The 16th International Conference on
Solid-State Sensors, Actuators and Microsystems
June 5-9, 2011, Beijing, China

MAGNETIC MICROHEATERS FOR CELL SEPARATION, MANIPULATION, AND LYSING

Angelo Gaitas[1,2], *Paddy French*[2]
1. PicoCal Inc., 333 Parkland Plaza,
Ann Arbor, Michigan, USA
2. EI/EWI-DIMES, Delft University of Technology,
Mekelweg 4 2628CD Delft, the Netherlands

ABSTRACT

Precise heating is important for biological culturing, biological characterization, and thermal lysis, while cellular manipulation has been an area of significant interest and has been explored by a variety of methods. In this work, we present a preliminary study of the use of metallic thermal probes. The probes were used for magnetophoresis and micromanipulation of magnetically labeled HeLa cells. The probes were also used for rapid thermal lysis of magnetically labeled cells in liquid. The ultimate goal of this work is to combine thermal probes with magnetic tweezers for sample enrichment, magnetophoresis, manipulation, and thermal applications.

KEYWORDS

Magnetic Tweezers, Microheaters, Thermal Probes, Cell Separation, Cell Manipulation, Cell Lysis, Magnetophoresis.

INTRODUCTION

Precise heating is important for biological culturing and characterization [1]. For example, surfactants are often used to induce osmosis-based release of nucleic acids or proteins of interest from cellular structures to allow for testing of the nucleic acids. Microheaters can also be integrated into fluidic systems for biological work. Recently, microheaters [2] were used for high-speed liquid pumping, mixing and particle entrapment in thin layers of oil and water. Rapid thermal lysis was performed on a droplet containing cells deposited on a microheater [3].

A critical step in many nucleic acid analysis protocols is enrichment of nucleic acids or cell population through magnetic separation of magnetically labeled cells. Magnetic particles, which can range in size from the nanometer to the micrometer scale, have become a standard process in sample enrichment with application to clinical diagnostic labs and biological research [4]. Their use spans many fields [5]. Another application is the use of magnetic beads for labeling cells of interest and magnetically separating the cells from a heterogeneous sample, such as whole blood [6], for enumeration purposes. This application has been useful in clinical labs, where enumeration of circulating tumor cells have been approved in the US for diagnoses and prognosis of breast and prostate cancer [6]. Magnetically labeled cells have also been an important component in the field of magnetic tweezers [7].

Cellular manipulation and characterization has been an area of significant interest and has been explored by a variety of methods, including optical tweezers, magnetic tweezers and atomic force microscopy [8, 9]. While individually these techniques are extremely useful and broadly implemented, there are many potential advantages to combining them. For example, magnetic tweezers and optical tweezers have been combined to increase the controlled degrees of freedom of trapped bodies [10]. While there have been combinations of optical tweezers and magnetic tweezers, there is limited work on the idea of combining heating and magnetic properties into a single microprobe, especially for manipulation and characterization of magnetically labeled cells.

In this work, we present a preliminary study of the use of metallic thermal probes, including photoetched Ni probes and bent wire Ni-Cr probes. The Ni probe was used for magnetophoresis and micromanipulation of magnetically labeled HeLa cells. The 75 μm diameter Ni-Cr bent wire probe was used for rapid thermal lysis of magnetically labeled cells in liquid. The ultimate goal of this work is to combine thermal probes with magnetic tweezers for sample enrichment, magnetophoresis, manipulation, and

thermal applications (e.g. thermal characterization, heating, thermal lysis and thermal cycling).

Silicon micromachined heaters have been used for some applications [3]. These heaters are relatively expensive to fabricate since they require several clean-room steps. In addition, conventional silicon micromachining does not lend itself for metal probe fabrication. Furthermore, deposition of metallic thin films results in weak magnetization. Fabrication methodologies such as micro-electric discharge machining (μEDM) and photo-etching can be used to reduce cost and enable the fabrication of metallic microheaters. Therefore, one more goal of this paper is to explore novel and inexpensive ways to fabricate a microheater using photo-etching.

MICRO-HEATERS

The Ni probes were manufactured using photochemical machining. Photochemical machining includes chemically etching the metal while a photographically prepared mask is used to protect the metal that is to remain after the etching process. The probes are shown in Figure 1a. Fig. 1b shows the tip of the probe, which is < 200nm. Simulations were conducted to determine the optimal designs in order to maximize heating at the tip area. The part of the probe closer to the tip was half etched to increase the heating at the tip point. Electropolishing was used to further thin the microheater and make the surface reflective allowing the probe to be compatible with atomic force microscopes. The probe has resistance of 2 Ohms.

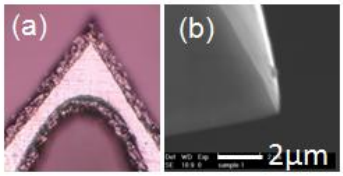


Figure 1. *(a) Optical microscopy image of the nickel probe after photochemical machining (b) A scanning electron microscopy image taken at a 40° angle. The tip is < 200 nm.*

An additional microheater was constructed using off-the-shelf components, which allowed for further investigation of rapid thermal lysis. This microheater was constructed by using a custom built wire bending setup for bending a 75 μm Ni-Cr wire.

CELL PREPARTION

HeLa cells were prepared by standard cell culturing protocols, cultured in DMEM in a 5% CO_2 humidified environment. Streptavidin magnetic beads with a 2.8 μm (M-280s, Invitrogen, Inc.) were rinsed several times in DMEM. These particles were then were mixed at 1 Hz with end-over-end rotation at a concentration of cells of 10^6 cells/mL. Cells were mixed with beads suspended in DMEM at 1/5 the concentration of the stock solution. 50 μL of the diluted beads were mixed with 450 μL of HeLa cell solution. Measurements with the nickel thermal probe did not utilize end-over-end incubation. The end result of the binding protocols was HeLa cells with attached magnetic beads, with a sufficient number of beads that would allow for magnetic micromanipulation. To detect cell death and cell lysis, Propidium Iodide stock solution (1 mg/mL) was added to the HeLa cells at a concentration (10 μL of stock was added for every 1 mL of cell solution). Cell preparation was conducted at Prof. Raoul Kopelman's laboratory by Dr. Brandon McNaughton.

EXPERIMETAL SET-UP

A one inch conical NdFeB magnet (Engineered Concepts Birmingham, Alabama) was used to magnetize the tip of the microheater. The tip of the microheater was held in contact with the tip of the conical magnet. This resulted in the microheater tip having a permanent magnetic dipole similar to that of a translational unipolar tweezer. The microheaters were mounted on a micromanipulator (fig. 2). A Zaber motorized stage was used to move the sample in relation to the microheater. An inverted microscope was used to record the experimental measurements. Cellular magnetophoresis was measured with the ImageJ plugin MultiTracker.

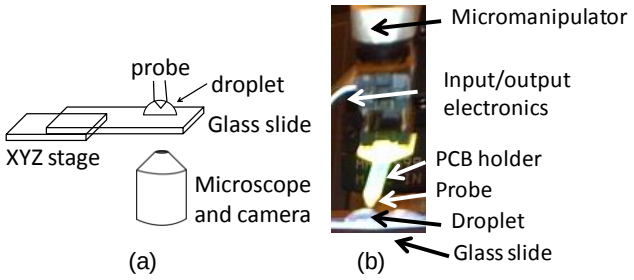


Figure 2. *(a) Schematic Illustration of setup and (b) image of the probe attached to a pcb and micromanipulator in a 100 μL droplet containing magnetically labeled cells.*

THERMAL CHARACTERIZATION

A 12μm thermocouple was used to characterize the nickel probe in liquid. Biologically relevant temperatures could be achieved with a power of 1.75 W resulting in a maximum temperature of 35 °C. Higher temperatures can be achieved by increasing the resistance of the microheater which can be accomplished by changing the material and physical dimensions of the micro-heater. Temperatures at distances of up to 1 cm from the micro-heater were also taken at a power of 1W and were found to be

reduced by 3.1 °C from 30°C in contact in liquid. These results demonstrate that a magnetic microheater can be used manipulate cells and to control the local environment. Temperature versus power curves were taken in PBS solution. The temperature of the microheater must be held below the Curie temperature, T_c . The Curie temperature (T_c) for nickel is 360 °C. Above that temperature the microheater would become paramagnetic and would only work as a magnetic tweezer in the presence of an external magnetic field. Here we focus on the ferromagnetic case $<T_c$, resulting in the micro-heater having a permanent dipole moment.

For rapid thermal lysis measurements, an additional probe was constructed using a 75 µm Ni-Cr wire, which allowed for further investigation of rapid thermal lysis. Figure 3 shows the thermal calibration of the magnetic Ni-Cr wire probe. The microheater was immersed into approximately 100 µL of water and powered at different currents. The effect of altering the current was measured with a small thermocouple. Once the micro-heater was powered at higher currents thermal expansion was observed. We placed the thermocouple on the outer side of the bent wire so that it remained in contact with the thermocouple. As it can be seen from Figure 3, temperatures as high as 73.5 °C could be reached at a power of 2.9 W. While these measured temperatures are not at the boiling point of water, we did observe significant boiling. This is most likely a result of the thermocouple not measuring the temperature at the microheater-water interface. However, these temperatures were sufficient to induce cell death and cause cell lysis as is described below.

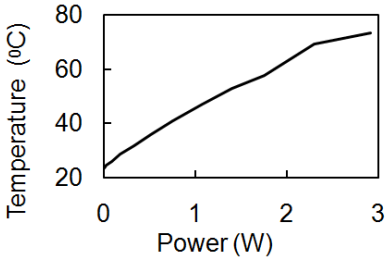
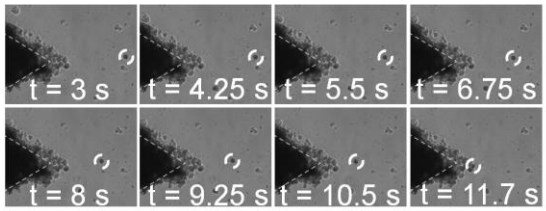


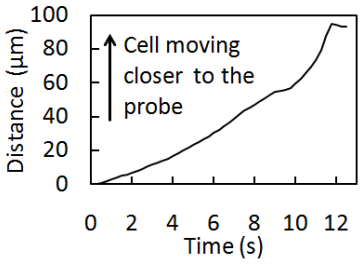
Figure 3. *Thermal calibration of the 75 µm Ni-Cr bent-wire probe using a thermocouple. The probe was immersed into a 100 µL droplet of water and powered at different currents. Temperatures up to 73.5 °C were observed at 2.9 W.*

CELL MANIPULATION

Figure 4 shows the magnetophoretic response of a single HeLa cell. The nickel probe has many cells already attached to its surface, which resulted from translation in the sample. After the Ni probe was held in a stationary position, several cells had a large enough magnetophoretic response to translate across the surface of the slide. The location of the cell was tracked in Figure 4b demonstrating that the cell is moving as a result of magnetic force rather than some other fluidic motion, such as convection. Also, the acceleration is indicative of the force gradient created by the magnetic probe. The device was not heated while observing magnetophoresis. The magnetic force was large enough to quickly transport cells within the droplet or completely remove cells from the fluid and place them into a different droplet. This is similar in concept to performing magnetic separation by placing the magnet inside the fluid (as it is done with the commercially available magnetic PickPen), but at the microscopic level. Furthermore, magnetic beads can be functionalized for affinity-based specificity, which is a potential alternative to functionalizing a micro-heater itself [3].



(a)



(b)

Figure 4. *(a) Microscopy images of thermal magnetic probe attracting a single magnetically labeled HeLa cell. Dotted lines indicate location the cell. (b) Data from the image analysis of images, where starting distance is from the starting location of the cell. The cell is accelerating toward the probe. The cell moves with an average speed of 7.8 µm/s.*

Cells that were magnetically attached to the microheater were manipulated. Manipulation was controlled with a xyz Zaber motorized stage. The maximum translational velocity used was 50 µm/s. We did not observe detachment of cells from the micro-heater. To test if there were conditions that could remove the cell, we manually oscillated the direction of translation, which was sufficient to release the magnetically bound cells. This also indicates that there was not significant interfering non-specific binding between the cell and the microheater. Similarly, for the 2.8 µm magnetic beads, the maximum translational

velocity did not produce enough drag to overcome the magnetic interaction. Removal was only accomplished by abrasively removing the beads with a pipette tip.

Cursory, we observed that the nickel probe could be heated to induce convection, which then in turn increased the number of magnetically labeled cells that were close enough to be magnetically attracted to the microheater. These preliminary measurements suggest that higher currents cause higher temperatures and significant convection, resulting in an increased number of cells attaching to the microheater. Without convection the number of cells at the tip remained constant. Further experimentation and development are currently needed to observe either of these effects with our micro-heaters.

RAPID THERMAL LYSIS

To further demonstrate the possibility of using thermal magnetic microheaters to both isolate and thermally characterize magnetically labeled cells, we used a 75 µm Ni-Cr bent-wire micro-heater to investigated rapid thermal lysis. Figure 5 shows the effects of local heating on magnetically labeled HeLa cells. The probe is heated by resistive heating with a current of 400 mA. The black part of the image is the Ni-Cr probe. After 5 seconds of heating, most of the cells in the image are still healthy and appear to not be lysed. As described in [3] cell death is first indicated by fluorescence originating from the nucleus of the cell and lysis is indicated by uniform fluorescence from the entire cell. After 25 seconds of heating, both cases can be seen in Fig. 5. This indicates that the 75 µm Ni-Cr bent-wire probe can be used to locally heat magnetically labeled HeLa cells to induce both cell death and cell lysis. Furthermore, this occurred on the time scale as previously reported by other rapid thermal lysis probes [3]. We anticipate that with small micro-heaters, the temperature gradients will be more dramatic allowing for selective lysis of individual cells.

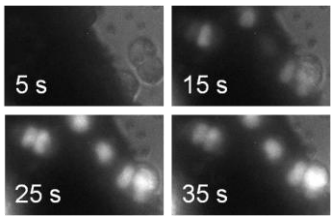


Figure 5. *Combined bright-field and fluorescence image of the Ni-Cr wire probe and HeLa cells.*

CONCLUSION

In conclusion, the designed magnetic thermal micro-heaters were fabricated out of nickel or by bending 75 µm Ni-Cr wire. The nickel micro-heaters were used for magnetophoresis, for micromanipulation of magnetic beads and magnetically labeled HeLa cells. The Ni-Cr micro-heaters were used to induce cell death and cause rapid thermal lysis. The ability to combine magnetic properties and heating in this manner could have applications for cell characterization or for selective cell lysis.

ACKNOWLEDGMENTS

The authors thank Dr. Brandon McNaughton for cell preparation and consulting. We thank Prof. Raoul Kopelman, Prof. Yogesh Gianchandani, and Dr. James Deatherage for their help and support. This research was funded by NIH grant number R43GM084520 (A.G.).

CONTACT

Angelo Gaitas, 333 Parkland Plaza, Ann Arbor, MI48103, USA, (734) 972-9348, angelo@picocal.com

REFERENCES:

[1] D.H. Kim, P.K. Wong, J. Park, A. Levchenko, Y. Sun, Microengineered Platforms for Cell Mechanobiology, Annual Review of Biomedical Engineering 11 (2009) 203-233.

[2] A.S. Basu and Y.B. Gianchandani, Virtual microfluidic traps, filters, channels and pumps using Marangoni flows, Journal of Micromechanics and Microengineering 18, 115031 (2008).

[3] N. Privorotskaya, Y.S. Liu, J. Lee, H. Zeng, J.A. Carlisle, A. Radadia, L. Millet, R. Bashir, W.P. King, Rapid thermal lysis of cells using silicon–diamond microcantilever heaters, Lab on a Chip 10 (2010) 1135.

[4] H.H. Kessler, G. Muhlbauer, E. Stelzl, E. Daghofer, B.I. Santner, and E. Marth, Fully Automated Nucleic Acid Extraction: MagNA Pure LC, Clinical Chemistry 47, 1124 (2001).

[5] Q.A. Pankhurst, J. Connolly, S.K. Jones, and J. Dobson, Applications of magnetic nanoparticles in biomedicine, Journal of Physics D Applied Physics 36, 167-181 (2003).

[6] S. Riethdorf, H. Fritsche, V. Müller, T. Rau, C. Schindlbeck, B. Rack, W. Janni, C. Coith, K. Beck, and F. Jänicke, Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system, Clinical Cancer Research 13, 920 (2007).

[7] A.R. Bausch, F. Ziemann, A.A. Boulbitch, K. Jacobson, and E. Sackmann, Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry, Biophysical Journal 75, 2038-2049 (1998).

[8] M. Tanase, N. Biais, and M. Sheetz, Magnetic tweezers in cell biology, Methods in Cell Biology 473-

493 (2007).
[9] K.C. Neuman and A. Nagy, Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy, Nature Methods 5, 491-505 (2008).
[10] L. Sacconi, G. Romano, R. Ballerini, M. Capitanio, M. De Pas, M. Giuntini, D. Dunlap, L. Finzi, and F.S. Pavone, Three-dimensional magneto-optic trap for micro-object manipulation, Optics Letters 26, 1359-1361 (2001).