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Organotypic Hippocampal Slice Culture PROTOCOL

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ABSTRACT

Neurodegeneration due to neurotoxicity is one of the phenomena in temporal lobe epilepsy. Experimentally, hippocampal excitotoxicity process can occur due to kainic acid exposure, especially in the CA3 area. Neuronal death, astrocyte reactivity and increased calcium also occur in hippocampal excitotoxicity, but few studies have investigated immediate effect after kainic acid exposure. The organotypic hippocampal slice culture (OHSC) is a useful model for studying the neurodegeneration process, but there are still many protocol differences. In this study, minor modifications were made in the OHSC protocol.

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Preparation of Materials

1 Prepare your Equipment and Reagents

Equipment

1.1

- Minisart sterile syringe filter (0.22 µm), lot: 81040103, Ref 16534, Sartorius
- Sterile culture plate (six well multidishes)
- Disposable syringe one-med, Lot 02121988, 10 ml
- Millicell cell culture inserts 0.4 µm, diameter 30 mm, **Merck**, Ref: PICM03050, lot: R0DB59407
- Confetti, LCR Filter type 0.45 µm PTFE Membrane, 13 mm, **Omnipore**, Ref: JHWP01300, Lot: R0MB18744
- Tissue slicer
- Stereomicroscope (**Euromex**)
- Conical tube 15 ml, SPL, 50015, 50/sleeve
- Centrifuge tube 50 ml, 50 pcs/pack, Shandong
- Tube 100 mL
- Petri dish
- Stainless steel blade
- Scissors for initial dissection
- Sharp forceps for holding head
- Thin scissors for removing skin
- Angled scissor for cutting skull
- Blunt forceps for removing skull
- Blunt, rounded spatula for removing brain
- Scalpel and blade for dividing brain
- Rounded spatula for transferring brain
- Laminar Air Flow (**ESCO**)
- Inkubator CO_2 (**ESCO, CCL-170/240 L**)
- Micropipette 200-1000 µL and 20-200 µL

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- Blue tip (Biologix, Lot#:J00820Z1440-05)
- Yellow tip (Biologix, Lot#:JH96170003Z1180-03)
- Autoclave
- Beaker with ice

Reagents

1.2

- Minimum Essential Medium Eagle (MEM), Sigma, M0769-10x1L, Lot SLBS6945
- Earle's Balanced Salt Solution (EBSS) 10X, Sigma, Lot # RNBG8086, 500 ml
- Earle's Balanced Salt Solution (EBSS) 1X, Gibco, Lot 2914813,Ref 24010-043 500 ml
- D-Glucose (Dextrose), Merck, CAS-No: 50-99-7, 250 g
- Penicillin-Streptomycin, Gibco, Ref 15070-063, Lot 2145472, 100 ml
- Horse serum, Gibco, Ref 16050-122, Lot 2208948, 500 ml
- Nystatin, Abcam, ab141118, Lot GR305852-5, 5MU
- HEPES, Sigma, H3375, Lot SLBB55267, 25 G
- Natrium bicarbonate, Merck, CAS-No: 144-55-8
- Amphotericin B, Gibco, Ref 15290-026, Lot 2090190, 50 mL
- L-Glutamine
- Wida W1 Unicap sterile water for irrigation, no reg. DKL0430503441A2, 1000 ml

Sterilization of Tools and Materials

2 The tools and materials that need to be sterilized inside the autoclave are as follows:
•Millicell cell culture inserts that have been cut into 3, then put in a petri dish
•Surgical tools
•Blue tip and yellow tip
•Conical tube 15 mL, Centrifuge tube 50 mL, and tube 100 mL

Preparation of Solutions

3 EBSS and D-glucose (for culture medium)
Prepare by dissolving 32.5 g glucose in 250ml EBSS, sterilize via syringe filtration through a 0.2 µM filter and store at -20^{o} C as 6 ml aliquots.

Penicillin/streptomycin (for culture medium)
From 5,000 U ml^{-1} stock solution, store at -20^{o}Cas 2 ml aliquots.

Horse serum (for culture medium)
Store at -20^{o} C as 26ml aliquots.

Nystatin (for culture medium)
From 10,000U ml^{-1} stock solution, store at -20^{o} C as 0.2 ml aliquots.

Slicing medium
1 M HEPES+EBSS solution. Prepare the solution by dissolving 71.49 g HEPES in 300 ml EBSS. Stir using a

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magnetic flea to dissolve. Sterilize via syringe filtration through a 0.22 µm filter and store at -20^{o}C as 14 ml aliquots. These aliquots can be stored at -20^{o}C until use.

Culture medium (for 100 ml)
50 ml MEM (Eagle) with Glutamax-1, 18 ml EBSS, 5 ml EBSS+D-glucose (see stock solutions), 1 ml penicillin–streptomycin, 25 ml horse serum and 0.06 ml Nystatin.

Preparation of The Culture Plate

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- Remove the lid of the plate and place 1 ml culture medium into each well.
- Open the packaging of the culture inserts. Place an insert into each of the six wells using sterile forceps. The culture medium will wet the insert membrane and aid adherence of the confetti to the surface of the membrane.
- Place the autoclaved confetti discs in a Petri dish. Remove the protective blue coating using sterile forceps and cut the confetti into halves using a sterile scalpel.
- Place three halves of confetti into position on each insert. None of the pieces should overlap.
- Place the plate into a 37 1C5% CO2 incubator to enable the medium to warm up and condition before platting

Preparation for the Dissection

5

- Place 10 ml slicing medium into a 50-ml conical tubeand keep it in a beaker with ice. Bubble the medium with 5% CO2/95%O2 for 10 minutes. This is the cold slicing medium to cool the brain after removed from the skull, before hippocampal isolation.
- Spray the walls and the floor of the flow cabinet with ethanol. If available, use the UV irradiation facility to sterilize the hood.
- Clean the slicing blade with acetone and subsequently with 100% ethanol before mounting onto the slicer. Fix into the slicer and spray again with 100% ethanol and leave to dry.
- Flame-sterilize the tools over a Bunsen burner and place them onto the 90-mm Petri dishes. The flow cabinet should be equipped with one slicing bath, rinsed in 70% ethanol and then left to dry, and one beaker half-filled with 70% ethanol (to place tools after use and aid in cleaning for the next preparation) and the cyanoacrylate.
- Place a 60-mm Petri dish filled with 5 ml slicing medium into the hood. This will be used to hold the brain during dissection and should be kept on ice

Dissection and Slicing

6

- Swab the skin around the neck and skull of the animal with 70% ethanol.
- Kill animals by cervical dislocation and then decapitate using scissors.
- Cut the skin and expose the skull. Open the skull by cutting from side to side along the interaural line and then along the sagittal suture with small scissors. Scoop out the brain quickly with a rounded spatula and place it in cold slicing medium to chill for1 minute. The brain should be covered with cold slicing medium
- Pour 10 ml of cold slicing medium to the slicing dish. Transfer the brain to slicing dish
- Under the dissecting microscope: Place the brain and hold it at the midline with the dissecting forceps pressed to the bottom of the 60 mm dish. Use the hippocampus dissecting tool to separate the

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hemispheres leaving out the midbrain. The hippocampi are then exposed on each hemisphere. Then gently scoop the hippocampus out with the hippocampus dissecting tool

- Using a snipped tip of a P1000 filter pipette tip, gently aspirate the hippocampus and transfer it to the Teflon sheet on the tissue slicer. Position the hippocampus on its concave side.
- Align the hippocampi perpendicular to the blade to obtain coronal sections and drain excess of liquid.
- Slice the hippocampi every 350 µm. Pour 10 mL cold slicing medium into a 60 mm dish and transfer sliced hippocampi from the slicer using another snipped P1000 filter tip and cold slicing medium. Avoid making bubbles.
- With the help of an iris spatula and a straight spatula gently separate the slices from each other.
- Separate well defined and undamaged slices from damaged slices

Platting Slice

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- Place a Petri dish into the flow cabinet.
- Take the tube containing the slices and swirl gently for a few seconds to resuspend the slices in solution. Once suspended, pour all of the content into the petri dish.
- Using a sterile wide-end pipette, take a slice from the dish. The slice should be located near the tip of the pipette to aid plating.
- To plate, squeeze the teat until one drop of solution containing the slice is in the center of the confetti. Using the sterile thin-end pipette, remove excess slicing medium from the insert. The slice should lie flat on the confetti.
- Once all the slices have been plated, place the plate back into the 5% CO2 incubator with temperature 37 ^{o}C.

Feeding the Cultures

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- The culture medium is changed every 2–3 days, performed in sterile conditions under a flow cabinet.
- Take the plate from the incubator. Pipette the amount of medium needed (calculating 1 ml per well plus 1 ml extra) into a canonical tube and allow it to condition in the incubator.
- Place two disposable sterile plastic pipettes onto a support. The first pipette will be used to remove the old medium and the second one to add fresh medium.
- Flame-sterilize the forceps and place onto a support.
- Take the plate from the incubator and pick up each insert by holding the plastic edge using forceps. Usingfree hand, remove and dispose of the old medium from the well. Then, add 1.3 ml of new medium and place the insert back into the well. Perform the same procedure for all six wells. Make sure no air bubbles are trapped between the slice and the medium

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