

Hybrid Silicon/Silicone (polydimethylsiloxane) Microsystem for Cell Culture

Jennifer Blain Christen and Andreas G. Andreou

Abstract— We discuss the design, fabrication and testing of a hybrid microsystem for stand-alone cell culture and incubation. The micro-incubator is engineered through the integration of a silicon CMOS die for the heater and temperature sensor, with multilayer silicone PDMS (polydimethylsiloxane) structures namely, fluidic channels and a 4mm diameter, 30 μ L, culture well. A 25 micron thick PDMS membrane covers the top of the culture well, acting as barrier to contaminants while allowing the cells to exchange gases with the ambient environment. The packaging for the microsystem includes a flexible polyimide electronic ribbon cable and four fluidic ports that provide external interfaces to electrical energy, closed loop sensing and electronic control as well as solid and liquid supplies. The complete structure has a size of (2.5 \times 2.5 \times 0.6cm³). We have employed the device to successfully culture BHK-21 cells autonomously over a sixty hour period in ambient environment.

I. MICROSYSTEMS FOR CELL CULTURE

Cell culture is one of the most important tools for study of cell function, tissue engineering and pharmacology. Adaptation of traditional silicon-based technologies, advances in bio-materials [1] and soft lithography [2] have been applied to cell culture to produce microfluidic structures that allow for highly parallel testing of many small, varied populations of cells on a single platform with effort comparable to a single traditional experiment including study of growth, secretion or gene expression [3]. However, structures are typically fabricated on passive silicon dioxide (glass) substrates [4][5][6]. To give these systems more advanced functionality, they must go beyond passive structures to systems that incorporate sensing, actuation and control. One of the first examples of hybrid system integration involved a commercial CMOS APS (active pixel sensor) imager overlaid with microfluidic channels [7]. Note, that a custom fabricated silicon die for each use is not feasible considering the size of the die necessary to accommodate the fluidic geometries, spacing and interfaces particularly in a field that relies to great extent on disposable technologies. It is possible to both conceptually and physically divide the functionality required in active fluidic systems into centimeter dimension structures for transporting fluids, gases and matter and millimeter size devices for active environmental control, detection, amplification, signal conditioning and communication. A paradigm is presented, exemplified by the micro-incubator, for scalable, cost-effective system integration using soft lithography to create disposable microfluidics that incorpo-

rate reusable functional silicon blocks. Specifically, incorporating basic CMOS integrated circuits with multi-layer structures fabricated in PDMS to control and manipulate liquids and gases. The approach is aimed at simple, inexpensive fabrication methods for disposable components with elementary fluidic functions for use in tandem with expensive, highly functional components for reused many times in various systems.

II. MICRO-INCUBATOR SYSTEM ARCHITECTURE

Standard cell culture relies upon incubators to provide an environment with strictly controlled temperature, humidity and gas (pH) content, 37°C, 5% CO₂, and 100% RH. The micro-incubator mimics these conditions using a three-dimensional architecture while reducing the external inputs to a simple computer interface and 4 disposable syringes. The architecture for the micro-incubator (see Figure 1) is impervious to many of the most popular problems encountered during cell culture in microfluidic structures: heating/perfusion systems with sheer stress, gas exchange, bubbles, and cell growth in enclosed channels. These advantages and the extremely high tolerance on the alignment of the layers yield a system that is robust, simple to fabricate and a highly functional tool for research and commercial applications.

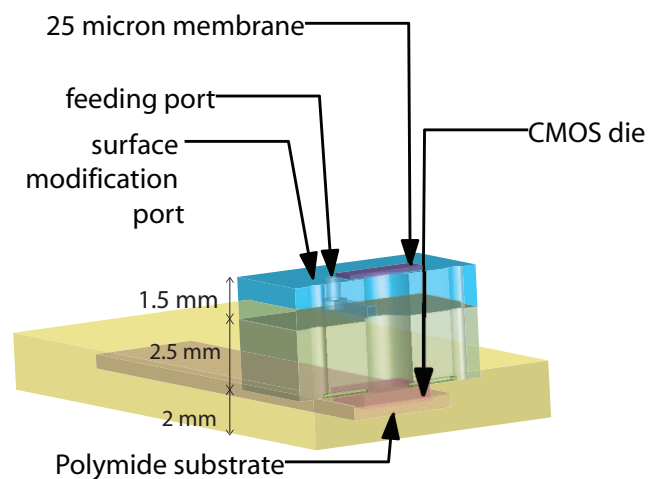


Fig. 1. Drawing of the cell-culture micro-incubator shown in cross-sectional perspective with simplified ports. The layers from bottom to top are electronics encapsulation (yellow) including polyimide substrate (orange) and CMOS die (pink), surface modification (green), feeding (blue), and breathing (purple)

J. Blain Christen and A.G. Andreou are with the Department of Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD 21218 USA blain, andreou}@jhu.edu

1) *Perfusion systems and cell shear stress*: Devices reported in the literature provide an environment quite different from the traditional culture vessels. The amount of media per surface area is much less than in traditional culture, requiring frequent or constant media exchange. Here we present an architecture that, is a scaled model of traditional vessels employing a three dimensional structure to obtain the same media per surface area as traditional culture vessels eliminating the need for an automated perfusion system. Devices requiring media exchange employ fluidic channels in a single PDMS layer flowing into the cell culture area. This flow causes shear stress necessitating complex feeding systems. A three dimensional architecture allows fluid to enter the culture area from above, changing the direction of the stress from shear to perpendicular, much more amenable to cell growth [8].

2) *Gas exchange*: A unique feature of the system discussed here is that the cell-culture area is capped with a thin, 25 micron layer of PDMS. This membrane serves as a breathing layer that encloses the cell-culture well, allowing gas exchange with the environment while the hydrophobic surface limits evaporation.

3) *Air gaps (bubbles)*: Another common problem in microfluidic systems is the formation of bubbles in the channels. The multilevel system in this paper does not suffer from formation of air bubbles, since the cells are allowed to grow exclusively in the well. The static system retains bubbles in the channel where they form. If bubbles form in the well, the height of the media and air pocket above allow the bubble to rise at the surface and dissipate.

4) *Cell Growth in channels*: The micro-incubator includes two sets of channels, the adhesion factor channels in the surface modification layer and the plating/feeding channels in the feeding layer. The adhesion factor channels have a height of $11\mu\text{m}$ and width of $20\mu\text{m}$; they directly interface the cell culture substrate, being formed directly above the CMOS die. Their location limits the adhesion factor to the bottom of the well ensuring cells proliferate there exclusively. The feeding channels are formed above the surface modification layer, 2.5-3mm above the well base and have a height of $150\mu\text{m}$ and width of either $200\mu\text{m}$ or $500\mu\text{m}$. These channels are used for both plating and feeding the cells. Since plating and surface modification are performed in separate channels, the cells do not adhere within any channel and are easily removed by flushing. It is important that the cells are not allowed to remain within the channels since the architecture depends on cell presence exclusively at the base of the well. The larger cross-sectional area of the plating/feeding channels not only allows cells pass through easily but also require very little pressure to create flow for feeding.

III. FABRICATION AND ASSEMBLY

A. Silicon Heater and Temperature Sensor

The heater and the temperature sensor were fabricated in a $1.6\mu\text{m}$ single polysilicon levels, two metal level CMOS technology with a die size of $4.6\times 4.7\text{mm}^2$. The heater and temperature sensor are partial loops of polysilicon formed

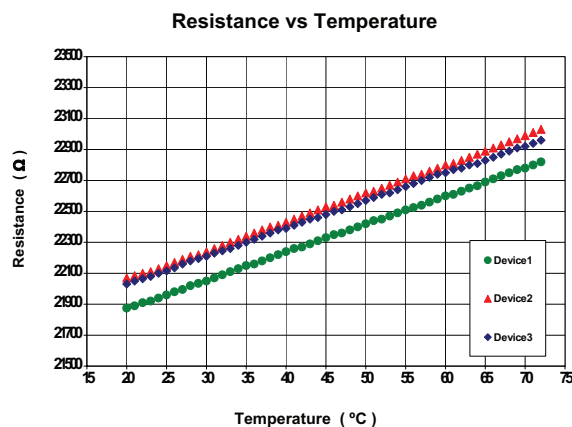
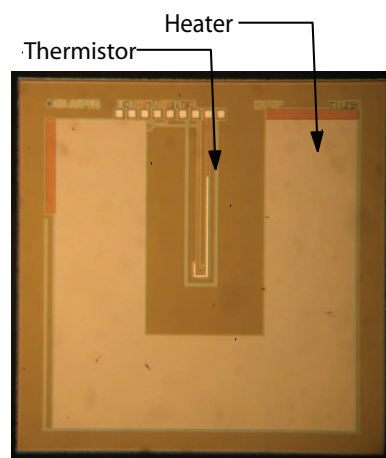


Fig. 2. Photomicrograph of the CMOS heater and temperature sensor (top). Experimental data for calibrating the polysilicon thermistor element (bottom).

along the perimeter and interior of the chip respectively. The heater resistance is 160Ω and the temperature sensor resistance is $20\text{-}25\text{k}\Omega$. Calibration was performed by heating the chip to known temperatures and measuring the resistance of the temperature sensor. It was determined empirically that a single calibration point is adequate to determine the temperature within half a degree, (Figure 2).

B. Soft Lithography with PDMS

1) *Master Mold Fabrication*: Master molds were fabricated by patterning photoresist on a three inch silicon wafer using 5000dpi transparencies masks printed by PageWorks. Three types of master molds were used: blank (Shibley S1813), positive (SJR 5740) and negative (SU8 2100). These molds were used to create the breathing layer, surface modification layer and feeding layers respectively. Lithography was performed as directed by manufacturer with the following exceptions. In addition to use as a master mold, blank masters were used as a substrate for both the positive and negative masters. Positive and blank masters were baked at 200°C overnight. In the first case to reflow the features and in the second case to withstand the SU8 development.

2) *Replica Molding*: Replica molding, a soft lithography technique, was used to pattern PDMS by simply pouring uncured PDMS over a patterned master mold, curing, and

peeling a permanently patterned negative from the master. General Electric RTV 615 part A and B pre-polymers were used and mixed in a 10 A to 1 B ratio by weight. Master molds were secured in flexible plastic containers and the pre-polymer mixture was cast before curing in a Blue-M oven. The thickness of the surface modification, 2.5-3.0mm, and feeding layer, 1.5-2.0mm, determined the height of the media and air pocket above the media respectively. The blank molds were used to create 25 μ m thick layer of PDMS by spinning 1.5mL of PDMS pre-polymer at 3000rpm for 45 seconds.

3) *Embedding Devices in Three Dimensional Structures:* Additional devices can be easily embedding in the PDMS to create three dimensional structures. Embedding simply involved placing the structure in the container with the mold before casting the PDMS. Device height can be controlled by creating a single layer in multiple steps: adding a portion of the PDMS, lightly curing, positioning the device adding the remaining PDMS and curing. For the micro-incubator a type J IRCO-010, Omega thermocouple was embedded in the surface modification layer to provide a second measurement of the well temperature.

4) *Trimming and Punching Fluidic Ports and Wells:* Once the PDMS was cured and peeled from the master mold, it was placed channel side up on a soft rubber cutting mat and trimmed with a razor blade. The well and ports were punched from the PDMS in the proper location, visible in the pattern. Either 4mm diameter brass tubing (well) or 23 gauge blunt end needles (ports) was punched through the PDMS perpendicular to the surface. The PDMS and punch were then lifted and the cut PDMS removed from the interior of the punch with a pair of tweezers.



Fig. 3. Photograph of the assembled two layer structure with wells, ports and interconnects formed (left) and the complete micro-incubator (right).

IV. INTEGRATION

The CMOS die was wire-bonded to a flexible polyimide coated substrate after cleaning with ethanol. The wire bonds were protected with acrylic adhesive and covered with a thin layer of PDMS. PDMS was then cast until just level with the top of the chip and lightly cured. As previously mentioned, it was important that layers were punched in the correct order. The feeding layer containing the upper level channels was punched first. Then, the well was punched in its companion surface modification layer. The wells of the two layers were roughly aligned and assembled. PDMS layers can be separated and re-assembled until the results are acceptable before curing. The alignment was unimportant to the function of the CMOS incubator, they need only

to overlap. The assembled layers were then lightly cured to seal the interface. Once the two layers were secured, the ports for the surface modification layer were punched through the entire structure. This formed the self-aligned port and interconnect through the feeding layer. Once the ports were punched, the assembled layers were placed atop the electronics layer with the well over the heater. Finally, the breathing layer was assembled above the well. This layer was trimmed generously cover the well, but not the ports. The final cure was performed in overnight at 65°C.

The connections from the fluidic ports were made with 23 gauge hypodermic tubing connected to flexible PVC Micro-Bore tubing fit over 23 gauge luer hub needles. The fluid supplies were provided through the luer hub connector using disposable syringes.

V. CELL CULTURE IN THE MICRO-INCUBATOR

Cell culture was performed using standard procedures with the BHK-21 cell line in CO₂-free media [9]. The micro-incubator was sterilized with ethanol. The interior of the assembly was flushed with ethanol, deionized water, air, deionized water and air. After the cleaning, fibronectin was applied to the cell culture area by flowing through the surface modification layer channels, left to stand for one to two hours and flushed with 1mL of PBS. Cells suspended at 10⁶/mL were then plated through the feeding layer channels and allowed to attach for approximately one hour. Although cells were present in the feeding channels they did not attach due to the absence of the adhesion factor. The channels were flushed to clear unattached cell, then the chamber was filled with media. Finally, the system was connected to the computer controlled test setup for incubation. The complete micro-incubator is shown in figure 3.

The temperature of the thermocouple was measured using a USB temperature measurement module with built in differential thermocouple measurements with cold junction compensation. The thermistor was controlled by a data acquisition card. The thermocouple was initially calibrated to the thermistor due to its distance from the cells. Subsequently, the reading from the embedded thermocouple was used to maintain the temperature of the incubator. The results for the final experiment are shown in figure 4. The chip was re-used to display the ability to use the CMOS die in multiple experiments. The bubbles that are visible in the micrographs dissipated over time.

VI. DISCUSSION AND FUTURE WORK

The use of multilevel PDMS in the design of the micro-incubator allows for a more sophisticated design with elevated microfluidic channels. The microfluidic channels and culture vessel architecture reduces shear stress on the cells and eliminates the need for complex feeding mechanisms [10]. The microelectronic substrate opens up a wide variety of capabilities in stimulation, sensing and actuation [11][12] as demonstrated in unheated devices.

Future designs will incorporate more sophisticated active CMOS circuits including amplifiers and sensors (e.g. PTAT)

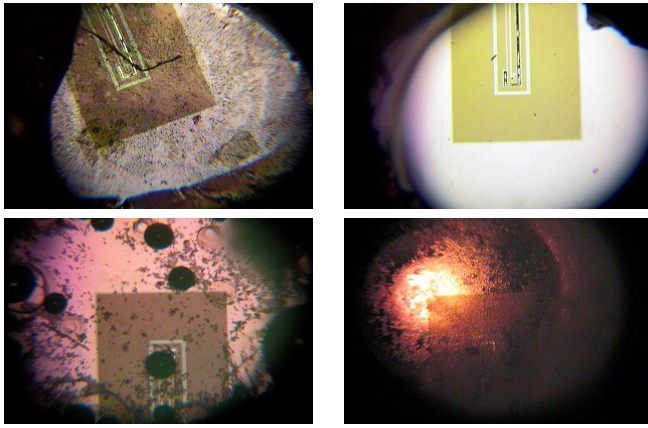


Fig. 4. Micrographs of micro-incubator during cell culture. Before and after cleaning (top) and cell growth after 12 and 60 hours (bottom).

and close loop control of the temperature such as the device reported in [13] for short term cell maintenance after culture in a standard incubator. While the work presented incorporates the single heater CMOS die, work is in progress to integrate a heater array in a PDMS micro-incubator and preliminary results were presented here [14].

Imaging the progress of cell culture was difficult, this is evident by the poor quality of the pictures in Figure 4. Future designs, will explore the use of a transparent CMOS substrate using xenon difluoride etched chips [15] and Silicon-on-Sapphire (SOS) CMOS technology. A wireless heater and temperature sensor has been designed, fabricated and tested [16] and it will be tested in future design of the micro-incubator.

VII. CONCLUSION

We have discussed a system design for an autonomous micro-incubator using commercially fabricated CMOS integrated circuit for heating and sensing. Multilevel PDMS channels and a well form the structures for culturing, testing and maintaining cells. Fabrication requires no special facilities or equipment other than a one time lithography step to create master replicas for the PDMS channels. All processing and integration steps, are amenable to batch fabrication and integration and hence the approach taken is viable for large scale industrial production and commercialization.

The laptop computer interface for the device makes it amenable for field testing and in a laboratory setting for long term cell culture studies, persistent electrical or optical observation, stimulation, or measurement while maintaining the incubation environment. The simplicity of both the fabrication and use of the system and the virtually unlimited capabilities offered by the CMOS substrate result in a microsystem with capabilities beyond any previously demonstrated.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grant ECS-0225489, "Cell Clinics On a Chip", a National Science Foundation Graduate Research Fellowship

and a National Science Foundation G K-12 Fellowship. The authors would like to thank Francisco Tejada for his assistance with wire bonding and Jill Blain for her assistance with photography. CMOS chip fabrication was provided by MOSIS. We thank Prof. Murray Sachs, director of the Whitaker Biomedical Engineering Institute and Prof. Jennifer Elisseeff for graciously allowing the use of the shared tissue engineering laboratory in Clark Hall. The soft-lithography processing was done in the Whitaker Institute Fabrication and Lithography Facility at Johns Hopkins University.

REFERENCES

- [1] R. Langer and D. A. Tirrell, "Designing materials for biology and medicine," *Nature*, vol. 428, pp. 487–492, April 2004.
- [2] Y. Xia and G. Whitesides, "Soft lithography," *Angewandte Chemie International Edition*, vol. 37, no. 5, pp. 550–575, Dec. 1998.
- [3] N. Li, A. Tourovskaia, and A. Folch, "Biology on a chip: Microfabrication for study of the behavior of cultured cells," *Critical Review in Biomedical Engineering*, vol. 31, no. 5 and 6, pp. 423–488, 2003.
- [4] N. Cohen, "The development of spontaneous beating activity in cultured heart cells: From cells to networks," Ph.D. dissertation, Israel Institute of Technology, Kislav, 5761, Haifa, Israel, December 2000.
- [5] W. Gu, X. Zhu, N. Futai, B. Cho, and S. Takayama, "Computerized microfluidic cell culture using elastomeric channels and braille displays," *Proceedings of the National Academy of Sciences*, vol. 10, no. 45, pp. 15 861–15 866, 2004.
- [6] P. Hung, P. Lee, P. Sabouchi, N. Aghdam, R. Lin, and L. Lee, "A novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array," *Lab on a Chip*, vol. 5, no. 44–48, pp. 44–48, 2005.
- [7] M. Adams, S. Quake, and A. Scherer, "On-chip absorption and fluorescence spectroscopy with polydimethylsiloxane (PDMS) microfluidic flow channels," *2nd Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine and Biology*, vol. 1, no. 1, pp. 369–373, May 2002.
- [8] G. Walker, H. Zeringue, and D. Beebe, "Microenvironment design considerations for cellular scale studies," *Lab on a Chip*, vol. 4, pp. 91–97, 2004.
- [9] J. Blain Christen and A. Andreou, "Integration of silicon CMOS with multilayer silicone (PDMS) fluidics into a Micro-Incubator for cell culture studies," *Transactions on Circuits and Systems Part I: Regular Papers: Special Issue Life Science Systems and Applications*, (submitted).
- [10] M. Shin, K. Matsuda, O. Ishii, H. Terai, M. Kaazempour-Mofrad, J. Borenstein, M. Detmar, and J. Vacanti, "Endothelialized networks with vascular geometry in microfabricated poly(dimethylsiloxane)," *Biomedical Microdevices*, vol. 6, no. 4, pp. 269–278, 2004.
- [11] M. Hutzler and P. Fromherz, "Silicon chip with capacitors and transistors for interfacing organotypic brain slice of rat hippocampus," *European Journal of Neuroscience*, vol. 19, pp. 2231–2238, 2004.
- [12] Y. Liu, E. Smela, N. Nelson, and P. Abshire, "Cell-Lab on a chip: A CMOS-based microsystem for culturing and monitoring cells," IEEE EMBS. San Francisco CA, USA: IEEE, September 1-5 2004.
- [13] B. Debusschere and G. Kovacs, "Portable cell-based biosensor system using integrated CMOS cell-cartridges," *Biosensors and Bioelectronics*, vol. 16, pp. 543–556, 2001.
- [14] J. Blain Christen and A. Andreou, "CMOS heater array for incubation environment cellular study," *Proceedings of the 48th Midwest Symposium on Circuits and Systems*, August 2005.
- [15] J. Blain Christen, C. Davis, M. Li, and A. Andreou, "Design, double sided post-processing, and packaging of CMOS compatible Bio-MEMS device arrays," *IEEE International Symposium on Circuits and Systems*, 2002, vol. 1, pp. 665–668, May 2002.
- [16] E. Culurciello and A. Andreou, "3D integrated sensors in silicon-on-sapphire CMOS," *Proceedings of the 2006 IEEE International Symposium on Circuits and Systems*, 2006.