Developing a Portable Heating Platform for Microfluidic In Vitro Devices for Assisted Reproductive Technology

Capstone Report

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Abstract:

Infertility affects many couples worldwide, increasing the need for advancements in Assisted Reproductive Technology (ART). This capstone project aims to develop a portable heating platform for microfluidic in vitro devices to maintain precise and uniform temperature control during embryo culture outside traditional incubators. The platform is designed for NUNC 4-well dishes, features a 3D-printed PLA+ holder and uses one of two heating technologies: a Peltier element and an indium tin oxide (ITO)-based microheater fabricated via Magnetron Sputtering.
The Peltier element achieves the target temperature of 37°C with low power consumption, but its thermal uniformity requires further evaluation. The 135 nm ITO microheater, with a sheet resistivity of 233.03 Ω/□, achieved steady state at around 37°C in 89 seconds with 5V and 0.5 A of power supply, while 13 V and 0.5 A of power supply choice can be used for rapid heating with the necessary help of active control. The temperature monitoring was done using a Arduino and DS18B20 sensor, which has a ±0.5°C accuracy.

Contents

Chapter 1

Introduction

Infertility, disease described as inability to achieve pregnancy even after 12 month or more of unprotected sexual intercourse due to male, female or unknown causes [1, 2]. In a world where 1 in 6 couples struggle with infertility, developing assisted reproductive technologies became a worldwide necessity [3]. Assisted Reproductive Technology (ART) refers to medical procedures used to overcome fertility, involving the manipulation of sperm, egg or growing embryos outside of human body [4]. One of the most popular method of ART is In Vitro Fertilization (IVF), where female mature egg cells are fertilized with a sperm in laboratory controlled conditions [5, 6, 7]. IVF has been advancing rapidly since the first ever test-tube baby was born over 45 years ago [8, 9, 10, 11]. Yet, there are still some challenges in mimicking the accurate conditions necessary for successful embryo culture, temperature regulation being one of the most important [12, 13]. Since embryos are highly sensitive to thermal fluctuations, any deviations from 37°C core body temperature can affect it. The role of incubator is crucial when maintaining needed temperature in low tolerance [14, 15]. However, there are cases where procedures cannot be performed inside incubators, but still require precise core body temperature environment. During the most of these procedures the embryo growing dishes are carried using aluminum holders without any heating mechanisms. The heat loss and uncontrolled temperature in these precise technologies can be detrimental for the embryo development. Maintaining a stable and controlled thermal environment during these procedures is a critical challenge.

This capstone project aims to address this issue by developing a portable heating platform for microfluidic in vitro devices. The proposed solution includes designing and 3D printing a custom platform, fabricating a heating element, and integrating a suitable temperature sensor.

1.1 Ethical and Professional Responsibilities

- **Ethical Responsibility:**
  Developing any kind of biomedical equipment involves significant responsibilities, especially in reproductive healthcare. The main idea of my capstone project is developing a heating platform for embryos during Assisted Reproductive Technology (ART) procedures outside of IVF incubators. Thus, any major deviation from the required temperature may potentially harm the embryo, leading to potential failed pregnancies or development abnormalities. This makes the responsibility for health of embryo, project's primary ethical concern . Proper way of handling this responsibility is to develop strict quality control procedures. To ensure minimal errors, lots of rigorous test would be done, each time configuring the model close to perfection.
  Consent is also another crucial point we should take into account. When testing new device in clinical settings, every patient should be aware of the experimental nature of the device, and correlated risks. Violating the consent of the patient and experimenting with new device without valid authorization, may lead to potential lawsuits and loss of trust in our work. Informed consent is fundamental ethical principle in any medical research. Every patient has a right to know everything about their treatment, and we cannot disclose and relevant information on this matter. To avoid these risks we should establish a clear communication with all parties and transparency in our work ensuring that patients would be all-aware of the device's risk and potential.

- **Informed Judgments:**
  When dealing with any healthcare system it is vital to do well informed decisions. Because, every decision we make may be harmful and have a potential disaster behind it. To ensure that judgments made during development of the heating platform for microfluidic in vitro devices in Assisted Reproductive Technology (ART) are well-informed, a multidisciplinary approach would be used. Firstly, thorough research would be done on the technical requirements of the project, namely the range of possible allowed thermal deviations, level of harm with respect to the level of thermal deviation and etc. This way we would first understand the problem theoretically and explore the possible limitations we would have. Then, lots of tryout tests would be conducted to explore the device's limits and risks. Last but not least, we would consult proper professional of the field and ask for their opinion on the work done. The feedback from professional would further increase our knowledge on the matter, subsequently device would be improved.

- **Global Context:**
  The development of a portable, heating platform for microfluidic in vitro devices in Assisted Reproductive Technology (ART) holds significant global relevance, because infertility affects 1 in 6 couples worldwide as stated in [3]. However, the relevance is varying over the world, depending on the healthcare systems, availability of healthcare, political factors, economical factor and socio-cultural factors.
  In regions where healthcare system is advanced and economic well-being is on good level such as Western Europe, North America, the development of ART would thrive, making the development of the projects similar to this more frequent and on demand.
  According to [8], the only countries that met expected demand for ART, are those who managed to minimize cost of the ART procedures for their people. It means on top of having good economical situation, state needs to have favorable policy regulations regarding ART, making it more affordable to the people.
  However, this means in the regions were the economical well-being of the people are not in good shape, the impact of the project would be minimal. The focus of the nation would not be aimed towards development of Assisted Reproductive Technologies, rather more towards primal needs to survive.
  Another factor that needs to be included is socio-cultural norms in the country. Whether it is acceptable and encouraged to use Assisted Reproductive Technologies is detrimental to development of the infrastructure.

- **Economic Impact:**
  The development of a portable heating platform for microfluidic in vitro devices in Assisted Reproductive Technology (ART) has both short-term and long-term economic effects. In the short term, the effectiveness and cost-effectiveness of the ART procedures would be up. Traditional IVF procedures require expensive, heavy equipment. Our project will help to lower expenses of the lab experiments and possible enhance their effectiveness. In short term we aim to make ART more affordable. In the long term, the economical benefit of the project can be crucial. By developing a portable, relatively cheap heating platform, we would contribute to the cost reduction trend of the procedures in the future. Effective, low cost procedures leads to more attention to ATR from couples wanting to be assisted and from investors, which means in long term we would be able to attract money to the industry. This expansion of the industry could lead to creation of more jobs in medical facilities, research and development, manufacturing sectors.

- **Environmental Impact:**
  Developing a portable heating platform for microfluidic in vitro devices for

Assisted Reproductive Technology (ART) have several environmental effects. Using a portable, multi-use platform contributes to the sustainable energy saving trend. The heating of the platform expected to be more energy-efficient rather than industrial incubators. These incubators are relying on significant energy for constant operation, contributing to a high carbon footprint of the fertility clinics. Moreover, portability characteristic of the platform could be crucial, in logistics matters. The relatively small sized platforms can be easily transported instead of bulky incubators. To ensure more environmental sustainability of the project, eco-friendly materials can be chosen for fabrication of the device. The holder itself would be fabricated using non-harmful material to ensure safety of procedures and environment together.

- **Societal Impact:**
  The biggest impact of the project can be seen from societal perspective. In a world where millions of people suffer from infertility, any type of research, innovation, investments directed into reproductive care system help to address this big issue. Infertility often comes with significant emotional, psychological, and social stress. By creating a efficient portable heating platform for ART procedures, this project is contributing to improving the success rate of assisted reproductive technologies. Thus, offering hope and relief to the people suffering from infertility.
  Further developing fertility treatments, has the potential to make these treatments more accessible to underdeveloped regions of the world. Improving affordability of fertility treatments can reduce the gap between wealthy and poor sectors of the world in health care availability terms. Nowadays, due to relatively high cost of the treatments in most countries, fertility assistance procedures are exclusive for wealthier portion of the population. High cost of the treatment limits access for those who cannot afford it, creating a inequality in fulfilling a basic human need for reproduction. Because, it is fundamental instinct and need for human being, limiting access to fertility treatments raise ethical and sociological concerns. Reducing the costs could bridge the gap between poor and rich.

Chapter 2

Methodology

2.1 Platform Design

The platform design process began with proper identification of the functional requirements. The main goal was to heat a NUNC 4-well dish uniformly while maintaining precise temperature control to support in vitro conditions for Assisted Reproductive Technology (ART) operations. The 3D model design was created in SolidWorks software, with a focus on developing a holder suitable for the NUNC 4-well dish and the selected heating element.

2.2 Heating element

The heating element is a core component of the this platform, designed to provide uniform and precise temperature for the NUNC 4-well dish. As a heating element of platform, we considering either indium tin oxide (ITO) Micro-heater or Peltier element. The preliminary choice of heating source is Peltier element, thermoelectric module, created by inserting P and N-type semiconductors between ceramic substrates [16]. Due to its compact dimensions and calm thermoelectricity, it has already found its use in biomedical environment as can be seen from [17, 18, 19, 20]. However, after further evaluation, the ITO-coated glass microheater was selected as the final heating element due to its superior thermal uniformity and suitability for precise temperature control in clinical applications.

2.2.1 Peltier element

The main advantage of choosing Peltier element is its simplicity of implementation into the project. Main characteristics needed for implementations are, input power required for the element to operate at desired temperature. To determine the voltage and current required for the Peltier element to reach a target temperature of

37°C, experimental measurements were conducted using a PT1000 resistance temperature detector (RTD) and a regular multimeter. The PT1000 sensor's resistance was measured at various operating conditions of the Peltier element using resistance mesureing mode of multimeter, and the corresponding temperatures were calculated using the standard PT1000 resistance-temperature formula [21].

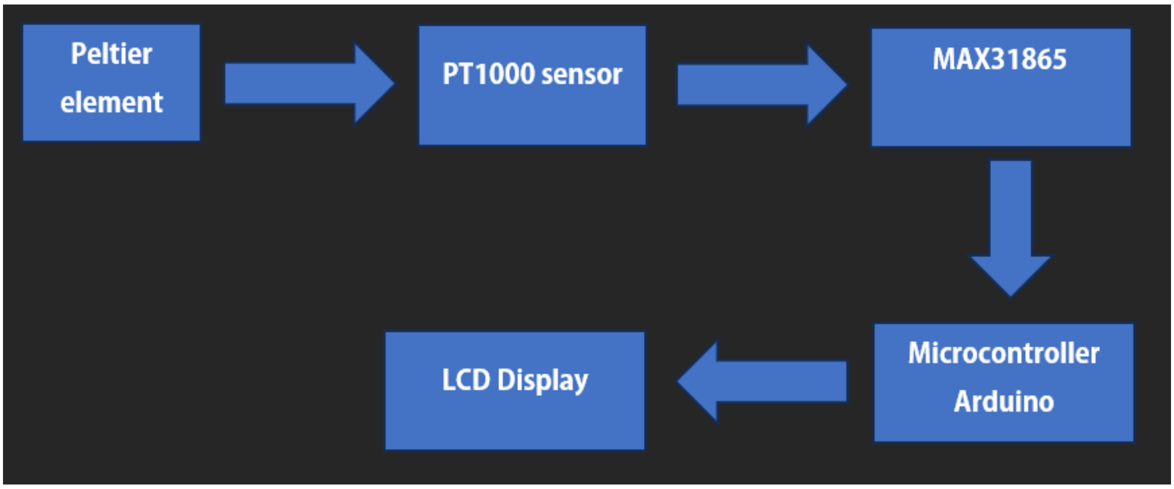


Figure 2.1: Experiment setup

The relationship between the temperature and resistance of the PT1000 is given by equation :

$$R_t = R_0 \cdot (1 + A \cdot t + B \cdot t^2), \tag{2.1}$$

where:

- R_t is the resistance at temperature t (in °C),
- R_0 is the nominal resistance at 0°C (1000 Ω for a PT1000 sensor),
- $A = 3.90802 \times 10^{-3}\,°\mathrm{C}^{-1}$ is the first-order temperature coefficient,
- $B = -5.80195 \times 10^{-7}\,°\mathrm{C}^{-2}$ is the second-order temperature coefficient.

After rearranging Equation 2.1 to calculate the temperature t from the measured resistance we get R_t:

$$t = \frac{-A + \sqrt{A^2 - 4B \cdot (1 - R_t/R_0)}}{2B}. \tag{2.2}$$

2.2.2 ITO based microheater

Indium tin oxide is a transparent conductive material,which dissipates heat due to its resistance when an electric current passes through.

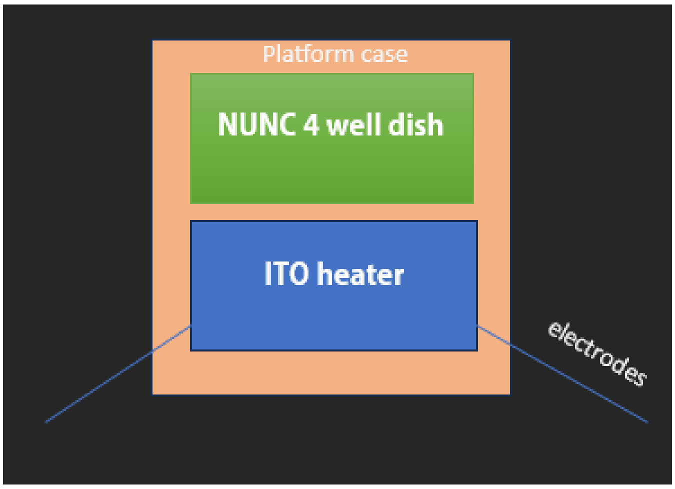


Figure 2.2: Cross-Sectional View of the Heating Platform Prototype

The fabrication of the microheater begun with the deposition of thin film Indium tin oxide on a substrate glass material, using Magnetron Sputtering - KJLC LAB-18. In this tool, we sputter indium tin oxide with the 40 W power into glass substrate in vacuum condition. To ensure even distribution of ITO onto substrate, glass is rotated at 10 rpm speed. The ITO deposited glass need to have wires connected to power supply. Thus, we glued copper wires to the long sides of the glass using silver paste and let it dry in 60°C oven for couple of hours [22].

Figure 2.3: Magnetron Sputtering - KJLC LAB-18

As a microheater, this approach relies heavily on the resistance of the applied ITO film. The resistance depends on various factors, but the parameter we can directly control in our case is the ITO film thickness, which is set in the Magnetron Sputtering tool settings. A comparative analysis was conducted with two samples: one with a thickness of 50 nm and another with a thickness of 135 nm. To characterize the electrical properties of the ITO glass, resistivity and other parameters were measured using the 8400 Series Hall Measurement System (HMS) in the laboratory at Nazarbayev University, ensuring the film's suitability for uniform heating. The characterization process involved cutting the ITO-coated glass into 10 mm × 10 mm dimensions to fit the sample into a 10 mm prober pin sample card. The card with the sample was then placed into the HMS chamber, where a magnetic field was applied, and resistivity was measured using a four-probe configuration.

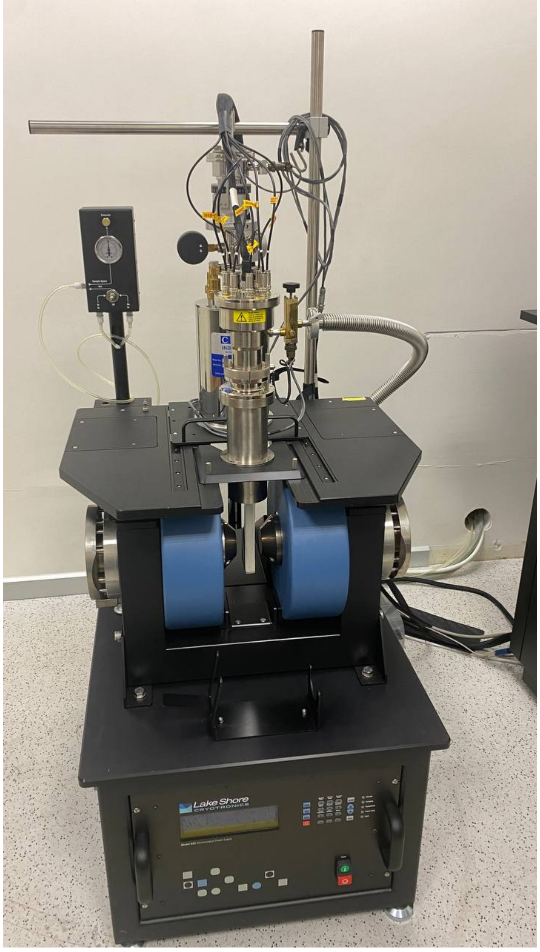


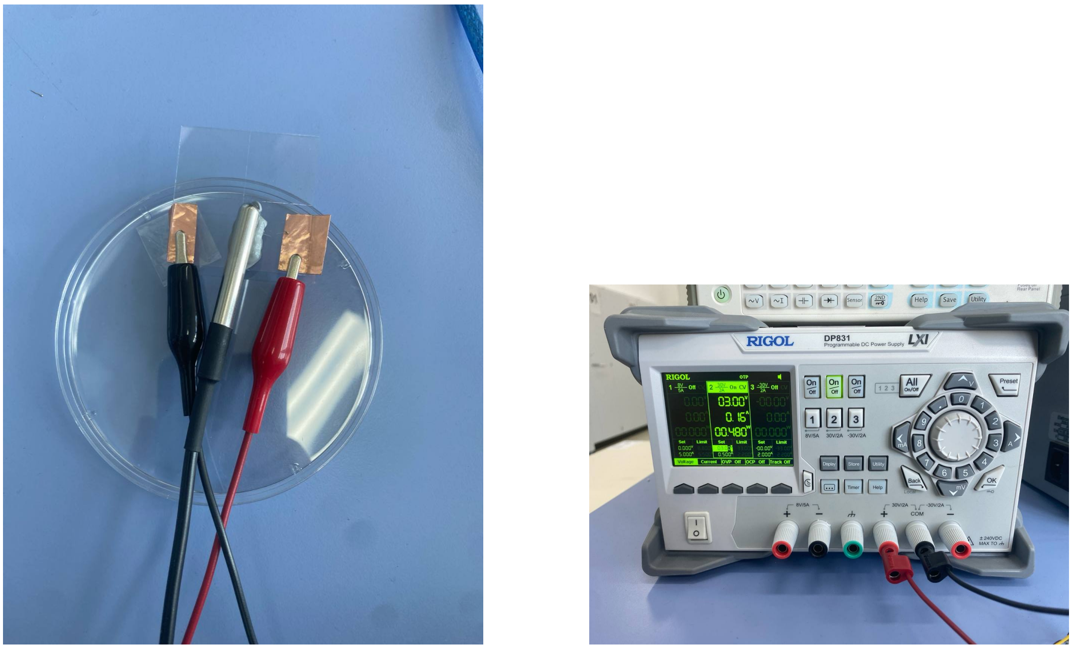
Figure 2.4: 8400 Series Hall Measurement System (HMS) used for ITO glass analysis

(a) ITO-coated glass sample on prober pin sample card (view 1)

(b) ITO-coated glass sample on prober pin sample card (view 2)

Figure 2.5: ITO-coated glass samples prepared for HMS characterization

To find the required power supply for the resultant ITO-glass sample to reach a target temperature of 37°C, experimental measurements were conducted using programmable laboratory power supply Rigol DP831 and a DS18B20 temperature sensor. The DS18B20 temperature sensor was glued to the sample via thermal paste to ensure maximum heat transfer to the sensor. Arbitrary constant current I = 0.5 A was taken to align the experiment conditions with Peltier element testing settings. Temperature and corresponding time values were recorded for 5 different voltage values (5 V, 7V, 9 V, 11 V, 13 V).



(a) Power supply and Temperature sensors connected to ITO glass 135nm sample

(b) Programmable laboratory power supply Rigol DP831

Figure 2.6: Experimental setup for ITO heater

2.3 Temperature sensor

Accurate temperature monitoring is critical when working with sensitive bio materials. PT1000 resistance temperature detector (RTD) was used for temperature sensing due to its high accuracy, reliability.

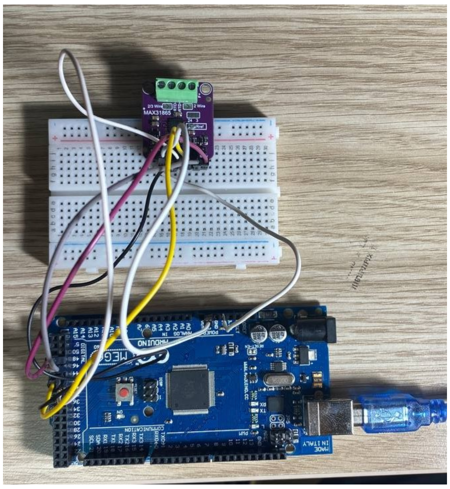


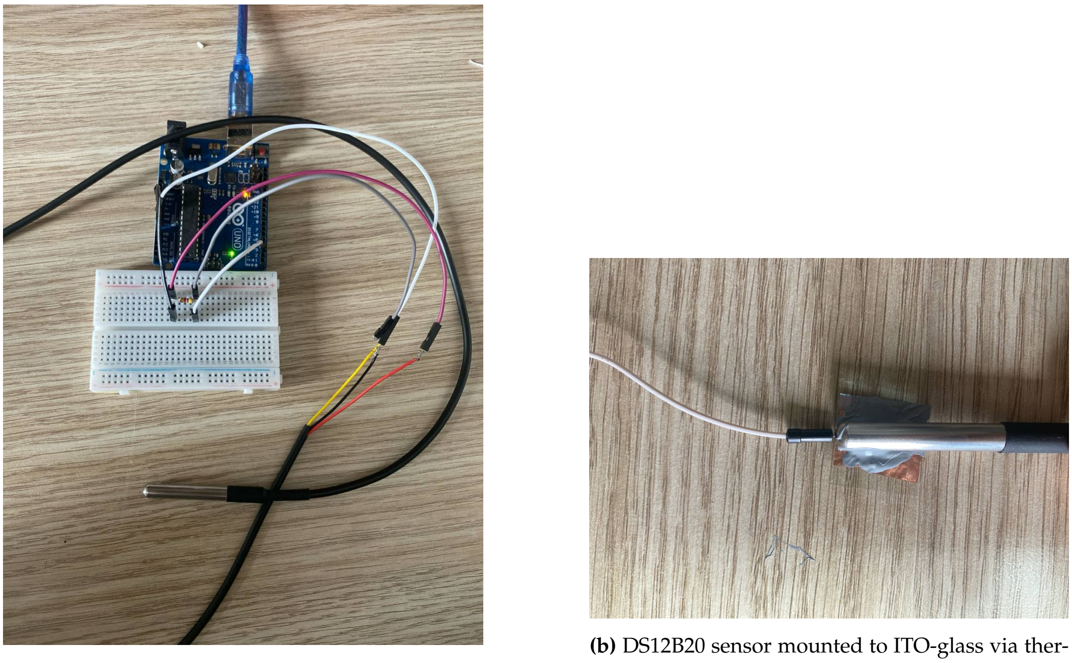
Figure 2.7: MAX31865 module in conjunction with Arduino Mega 2560

To amplify the RTD's small resistance changes, a MAX31865 module was utilized. This module converts the RTD's resistance data into a readable digital signal. Digital signal is then processed in Arduino Mega 2560 micro controller. The Arduino Mega 2560 reads temperature data from the MAX31865 module via the SPI interface. The pin connection between module and microcontroller is as following:

- VIN pin of module is connected to 5 V of Arduino
- GND is to Ground of Arduino
- CLK (Clock) pin is to Digital 40 of Arduino
- SDO (Serial Data Input) pin is to Digital 42 of Arduino
- SDI (Serial Data Output) pin is to Digital 44 of Arduino
- CS (Chip Select) pin is to Digital 46 of Arduino

The temperature values are then displayed on an LCD keypad shield mounted on the Arduino. The LCD provides a real-time visual representation of the temperature.
However, after conducting a couple of tests with the PT1000, it was found to be overly sensitive to electrical noise from the microheater's operation and overly

reliant on the error of resistors used in the circuit, resulting in temperature readings fluctuating by ±1°C. Consequently, the temperature sensor was switched to the DS18B20, a digital waterproof sensor that communicates via the 1-Wire protocol. The DS18B20 provides a direct digital temperature output, eliminating noise-related issues, and offers an accuracy of ±0.5°C over the range of -55°C to 125°C, which is sufficient for maintaining the target temperature of 37°C [23]. The DS18B20 enables fine temperature monitoring and features simple wiring: a single data line, power, and ground, thereby facilitating integration with a microcontroller such as an Arduino. The DS18B20 was mounted on the glass substrate near the ITO microheater with thermal paste to ensure accurate temperature readings.



(a) DS18B20 sensor connected to Arduino board

(b) DS12B20 sensor mounted to ITO-glass via thermal paste)

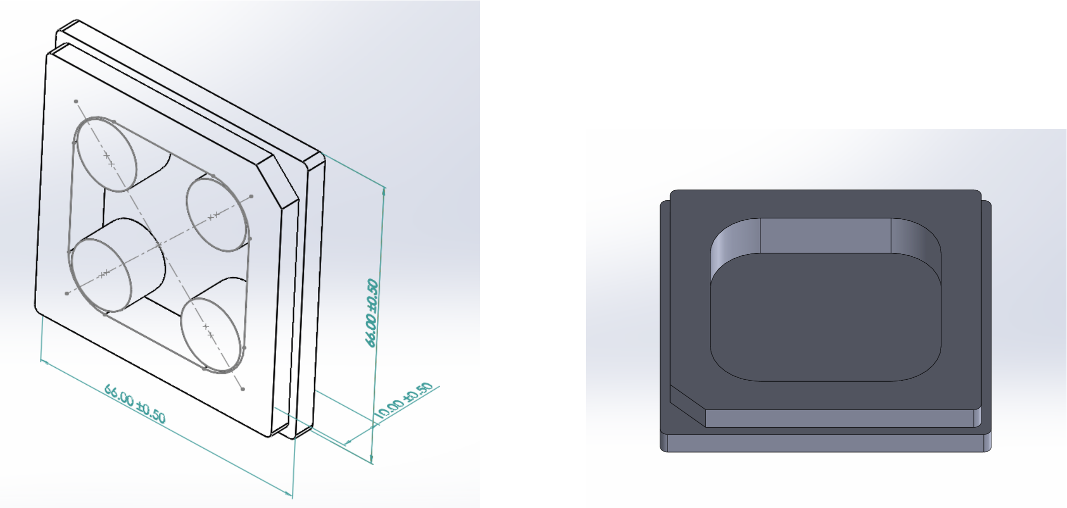
Figure 2.8: DS18B20 sensor

Chapter 3

Results and Discussion

3.1 Holder design

The aforementioned NUNC 4-well dish, is commonly used in the clinics for embryo culturing, and has the dimensions of 66 x 66 x 10 mm.

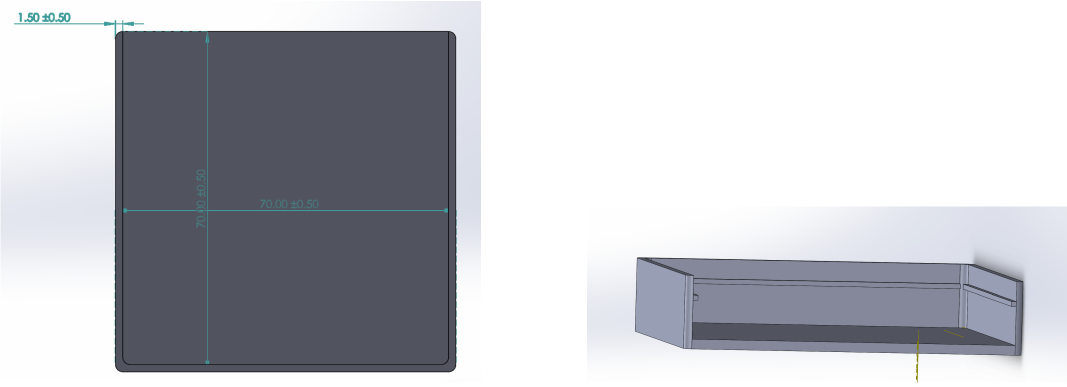


(a) NUNC 4 well dish dimensions

(b) CAD model of NUNC 4 well dish

Figure 3.1: NUNC 4 well dish

The holder design was successfully designed to fit the NUNC 4-well dish and allowed room for placing a heating element. The dimensions chosen (70 x 70 x 20 mm) offer a functional design while being relatively easy to fabricate. The inclusion of 10 mm space for the heating element ensures that both the Peltier element and ITO-based microheater can be easily implemented. Additionally, the 1mm edge acting as a shelf helps to sustain the dish above microheater.



(a) Platform front view with dimensions

(b) CAD model of Holder Platform

Figure 3.2: Holder Platform

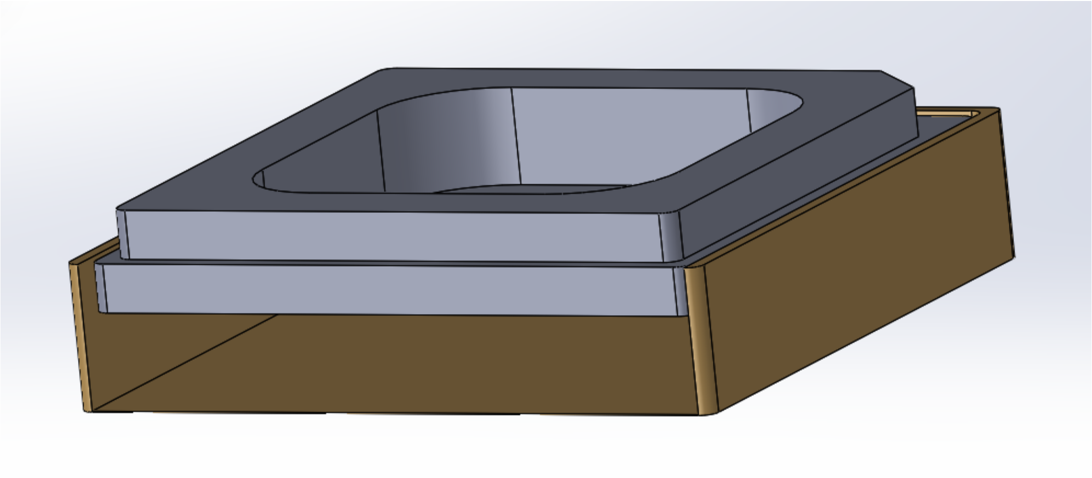


Figure 3.3: 3D Model of the NUNC 4-Well Dish Mounted on the Heating Platform

After designing the model, it was decided to 3D print it using FABLAB provided 3D printers. The 3D model was printed using PLA+ (Polylactic Acid Plus) filament, mainly selected due to its affordability and ease of printing, requiring low temperatures (200–220°C) that minimize common issue of warping of models in 3D printers and ensure accuracy of dimensions given.

(a) Top view of the 3D-printed holder showing its structure

(b) 3D printed holder with NUNC 4 well dish mounted on top

Figure 3.4: 3D-printed holder for the ITO-based microheater, fabricated using PLA+

3.2 Peltier element

The Peltier element was tested with various power supply values, to find out exact values to achieve desired 37 °C. However, without automated control, manual adjustments were required to maintain the target temperature. The temperature-voltage relationship is shown in Figure 3.5. The current was fixed at 0.5 A, and we varied the voltage of power we supplying. The desired 37 °C was achieved at around 1.6 V. Meaning that, if we pick Peltier element as a heater of the platform, we have to implement a 1.6 V of power supply to the system. Results from this experiment, confirms that the Peltier element can meet the heating requirements with relatively low power consumption. However, since the uniformity of the heating is unknown, we can not rely on Peltier. In clinical applications where precision is paramount, uniformal distribution of heat is one of the key feautures we are looking for.

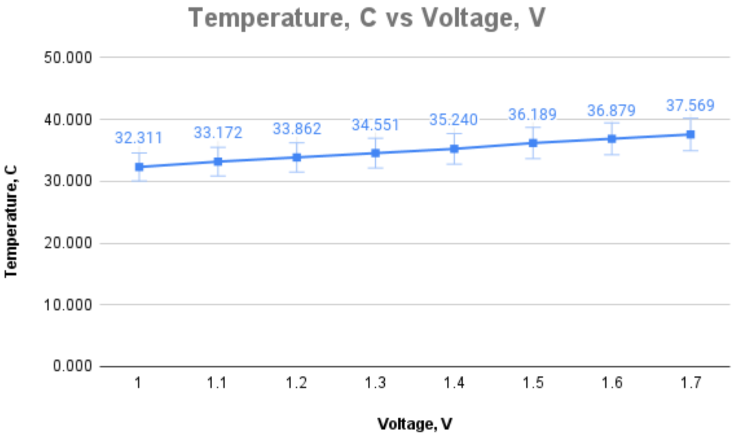


Figure 3.5: Dependence of Temperature on Voltage at Fixed Current (I = 0.5 A, n = 3, Mean of 3 Trials)

3.3 ITO Film Characterization

The other choice of microheater element was ITO thin film. This approach provided a promising alternative to the Peltier element, particularly for aforementioned applications requiring highly uniform temperature distribution across the surface. However, the thermal performance of the microheater is dependent on the film's thickness, resistance, adherence to the substrate and other numerous factors. Thus, the development of the ITO-based microheater for IVF application, required careful analysis of the ITO thin films. Two ITO glass samples with thicknesses of 50 nm and 135 nm were fabricated using Magnetron Sputtering - KJLC LAB-18 and analysed using the 8400 Series Hall Measurement System (HMS).

(a) 50 nm thin ITO deposited glasses with copper wires, next to a regular glass

(b) 135 nm thin ITO glass sample

Figure 3.6: Fabricated ITO glass samples

The results, summarized in Table 3.1, show significant differences in electrical properties that directly impact the microheater's performance. The Ohmic checks for both samples confirmed good contact quality, with Sample 1 showing a worst-case correlation of 0.9999968 and Sample 2 at 0.9994873. The relationship between sheet resistivity and thickness follows a positive correlation, indicating that thicker films not only reduce resistance due to increased thickness but also enhance the film's conductivity, most likely due to fewer defects in the film structure. High sheet resistivity of Sample 1 (3929.98 $\Omega/\square$) indicates that it is less suitable as a microheater, as it will require high voltages and can lead to non-uniform heating. Sample 2, with a sheet resistivity of 233.03 $\Omega/\square$, is better choice for uniform and efficient heating.

Table 3.1: Comparative Analysis of ITO Film Resistivity from HMS Measurements

Sample	Thickness (nm)	ρ ($\Omega \cdot$ m)	ρ_{sheet} ($\Omega/\square$)
Sample 1	50	1.965e-4	3929.98
Sample 2	135	3.146e-5	233.03

To further investigate thermal properties of ITO film, power supply experiment was done on 135 nm ITO glass sample. It determined the rate of temperature changes for various voltage values. The current is fixed at I = 0.5 to ensure experimental setup alignment with Peltier element testing setup. Figure 3.7 shows that 13 Volt power supply can reach the required temperature fastest at around 26 seconds. At 11 V, the results were similar, reaching the 37°C at 29 seconds. The

9 Volt test achieved the desired temperature after 36 seconds, while 7 Volts power supply required 45 seconds. The slowest rate happened at 5 Volt power supply, which needed 89 seconds to reach the desired 37°C.

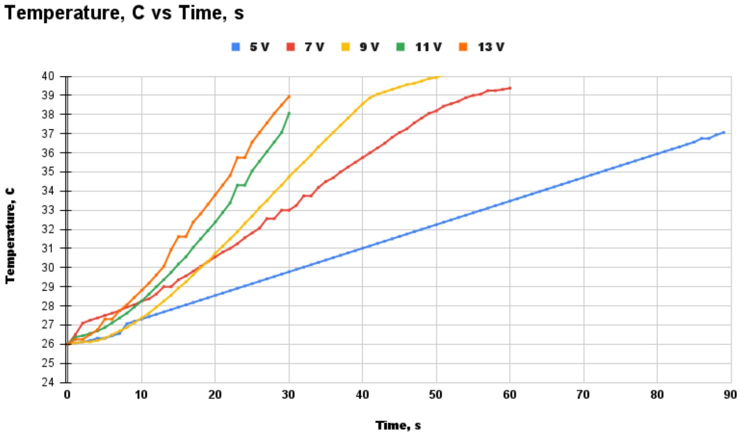


Figure 3.7: Time vs Temperature Profile for Varying Voltages with constant current I = 0.5 A

The results of ITO glass heating experiment, indicated that higher voltage correlate to fastest heating; however the danger of overshoot is present, as seen at 13 V, which is still to be stabilzed at some temperature. This could be mitigated with active temperature control in the future. Thus, the suitable Voltage needed to supply is approximately 5 V, which acheives steady-state at around 37°C. while 13 V can be used for rapid heating with appropriate temperature control to prevent exceeding the temperature.

Chapter 4

Conclusion

This capstone project aimed to develop a portable heating platform for microfluidic in vitro devices used in Assisted Reproductive Technology (ART). The project addressed a critical challenge in maintaining precise and uniform temperature control for embryo culture outside conventional incubators. The designing phase resulted in a 3D-printed holder, fabricated using a PLA+, which can fit both NUNC 4-well dish and chosen heater. Both Peltier elements and ITO-based microheaters were analysed as potential heating technologies, with results highlighting the strengths and limitations of each approach. The Peltier element proved to be capable of achieving the target temperature of 37°C at relatively lower power consumption, thereby proving its potential as a simple and efficient heating concept. However, it needs further investigations toward the exploring of thermal homogeneity, an essential factor for clinical applications.

The ITO-based microheaters of 50 nm and 135 nm film thickness, were fabricated by Magnetron Sputtering. Hall Measurement System (HMS) analysis revealed a low sheet resistivity of 233.03 Ω/□ of 135 nm ITO film, making it suitable for efficient heating. Temperature experiments on ITO-glass samples were conducted, using the DS18B20 sensor, integrated with an Arduino, which provided reliable temperature monitoring with an accuracy of ±0.5°C [23]. Results showed that ITO-glass heater with 5V and 0.5 A of power supply can achieve steady state at around 37°C in 89 seconds, while 13 V and 0.5 A can be used for rapid heating with the need for active temperature control to prevent overshoot of temperature.

Overall, portable heating platform demonstrated its potential for biomedical applications with efficient heating. Future work should focus on verifying heating uniformity, long-term performance of heaters and implementing a suitable temperature control to maintain 37°C.

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Appendix A

Arduino Code for DS18B20 Temperature Sensor

```
#include <OneWire.h>
#include <DallasTemperature.h>

#define ONE_WIRE_BUS 2
OneWire oneWire(ONE_WIRE_BUS);
DallasTemperature sensors(&oneWire);

void setup(void)
{
        Serial.begin(9600);
        Serial.println("Arduino␣Digital␣Temperature␣//␣Serial␣
            Monitor␣Version");
        sensors.begin();
}

void loop(void)
{
        sensors.requestTemperatures();
        Serial.print("Temperature␣is:␣");
        Serial.println(sensors.getTempCByIndex(0));
        delay(1000);
}
```