires were glued to the metal pads of the sensor and it is cured for two hours at 120⁰ Centigrade. Resistance is checked at the free end of the glued wires, using a Digital multimeter. If the resistance value is almost equal to the value measured from the metal pads, the glue is cured. Otherwise, it is cured further, accordingly. The following fig 2.13 illustrates how the sensor forms a part of the bridge and the signal is used to monitor temperature.

The sensor forms one of the arms of the Wheatstone bridge, a potentiometer forms two other arms and a resistor forms the fourth arm. The potentiometer and the resistor both have a value comparable to that of the temperature sensor (resistor). The Wheatstone bridge gives a voltage output for the temperature signal picked up by the temperature sensor (one of the arms). The circuit for the Wheatstone bridge is shown below.

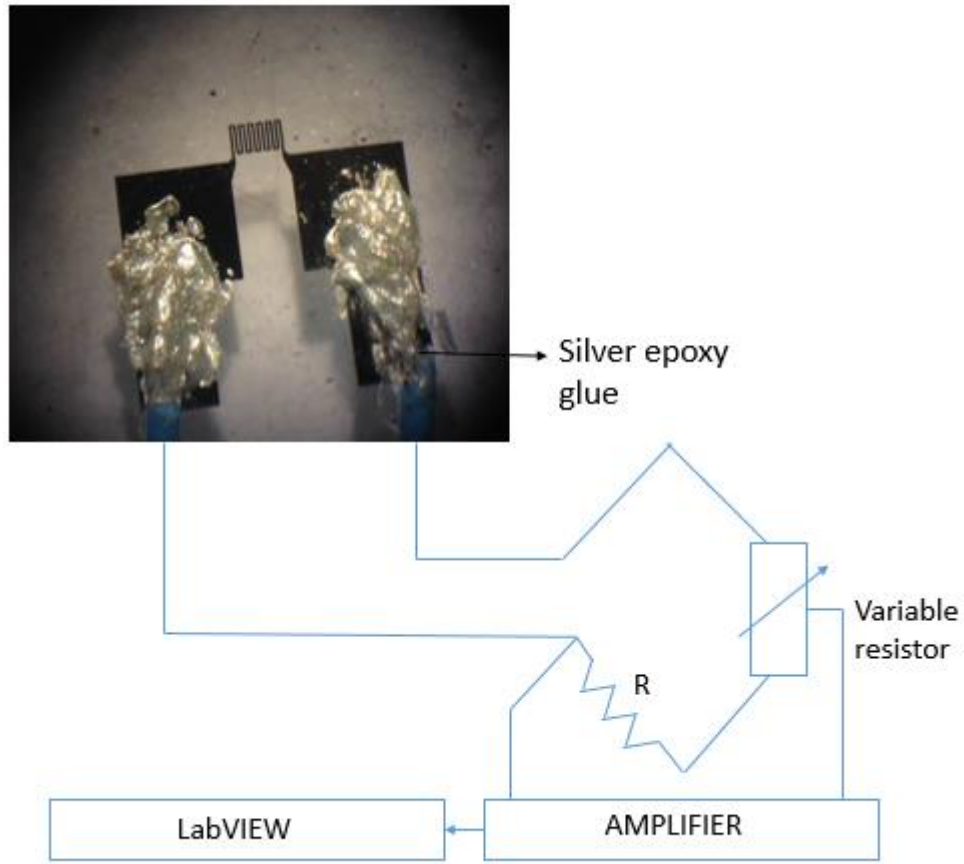


Fig 12 : Temperature sensor as a part of Wheatstone bridge

2.4 Thin PDMS layer on the substrate:

The substrate (with fabricated sensors) was coated with a thin layer of PDMS. The PDMS prepolymer (Slyguard 184) and curing agent were mixed in the ratio of 10:1 was degassed, to remove any air bubbles that result from the mixing process, and carefully poured onto the substrate avoiding further air bubbles. This was cured for two hours at 60°C.



Fig 13: Substrate coated with a thin PDMS layer

The substrate was coated with the PDMS layer in order to avoid direct contact of culture media with the metal.

2.5 Fabrication of microchannel

The microchannel shown in the fig 14 was fabricated by Soft lithography of Polydimethylsiloxane (PDMS). Soft Lithography is fabricating microfluidic devices using a soft polymeric mold such as PDMS replica from a master design. ^[23] The dimensions of the channel were according to the designed photomask such that the integrated sensors are within the microchannel to get a local measurement of temperature.

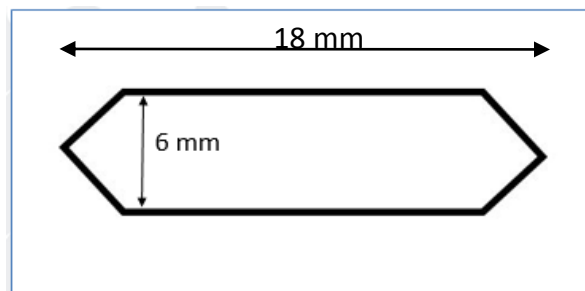


Fig 14: Design of the microchannel

2.5.1 Negative master design:

The negative master design required to fabricate the microchannel was made by a thin film cut using a Silhouette printer shown in fig 15(a). The Silhouette printer is a commonly available desktop printer that is used to cut thin films. Thin film is stuck on to the cutting mat and fed into

the printer as depicted in fig 15(a). The blade shown in fig 15(b) cuts the film when the desired pattern is sent to the printer using the compatible software. The patterns are carefully peeled out of the cutting mat and placed in a petri dish. This serves as a negative master design for the microchannel. The thickness of the film used was 100 μm .

Silhouette printer is advantageous in ways that it is simple to use, thus offers flexibility to modify the design without consuming much time. It can cut complex patterns with a resolution of 1mm. One of the major advantages is that it allows to avoid the design of negative pattern by using SU-8 and going through all the steps of Photolithography which is time consuming and not very flexible. Therefore, Silhouette printer is a very convenient tool when the design required is of the order of few millimeters.

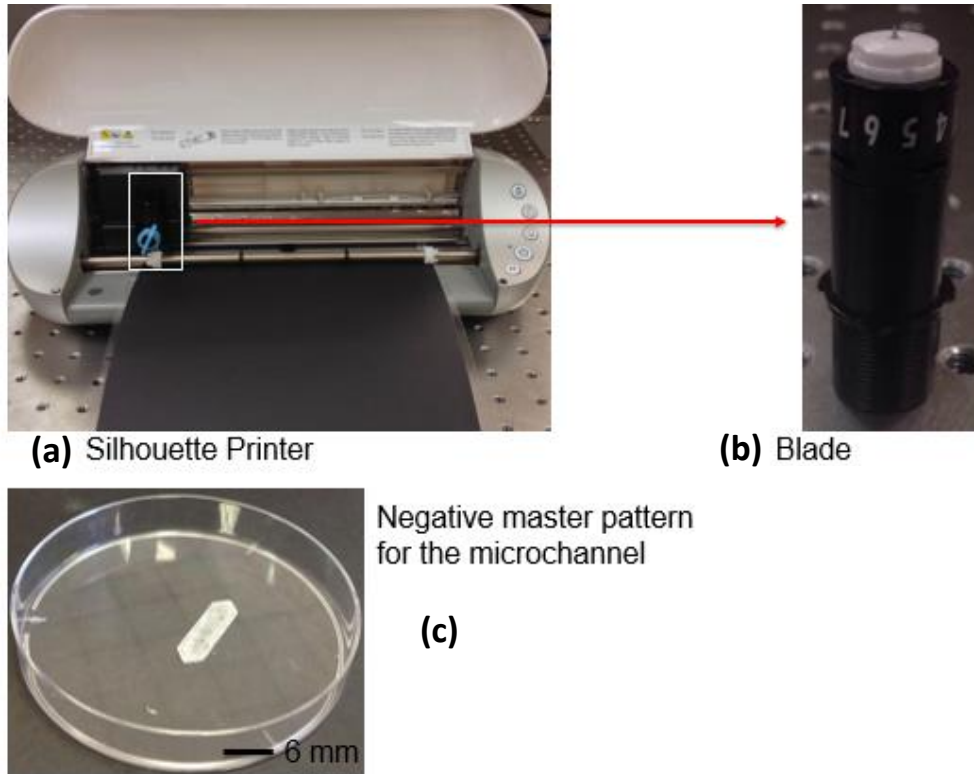


Fig 15 (a) Silhouette printer (b) Blade that cuts thin films fed into the printer (c) Negative pattern of the microchannel placed in a petri dish.

Soft Lithography of PDMS involves three steps:

1. Casting
2. Curing and
3. Releasing

2.5.2 Casting and Curing:

The PDMS polymer and the curing agent were mixed in a ratio of 10:1, measured by weight. This mixture was then placed in a degassing chamber for about 30 minutes to remove any air bubbles that form during the mixing process. This mixture was then poured into the petri dish with the films aligned. The petri dish was placed in an oven for three hours at 60°C.

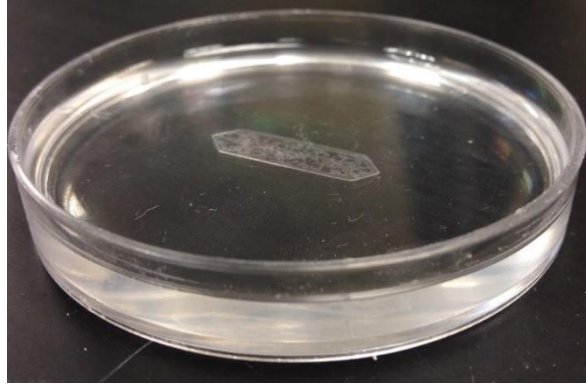


Fig 16: PDMS poured on the negative master design

2.5.3 Releasing

After three hours, the cured PDMS was carefully peeled out from the petri dish and cut as required using a scalpel. Fig 17 shows the microchannel.



Fig 17: Microchannel

2.6 Bonding microchannel to the substrate:

The microfluidic cell culture device has a microchannel and substrate with temperature sensors on it. The microchannel fabricated as explained in section 2.5, is bonded to the substrate (section 2.1)

using Oxygen Plasma. Fig 18 shows the arrangement of the components in order to build the microfluidic device.

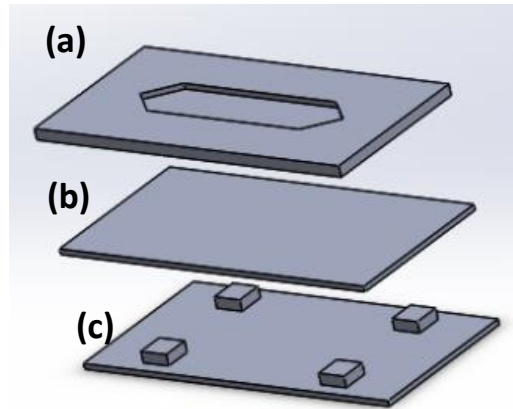


Fig 18: (a) Microchannel, (b) Thin PDMS layer coated on the substrate (c) Substrate with temperature sensors

The substrate with PDMS layer and the microchannel were subjected to Oxygen plasma treatment. This makes the PDMS surface hydrophilic, which is inherently hydrophobic. In other words, oxygen plasma chemically activates the PDMS surface such that it can bond to another surface (PDMS here) ^[30]. The microchannel and the substrate were first cleaned with Isopropanol to remove any dust on the surface. These were then placed in the oxygen plasma chamber with the bonding surfaces facing upwards, for 45 seconds. The microchannel was then aligned such that the sensors were within width of the channel and was bonded to the substrate. Fig 19 shows the microfluidic cell culture device.



Fig 19: Microfluidic cell culture device

2.7 Design of Hot Plate:

The next component needed in order to achieve the goal is a custom built hotplate that can provide a temperature gradient. The equipment used to build the hot plate is described below.

1. Aluminum plates - surface of the hot plate on hot and cold sides
2. Peltier cooler - source of heat

Peltier cooler operates based on thermoelectric effect. It states that when an electrical current is applied across the between two conductors of a semiconductor material, a temperature difference is created ^[31]. One side of the Peltier cooler is heated up and the other side is cooled down when a current is applied. Fig 20 shows a Peltier cooler.

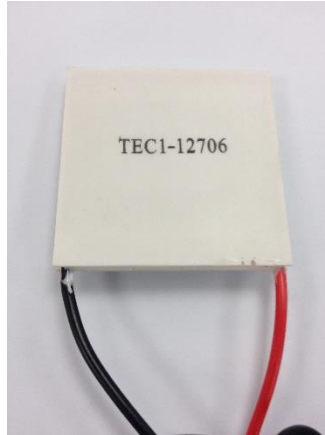


Fig 20: Peltier cooler

3. Analog temperature sensor TMP 36: This is used to measure the temperature on the surface of the hotplate. It is a commercially available temperature sensor that operates on low voltage [26]. It provides voltage output that is linearly proportional to the temperature in °C [32]. Fig 21 shows a TMP 36 sensor.



Fig 21: TMP 36 sensor

An aluminum plate is cut into two, and are placed separated to each other at a distance of about 2 mm. A Peltier cooler is glued to the bottom of each plate using a thermally conductive glue. This is glued to another bottom plate and this setup collectively serves as a hotplate. The TMP 36 sensors are placed on the top of the plate in order to get a measure of the surface temperature, accurately. The fabricated microfluidic cell culture device was placed symmetrically on both the

plates such that half of the microchannel was on the hot side and the other half on the cold side.

Fig 22 shows the hot plate with the microfluidic device place on its surface.

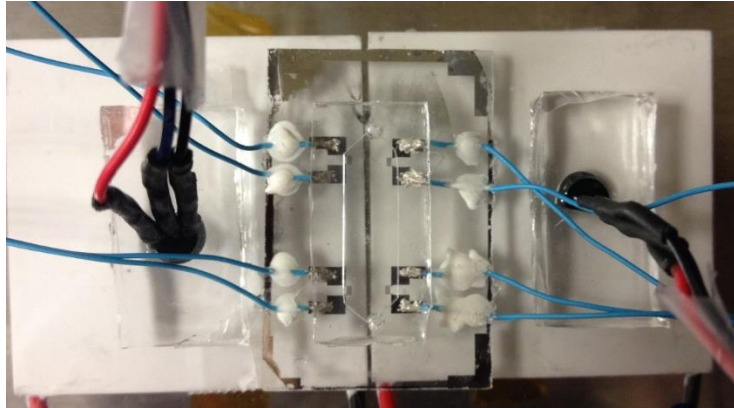


Fig 22: Custom built hotplate with the microfluidic device place on its surface

The Peltier coolers are connected to a heater circuit which controls the current supplied, based on the LabVIEW PI control output.

Chapter-3

Experiments and Results

3.1 Calibration of sensors:

The fabricated temperature sensors were calibrated prior to the experiment. Voltage values of the sensors were recorded at different temperatures using a program written in LabVIEW. A hotplate was used to change the temperature and corresponding voltage values of the sensor were recorded from LabVIEW output.

The sensor on the cold side is named S_A and the sensor on the hot side is named S_B . S_A and S_B were calibrated to establish a relation between the voltage and temperature in the range of 20°C to 45°C .

Fig shows the sequence of steps to calibrate the temperature sensors is schematically shown in fig 23.

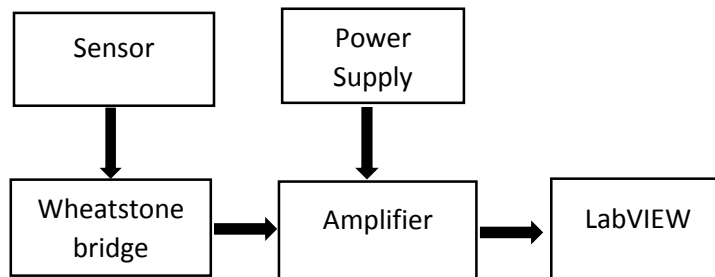


Fig 23: Block diagram showing the process flow of sensor calibration

The device with integrated temperature sensors was positioned firmly on a hotplate. One sensor was calibrated at a time. An IR Temperature Gun was used to measure the surface temperature of the hotplate. As the temperature on the hotplate was increased, the resistance offered by the metal

pattern (temperature sensor S_A) increased. This increase in resistance of sensor S_A was recorded as an increase in voltage (change in resistance is measured as voltage output of the Wheatstone bridge) using LabVIEW. The output was recorded from LabVIEW using NI DAQ. Similarly, sensor S_B was also calibrated and a relation between temperature and voltage was obtained.

Fig 24 shows the calibration of sensor S_A and fig 25 shows the calibration of sensor S_B

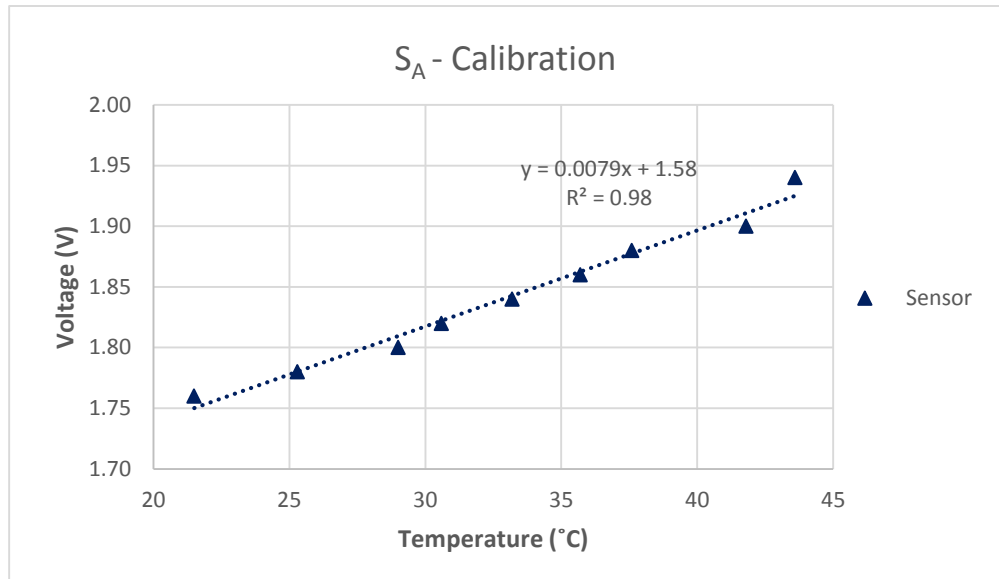


Fig 24 Calibration of sensor S_A

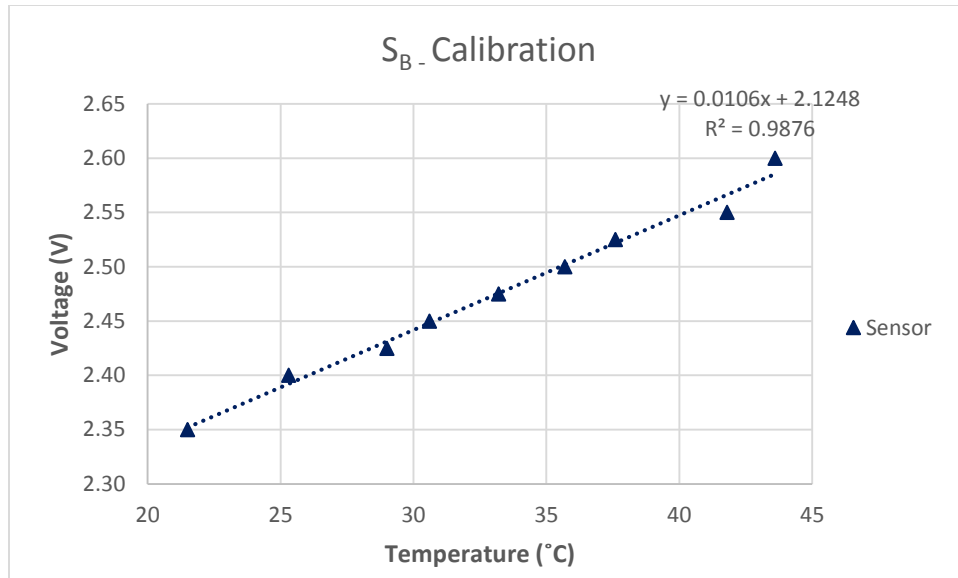


Fig 25 Calibration of sensor S_B

3.2 Experimental procedure

The experimental setup is schematically illustrated in fig 26.

The fabricated cell culture device was placed on the custom built hotplate symmetrically, such that half of the microchannel was on the hotter side and the other half on the colder side. The sensor S_A on the cold side with resistance $3.5 \text{ k } \Omega$, was connected to a Wheatstone bridge circuit with resistances of comparable value. The output of the bridge was connected to an Instrumentation amplifier with gain 252, in order to amplify and this output was read on a PC using LabVIEW. A feedback was given by LabVIEW to the heater circuit such that the current provided by the heater to the Peltier cooler was controlled.

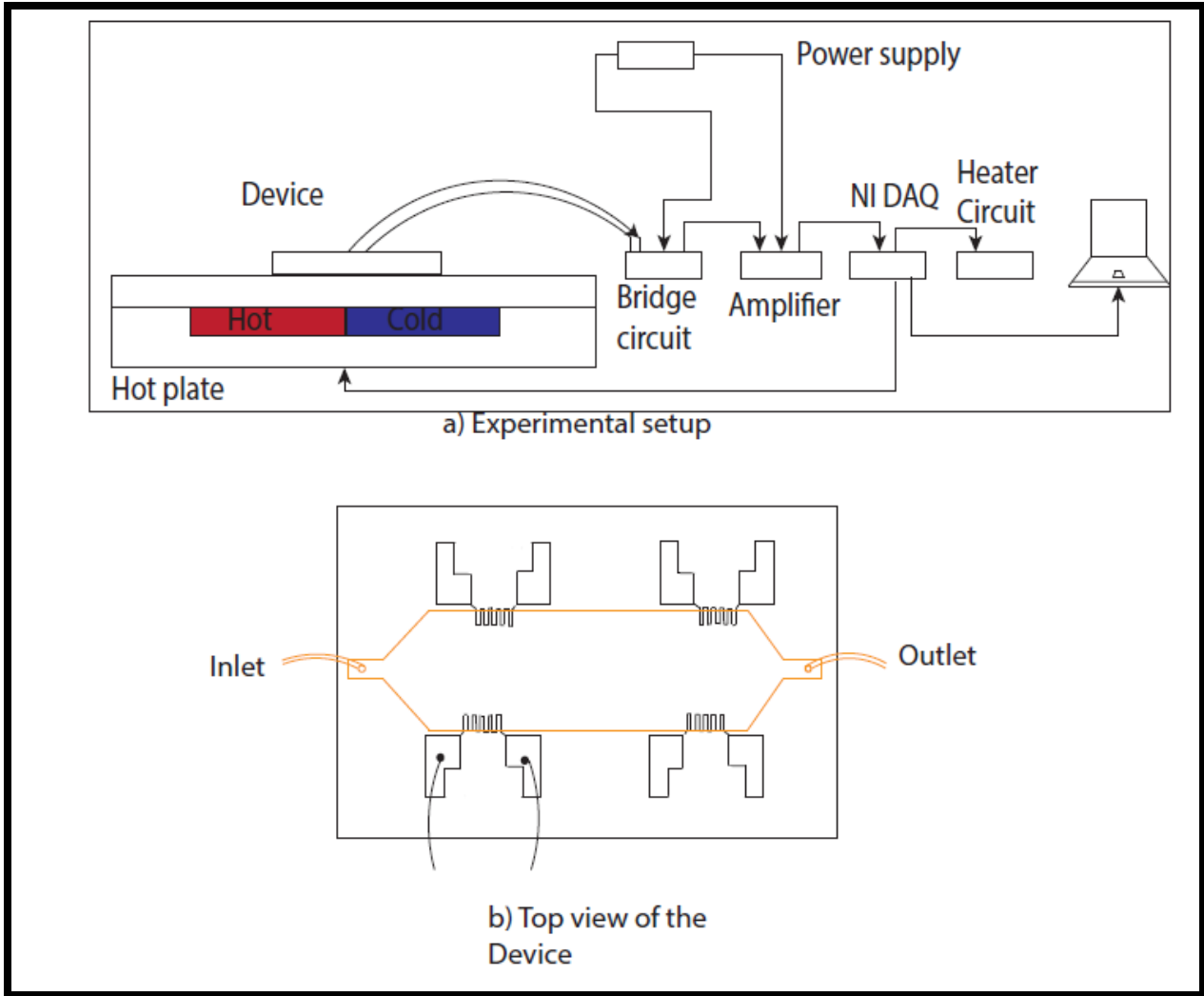


Fig 26: Schematic of the experimental setup

In order to maintain a temperature gradient and control the current provided by the heater circuit, a Proportional Integral (PI) control loop was used. A program for PI Control was written in LabVIEW, whose output controls the current provided by the heater circuit to the Peltier cooler.

3.2.1 PI Control ^[33]

Fig 27 explains the working of a PI Control loop.

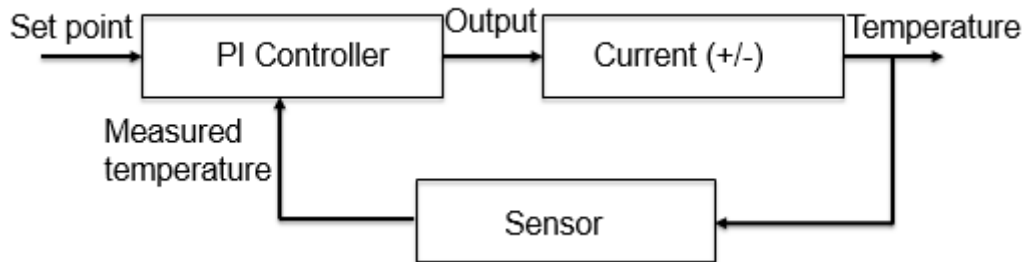


Fig 27: PI control loop

The setpoint is the desired value at which the temperature has to be maintained. Measured temperature is the value that the sensor reads. If the set point and the measured temperature are equal, then the output of the controller is zero. If the measured temperature is different from the set point, there is an error and the current being supplied to the heater has to be changed.

$$\text{Error} = \text{Set point} - \text{Measured temperature}$$

If the measured temperature is lower than the set point, the error is positive. The controller gives a positive output which increases the current supply to the Peltier cooler and the temperature on the hotplate increases. This change in temperature is sensed by the integrated temperature sensor and current is supplied until the measured temperature reaches the value of the set point.

When the measured value and the set point are equal, the current becomes zero. The current increases again when the measured temperature becomes lower than the setpoint. This loop repeats and the temperature is maintained around the value of the setpoint.

Proportional term: The proportional term depends on the current error value. It gives an output proportional to the error. The output can be adjusted by multiplying the error by a proportional constant (K_p).

Integral term: The integral term integrates the error value over time. A change in error will cause the integral term to change. It sums up and gets added as an offset to the error term in order to give a corrected output. The error is multiplied by Integral gain (K_i) and added to the output.

The values of K_p and K_i need to be optimized in order to get the desired output – less overshoot from the set point.

Using the PI Control loop, a gradient of 3°C was maintained in the microchannel across a width of 6mm, with the hot side at 40°C and the cold side at 37°C , on the hotplate.

Fig 28 shows the experimental setup that was used to perform the experiments.

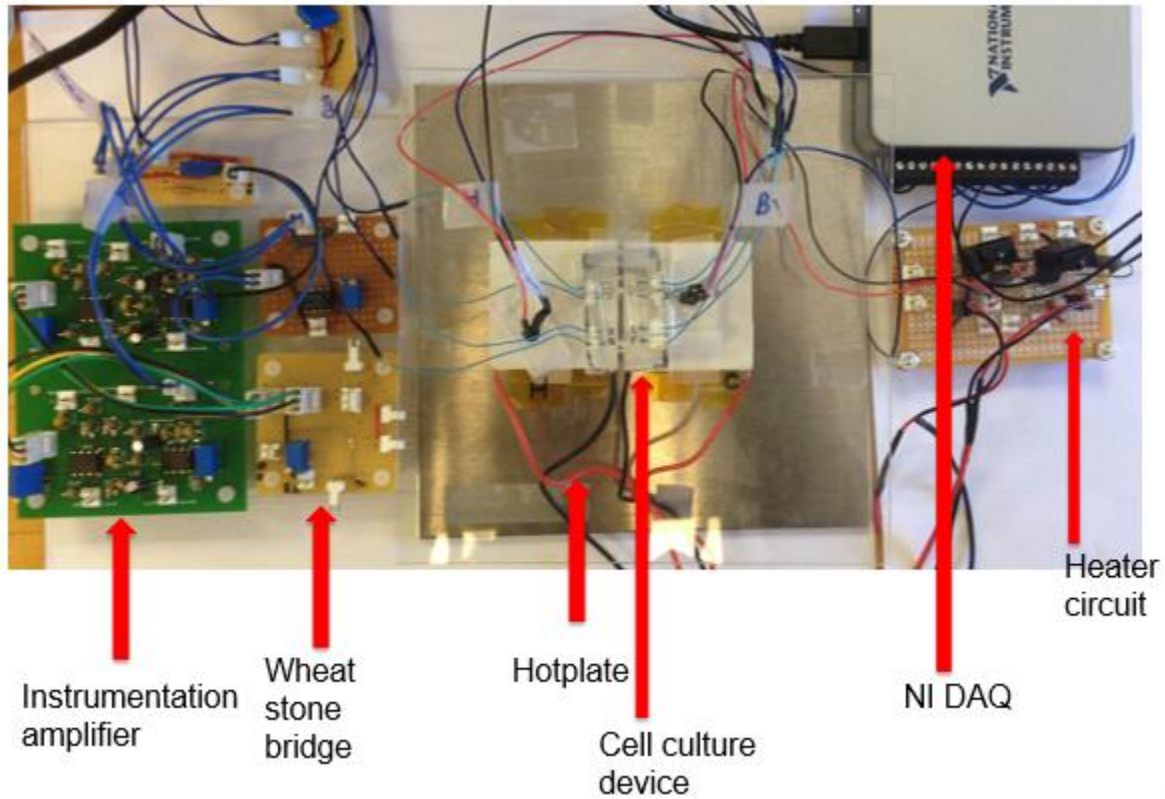


Fig 28: Experimental setup

The electrical circuitry used in the experimental set up are

1. Wheatstone bridge – gives a voltage output for a given resistance value of the sensor
2. Instrumentation amplifier – amplifies the bridge output
3. Heater circuit – controls the current being provided to the Peltier cooler in order to maintain the temperature gradient
4. Voltage supply

The experimental process flow is illustrated in the following fig 29

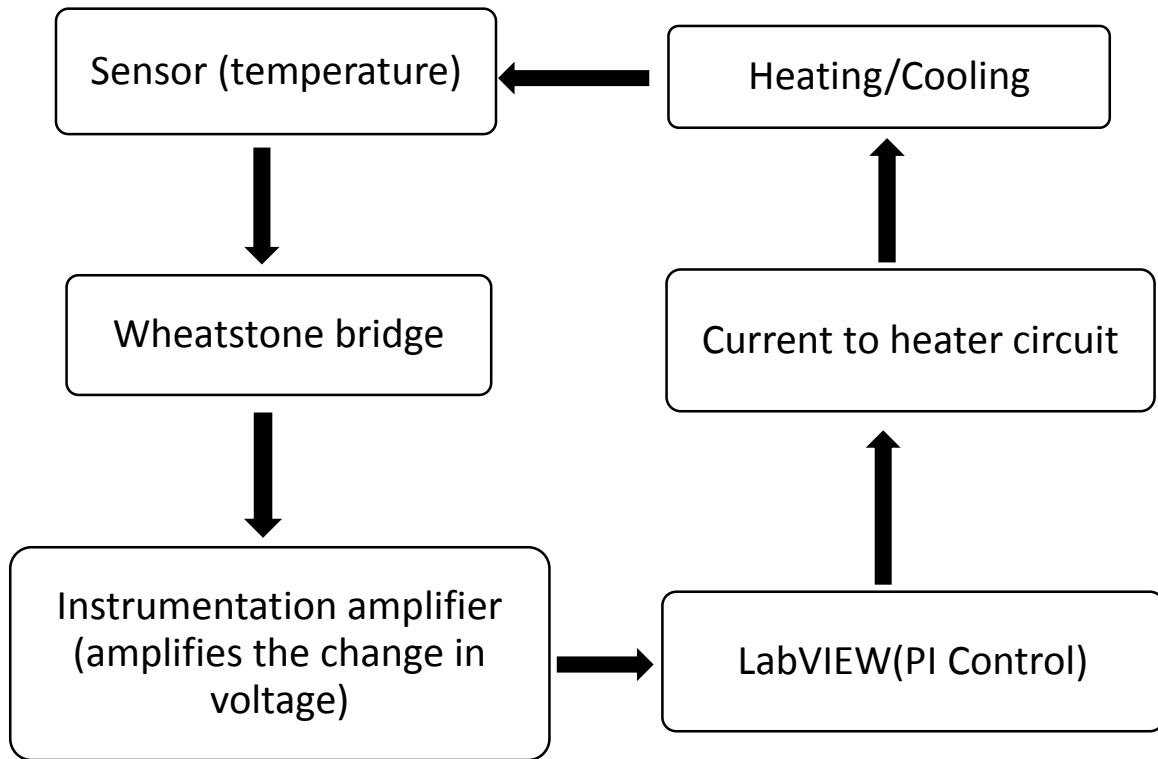


Fig 29: Experimental process flow

Initially, when the power was turned on, the whole surface of the hotplate was at a uniform temperature (room temperature). This temperature is sensed by the integrated sensor and the change in temperature which is the output from the bridge circuit in terms of voltage, is amplified by the instrumentation amplifier. This amplified output is compared with the set point of temperature. Depending on the error value, the PI controller gives a positive output if the temperature is lower than the set point and a negative output, if the temperature is higher than the setpoint. The output changes the amount of current supplied by the heater circuit to the Peltier cooler. Thus, the temperature is maintained on hot and cold sides of the hotplate. The temperature values were automatically saved to a text file by LabVIEW.

Two cases have been tested with different K_p and K_i values in each case, to evaluate the performance of the hotplate and observe the temperature gradient within the microchannel.

3.2.2: Testing

Case-1:- Hotside - 40 °C and coldside 37 °C with proportional gain K_p [V/ °C]= 3 and integral gain $K_i = 0.001$ [V/ (°C.s)]

The output of the sensor for case 1 is shown in fig 30.

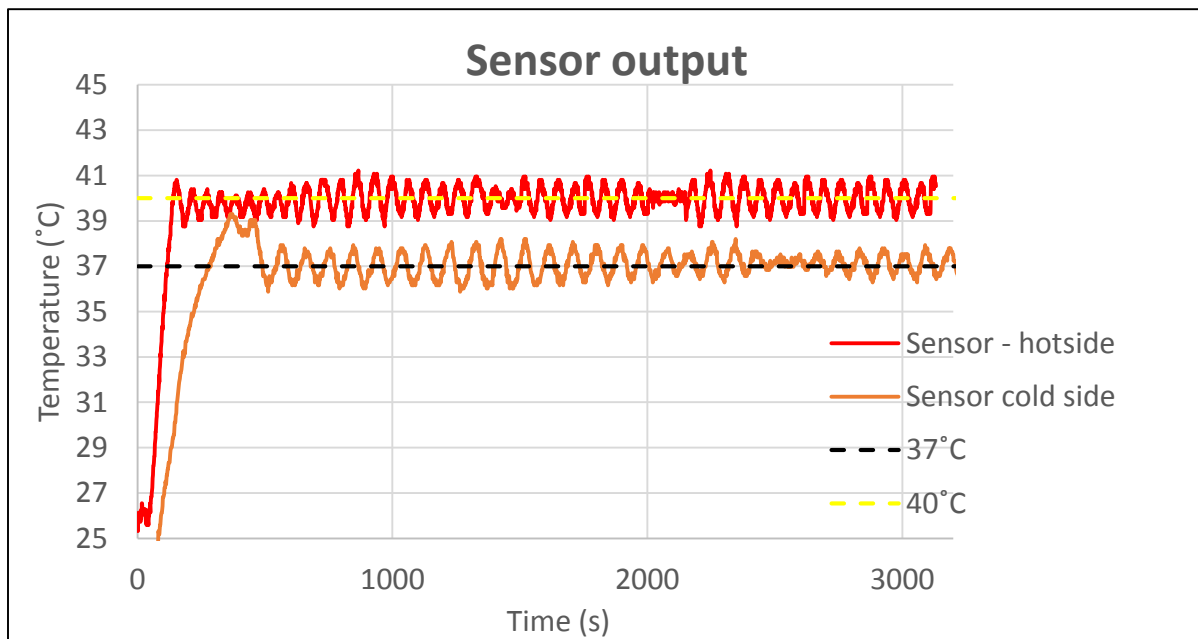


Fig 30: Sensor output in case 1 ($K_i = 0.001$ [V/ (°C.s)] and K_p [V/ °C] = 3, $\Delta t = 200$ milliseconds

)

The above plot shows the output of the sensor for about 50 minutes. The temperature on the hotside was maintained at 40°C and the temperature on the cold side at 37°C. The fluctuations observed

were $\pm 1^\circ\text{C}$ on both the hot and cold sides. Δt in this case is 200 milliseconds, which is the time taken by each loop in PI control.

Case-2 :- Hotside - 37°C and coldside 34°C with proportional gain K_p [$\text{V}/^\circ\text{C}$] = 3 and integral gain K_i = 0.001 [$\text{V}/(^\circ\text{C}\cdot\text{s})$]

Fig 31 shows the sensor output in case 2.

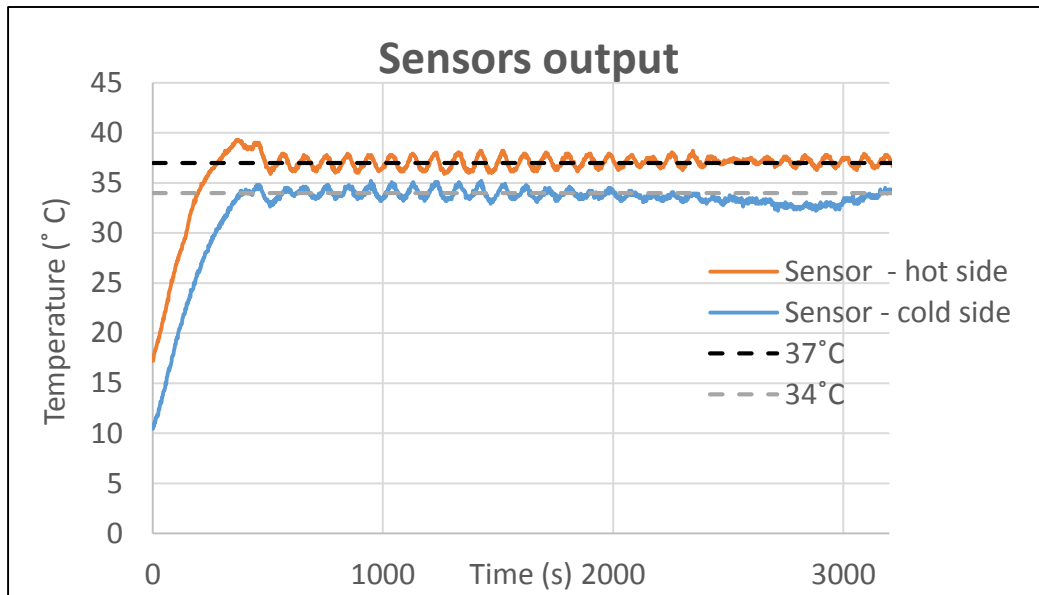


Fig 31: Sensor output in case 2 ($K_i = 0.001\text{V}/(^\circ\text{C}\cdot\text{s})$ and K_p [$\text{V}/^\circ\text{C}$] = 3, $\Delta t = 200$ milliseconds)

The above plot shows the output of the sensor for about 50 minutes. The temperature on the hotside was maintained at 37°C and the temperature on the cold side at 34°C . The fluctuations observed were $\pm 1^\circ\text{C}$ on both the hot and cold sides. Δt in this case is 200 milliseconds.

3.3 Experiment – monitoring temperature gradient across the microchannel

The temperature gradient across the microchannel was monitored for about 7 hours. Considering the fluctuations in temperature measurement of the sensor in section 3.2.2, the value of K_p was

reduced to 1. The experiment was performed at K_p [V/ °C] =3 and K_i = 0.001 [V/ (°C.s)]. (Δt is 100 milliseconds). This gave a relatively stable temperature measurement and significantly reduced the fluctuation. The sensor output is shown in fig 32 and fig 33.

Fig 32 shows the sensor output when the temperature on the was maintained at Hotside 40 °C and coldside at 37 °C . The gradient in temperature was monitored for about 7 hours.

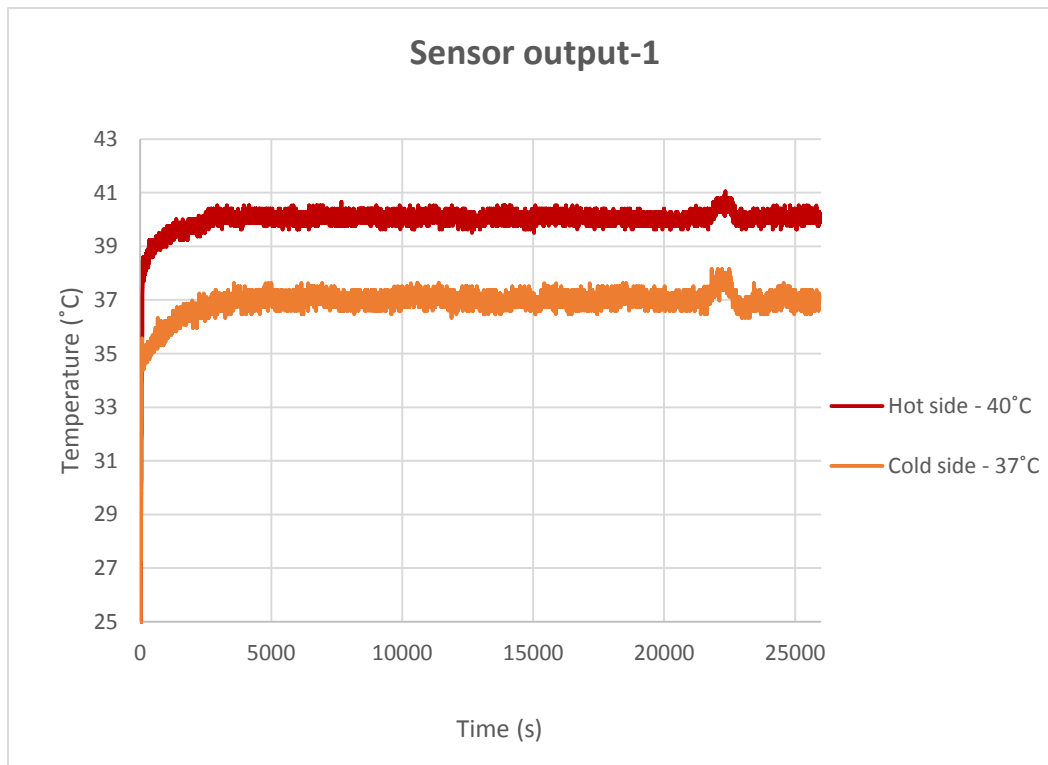


Fig 32 shows the output of the sensors in case 1 (K_i = 0.001 [V/ (°C.s)] and K_p [V/ °C] = 1,

Δt =100 milliseconds)

The graph shows the temperature on the hotside and coldside. Initially, when the power is turned on, the temperature rises from ambient level i.e. 25 °C and reaches 40 °C on the hot side and 37 °C on the cold side. The PI control loop helps maintain the temperature gradient of 3 °C across a width of 6mm. The temperature was monitored for 7.2 hours and the maximum fluctuation observed on

the hot side was $40\pm 0.5^\circ\text{C}$ and that on the cold side was $37\pm 0.5^\circ\text{C}$. This shows that the temperature was consistent on hot and cold sides and the gradient was effectively maintained for 7.2 hours. In other words, this device can be used to perform cell experiments that last for hours.

Fig 33 shows the sensor output when the temperature on the hot side was maintained at 37°C and cold side at 34°C . The gradient in temperature was monitored for about 7 hours.

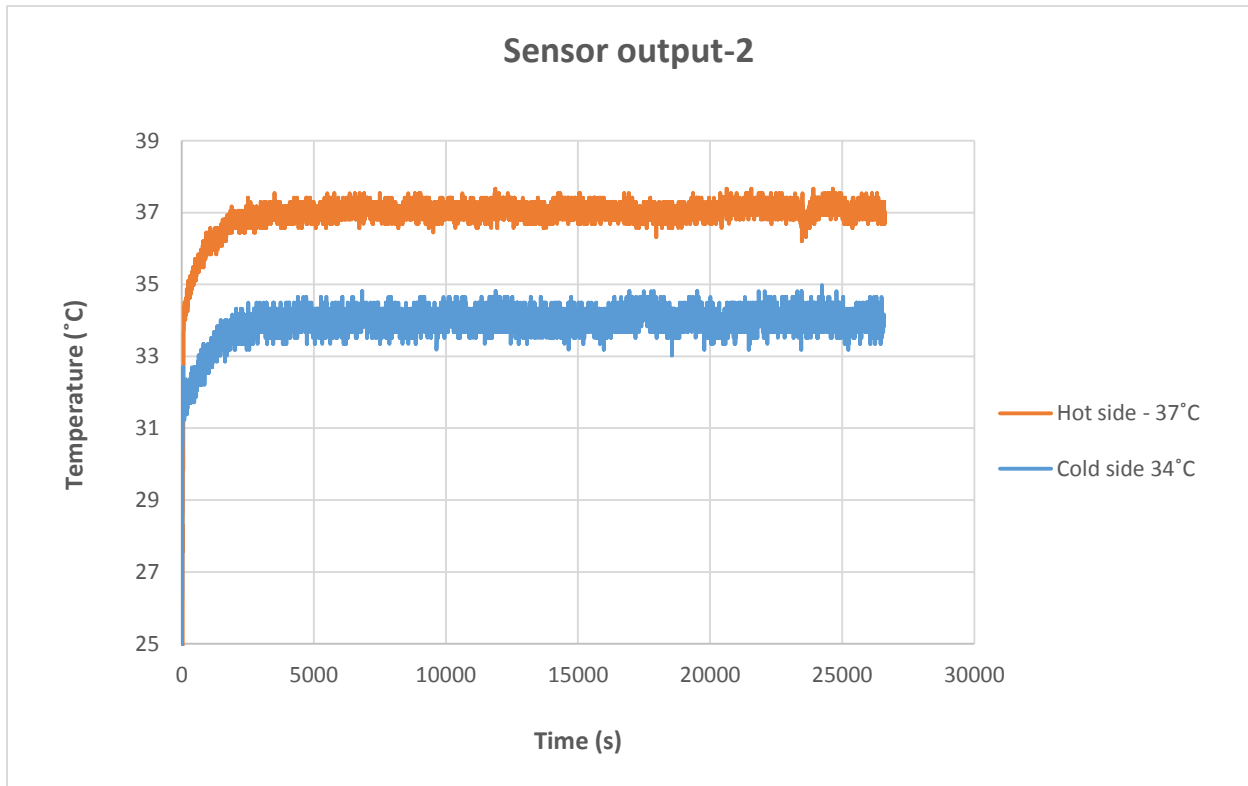


Fig 33: shows the output of the sensors in case 2 ($K_i = 0.001[\text{V}/(^\circ\text{C}\cdot\text{s})]$ and $K_p [\text{V}/^\circ\text{C}] = 1$, $\Delta t=100$ milliseconds)

The graph shows the temperature on the hot side maintained at 37°C and on the cold side at 34°C . Initially, when the power is turned on, the temperature rises from ambient level i.e. 25°C and reaches 37°C on the hot side and 34°C on the cold side. The PI control loop helps maintain the temperature gradient of 3°C across a width of 6mm. The temperature was monitored for 7.2 hours

and the maximum fluctuation observed was $\pm 0.5^{\circ}\text{C}$ on both hot and cold sides. This shows that the temperature was consistent on hot and cold sides and the gradient of 3°C was effectively maintained for 7.2 hours.

3.4 Cell experiment:

A cell experiment was performed in order to test the compatibility of cells in the fabricated device with the set conditions. The system was allowed to stabilize with temperature at 37°C on the hot side and 34°C on the cold side.

The inlet of the microchannel was connected to a 1ml syringe filled with DMEM media and the outlet to another 1 ml syringe with PBS. A pump was used at the outlet syringe to draw in the medium. MCF-7 breast cancer cells were acquired from (Dr. K. Claffey's lab, University of Connecticut Health Centre). The concentration of the obtained cells was at 1 million cells/ml. Considering $80,000\text{cells}/\text{cm}^2$, 250,000 cells (0.025ml) were resuspended in $50\ \mu\text{l}$ of media containing 25mM HEPES. The channel was initially filled with sterile Phosphate Buffer Saline (PBS) solution. The cells were then introduced into the channel using a pipette, under the hood. The microdevice was then placed on the custom build hotplate and after two hours, the media was flown through the microchannel at a rate of $100\ \mu\text{L}/\text{hour}$, for about 4 hours.

Fig 34(a) shows the microchannel filled with culture medium and 34(b) shows the microscopic image of the microchannel.

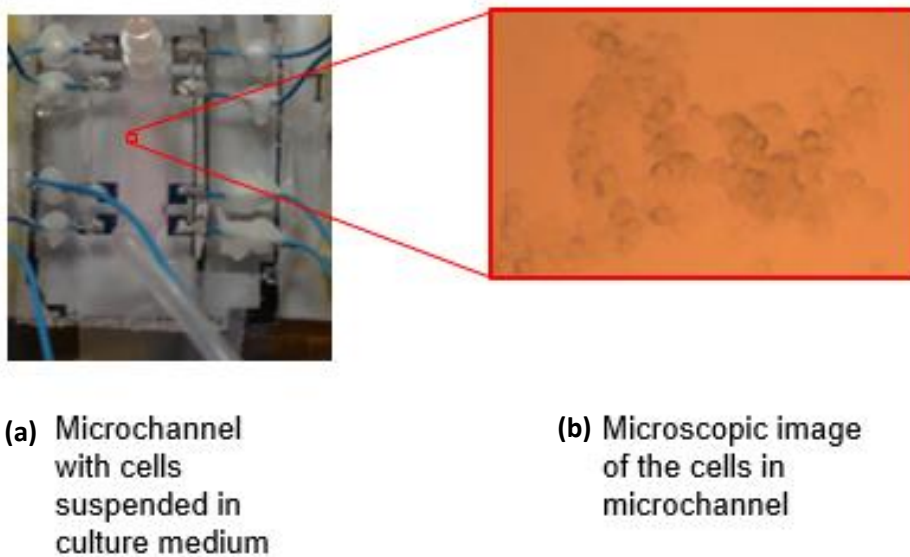


Fig 34: (a) Microchannel with cells (b) Microscopic image of the cells in the microchannel

The above test proved that the cells were compatible with the fabricated device since there was no visible difference in the cells at the end of 6 hours, and long term cell experiments can be performed in order to study the effects of temperature gradient on cancer cells.

3.5 Integration of the fabricated device with an imaging setup:

The fabricated device can be integrated with an imaging setup to perform live cell imaging. This helps to observe the effect of temperature gradient on cells and the changes that they undergo. Fig 35 shows an illustration of the cell culture device with an imaging objective.

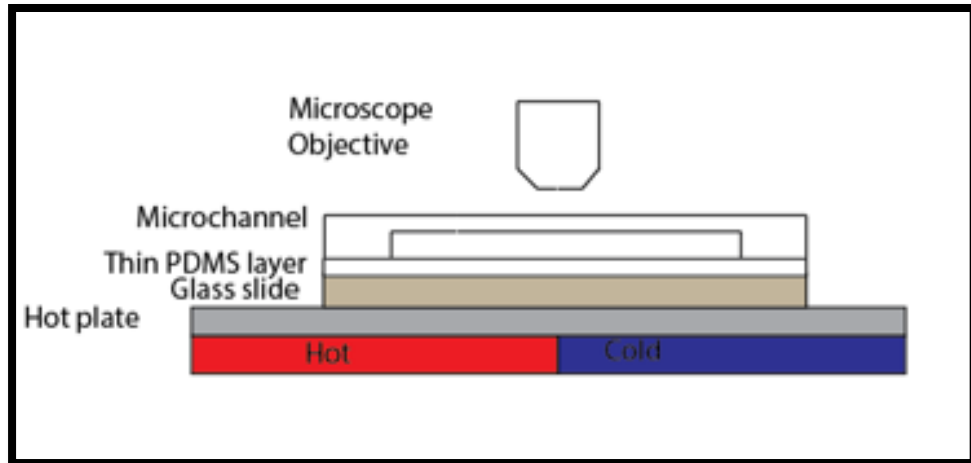


Fig 35: Fabricated device can be integrated with an imaging setup

Chapter-4

Conclusion

A microfluidic cell culture device was fabricated that can help study the effect of temperature gradient on cell cells. The device consisted of a microchannel that can hold cells suspended in culture medium, and integrated temperature sensors which can provide localized temperature measurements from within the channel. A custom built hot plate was designed in order to provide a temperature gradient. Chromium metal was used to fabricate temperature sensors since it is easy to fabricate, inexpensive and highly resistive. PDMS was used to fabricate the microchannel because of its non-toxicity, bio-compatibility, and optical compatibility.

The device was evaluated by monitoring a temperature gradient of 3°C across the microchannel of width 6mm for about 7 hours. The results showed that the thermal gradient was consistent throughout the course of 7 hours with a fluctuation of ± 0.5 °C. This proves that the device was efficient in maintaining the thermal gradient. The device was also tested to check if the cells were able to survive in the set conditions. Comparing images taken at the beginning and end of a 6 hour experiment, there was no visible difference in the cells. This shows that the cells will be able to survive in the fabricated device with the provided conditions for hours and effect of thermal gradient can be studied.

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