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Culture protocol for dissociated cultures

Version 2.2

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1. Important precautions

In order to preserve the functioning and integrity of the Biochips, we strongly recommend you follow the precautions listed below when handling the chips.

- Touching the active area of the BioChips may damage the chips. In particular, objects (e.g. metal tools) can scratch or damage the electrodes. In case you need to mechanically clean the active area of the chips, use a soft paintbrush and gently clean the active area with a water solution.

- When cleaning or sterilizing, avoid immersing the entire BioChip in water or ethanol. Prolonged immersion in water might cause oxidation of the contact pads. Ethanol might cause some parts of the BioChip to deteriorate. As a general rule, while the chamber can be wet, the rest of the chip should remain dry.

- Take care to avoid accidentally short-circuiting the gold contact pads. Touching the gold contact pads with bare fingers should also be avoided. The use of gloves to handle the chips is highly recommended.

- Avoid using autoclaves, ovens or UV-lights for sterilization, as these methods could cause the packaging glue to deteriorate, decreasing the lifetime of the chip.

- Avoid using media with a high concentration of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) during recordings as it can interfere with the electrodes and make the chip unstable.

- The pH of the culture media should be kept at physiological conditions (7 - 7.5). Significant changes in the pH of the media culture (e.g. induced by evaporation during cell culture) might damage the electrodes.

2. Protocol

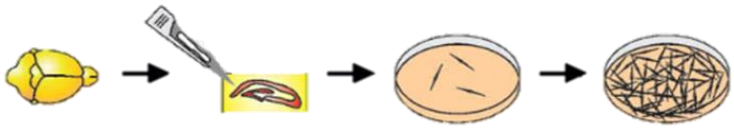
2.1. CELL TYPE

Hippocampal or cortical neurons from Sprague Dawley rat embryos at E17-18.

2.2. DISSOCIATION

Embryos are removed and dissected under sterile conditions using published protocols, then the cortex and hippocampi are dissociated via enzymatic digestion by using trypsin or papain for 15-20 minutes at 37°C. Lastly, cortex and hippocampi are triturated by using a fire-polished Pasteur pipette.

(see G. Banker, K. Goslin, Culturing Nerve Cells. (Cambridge, Massachusetts, ed. MIT Press, 1991)).

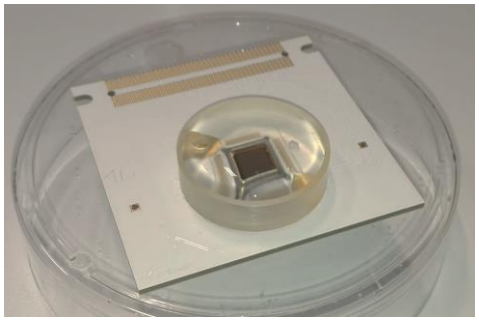


2.3. STERILIZATION

Before sterilization, and only if the chips have been dry for a long period, we suggest filling the chambers with DDW water and re-hydrating them for 1-2 hours. To guarantee a higher level of sterilization, the external area around the chamber of the chip is cleaned with a tissue soaked in pure ethanol and dried under a hood in a sterile petri dish for about 1 minute. The BioChip chamber is then filled with 70% ethanol for twenty minutes, rinsed with sterile DDW 4-5 times and dried under a laminar hood. UV or autoclave sterilization methods are not to be used because they could damage the BioChip. Immersing the whole chip in ethanol should also be avoided as this could affect the device substrate. Longer ethanol sterilization or the use of higher ethanol concentrations may affect BioChip functionality.

2.4. CHIP COATING

Suggestion: <to increase chip hydrophilicity and cellular adhesion, before coating and after sterilization, fill the chamber overnight with the sterile medium used to grow the neurons. On the following day, dry the chamber completely without rinsing it with water and proceed with the coating.>



Four different coatings are suggested:

1. single layer coating with poly-d-lysine: the active surface of the Biochip is coated with a drop of poly-d-lysine (Sigma P-6407) at 0.1 mg/ml* dissolved in DDW (overnight in the incubator).

2. double layer coating with poly-d-lysine and laminin: the active surface of the BioChip is coated with a drop of laminin (Sigma L-2020) for 3-5 hour 0.1 mg/ml* dissolved in DDW, then the drop is removed without rinsing and the surface is coated again with a drop of poly-d-lysine (Sigma P-6407) (overnight in the incubator) at 0.1* mg/ml.
   (see L. Berdondini et al., Lab On A Chip 9, 2644 (2009).)

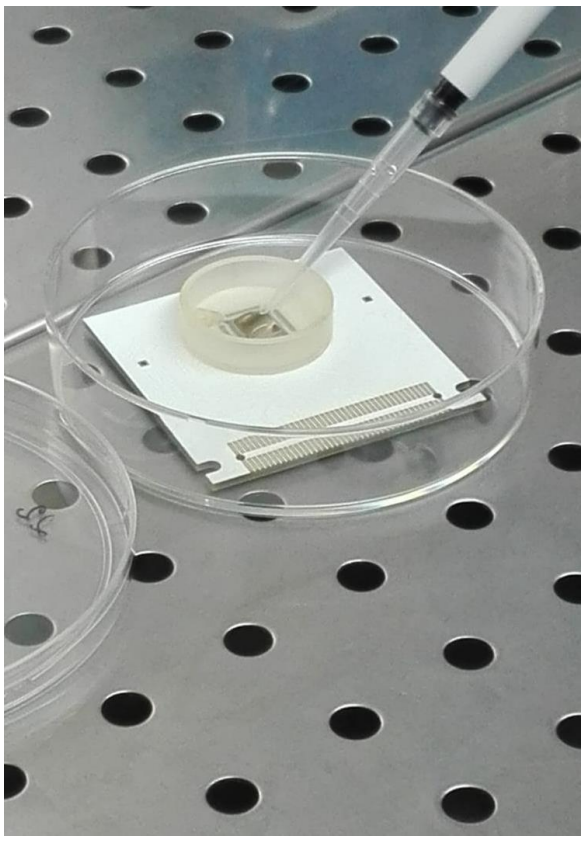
3. single layer coating with PEI (Poly-ethylenimine): the active surface of the Biochip is coated with a drop of PEI (Sigma 482595, 1:500 dissolved in borate buffer) (overnight in the incubator).

4. Poly-DL-ornithine: the active surface of the BioChip is coated with a drop of PDLO 50 μ g/ml (Sigma P0671) dissolved in borate buffer (overnight in the incubator).
   (see http://journal.frontiersin.org/article/10.3389/fnins.2016.00121/full)

The suggested drop sizes for coating are 30 μ L for Prime and 90 μ L for Arena and Stimulo chips. The ideal coating depends on the model and cell concentration the lab wants to use.

On the following day, regardless of the coating used, the active surface is gently rinsed three times with a drop of DDW and dried under a sterile hood. Once dry, the cells are immediately seeded onto the treated surface of the chip.

* lower concentrations as 0.05 or 0.025 mg/ml have also been successfully tested.

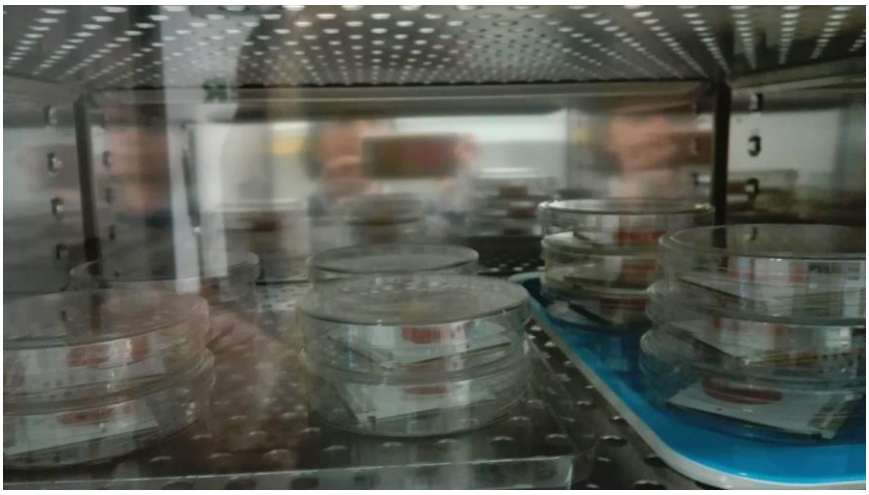


2.5. SEEDING

For standard cultures, neurons are plated onto the active area of the Prime in 30-40 μ L drops containing 30,000 to 60,000 cells (~1,000-1,500 cellc/ μ L). For Arena and Stimulo, the drop should be 80-90 μ L with a similar concentration, i.e. ~1,000-1,500 cells/ μ L. The BioChip is kept in the incubator and after 1 hour, the chamber is filled with ~1.5 mL of medium (1% Glutamax, 2% B-27 supplemented Neurobasal Medium from Invitrogen). Recently the use of Neurobasal + B27 in the protocol has either been completely replaced by the new formulation Neurobasal Plus -B27 Plus (Catalog number: A3653401) produced by Thermo Fisher, or kept only for the first 4 days after plating and then replaced with Brain Phys Neuronal medium + SM1 supplement (KIT code 05792), produced by StemCell Technologies.
The formulations of these new media and supplement ensure a greater survival of cultured neurons and a richer synaptic expression as reported in the paper of Bardy C et al. “Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro” Proc Natl Acad Sci U S A. 2015 May 19; 112 (20): E2725-34.

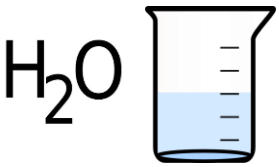
2.6. CULTURE MAINTENANCE

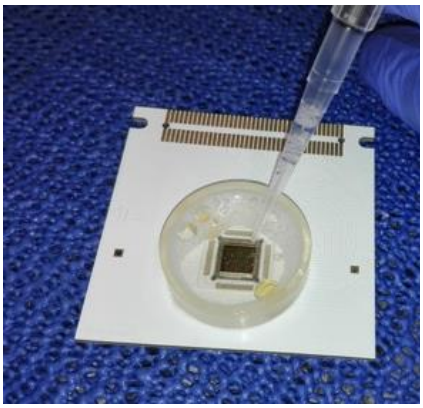
Cultures are kept in the incubator at a humidified temperature of 5% CO2, 95% air at 37°C. One-third to one-half of the media is changed every week to balance evaporation (once cultures mature, the media can be changed twice a week). To avoid the osmolar unbalancing caused by strong evaporation, the BioChip can be closed in a petri dish together with a smaller open petri dish containing 2 mL of sterile water (additionally the enclosing petri can be sealed with a thin layer of parafilm around the edge). The pH is visually checked every 2-3 days; if the color of the media indicates an acidification process (shifting from pink to orange-yellow), the media should be changed.

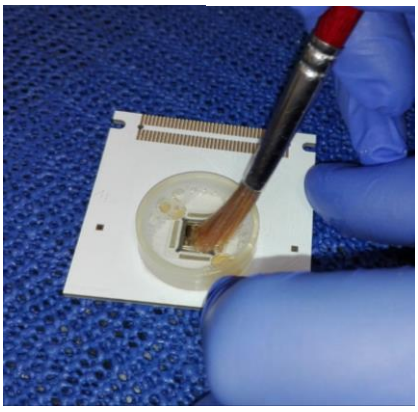


2.7. CLEANING

After recording, the BioChip is rinsed with DDW and gently cleaned using a soft brush with highly diluted (e.g. 10x the suggested concentration detergent like WPI-Enzol (WPI) or Terg-A-zyme (Alconox)). Finally, the BioChip is thoroughly rinsed with DDW (it is really important be sure to have removed all the soap residual). Once the BioChip is dry, it can be stored in a closed box in order to protect the recording area from dust and dirt.







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