3Brain GmbH
Schulstrasse 76
7302 Landquart
Switzerland

Standard protocol for dissociated cultures on BioChips

Date: 24/01/2013

Version: 1.2.1

Author(s): Alessandro Maccione – alessandro.maccione@iit.it, Luca Berdondini luca.berdondini@iit.it

in collaboration with Italian Institute of Technology - www.iit.it

1. Important precautions

We strongly recommend to follow the listed important precautions on chip handling in order to preserve the functioning and integrity of the Biochips.

- Touching the active area of 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 brush and gently clean the active area in water solution.
- For cleaning or for sterilization avoid immersing the entire BioChip in water or ethanol. Prolonged immersion in water of the entire BioChip might cause oxidation of the contact pads. In ethanol, some parts of the BioChip might be deteriorated. As a general rule only the chamber can be wet while the rest of the chip should stay dry.
- Avoid to short-circuit the gold contact pads accidentally. A proper handling of the BioChip requires to avoid touching the gold contact pads with bare fingers too.
- For sterilization do not use autoclaves, ovens or UV-light, since these methods could deteriorate the packaging glue.
- Avoid using HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) in your solutions during recordings, since we observed that it can interfere with the electrodes making the chip unstable.
- The pH of the culture media should be kept at physiological conditions (7 - 7.5). Important 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 condition using published protocols[1], then cortex and hippocampi are dissociated via enzymatic digestion by using tripsin or papain for 15-20 minutes at 37°C. Finally they are triturated by using a fire-polished Pasteur pipette.

2.3. Sterilization

Before sterilization and if chips have been dried for long periods, we suggest to fill the chambers with DDW water and to re-hydrate them for 1-2 hours.

BioChip chamber is filled with ethanol 70% for twenty minutes, rinsed with sterile DDW 4-5 times and then dried under laminar hood. UV or autoclave sterilization methods are not used because they could damage the BioChip. Also avoid immersing the whole chip into ethanol that could affect the device substrate. Longer ethanol sterilization or the use of higher ethanol concentrations may affect the BioChip functionality.

2.4. Chip coating

Three different coatings are suggested:

1. *single layer coating with polylysine*: active surface of the Biochip is coated with a drop of poly-d-lysine (Sigma P-6407) (overnight into the incubator). The day after, active surface is kindly rinsed with a drop of DDW and dried under sterile hood.
2. *double layer coating with polylysine and laminin*: active surface of the BioChip is coated with a drop of laminin (Sigma L-2020) for 3-5 hour, then the drop is removed without rinsing and the surface is coated again with a drop of poly-d-lysine (Sigma P-6407) (overnight into the incubator). The day after, the active surface is kindly rinsed with a drop of DDW and dried under sterile hood[2].

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

[2] L. Berdondini et al., Lab On A Chip 9, 2644 (2009).

3. *single layer coating with PEI (Poly-ethylenimine)*: active surface of the Biochip is coated with a drop of PEI (Sigma 482595) (overnight into the incubator). The day after, active surface is kindly rinsed with a drop of DDW and dried under sterile hood.

Suggested drop sizes are of 30 µL for BioChip4096S and of 90 µL for BioChip4096S+ and BioChip4096E.

2.5. Seeding

For standard cultures, neurons are plated onto BioChip4096S in 30-40 µL drop containing nominally 30.000 to 60.000 cells (~1.000-1.500 cell/ µL) on the active area. For BioChip4096S+ and BioChip4096E the drop should be of 80-90 µL and at similar concentration, i.e. ~1000-1.500 cell/ µL. BioChip is then kept into the incubator and after 3-5 hours, the chamber is filled with ~1.5 ml medium (1% Glutamax, 2% B-27 supplemented Neurobasal Medium from Invitrogen).

2.6. Culture maintenance

Cultures are kept into the incubator at a humidified temperature of 5% CO_2, 95% air at 37°C. One third to half of the media is changed every week to balance evaporation. To avoid that strong evaporation produces osmolar unbalancing, the BioChip can be closed in a Petri dish together with a smaller opened Petri dish containing 2 mL of sterile water (additionally the enclosing Petri can be sealed with a thin layer of parafilm around the edge). PH is visually checked every 2-3 days; if media colour indicates an acidification process, (shifting from pink to orange-yellow) media change is anticipated.

2.7. Cleaning

After recording, BioChip is rinsed with DDW then it is gently cleaned using a soft brush with a detergent as WPI-Enzol (WPI) or Terg-A-zyme (Alconox). Finally BioChip is abundantly rinsed with DDW.

Once the BioChip is dried it can be stored in a closed box in order to protect the recording area from dust and dirtiness.

3Brain GmbH · Schulstrasse 76 · 7302 Landquart · CH