Development of a Heating System for Point-of-Care Microfluidic Applications

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Abstract

In this work, a heating system envisioned to be used for microfluidic devices was designed and fabricated. The area of microfluidics is one of intense growth and relevant to various fields of study, sometimes requiring heating capabilities. For this endeavour, simulation work was conducted to plan the microfabrication of microheaters to investigate both appropriate designs and materials such as ITO and TiW. For the final heaters, of Generation II, a maximum temperature uniformity of 1.33% and 2.33% for TiW and ITO was obtained, in addition to a maximum temperature instability of 0.81 °C. For microchannel temperature assessment, fluorescence thermometry with Rhodamine B was used and a calibration reaching 100°C with a sensitivity of 1.07%/°C was obtained, which allowed the modulation of temperature for a DNA chip and a cell chip. Finally, this understanding was applied to a real device, a cell chip, and the developed methodology was validated as appropriate for usage as a microheater for cell culture growth.

Keywords: Heating System, Microfluidics, Fluorescence Thermometry, DNA Isothermal Amplification, Cell culture chip, Point-of-Care.

1. Introduction

Point-of-care testing is a developing variant of quick, repeatable and efficient medical testing that can be made available to both developed and developing countries for swift detection of various analytes, from glucose to bacterial infections [1]. These solutions allow the replacement of bulky and complicated laboratory testing equipment by transportable chips with fit for purpose sensitivity and short turnaround times.

This type of technology is highly associated with microfluidics in which diminish amounts of volume both of reagent and analyte are required to have an answer. There are many phases to an arbitrary device, such as sample extraction, preparation, processing and transduction to a result, which are done within microchannels. A way of fabricating these microchannels is with polydimethylsiloxane (PDMS), a transparent, gas permeable polymer, that is compatible with these objectives and allows rapid prototyping. Examples of point-of-care applications include bacterial detection chips where DNA amplification is undertaken (both isothermal and anisothermal) and cell culture chips for the analysis of various growth conditions.

Both of these applications require some form of heating which, for a point-of-care solution, demand that it is equally mobile and of reduced proportions. The field of microfabrication has expanded ludicrously in the past half century and offers so many applications for a great array of needs, from processors to microchips for the analysis of various growth conditions.

In terms of soft-lithography for the fabrication of the PDMS microchannels, the cleanroom was used to create the Aluminum microheaters and k-type Model 5TC-TT-KJ-40-1M, PFA insulated thermocouples (Omega Engineering, Inc., Norwalk, USA) connected to a DT9828 terminal (Data Translation, Marlborough, Massachusetts, USA) were used to measure the surface temperature. The heaters were powered by a DC Power Supplier E361A from Hewlett Packard.

In terms of physics, the Joule Heating module was selected, which includes heat transfer in solids, electric currents and an integrating multiphysics function of temperature coupling, boundary electromagnetic heat source and non-isothermal flow.

The information retrieved from these simulations was treated with the graphical capabilities of the software.

2.2. Heater Microfabrication

For the microfabrication of the devices, a cleanroom Class ISO4 and ISO5 was used. For depositions, the magnetron sputtering machines Nordiko 7000 (Nordiko Technical Services Ltd, Havant, Hampshire, UK) and Alcatel were used, for lithography a Direct Write Laser setup with a 405 nm laser (DWL lithograph, Heidelberg Instruments, Heidelberg, DE) was used and the photore sist spincoating and development was done in an SVG resistcoater and developer track (Silicon Valley Group Inc., San Jose, CA/USA). For patterning, a wetbench was used for lift-off with the aid of Microstrip 3001 (Fujifilm, Tokyo, JP) whereas wet etching was conducted by using Aluminum etchant solution (TechniEtch A180, Microchemicals, Ulm, DE). The cleaning of the device was done at many stages by using acetone, isopropyl alcohol (IPA) and deionized water (DI water).

For their characterization, a FLIRONE infrared camera from FLIR SYSTEMS was used to visualize the heat profile of the microheaters and k-type Model STC-TT-KI-40-1M, PFA insulated thermocouples (Omega Engineering, Inc., Norwalk, USA) connected to a DT9828 terminal (Data Translation, Marlborough, Massachusetts, USA) were used to measure the surface temperature. The heaters were powered by a DC Power Supplier E361A from Hewlett Packard.

2.3. Intrachannel Calibration

In terms of soft-lithography for the fabrication of the PDMS microchannels, the cleanroom was used to create the Aluminum hard masks for the fabrication of the SU-8 molds. These were done on a silicon substrate and with the positive SU-8 photoresist by using a hot plate, a vertical laminar airflow cabinet (FASHERS-SC EN, Cornaredo, IT), a spinner (Laurel Corp., North Wales, PA/USA) and a 254 nm, 400 W UV lamp (UV Light Technology Limited, Birmingham, UK). From this structure, PDMS structures were molded by creating a mixture 10:1 of liquid Sylgard 184 PDMS (Dow Corning, Midland, MI/USA) and the curing agent KIT (Dow Corning, Midland, MI/USA). This
mixture was dessicated in vacuum conditions and put to cure in an oven (loading model 100-800, Memmert, Schwabach, DE). After curing, the inlet and outlet were pierced with 20 ga x 1/2” Luer Stub syringe tips from Instech. For irreversible sealing, treatment was done with an Oxygen Plasma Cleaner PDC-002-CE (Harrick Plasma, Ithaca, NY/USA) on both surfaces (PDMS structure and membrane) which are then put together.

For temperature testing, a Rhodamine B solution with a concentration of 25 mg/L was prepared by adding 5 mg of Rhodamine B base with 97% Dye content (Sigma-Aldrich, USA) to 200 mL of DI Water. This was flowed in the microchannels using a syringe pump NE-1002X (New Era Pump Systems, Inc., Farmingdale, NY/USA) and the fluorescence analyzed with a LEICA non-inverted fluorescence microscope with a 100 W mercury short-arc UV lamp, 10x magnification and a CCD camera. The obtained pictures were treated with the ImageJ software. As control, the thermocouples used during heater characterization were also utilized for this section.

2.4. Cell Chip Validation

The final section of cell chip validation used the microheaters constructed in the previous sections, the thermocouple setup, the fabricated microchannels, a syringe pump and the installations of the Faculty of Pharmacy. To test whether cells adhere to the bottom of the microchannel, HCT-116 (Middlesex, UK) human colon cancer cells were procured. This preparation was done in a sterile environment using a standard procedure within a Class II Type A 2 Biological Safety Cabinet (Esco Micro Pte. Ltd., SG). Here, a sample of cells was extracted after the passing of the main cell culture and Trypsin 0.25% 1x (Hyclone Laboratories, Logan, UT/USA) was applied so as to compromise the adhesion of these cells to the surface and allow them to resuspend and consequently be manipulated. An Haemocytometer Neubauer Improved Counting Chamber 0.0025 mm² from GmbH & Co Kg (Wertheim, DE) was used for cell counting. For the experiment, there were three main components: HCT-116 cells, their medium, McCoy’s 5A from Gibco ThermoFisher Scientific (Paisley, UK) and Collagen I, Rat Tail, 100 mg, Corning (Bedford, MA/USA). This structure was also different, in which the PDMS slab in sealed on a mini polystyrene petri dish by combining oxygen plasma with a solution of 0.2 mL of 3-Aminopropyltriethoxysilane (APTES) 99% (Acros Organics, UK) and 20 mL of DI Water, which leads to an irreversible bonding.

3. Microheater Simulations

3.1. Small and Large Scale Heating

This section is focused on determining the most adequate designs to fulfill the heating requirements of the microfluidic systems considered for this project, namely DNA chips (for DNA isothermal amplification) and cell culture chips. Both of these applications require a stable temperature at 37°C and temperature uniformity across their working area. As a benchmark geometry guide for the heaters development, a chemical lysis chip connected to an amplification chamber, henceforth referred to as the Origin Microchannel, was used. This microfluidic structure exhibits two heating areas of interest, the core and the chamber (Figure 1).

Figure 1: Figure of the Origin Microchannel, with the relevant heating areas of the Core and the Chamber.

Due to the dimensions of this design, the need for strictly controlled temperature for the chamber (to ensure ideal conditions for the polymerase and ligase to undertake their function) and uniform distribution of heat for the core, though less stringent, it was necessary to observe the requirement for a smaller scale heater with the capacity to respond quickly to current imposition and for a larger scale heater that satisfies the greater area that composes the core.

The first was narrowed down to an altered design based on an existing design by Domenico et al [8]. These alterations were two-fold, firstly, a downsizing of the design was done to better respect the dimensions of the chamber and, secondly, the area in the middle was freed to allow visual access to the PDMS microchannel. To test the appropriateness of this variant, a design was done in indium tin oxide (ITO), that took advantage of its transparent nature by fully covering the chamber, and another in Titanium-Tungsten alloy (TiW), in which the center material was replaced by an outer rim (or ring) which allowed access to the chamber. This can be observed in Figure 2.

Figure 2: On the left, the original microheater [8] is depicted and followed, on the right, by the adaptations done for the TiW and ITO versions.

By simulating both materials and their respective adaptation of the original microheater, in Figures 3 and 4, it was possible to isolate their differences in performance. The contact pads were chosen to be Aluminum.

Figure 3: TiW Chamber Heater at 1 V.

Figure 4: ITO Chamber Heater at 1 V.
In Figures 3 and 4, we observe the performance of this microheater for both TiW and ITO, where the latter’s chamber heater was a ring-like structure (due to the need to optically access the chamber) and the former was a fully circular heater (to take advantage of the material’s transparency). As expected, the metallic alloy TiW portrays stronger heating capabilities with the same voltage when compared to the conductive oxide, ITO. Nonetheless, both exhibited an area of approximately isothermal heating in their center. These results allowed the classification of these structures as adequate for the chamber heater.

For the large scale heater, a grid structure was found to be preferable for heating the core due to its comparable temperature uniformity to a regular plate design but having the advantage that quick cooling was also possible. Due to the size of the tracks, it was also a secure bet in terms of reliability and durability. This structure was equally appropriate for the materials of ITO and TiW. In Figure 5, one may observe the performance of this solution - the straight drift plate.

With these two structures in tow, it is necessary to study their interaction and adjust the thickness of the films to grant the range of temperatures desired.

3.2. Integration and Final Designs

Integrating the small heaters in Figures 3 and 4 with the large heater in Figure 5, a new microheater is created. By doing this, the objectives are to have two different points that can be instantly actuated - the chamber, where strict temperature control is necessary and the core, where a large area should be stable. At the same time, both of them should display a uniform heat profile.

3.3. First and Second Generations

Due to the existence of a section of the microchannel that is beyond the range of the heaters, two designs were envisioned. One with only one chamber heater and another with two micro-heaters for a larger uniform heating area. For Generation I, four microheaters per material were planned (two for Design 1 and two for Design 2) which originates eight heaters in total. The simulation was adapted to the final design for Generation I and, as an investigatory effort, the full construct including PDMS microchannel was placed on the heater TiW1-1. This allowed the extraction of graphs relating the heat outflow from the heaters to the heat inflow from the microchannels. This simulation can be found in Figure 6.

During the experimental assessment of microchannel temperatures, however, it was necessary to lengthen the contacts to ensure a more reliable electrical connection through crocodile clips. For this endeavour, due to the structural alteration of the device, the simulation was adapted to ensure its functionality (i.e heat profile) is maintained. While the whole simulation includes the PDMS structure over the heater, only the relevant microchannel and heater surfaces were considered as areas of interest.

Form these trials, it was possible to extract the heat flows of the system and evaluate, albeit with limited detail due to computational constraints, if indeed the contact geometry change would defeat the purpose of this design.

The base simulation of a specific heater, TiW2-1, is depicted in Figure 7. For other heaters, it was a matter of exchanging the geometry module of the file and applying the remaining functions identically.

In this way, it was successfully concluded that this adjustment to increase experiment quality did not compromise device performance.

3.4. Temperature Variation in a Microchannel

To evaluate the temperature variation within a microchannel, from top to bottom, a simple simulation utilizing laminar flow, heat transfer in fluids and heat transfer in solids, was used as seen in Figure 8. This study was important to ensure that the temperature difference between the fluid near the lower boundary and the fluid at the top boundary was low, in addition to studying the effect of flow rate on heat dissipation.
It was found that, at these dimensions, variations of flow rate (10 µL/min to 100 µL/min) do not entail a change greater than 1°C in average microchannel temperature. Moreover, within the microchannel itself, the difference between top and bottom of the channel does not exceed 0.03°C (Figure 9).

With the simulations of this section, it was possible to determine a guiding path for the microfabrication of the microheater and, later, for its testing alongside a microfluidic system.

4. Heater Microfabrication

Having determined the appropriate design of the microheaters via simulation, these could advance into the microfabrication stage. This process was done in a cleanroom, where the aluminum hard masks for soft lithography were also fabricated. For a schematic of the microfabrication of the heaters, the hard masks and of the PDMS microchannels, consult Figure 10.

Using two depositions (TiW/ITO + Aluminum), two lithographies (one before heater deposition and one after contact deposition), one lift-off (to pattern the heater) and one wet etch (to pattern the contacts), it was possible to create the Generation I heaters for TiW and ITO. In total, these are 4 heaters of TiW and 4 heaters of ITO.

For the first layer, a lithography was done with a non-inverted mask which was followed by the deposition of either ITO or TiW. For the second layer, the deposition of Aluminum was done and then an inverted mask was used for the direct write laser lithography. This was followed by wet etch procedure which, for the case of TiW, was adequate but, for the ITO heaters, led to some damage in the first layer. The first layer of TiW can be found depicted in Figure 11.

While the first layer was the same for both Generations, the contacts were different in terms of their length. After the deposition and patterning of the second layer of Aluminum, the heaters were ready for dicing. In Generation I, there were four devices per sample (Figure 12).

For the finalized second generation, there were two devices per sample and the difference in contact pad length is evident as depicted in Figure 13.
During the microfabrication process, metrology steps were undertaken to ensure the purity of the films and if their desired thickness was respected during the depositions. Glass calibration bars were used for the measurement of resistance and consequent calculation of resistivity, whereas the uniformity of the films was measured directly on the samples. These results can be found in Tables 1 and 2.

In terms of film thickness and uniformity, one may evaluate Table 1 where the averages of the measurements at the relevant points from Figure 13 are displayed.

Table 1: Heater Thickness Uniformity for Generation II Heaters

<table>
<thead>
<tr>
<th>Material</th>
<th>Desired Thickness (Å)</th>
<th>Average (Å)</th>
<th>Non Uniformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiW</td>
<td>1800</td>
<td>1834</td>
<td>8.9%</td>
</tr>
<tr>
<td>ITO</td>
<td>5000</td>
<td>4730</td>
<td>11.8%</td>
</tr>
<tr>
<td>*ITO</td>
<td>5000</td>
<td>6258</td>
<td>9.4%</td>
</tr>
<tr>
<td>AlSiCu</td>
<td>5000</td>
<td>4985</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

From Table 1, it is evident that the depositions done in the Nordiko 7000 (TiW and AlSiCu) portray more uniform films (in which non-uniformity was calculated from the greatest maximum relative error between the average of all measurements and each measurement) whereas both ITO and *ITO show a greater degree of roughness throughout the thin film. Additionally, *ITO exhibits greater thickness than expected, which is a complication of the deposition.

These two ITO samples (ITO and *ITO) refer to Generation II, where the former is from a first run and the latter from a second run.

Table 2: Resistivities for the materials used in the heaters, from calibration bars used in Generation II depositions.

<table>
<thead>
<tr>
<th>Material</th>
<th>Measured Resistivity (Ω/m)</th>
<th>Expected Resistivity [9] (Ω/m)</th>
<th>Expected Type of Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiW</td>
<td>3.68 × 10^−6</td>
<td>10^−6</td>
<td>Metal</td>
</tr>
<tr>
<td>rITO</td>
<td>1.97 × 10^−6</td>
<td>10^−6</td>
<td>Oxide</td>
</tr>
<tr>
<td>ITO</td>
<td>3.96 × 10^−6</td>
<td>10^−6</td>
<td>Oxide</td>
</tr>
<tr>
<td>AlSiCu</td>
<td>4.75 × 10^−6</td>
<td>10^−6</td>
<td>Metal</td>
</tr>
</tbody>
</table>

In terms of the resistivities, in Figure 2, TiW and AlSiCu refer to the quality of the heater deposition of Generation II, rITO refers to the quality of the depositions of this oxide for Generation II. Moreover, TiW seems to have higher resistivity than expected, which was a process engineering decision done by increasing the N₂ concentration during the magnetron sputtering deposition.

5. Heater Characterization

With the heater microfabrication concluded, it was necessary to characterize their performance. This was done, in Generation I, by using an Infrared (IFR) Camera and, in Generation II, by combining both an IFR Camera and thermocouples. It was noted that the IFR camera is an appropriate tool to analyze the heat profile of a device whereas the thermocouples provide a more accurate temperature reading.

Following the schematic in Figure 14, the heater codes (e.g. TiW1-1) represent the Generation and Design used for the heater. TiW1-1 corresponds to Generation I, heater design 1 and TiW1-2 corresponds to Generation I, heater design 2. Additionally, consider that TiW1-4 is a copy of TiW1-1 and TiW1-3 is a copy of TiW1-2.

Table 3: Generation I Heater Functional Results

<table>
<thead>
<tr>
<th>Heater</th>
<th>Voltage Used (mV)</th>
<th>Temp. Stability (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core — Chamber</td>
<td>TiW1-1</td>
<td>1.6 (72 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>TiW1-2</td>
<td>1.5 (70 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>TiW1-3</td>
<td>1.5 (72 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>TiW1-4</td>
<td>1.6 (69 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>ITO1-1</td>
<td>2.4 (46 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>ITO1-2</td>
<td>2.2 (40 mA)</td>
</tr>
<tr>
<td>Core — Chamber</td>
<td>ITO1-4</td>
<td>2.4 (43 mA)</td>
</tr>
</tbody>
</table>

In an approximately 20-minute long steady-state, from Table 3, it is possible to conclude that all varieties of heaters were extremely stable with oscillations below 1°C. Moreover, the difference between chamber and core temperature does not seem significant, which shows a robust uniformity across all heaters, particularly TiW1-1 and TiW1-4. Due to damage withheld during testing, results could not be extracted from heater ITO1-3.

For Generation II, thermocouples were placed beneath the microheater while the IFR recorded its surface. A test was conducted to evaluate whether the temperature at the top surface of the heater (metallic side) would be the same as beneath the substrate (glass side) and it was shown that there is no great temperature difference between the two. The core exhibited the same temperature on both faces of the heater while the chamber was around 2°C colder on the bottom face. Furthermore, comparing the thermocouple measurements with the IFR acquisitions, it was noted that the latter’s measurements were higher than expected which suggests that the black paint used to increase the emissivity was not of ideal quality and that the camera did not display the spatial resolution necessary to extract the temperature of these fine structures.

In Table 4 the obtained temperature stability values at 37°C are presented as it is the relevant temperature for cell cultures, particularly TiW1-1 and TiW1-4. Due to damage withheld during testing, results could not be extracted from heater ITO1-3. For Generation II, thermocouples were placed beneath the microheater while the IFR recorded its surface. A test was conducted to evaluate whether the temperature at the top surface of the heater (metallic side) would be the same as beneath the substrate (glass side) and it was shown that there is no great temperature difference between the two. The core exhibited the same temperature on both faces of the heater while the chamber was around 2°C colder on the bottom face. Furthermore, comparing the thermocouple measurements with the IFR acquisitions, it was noted that the latter’s measurements were higher than expected which suggests that the black paint used to increase the emissivity was not of ideal quality and that the camera did not display the spatial resolution necessary to extract the temperature of these fine structures.

In Table 4 the obtained temperature stability values at 37°C are presented as it is the relevant temperature for cell cultures and isothermal DNA amplification (such as Rolling Circle Amplification). In Table 5, the results regarding the tests at 60°C are presented, which is the temperature for DNA denaturation, another important biological process.
Using this structure, the RhB solution at a concentration of 25 mg/L (as it has highest sensitivity according to literature [10]), a syringe pump and a fluorescence microscope, it was possible to calibrate the fluorescence variation with temperature and organize it as a curve, in Figure 16.

An important value to take from this calibration is its sensitivity, which is calculated according to Equation 1.

\[ S = \frac{1}{I_0} \frac{dI}{dT} \times 100 \]  

The value obtained for the sensitivity of the experimental data was 1.70%/°C, in comparison with the literature's 1.68%/°C [10]. This expresses that for an increase of 1 °C, 1.70% of the fluorescence is lost which is slightly more sensitive than the value in literature. The disadvantage of this first calibration curve, however, is its limit of approximately a 50 °C temperature before the emergence of expanding microbubbles and RhB diffusion into the PDMS matrix becomes excessive. These challenges will compromise lengthy assays and introduce artifacts into the data.

6.2. Challenges

The two main challenges of these experiments are the RhB’s tendency to passively diffuse into the PDMS and the formation of microbubbles via evaporative events. Understanding these phenomena will enable the development of a methodology to compensate for them.

6.2.1 Bubble Formation

For this hurdle, it was necessary to understand that bubble formation is an issue that can be introduced into a microfluidic system due to, generally, improper sample loading [11]. This introduction of air bubbles, in combination with temperature, will lead to their expansion which will affect the validity of the measurement areas.

In Figure 17, one can see how, during a RhB temperature assessment, a bubble shows a completely different fluorescent signal compared to the fluid.

![Figure 15: Reservoir Microchannel Full SU-8 mold and according PDMS structure, extracted from the corner section.](image)

![Figure 16: First RhB Calibration, limited in range by bubble formation and compared to literature [10]. Exposure time: 310 ms, Gain: 1x, Saturation: 1 and Gamma: 1.](image)

![Figure 17: Bubble Formation during a RhB temperature assessment.](image)
Apart from sample loading, there is also the issue of spontaneous formation of microbubbles due to solvent evaporation. The probability of this phenomenon increases as temperature is increased, which makes it extremely relevant for this work. As a microbubble forms in a nucleation spot (a roughness in the microchannel or a sealing imperfection), and temperature is increased, it will expand and possibly invalidate an experiment. At moderate temperatures, such as 50°C, it is not expected that water evaporates, however, due to the intricacies of the Maxwell-Boltzmann distribution (Figure 18), some molecules with higher than average kinetic energy can break the barrier to evaporation and thereby create microbubbles.

![Maxwell-Boltzmann distribution](image)

Figure 18: The Maxwell-Boltzmann distribution for several compounds. [12]

At these extremely limited volumes, this is a concern and can only be avoided by reducing the duration of the experiment, avoiding careless sample loading and choosing solvents with high boiling points.

### 6.2.2 Diffusion

In terms of diffusion, this is an event that happens immediately after inserting the RhB solution into the microchannel. This creates a glowing PDMS periphery of the microchannel, which, in combination with the solution itself, creates a higher signal than desired. On the one hand, in scenario 1, if the solution is continuously replenished, the diffusion will continue until most of the immediacy of the microchannel is saturated which can be a lengthy affair. On the other hand, in scenario 2, if the solution is left for a long enough time without replenishment, its concentration will be reduced as the RhB migrates to the PDMS. In a heating cycle (20 minutes of heating and 10 minutes of cooling), both the first and the last point should be room temperature. Firstly, the fluorescence should diminish and, upon cooling, it should increase. This would not be the case in either of the scenarios mentioned previously as in scenario 1 the fluorescence would continuously increase and in scenario 2 (depicted in Figure 19), due to loss of concentration of RhB in the microchannel and diffusion of it into the surrounding PDMS, the fluorescence would never return to room temperature levels.

![RhB diffusion](image)

Figure 19: RhB diffusion in static regime at room temperature, on the left at 0 minutes and on the right after 35 minutes.

To solve this, a methodology was developed by pre-treating the microchannels with RhB for a prolonged period of time.

### 6.3. Second Calibration

While the first calibration was a good result, the danger of the interference of the aforementioned challenges in the RhB fluorometry made it so trials were consistently diminished in quality. An experiment should be long enough to endure one heating cycle but, as temperature increased, the measurement of the fluorescence became more difficult. Therefore, the diffusion phenomenon was taken advantage of by leaving a RhB solution within the PDMS microchannels for 16 hours so as to ensure the direct vicinity of the microchannel contains a comparable concentration with enough thickness that it is measurable. This period of time, 16 hours, was determined to be sufficient for this endeavour, in accordance with the diffusion time established for RhB [13].

Moreover, the Origin Microchannel was used (Figure 1), the DNA chip for Rolling Circle Amplification (RCA) - an isothermal DNA amplification method. Its SU-8 mold along with the related microchannel are shown in Figure 20.

In terms of setup, it was necessary to construct a Poly-methyl methacrylate (PMMA) holder to ensure the heater and microchannel system did not move in regards to each other during insertion of RhB and observation under the microscope. This was done by designing the mask in Adobe AutoCAD and milling a PMMA substrate according to the dimensions of the heaters. In Figure 21, it is possible to observe the PMMA holders for design 1 and design 2.

![PMMA holders](image)

Figure 21: The fabricated PMMA plates, adapted for designs 1 and 2 by including crocodile connector entrances and microfluidic-sized drillings.

In this way, the schematic of the setup used for the second calibration is depicted in Figure 22.

![Setup](image)

Figure 22: Setup used for the Second Calibration.

The results extracted from this calibration, by taking a fluorescence picture at room temperature and then at every temperature step of 0.5°C, can be found in Figure 23.
An immediate observation to be done is the agreement with literature and even an extension of what exists which proves quite firmly that RhB absorbed into the PDMS has a similar fluorescent behaviour as when it is in solution. By using Equation 1 once again, a sensitivity of 1.07%/°C was obtained, which, while lower when comparing to the previous experiment and literature, at 1.68%/°C [10], is expected as there is a lower concentration of RhB in the PDMS walls when compared to solution. Nonetheless, this is a trade-off as, with this technique, it is possible to do temperature calibrations in microchannels that reach 100°C while avoiding microbubble-derived complications. Therefore, using this calibration curve, both the Origin Microchannel and a cell culture chip can be tested.

### 7. Applications to Microfluidic Systems

From the temperature tests done using thermocouples and the RhB solution, it was decided that the TiW heaters were to be used for the DNA chip due to their stability and the ITO heaters for the cell culture chip due to their transparency. This section sought to calibrate the heaters for both PDMS structures and to validate them for the usage with a cell culture chip.

#### 7.1. TiW Heater and the Origin Microchannel

For these experiments, the TiW2-1 heater was used with the objective of keeping the chamber at 37°C. Two approaches were conducted to evaluate the intrachannel temperature. The first was to use the RhB-doused PDMS structure with the 16 hour treatment to measure temperature differences at the wall. The second was to use a flow rate of 10 μL/min and measure the fluid itself. For these measurements, appropriate alignment between the chamber and the chamber heater had to be achieved, as depicted in Figure 24.

#### 7.1.1 Static Testing

Having ensured this, a heating power of 261 mW (90 mA, 2.9 V) was applied as this was determined to be the correct parameters for achieving a temperature closest to 37°C for isothermal amplification. Thermocouples were used as control for the temperatures at the heater surface, in the microchannel depth of the PDMS, at the top of the PDMS structure and room temperature. RhB was used to measure the temperature at the walls of the microchannel which were assumed to be at the same temperature as the fluid itself. These results can be consulted in Figure 25.

#### 7.1.2 Flow Testing

For the flow variant, possible to do with some reliability at these temperatures, RhB was flowed at 10 μL/min and put through a heating cycle. Using merely the RhB as a temperature sensor, it is possible to compare to the previous experiment. The results for this subsection, at the same heating power of 261 mW, can be found in Figure 26.
supports, such as the simulations suggested, that at these volumes flow rates have a limited impact of temperature distribution. Moreover, the graph illustrates what happens (as part of the red line) when there are bubbles contaminating the experiment. The difference in fluorescence can clearly be seen between the two pictures separated by the peak.

This concludes the calibration of the Origin Microchannel for usage with isothermal Rolling Circle Amplification at 37°C.

7.2. ITO Heater and the Cell Chip Microchannel

For the Cell Chip Microchannel, the third PDMS microchannel used in this work, the ITO heaters were used – namely, the ITO2-2 heater. Likewise, the SU-8 mold used for the curing of the microchannel can be found in Figure 27.

In the same way as was done for the Origin Microchannel, both the static and flow methods were used and showed no particular dissonance. In Figure 28, the static version temperature profiles at a heating power of 324 mW (90 mA, 3.6 V) are depicted.

This chip was more difficult to heat than the Origin Microchannel due to the fact that it is narrower, thereby having less contact area with the heater itself and the whole structure is taller which translates into a greater surface area leaking heat to the air.

Having calibrated this structure, there was an attempt to validate this heating system by studying if cells thrive in the conditions within it.

7.3. Cell Chip Validation

In this final section, human colon cancer cells from an immortal cell line were inserted into the cell chip and heated via the usage of the ITO heaters, due to their transparent property which allows the real-time observation of cells. Due to the various phases of cell adhesion and growth, it was necessary to create a system that replicated the environment within an incubator while attempting to isolate the temperature as the only variable. For these cells, the ideal temperature is 37°C ± 1°C. This experiment had three main phases: Preparation, Adhesion and Growth.

- Preparation - Firstly, the cell chip was prepared with a suitable environment for the cells to grow. This was done by adding collagen, McCoy's cell media and then the cells, sequentially, via microfluidic tubing and plugs (at 4 µL/min per two minutes each). The cells were, at that time, in suspension and were not adhered to any surface - a prerequisite for growth and a sign that they are in favorable conditions;

- Adhesion - From zero to four hours, it was expected that some cells began to adhere assuming temperature, media, humidity and gas exchanges were appropriate;

- Growth - Assuming adhesion was successful, media was imposed with a very slight flow rate (0.2-0.4 µL/min) for a prolonged period of time which may vary according to application, requiring that the heater was resilient and stable.

In order to stave off bubble formation, the microfluidic chip was halfway submerged in water within a petri dish to simulate the humidity present within an incubator. This was a technique that created additional stress on the heater as it was designed to heat smaller chips and not mini petri dishes with water. Nonetheless, the higher quality heater that was used for the final experiment, ITO2-1, did not show any degradation in structure or performance in spite of being made to function for up to 16 hours. To ensure that the temperature was adequate at microchannel level, a thermocouple was inserted during the curing process of the PDMS which allowed the calibration of the real temperature where the cells are placed during the assay. Moreover, this allowed the possibility of real-time temperature adjustments. This setup is depicted in Figure 29.

However, in spite of the preparations of the system to investigate the ideal power for 37°C of the microchannel, it was noted that this temperature did not seem to be ideal for the cells. This was due to the fact that their necessity for adhesion depends not on the temperature of the surrounding fluid but on the temperature of the sealing structure (in this case, polyethylene from the petri dish). Due to the thermocouple's positioning, it was recording the temperature of the upper wall of the microchannel and not of the fluid itself. In Table 6, the thermal characterization of the system during the final experiment is displayed.

<table>
<thead>
<tr>
<th>Thermocouple</th>
<th>Maximum Value</th>
<th>Minimum Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heater Surface</td>
<td>38.3 °C</td>
<td>37.5 °C</td>
</tr>
<tr>
<td>Microchannel</td>
<td>33.5 °C</td>
<td>32.7 °C</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>24.8 °C</td>
<td>23.1 °C</td>
</tr>
</tbody>
</table>

At a heating power of 187 mW (78 mA, 2.4 V), thus, it was possible to observe that the heater surface was on average, at 38°C and that the top of the microchannel it was, on average, at 33°C. According to the know-how developed during this project, it is a certain assertion to consider that the petri dish surface upon which cell adhesion would be undertaken portrayed a range of temperatures of 36.5-37.5°C which is concordant with the cells' ideal temperature. As evidence for this adequate heating, the cell fates are displayed in Figure 30.
It is clear that at the first hour, the cells themselves were rounded and not adhered. This is due to the fact that they are being placed in a new set of conditions, requiring time to adapt. As time goes on, particularly after hour 12, they display an elongated shape which is characteristic of adhesion and, consequently, having found an adequate environment to thrive. At hour 16, it is clear that the cells are confluent, numerous and thriving which proves that this heating system can be used for cell culture chips without the need for excessive optimization.

8. Conclusions

In this work, several milestones were achieved. Firstly, a simulation library was developed for Joule Heating of microfluidic systems that can be altered to support different projects requiring this module. Additionally, using the laminar flow module with fluid heat transfer, it was possible to model the variation of temperature within a microchannel. In the future, with more computational power, these two could be condensed into a single, powerful simulation tool with a denser mesh, granting even more accurate results. Secondly, the microfabrication underlined the importance of metrology for the detection of faulty thin film depositions and helped justify unexpected performance in the heaters. Moreover, the addition of $\frac{1}{2} V_2$ to the TiW deposition generated a more efficient device and should be considered for future applications. A metallization step would also confer more resistance to the contacts, impeding their degradation by the crocodile connectors. Finally, to prevent overetching of the ITO layer during the wet etching of the contacts, reactive ion etching should be considered as an alternative.

In terms of the microchannel temperature assessment, RhB proved to be an adequate compound to gain an understanding of what happens within the microchannel. While it was shown to be associated with complications when used with PDMS structures, the methodologies developed during this project allowed two separate ways of tackling the issues of bubble formation and diffusion. Furthermore, a calibration curve reaching high temperatures was produced by viewing diffusion as an opportunity instead of an obstacle. By using the calibration of RhB of the PDMS walls, it was possible to study high temperatures without fearing for the emergence of microbubbles, albeit with lower sensitivity. This section allowed the thermal calibration of an isothermal DNA amplification chip and a cell culture chip which expresses the usefulness of this methodology for the thermal modules necessary in complex microfluidic chips. This is further validated by the successful application of the heating module developed during this project to an existing cell chip which resulted in thriving cell outcomes, potentiating the usage of this module as a portable cell carrier or as part of the final design, thereby forgoing the need for an incubator. Perhaps more importantly, this whole thesis may serve as a comprehensive guide to aid the creation of heating modules that may be inserted into the scope of various projects.

With these considerations, it can be concluded that this thesis was successful in its proposed objectives, providing a useful and pragmatic contribution that can be adapted and readily applied to other ongoing projects requiring a heating component for their biological processes and consequent Point-of-Care applications.

References


Figure 30: Microscope pictures of the cell cultures at particular time points, showing relevant periods of cell adhesion and, in some cases, growth.