**Primary Neuron Culture Protocol**

9/4/20

Prepare solutions for plating

1. Borate buffer:
   1. 50mM, 3.09 g/L, pH 8.5
   2. Sterilize with 0.2 μm filter
2. Poly D lysine (PDL) hydrobromide (gibco, 0.1mg/ml, REF:A3890401. Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA):
   1. 2 mg/mL of 40x stock and 100 mg/50mL of pH 8.5 borate buffer (for 1x, dilute 40x with borate buffer)
   2. Sterilize with 0.2 μm filter
   3. Store in -20oC

Prepare plates

1. Wash 48 well plates (Thermo Scientific, NunclonTM Delta Surface. Made in Denmark. Thermo Fisher Scientific Nunc A/S. Kamstrupvej 90. P.O. Box 280 DK-4000 Roskilde. Denmark) 3 times with 0.5 mL distilled H2O (SIGMA Life Science, W3500-500ML, Water, sterile-filtered, BioReagent, suitable for cell culture, Lot#RNBK1827. SIGMA-ALDRICH, Co., 3050 Spruce Street. St. Louis, MO 63103 USA)
2. Treat each well with 0.5 mL PDL at 37oC for 1 hour
3. Wash 3 times with 0.5 mL distilled H2O
4. Place 0.5 mL plating media into each well at 37oC (should be done right before dissection)

Prepare solutions for dissection (all can use the same filter)

1. HBSS
   1. 490 mL of HBSS (ThermoFisher, gibco, Hanks’ Balanced Salt Solution 1X, REF14025-092. Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA), 6 mL of pH 7.4 HEPES, 3 mL of pen/strep, 6 mL of 100mM pyruvic acid, 100 mL of distilled H2O; total should be 600 mL
   2. Filter (VWR Vacuum Filtration 250ML 0.45um PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd. Radnor, PA 19087)
2. Enzyme solution
   1. 10 mL of HBSS, 200 μL of papain suspension (Worthington, LS003126. Worthington Biochemical Corporation, 730 Vassar Ave. Lakewood, NJ 08701. CAT#:LS003126, Conc.44.0mgP/ml), 2 mg of L-cysteine, 22 μL of 0.5mM EDTA
   2. Rotate for 0.5 hour (30mins)
   3. Filter (Millex-GP. Syringe-driven Filter Unit. 33mm, Pes Membrane, 0.22μm, Sterilized. Merck Millipore Ltd. Tullagreen, Carrigtwohill, Co. Cork, IRELAND. Rev. 07/20)
3. Plating media (containing neurobasal media, supplemented with 5% FBS, 1x B27 supplement (ThermoFisher,17504044), 0.5 mM L-glutamine, and 100 unit per mL of Penicillin-Streptomycin)
   1. 183 mL of neurobasal, (ThermoFisher, gibco, Neurobasal Media 1X, [-] L-Glutamine; [-] Phenol Red. REF:12348-017. Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA) 10 mL of FBS, 2 mL of GlutaMAX (100X, REF:35050-061. gibco, Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA), 4 mL of B27 (ThermoFisher,17504044), 1 mL of Penicillin-Streptomycin
   2. Filter (VWR Vacuum Filtration 250ML 0.45um PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd. Radnor, PA 19087) and keep in 4oC
   3. Warm overnight/2 hours before in incubator in T75 flask (Thermo Fisher Scientific, NuncTM EasYFlaskTM 25cm2 NunclonTM Delta Surface.Nunc A/S. Kamstrupvej 90. P.O. Box 280 DK-4000 Roskilde. Denmark)to calibrate pH
4. Neuronal/culture media (containing neurobasal media supplemented with B27 and 0.5 mM L-glutamine.)
   1. 193 mL of neurobasal, 2 mL of GlutaMAX (100x, REF:35050-061. gibco, Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA), 4 mL of B27
   2. Filter (VWR Vacuum Filtration 250ML 0.45um PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd. Radnor, PA 19087)
   3. Warm overnight/2 hours before in incubator in T75 flask to calibrate pH

Dissection

1. The hippocampi of 1-day postnatal CD1 mice pups were collected.
2. Clean surgical instrument with 70% ethanol (Sharp fine scissors 14060-11 F.S.T, *Sekmen Forceps,* 11008-15 F.S.T)
3. Cut off head and using scissors, cut skin of the top of the head laterally.
4. Stabilizing head with tweezers, use another set of tweezers to pull skin to the side
5. Cut through the bone
6. Using a spoon, scoop brain from underneath and place into culture dish
7. Separate the two hemispheres at the interhemispheric fissure from the brainstem
8. Cut off the olfactory bulbs
9. Carefully pull off the meninges but leave connected at the bottom
10. Flip so that the bottom of the brain is facing up
11. Completely pull off the meninges and associated tissues
12. Hippocampus should be visible and make two cuts at either end
13. Flip out hippocampus
14. Trim hippocampus so that additional tissue is discarded
15. Dissect hippocampus and using one pair of tweezers to push the tissue onto another, place hippocampus into the 15 mL tube with the 10 mL Hibernate-E media (ThermoFisher, gibco, Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA)

Cell culture

1. Wash two times with 10 mL HBSS by pipetting to aspirate and NOT vacuuming
2. Leave 1 mL HBSS in tube
3. After filtering the enzyme solution with rotator, add all 10 mL of solution to the tube
4. Incubate at 37oC for around 45 minutes and no longer than 1 hour
5. Remove media and leave 1 mL
6. Pipette 10 mL plating media and 50 μL DNAse (DNAse stock is 50 μg/mL) into a 15 mL tube and invert 2-3 times
7. Pipette 10 mL DNAse solution to the 1mL digestion solution with hippocampi and invert 2-3 times
8. Remove all media
9. Wash with 10 mL plating media
10. Wash two times with 10 mL HBSS by pipetting to aspirate and NOT vacuuming
11. Remove HBSS and leave 1 mL with HBSS with hippocampi
12. Resuspend hippocampi with P1000 filter tip and pipet up and down 20 times until obvious chunks disappear
13. Add 5 mL plating media
14. Pass cells through strainer with 40 μm mesh size
15. Count cells and plate 50,000 cells per well for a 48 well plate, and 25,000 cells per well for a 96 well plate
    1. Using cell counter
       1. Place glass slip on top of slide and pipette 10 μL cell solution under slip
       2. Count number of cells in one 4x4 grid (live cells appear round with dark ring and transparent inside) # x 104 cells/mL
    2. Make sure not to add a small volume of cells to a large volume of plating media, aim for 250 μL of cell solution per well for a 48 well plate
16. Leave plates in 37oC incubator for 12 hours.
17. The plating media was then removed and swapped with culture media containing neurobasal media supplemented with B27 and 0.5 mM L-glutamine.
18. The cells were cultured for an additional 7 days before use.

At day-in-vitro (DIV) 10, fibril strains were added to each well of neurons to a final estimated concentration of 0.62 nM (1 μg per mL).