Integration of Discrete Sensors and Microelectrode Arrays Into Open Microfluidic Hanging-Drop Networks

Conference Paper

Author(s):
Bounik, Raziyeh; Gusmaroli, Massimiliano; Misun, Patrick; Viswam, Vijay; Hierlemann, Andreas; Modena, Mario M.

Publication date:
2019-10-17

Permanent link:
https://doi.org/10.3929/ethz-b-000376523

Originally published in:
https://doi.org/10.1109/MEMSYS.2019.8870732

Funding acknowledgement:
171267 - Integration of CMOS sensors and circuits into microphysiological hanging-drop networks (SNF)
694829 - Microtechnology and integrated microsystems to investigate neuronal networks across scales (EC)

INTEGRATION OF DISCRETE SENSORS AND MICROELECTRODE ARRAYS INTO OPEN MICROFLUIDIC HANGING-DROP NETWORKS

Raziyeh Bounik[1], Massimiliano Gusmaroli[1], Patrick M. Misun[1], Vijay Viswam[1,2], Andreas Hierlemann[1] and Mario M. Modena[1]

[1]ETH Zurich, Dept. of Biosystems Science and Engineering, Bio Engineering Laboratory, Basel, SWITZERLAND

[2] MaxWell Biosystems AG, Basel, SWITZERLAND

ABSTRACT

This work presents a new versatile method to integrate discrete sensor chips into open-microfluidic hanging-drop networks. As proof of concept, we integrated an electrical-impedance-spectroscopy (EIS) sensor unit into a hanging-drop platform for monitoring the size of microtissues. The fabrication and assembly method enables the integration of a large variety of silicon-based sensors, such as chemical sensors, biosensors or microelectrode arrays (MEAs), in open-microfluidic hanging-drop networks to perform studies on three-dimensional microtissues.

INTRODUCTION

Motivation

Three-dimensional (3D) microtissues and organoids have been emerging as biomedical model systems to investigate complex tissues and organotypical functions in-vitro. Compared to two-dimensional (2D) cell cultures, these model systems more accurately recapitulate physiological conditions, such as real tissue organization and interactions between different cell types. Hanging-drop network platforms are microfluidic devices that can be used for the formation and culturing of 3D microtissues at large scales [1]. The perfusion features of these platforms enable to study the interaction between different types of microtissues in so-called body-on-a-chip configurations, as metabolites and secreted biomolecules can be exchanged and transported between the different tissue models by the common circulating liquid phase.

Recently, several attempts have been made to introduce sensor technologies into hanging-drop platforms, as complementary readouts to microscopy characterization. We previously developed an EIS unit [2] and electrochemical biosensors [3] to perform in-situ measurements with different types of microtissues. The EIS unit was used to measure organotypic functions like beating frequency of cardiac microtissues, while electrochemical biosensors were used to measure secreted and consumed biomolecules in the culturing medium. These sensors were fabricated on custom-designed glass plug-ins and were precisely aligned with the microfluidic structure to form a continuous fluidic network and to avoid liquid leakage.

To advance over previous work, we present here a new method to integrate discrete sensors and microelectrode arrays systems [4], which commonly feature dimensions in the millimeter range, into hanging-drop-network platforms. A new system with the following key features is presented:

1) the integration of independently fabricated and/or small, e.g., CMOS-based sensor units, into the fluidic system is enabled;

2) the same sensor units can be used in different fluidic networks without the need to modify the sensor dimensions and to alter the fabrication and assembly process.

Concept

Figure 1 shows the three main parts of our platform including a microfluidic hanging-drop network, a glass substrate, and two discrete sensors for EIS recordings. The microfluidic system consists of two fluidic networks formed by a center droplet on top of the sensor chip, to host the sample tissue, and two lateral droplets to provide extra medium and for hosting microtissues that are fluidically connected to the main sample of interest. Moreover, two rectangular openings were realized close to the center wells for the bond wires between the chips and the glass substrate. The glass substrate accommodates the metal leads to connect signals and supply lines from the sensor chip to the off-chip recording and data acquisition unit.

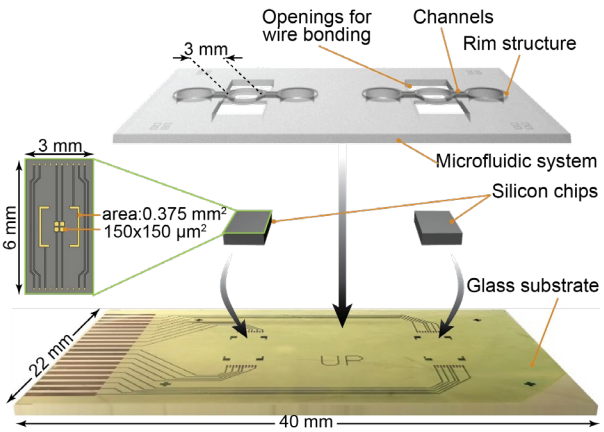

Figure 1: *Main components of the device: a microfluidic hanging-drop system in PDMS, two silicon chips with platinum electrodes (U-shapes and squares), and a glass substrate.*

Each sensor chip features four small square-shape inner electrodes and two U-shape outer electrodes (all electrodes are marked in yellow in the inset in Figure 1). This configuration of electrodes enables us to measure different parameters by EIS, including microtissue size and droplet height [5]. As the protection of the bond wires is one of the main challenges in the integration and

assembly steps, two shorted connections between pads were realized on each side of the chip to test the correct connection of the bond-wires to the pads.

METHODOLOGY & SYSTEM ASSEMBLY

The schematic of the microfluidic chip is shown in Figure 2. The fluidic chip is patterned on both sides to define the fluidic network at the top side and for accommodating the sensor units at the bottom side. The chip is made of polydimethylsiloxane (PDMS) and fabricated by soft lithography using a double-molding procedure obtained by flowing liquid PDMS between a bottom 3D-printed mold and a top silanized-PDMS mold.

To fabricate the sensor chips, platinum electrodes were patterned on a 4-inch silicon wafer by first depositing a silicon nitride isolating layer, followed by platinum deposition and patterning via standard lift-off photolithography. Finally, a silicon nitride passivation layer was deposited and patterned to protect and insulate the connections. Similarly, the glass substrate was fabricated by platinum patterning and silicon nitride passivation of a 4-inch borosilicate glass wafer.

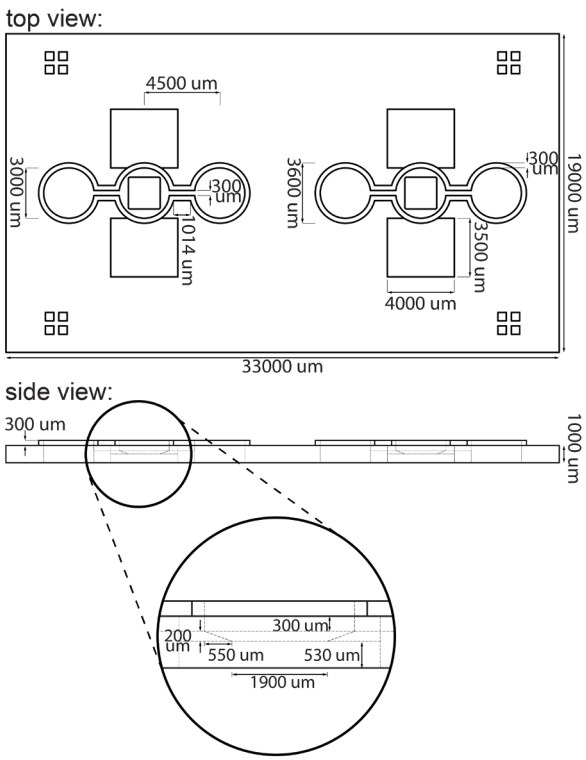

Figure 2: *Schematic of the microfluidic chip.*

Integrations of the sensor units was obtained by first attaching the sensor chips to the glass substrate by using a thin layer of a two-component epoxy (EPO-TEK H70E). Then, the microfluidic chips were aligned with the sensor units and plasma-bonded onto the glass substrate (30s at 50W, 0.4 mbar O_2, Diener Electronic, Ebhausen, Germany). Wire bonding was then performed to connect the pads on the sensor units to the pads on the glass substrate with a semiautomatic bonding machine (TPT HB16 wire bonder) operating in ball-ball ultrasonic bonding mode. Bond-wires were covered with epoxy (EPO-TEK 353ND) for protection, which was cured for 12 hours at 80°C. Faster curing at higher temperature could lead to strong epoxy shrinkage that may affect the bond-wire connections. Finally, fluidic inlets and outlets were realized by drilling holes in the glass substrate with a 800-µm-diameter diamond tip. A second PDMS layer containing two 1 mm holes was then bonded on the back side of the PCB to seal the tubing connections to the holes (Figures 3, 4).

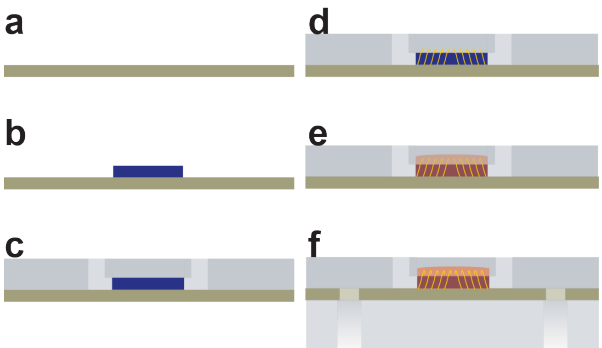

Figure 3: *Integration steps: a) Glass substrate, b) Chip attachment, c) Plasma bonding of the PDMS microfluidic system on the substrate, d) wire bonding, e) epoxy coverage of bond wires, f) Inlet/outlet drilling and backside PDMS bonding.*

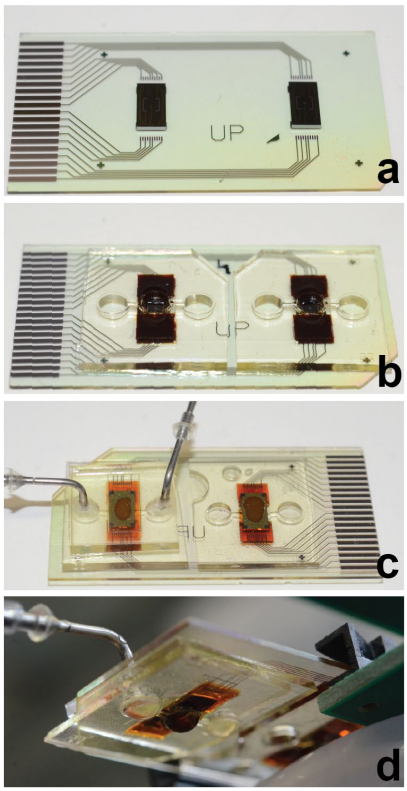

Figure 4: *Integration steps: a) silicon chips were glued onto the glass substrate using epoxy; b) PDMS microfluidic structures were bonded onto the substrate using plasma, and bond-wires were connected and covered with epoxy; c) fluidic inlet/outlets were made by drilling holes in the glass; d) the integrated system was connected to the recording unit and placed on the microscope stage. for operation.*

EXPERIMENTAL SETUP

The glass substrate was plugged in a custom-made printed-circuit board (PCB) for multiplexing the sensor signals and connecting them to the recording unit, an HF2 impedance spectroscope (Zurich Instruments AG, Zurich Switzerland). The control of the signal routing on the PCB was done via a custom-designed LABVIEW program.

The device was placed in a holder frame, which was located on the stage of an inverted microscope (NIKON Ti Eclipse). The experimental setup was tested using typical culture conditions, namely 37ºC and 95% humidity. Nemesys syringe pumps (Cetoni GmbH, Korbussen, Germany) were connected to the inlet and outlet of the chip to initiate fluid flow.

RESULTS

An optical feedback loop was used to measure the drop height by focusing on a glass bead inside the center droplet and to control the pumps to compensate for medium evaporation. Phosphate-buffered saline (PBS) solution has been used as the medium in the presented experiments. A constant flow of 0.3 µl/min was applied to replenish the solution while keeping the solution conductivity constant, which could be affected by salt upconcentration due to evaporation. Impedance measurements at 100 kHz were acquired by applying a 100 mV sinusoidal signal between the two inner small electrodes. Because of the small distance between these electrodes (200um pitch), the recorded impedance is almost independent of the drop size and mainly determined by the solution conductivity.

Figure 5 shows that we could arbitrarily select different drop heights using the optical feedback loop, while continuous perfusion of the solution enabled to maintain a constant medium conductivity between the electrodes.

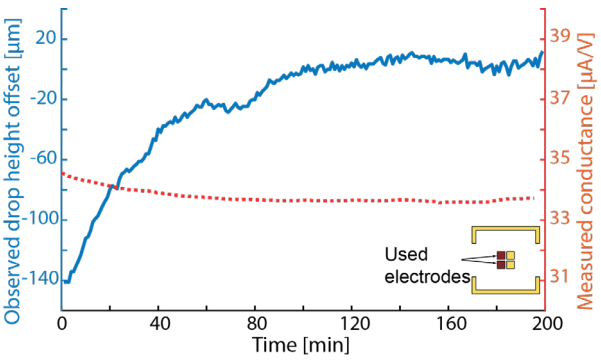

Figure 5: *Drop height, as measured by the autofocus system of the microscope, and solution conductance at 100 kHz, measured between the two inner electrodes.*

To investigate the relation between the recorded impedance and the electrode-to-sample distance, a 1-mm glass bead was placed in the center droplet, and EIS measurements between a center and a lateral electrode were conducted for different drop heights. Figure 6 shows that, as the drop height decreased and the bead approached the electrodes, the measured impedance significantly increased, as expected by the placement of a dielectric bead between the electrodes. Furthermore, as a control, measurements without glass beads were performed at 100 kHz to confirm that the detected current changes were caused by the bead (Figure. 6-b).

CONCLUSIONS

In conclusion, we developed a method to integrate discrete sensor units into hanging-drop network platforms. As a proof of concept, we integrated discrete EIS sensor chips into 3-drop microfluidic platforms. Experimental results showed that the system is stable in terms of fluidic network characteristics and electrical connections. The device enabled us to continuously monitor medium conductivity and to sense the electrode-to-sample distance. We anticipate that the integration strategy, presented here, will enable us to use multi-functional MEA sensor units for the characterization of 3D microtissues.

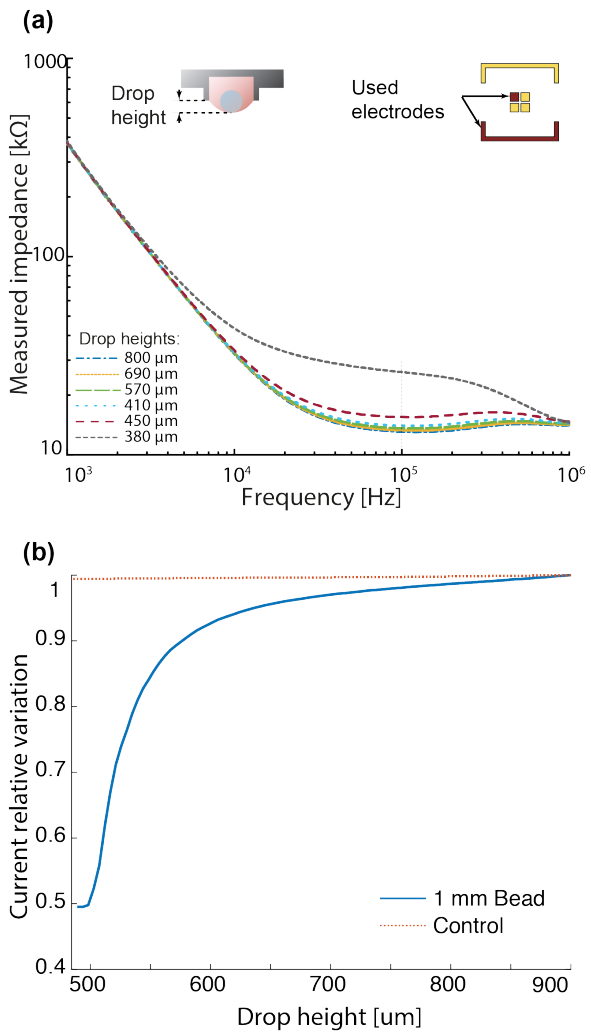

Figure 6: *a) EIS of 1-mm-glass beads inside a hanging drop, measured by using a center and a U-shaped electrode. b) Relative current variation at 100 kHz as a function of drop height.*

ACKNOWLEDGEMENTS

This work was supported by the Swiss National

Science Foundation through MHV-Grant 171267 as well as by the European Community through the ERC Advanced Grant "neuroXscales" under contract number AdG 694829.

REFERENCES

[1] O. Fey, P. M. Misun, D. a Fluri, J. G. Hengstler, and A. Hierlemann, "Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis," *Nat. Commun.*, 2014, vol. 5, no. 1, p. 4250.

[2] S. C. Burgel *et al.*, "Simultaneous impedance spectroscopy and stimulation of human iPS-derived cardiac 3D spheroids in hanging-drop networks," in *2015 28th IEEE International Conference on Micro Electro Mechanical Systems (MEMS)*, 2015, pp. 226–229.

[3] P. M. Misun, J. Rothe, Y. R. F. Schmid, A. Hierlemann, and O. Frey, "Multi-analyte biosensor interface for real-time monitoring of 3D microtissue spheroids in hanging-drop networks," Microsystems and Nanoengineering, 2016, vol. 2, pp. 91–94.

[4] J. Dragas *et al.*, "In vitro multi-functional microelectrode array featuring 59 760 electrodes, 2048 electrophysiology channels, stimulation, impedance measurement, and neurotransmitter detection channels," *IEEE J. Solid-State Circuits*, 2017, vol. 52, no. 6, pp. 1576–1590.

[5] Y. R. F. Schmid, S. C. Bürgel, P. M. Misun, A. Hierlemann, and O. Frey, "Electrical impedance spectroscopy for microtissue spheroid analysis in hanging-drop networks," *ACS Sensors*, 2016, vol. 1, pp. 1028-1035.

CONTACT

Raziyeh Bounik, tel: +41-61-387-3226;
raziyeh.bounik@bsse.ethz.ch