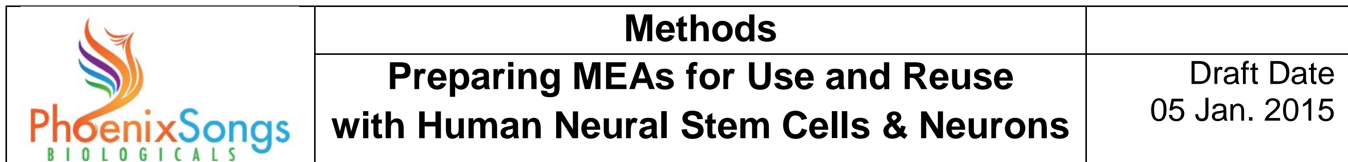

	Methods	
	Preparing MEAs for Use and Reuse with Human Neural Stem Cells & Neurons	Draft Date 05 Jan. 2015

Materials required for Using and Reusing the MEA

Solutions

Trypsin/EDTA Hank's based 0.05% with 0.5mM EDTA (PSB Catalog # 41004-100)
Sterile ultrapure tissue culture grade water (PSB Catalog #41003-1L)
Phosphate Buffered Saline (PBS) (PSB catalog # 41001-500)
1% Tergazyme solution w/v in sterile ultrapure water (Alconox catalog # 1304)
Poly-D-Lysine solution
Laminin solution
Neural Differentiation Media (PSB Catalog # 21004-250)

50µg/ml PDL Solution can be stored at 4° C for 2 years (sterile filter solution through .2µm filter unit)

100ml Ultrapure tissue culture grade water
5mg Poly-D-Lysine (Sigma # P7280-5mg)

20ug/ml Laminin Solution (sterile filter solution through .2µm filter unit)

50ml PBS (PSB catalog # 41001-500)
1mg Laminin

Other Materials

Sterile forceps
Sterile 250ml beakers
100mm x 25mm petri dishes or tissue culture dish
Human Neural Stem Cells (23002-009D HIP-009 NSCs for Differentiation)

Required Equipment

- All procedures should be conducted inside a biological safety cabinet such as a NuAire Class II Type A2.
- Human neural stem cells (hNSCs) can be maintained with an antibiotic if required in order to prevent bacterial contamination. All our lines have been tested with gentamycin (30µg/ml).
- Incubator should be low oxygen capable such as the NuAire 4950 with the following settings
    - 6% CO_2
    - 2-4% O_2
    - 95% humidity
    - 37°C

  NOTE: It is best to culture neural cells in low oxygen conditions for optimal differentiation of the hNSCs into neurons. Atmospheric oxygen incubators may be used but will result in slower growth of hNSCs and differentiated neural cultures with lower percentage of neurons and high percentage of astrocytes and oligodendrocytes.

1. Preparing the MEA for First Use

1.1. Remove the MEA from the package and transfer into an autoclave pouch then autoclave at 121°C for 30 minutes
1.2. Allow MEA to cool prior to handling then use forceps to aseptically remove the MEA from the autoclave pouch and place in a petri dish or tissue culture dish
1.3. Add 100µl PDL solution directly onto the electrode area of the MEA
    1.3.1. Incubate for 5 minutes at room temp

1.4. Remove PDL solution and rinse 3 times with sterile cell culture ultrapure water then aspirate dry.
1.5. Add 100 µl Laminin solution directly onto the PDL coated MEA surface and let set for 2 hours at RT
1.6. Aspirate the Laminin until the MEA looks dry.
1.7. Plate cells by adding 80ul of concentrated cells in media directly onto the center of the MEA surface. Determine cell density prior to plating on MEA. For PhoenixSongs HIP-009 NSCs it is best to have $2x10^5$ cells in 80µl Neural Differentiation Media
   1.7.1. Incubate for 30-60 minutes to allow for cell attachment
1.8. Add 1ml media down the side of the MEA and allow to spread over the surface area – the media from the cell drop will merge with the added media
1.9. On day 5-7 (preferably on a Monday or Friday) post plating remove half the media and replace with equal volume of fresh Neural differentiation media
1.10. Thereafter every Monday and Friday remove half the media and replace with an equal volume of Neural Differentiation Media to the MEA
1.11. These neurons will last in culture for greater than 60 days as long as half feeds are continued on Monday and Friday throughout the time in culture

2. Preparation for Reusing the MEA

2.1. Carryout all washing and rinsing of the MEA in the hood using aseptic techniques.
2.2. Remove the cells from the MEA by trypsin digestion for 20 minutes at 37°C – remove the media and add 1ml trypsin then return to the incubator.
2.3. Following trypsin digestion aspirate the cell/ trypsin solution from the MEA and pipet up and down approximately 10 times to dislodge cells from the MEA surface and rim then remove and rinse MEA with sterile ultrapure tissue grade water 3 times
2.4. Add 1 ml of 1% tergazyme solution to MEA and gently pipet up and down approximately 10 times to wash the entire surface and sides of the MEA then remove tergazyme solution and discard.
2.5. Rinse the MEA 3 times with sterile ultrapure tissue culture grade water.
2.6. Use forceps to aseptically pick up and transfer the MEA into a beaker with enough sterile ultrapure tissue culture water to completely submerge the MEA. Swirl a few times to move the water around.
2.7. Aspirate all the water from the beaker and make sure to remove all the water inside the MEA then fill the beaker again to completely cover the MEA. Repeat this rinse 20 times. (Only use sterile ultrapure tissue culture grade water for all rinses).
2.8. Following the final rinse, aspirate all the water from the MEA until dry. This entire wash/rinse/dry process should take approximately an hour. The MEA is glass so the water beads up and can be completely aspirated to dry the MEA. (Do not use compressed air to dry MEA as this air may contain tiny droplets of oil and debris from the compressor which can adversely affect the ability of NSCs to attach, differentiate and survive on the MEA).
2.9. Place in an autoclave pouch and autoclave for 30 minutes at 121°C.
2.10. Allow MEA to cool following sterilization then the MEA is ready to coat with PDL/Laminin as was done in section 1 above.