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MEA Application Note:
Cortical and Hippocampal Cryopreserved Neurons

MEA

Imprint

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A major part of this information is based on the instructions provided by members of the Neurochip consortium in Düsseldorf, Prof. Mario Siebler and Wiebke Fleischer; and the staff of QBM Cell Science, especially Dr. Babben Tinner-Staines.

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Introduction

About this Application Note

The intention of the MEA Application Notes is to show users how to set up real experiments with the MEA-System on the basis of typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes a complete protocol for the cultivation of *ready-to-use* cryopreserved primary neurons, suggestions for long term cultures, suggestions for MEA-System configurations, example data, and references.

Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us, especially the following persons:

Wiebke Fleischer

Babben Tinner-Staines

Material

Biological Materials

- *Ready to use* cryopreserved primary neurons (from hippocampus or cortex) from QBM Cell Science

Technical Equipment

- MEA-System (with amplifier and data acquisition, see Suggested MEA-System)
- Stimulus generator
- MEAs (microelectrode arrays)
- Sterile workbench
- Incubator set to 35 °C, 65 % relative humidity, 9 % O_2, 5 % CO_2
- Water bath at 37 °C
- Liquid nitrogen freezing tank
- Stereo microscope
- Inverted microscope
- Micropipettes and pipette tips (1000 µl)
- 15 ml BD Falcon tubes
- Teflon membranes (ALA Scientific Instruments)

Chemicals

- 70 % alcohol for disinfection
- Polyethylenimine (PEI)
- Laminin
- Glutamine
- Neurobasal Medium with B27 supplement (Gibco/Invitrogen)
- [Optional: penicillin/streptomycin]

Media

- Neurobasal medium
- B-27 supplement
- 0.5 mM Glutamine
- [optional: 50 U/ml penicillin/streptomycin] **This medium is recommended by QBM Cell Science, the provider of the cryopreserved neurons**

Methods

MEA Coating

Depending on the type of selected MEA, various coatings may be applied. Standard MEAs should be coated with Polyethylenimine or Laminin. Suggestions for coating methods can be found in the MEA manual available in the Download section of the Multi Channel Systems web site.

Thawing and Starting the Culture

Important: Do not centrifuge or vortex the cells. Keep the time between removing the vial from the liquid nitrogen tank and placing it into the water bath as short as possible.

1. Store the cells in liquid nitrogen until use.
2. Remove a vial with the cells from the liquid nitrogen and place it into a water bath pre-heated at 37 °C to thaw the cells.
3. After 2.5 minutes, remove the vial with the thawed cells and disinfect the outside of the vial with 70 % ethanol. Place it under a laminar flow hood. Proceed with the next step immediately after thawing.
4. Gently transfer 1 ml of the cells into a 15 ml centrifuge tube and immediately add 37 °C pre-warmed medium dropwise onto the cells, while rotating the tube by hand. This procedure should take approximately 2 minutes. We recommend addition of 4 ml medium resulting in a final cell density of 800.000 per ml.

Important: Do not add the whole volume of the medium to the cells at once. This may result in an osmotic shock. If the same vial is to be used for several different experiments in parallel, mix the cells by pipetting slowly up and down once, then aliquot the cells into the appropriate vessels.

5. Mix the cell suspension by inverting the tube carefully, twice.
6. Transfer the cell suspension into the MEA culture chamber, about 0.6 – 1.6 ml (depending on the maximum volume of the MEA culture chamber).
7. Incubate the cells in an incubator for 4 hours.
8. Remove the medium from the cells leaving a small liquid layer to ensure that the cells do not dry out and add 1.6 ml fresh, 37 °C preheated medium.
9. Incubate the cells at 37 °C with 95 % relative humidity and 5 % CO_2 until use.

Important: Cell death at the beginning of the cultivation should be considered normal. Generally, there are enough viable cells left for cultivation.

Maintenance

→ Change the medium at day 5 after thawing.

→ Replace 50 % of the medium with fresh, 37 °C preheated medium every 3 to 4 days. Warm an appropriate amount of medium to 37 °C in a sterile tube. Remove 50 % of the medium from the cell culture. Replace with the warmed, fresh medium and return the cells to the incubator.

→ Avoid repeated warming and cooling of the medium. Warm only the volume that is needed for a single procedure.

→ Compensation for media loss due to evaporation should be taken into consideration. Add additional media whenever necessary.

→ After 2 – 3 weeks of cultivation, the cells are ready for recording.

Longterm Culturing

In order to allow long term cultivation and recording, Multi Channel Systems MCS GmbH recommends the use of teflon membranes (fluorinated ethylene-propylene, 12.5 microns thick) developed by Potter and DeMarse (2001). The ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc., and distributed via the world-wide network of MCS distributors.

The sealed MEA culture chamber with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable membrane from Dupont that is selectively permeable to gases (O_2, CO_2), but not to fluid, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents
a dry-out of the culture.

Suggested MEA-System

System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from cultured neurons.

- **MEA2100-System**: For the MEA2100-HS60(HS2x60)-System, the same MEAs can be used as for the MEA1060 amplifiers, with the same advantages and disadvantages. In addition, for the MEA2100-HS120-System a 120-channel MEA layout is available (120MEA200/30iR), which provides a larger electrode field with a resolution identical to the 60MEA200/30iR. This might be interesting for a mapping application. The MEA2100-System allow to operate a maximum of two headstages with up to two 60 channel MEAs each. If all those slots are used with 6-well MEAs 24 wells can be tested simultaneously.
- **USB-MEA60-System**:60-channel MEA recording system for inverted or upright microscopes. The temperature controller **TC02** regulates the temperature of the MEA and of the perfusion fluid via the perfusion cannula **PH01**. A MEA1060 amplifier allows recording up to 60-channels from a MEA. This is the standard configuration for low-throughput academic research.

Microelectrode Arrays

Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see the Multi Channel Systems MCS GmbH web site or contact your local retailer.

Recommended MEAs include:

- **60 channel MEA** (60MEA200/30iR-Ti) with substrate-integrated reference electrode and TiN electrodes for establishing the cell culture and recording routine (for MEA1060 amplifiers and MEA2100-HS60 or MEA2100-HS2x60 headstages).
- **Economic MEA** (60EcoMEA or 60EcoMEA-Glass) for routine recordings with a medium throughput (for MEA1060 amplifiers and MEA2100-HS60 or MEA2100-HS2x60 headstages).
- **60ThinMEA**for high-resolution imaging. Thin MEAs are only 180 µm "thick" and mounted on a robust ceramic carrier. Tracks and contact pads are made of transparent ITO.
- **120 channel MEAs** (120MEA200/30iR-Ti) for MEA2100-HS120-System only**.**

References

1. F.J. Arnold, F. Hofmann, C.P. Bengtson, M. Wittmann, P. Vanhoutte, H. Bading, Microelectrode array recordings of cultured hippocampal networks reveal a simple model for transcription and protein synthesis-dependent plasticity, J Physiol 564 (2005) 3-19.

2. Hulata, E., R. Segev, et al. (2000). "Detection and sorting of neural spikes using wavelet packets." Phys Rev Lett **85**(21): 4637-40.

3. Hardingham, G. E., F. J. Arnold, et al. (2001). "A calcium microdomain near NMDA receptors: on switch for ERK-dependent synapse-to-nucleus communication." Nat Neurosci **4**(6): 565-6.

4. Potter, S. M. (2001). "Distributed processing in cultured neuronal networks." Prog Brain Res **130**: 49-62.

5. Potter, S. M. and T. B. DeMarse (2001). "A new approach to neural cell culture for long-term studies." J Neurosci Methods **110**(1-2): 17-24.

6. Segev, R., Y. Shapira, et al. (2001). "Observations and modeling of synchronized bursting in two-dimensional neural networks." Phys Rev E Stat Nonlin Soft Matter Phys **64**(1 Pt 1): 011920.

7. Streit, J., A. Tscherter, et al. (2001). "The generation of rhythmic activity in dissociated cultures of rat spinal cord." Eur J Neurosci **14**(2): 191-202.

8. Marom, S. and G. Shahaf (2002). "Development, learning and memory in large random networks of cortical neurons: lessons beyond anatomy." Q Rev Biophys **35**(1): 63-87.

9. Segev, R., M. Benveniste, et al. (2002). "Long term behavior of lithographically prepared in vitro neuronal networks." Phys Rev Lett **88**(11): 118102.

10. Wagenaar, D. A. and S. M. Potter (2002). "Real-time multi-channel stimulus artifact suppression by local curve fitting." J Neurosci Methods **120**(2): 113-20.

11. 0Eytan, D., N. Brenner, et al. (2003). "Selective adaptation in networks of cortical neurons." J Neurosci **23**(28): 9349-56.

12. Jimbo, Y., N. Kasai, et al. (2003). "A system for MEA-based multisite stimulation. " IEEE Trans Biomed Eng **50**(2): 241-8.

13. Otto, F., P. Gortz, et al. (2003). "Cryopreserved rat cortical cells develop functional neuronal networks on microelectrode arrays." J Neurosci Methods **128**(1-2): 173-81.

14. Segev, R., M. Benveniste, et al. (2003). "Formation of electrically active clusterized neural networks." Phys Rev Lett **90**(16): 168101.

15. Baruchi, I. and E. Ben-Jacob (2004). "Functional holography of recorded neuronal networks activity." Neuroinformatics **2**(3): 333-52.

16. Gortz, P., W. Fleischer, et al. (2004). "Neuronal network properties of human teratocarcinoma cell line-derived neurons." Brain Res **1018**(1): 18-25.

17. Gortz, P., A. Hoinkes, et al. (2004). "Implications for hyperhomocysteinemia: not homocysteine but its oxidized forms strongly inhibit neuronal network activity. " J Neurol Sci **218**(1-2): 109-14.

18. Hulata, E., I. Baruchi, et al. (2004). "Self-regulated complexity in cultured neuronal networks. "Phys Rev Lett **92**(19): 198105.

19. Segev, R., I. Baruchi, et al. (2004). "Hidden neuronal correlations in cultured networks." Phys Rev Lett **92**(11): 118102.

20. Wagenaar, D. A., J. Pine, et al. (2004). "Effective parameters for stimulation of dissociated cultures using multi-electrode arrays." J Neurosci Methods **138**(1-2): 27-37.

21. Wagenaar, D. A., Madhavan, R., Pine, J. and Potter, S. M. (2005). "Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation." J Neurosci 25(3): 680-8.

22. Evans MS, Collings MA, Brewer GJ (1998). Electrophysiology of embryonic, adult and aged rat hippocampal neurons in serum-free culture. Journal of Neuroscience Methods 79:37-46.