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in collaboration with



Standard protocol for dissociated cultures on Biochip

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1. Chapter

1.1. Cell type

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

1.2. Dissociation

Embryos are removed and dissected under sterile condition using published protocols (1), then cortex and hippocampi are dissociated via enzymatic digestion by using trypsin or papain for 15-20 minutes at 37°C. Finally they are triturated by using a fire-polished Pasteur pipette.

1.3. Sterilization

Biochip chamber is filled with ethanol 80% 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.

1.4. Chip coating

Two different coatings are suggested:

1. *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 rinse 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).
2. *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 rinse with a drop of DDW and dried under sterile hood.

1.5. Seeding

For standard cultures, neurons are plated onto Biochip in 30-40 μL drop containing nominally 30.000 to 50.000 cells ($\sim 1000\text{-}1500$ cell/ μL) on the active area and kept into the incubator. After 3-5 hours, the chamber is filled with ~ 1.5 ml medium (1% Glutamax, 2% B-27 supplemented Neurobasal Medium from Invitrogen).

1.6. 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 balancing evaporation. To avoid that strong evaporation cause osmolar unbalancing, Biochip can be closed in a Petri dish with a thin layer of parafilm on the sides and with a smaller open petri with 2 mL of sterile water inside. 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.

1.7. Cleaning

After recording, Biochip are rinsed with DDW then they are gently cleaned using a soft brush with a detergent as Terg-A-zyme (Alconox). Finally Biochip are abundantly rinsed with DDW.

1.8. References

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).