Intelligent Multi-Sensor Array for *In Vitro* Detection of Neurotransmitters Secreted from Neurons on-a-Chip

Teddy Zagardan[1], Roman Khourin[2], Gad Vatine[2] and Hadar Ben-Yoav[1]*
[1]Nanobioelectronics Laboratory (NBEL), Department of Biomedical Engineering,
Ben-Gurion University of the Negev, 8410501 Beer Sheva, Israel
[2]Regenerative Medicine and Stem Cell (RMSC) Research Center,
Ben-Gurion University of the Negev, 8410501 Beer Sheva, Israel

NanoBioElectronics Laboratory
Ben-Gurion University of the Negev

Introduction

Neurotransmitters are biomolecules secreted from neurons and can indicate the presence of various brain diseases. Therefore, detecting neurotransmitters can improve diagnosis, treatment and provide new insights to neurodegenerative diseases. Current methods are expensive, complex and require pre-treatment steps, while electrochemical sensors can provide an inexpensive and easily operatable analytical tool for the sensitive, rapid and selective determination of the electrochemically active neurotransmitters [1]. Here we present a microelectrodes array fabricated from glassy carbon (GC)—a biocompatible material, resistant to molecules adsorption, and has a wide electrochemical window [2]. The GC microelectrode are made of SU-8 photoresist that is pyrolyzed to produce carbon conductive microelectrodes. We successfully grow neurons on the array and measure a set of voltammograms generated by the neurons. By recording electrochemical signals from different locations in the same environment, we plan to monitor the spatiotemporal behaviour of neurons and their response to external stimulation.

Fabrication & Validation

Photolithography Process

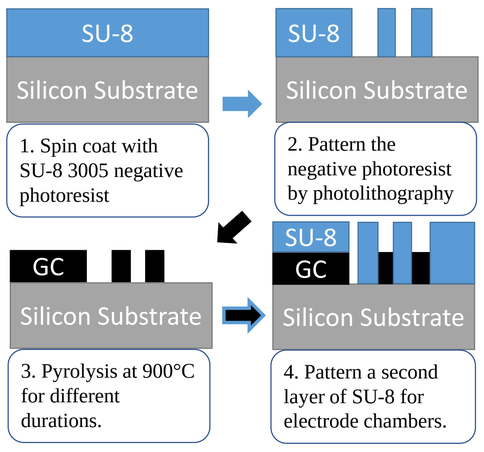

Figure 1: Lithography and pyrolysis process to fabricate glassy carbon microelectrode array.

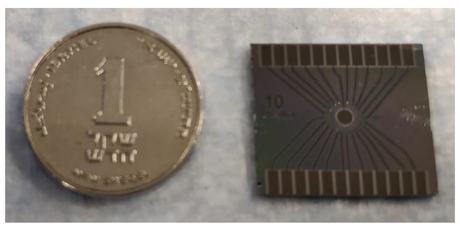

Figure 2: Fully fabricated microelectrodes array chip.

Details

- Spin coating at 3000[rpm] of SU-8 3005.
- Exposure time: 50[sec].
- Gap: 40[µm].
- Pyrolysis at 900°C with low pressure chemical vapor deposition "CTR-125".

Microelectrode Array Electrochemical Setup

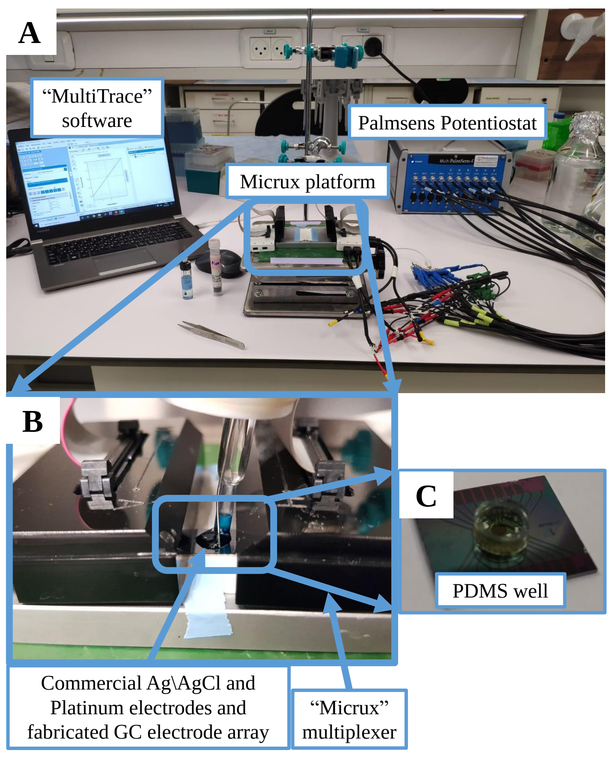

Figure 3: Multi electrode array measurement setup. General setup (A) close-up to measurement area (B) Polydimethylsiloxane (PDSM) well attached to the chip for measurement fluid storage (C).

Details

- Potentiostat: "MultiPalmSens4".
- "Micrux" multi-electrodes chip platform.
- Commercial Ag\AgCl reference electrode and platinum counter electrode.
- "MultiTrace" computer software.

Electrochemical Validation

Maximum and minimum electrochemical current were examined against pyrolysis parameters: 900°C dwelling time (1, 2 and 4 hours) and ramp rate (5, 10 and 15[C°/min]) at N_2 atmosphere. The optimization process showed that 4 hours and 10[C°/min] yielded the highest absolute values.

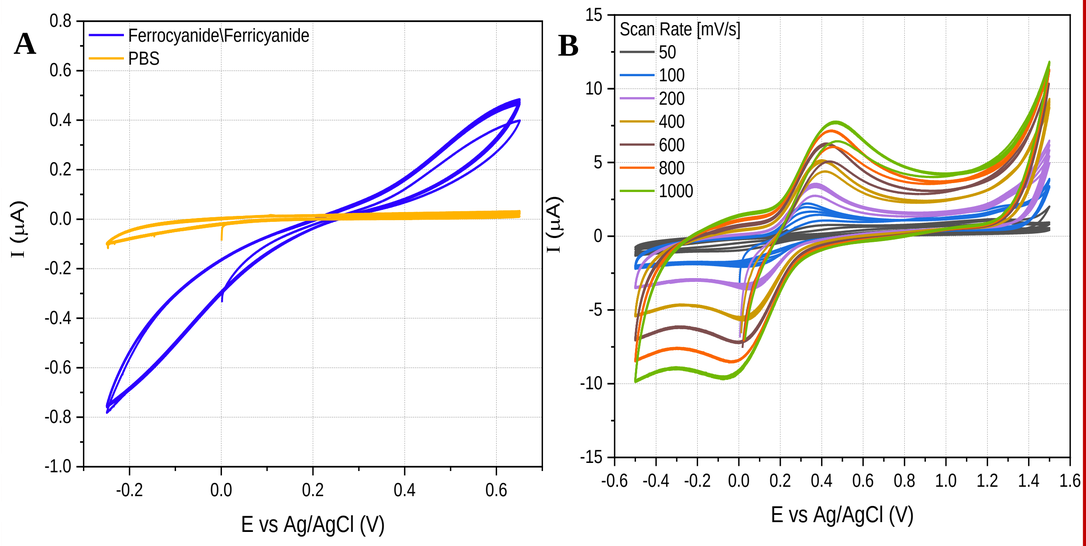

Figure 4: Electrochemical signal measured from either buffer (red) or ferrocyanide\ferricyanide solution (blue) by using the glassy-carbon microelectrode (A) Electrochemical signal measured from 5mM\5mM ferrocyanide\ferricyanide solution for different scan rates (B).

Results

- ✓ The electrodes display activity for a known analyte (ferrocyanide\ferricyanide).
- ✓ The GC electrodes electrochemical signals correspond to Randles-Sevcik equation.
- ✓ Longer dwelling time at 900°C yielded higher absolute current values.
- ✓ Ramp rate of 10[C/min] and four hours 900°C dwelling time were chosen as the optimal parameters.

Neurons on-a-Chip

Seeding & Measuring

Seeding process

1. Preparing surface: Poly-D-Lysine 0.1ug/ml
2. Supporting growth and differentiation: Laminin 50mg/ml
3. Adding neuronal differentiation medium with neurons
4. Fast scan cyclic voltammogram measurement

Figure 5: Neurons on-a-chip seeding process

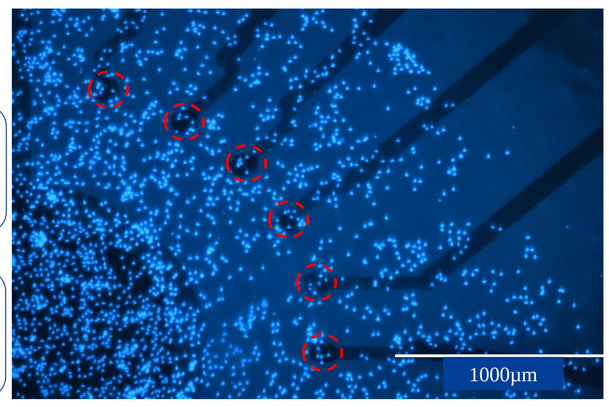

Figure 6: Fluorescent images of neuronal nucleus colored with DAPI on glassy carbon microelectrodes circled in red.

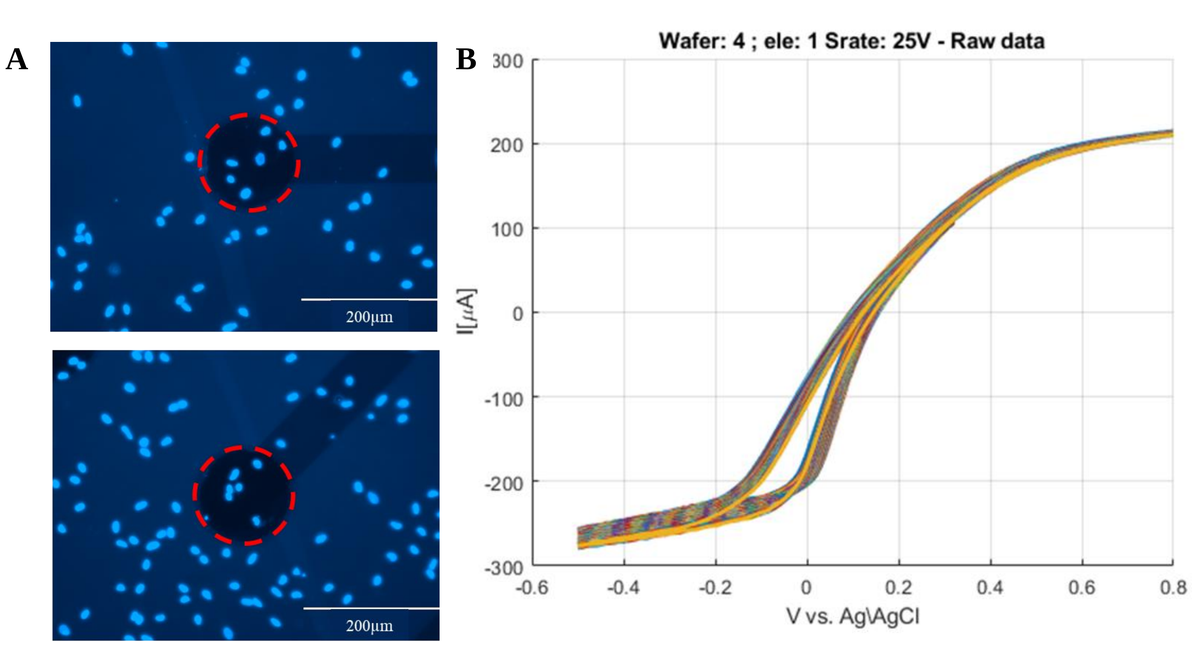

Figure 7: Measurement process (A) Fluorescent images of neuronal nucleus colored with DAPI on glassy carbon microelectrodes 1 (upper) & 2 (lower). (B) Fast scan cyclic voltammograms measurements of electrode 1 with neurons on a chip at 25V scan rate. (C) PCA scores of each cycle for each electrode. Shape and color associate each cycle to its electrode.

Analysis

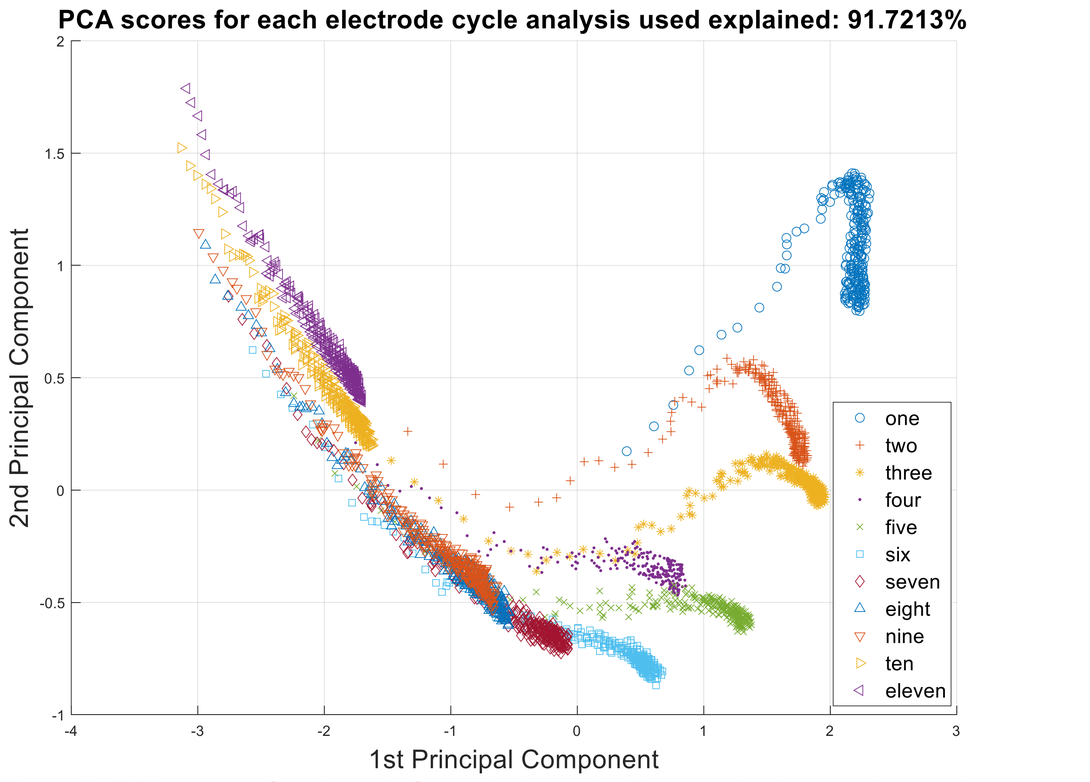

Figure 8: PCA scores of each cycle for each electrode. Shape and color associate each cycle to its electrode.

Conclusions

- ✓ Neurons were successfully seeded and grown on top of the fabricated microelectrode array lab-on-a-chip.
- ✓ The generated cyclic voltammograms correspond can be traced to the specific electrode.

Future Work

- Simultaneous measurement of the microelectrode array electrodes. Measuring simultaneously across 10 electrodes within the microelectrode array provide valuable insights on the interactions between the neurons and within the cell culture.
- Link the generated signals to the neurons aggregation on top of a microelectrode.
- Measuring the electrochemical in response to a chemical stimulant and differentiating between stimulated and passive electrochemical signals.

Acknowledgement

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References

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• Corresponding author: Dr. Hadar Ben-Yoav. Email: benyoav@bgu.ac.il