Peri.4U™ Application Protocol Multiwell-MEA

Measuring Neuronal Electrical Activity of Peri.4U™ (Axiogenesis AG) on the 24-well Multiwell-MEA System (Multi Channel Systems MCS GmbH).

Version 1.0: July 2017

1 Introduction

Peri.4U™ from Axiogenesis AG are human peripheral neurons derived from induced pluripotent stem cells. They built fully differentiated, homogeneous cultures and are suitable for electrophysiological applications. Peri.4U™ show stable spontaneous activity in burst-like activity patterns earliest at day 3 post thaw. It is recommended to perform experiments for compound testing between day 5 and 12 post thaw.

Multi Channel Systems MCS GmbH's microelectrode array (MEA) technology enables non-invasive, label-free measurements of local field potentials of electrically active cells including neurons, islets of Langerhans and cardiomyocytes and thus to decipher the function and dysfunction of the underlying ion channels.

Peri.4U™ can be cultured on MEA plates to form an electrically active neuronal network amenable to electrophysiological examination. Together, Peri.4U™ and the Multi Channel Systems MEA technology form an excellent, non-invasive platform for in vitro screening of compound efficacy and toxicity in human peripheral neurons.

This Application Protocol describes how to handle Peri.4U™ for use on the Multi Channel Systems Multiwell system using the 24-well MEA plate.

2 Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required for the handling of the Peri.4U™ neurons and the 24-well Multiwell-MEA plates.

Table 1: Overview of required equipment.

Item	Vendor	Catalog Number
Low adhesion pipette tips 10 µl	Carl Roth	PC91.1
Multiwell-MEA System	Multi Channel Systems MCS	
Storage box	Multiple Vendors	
37°C water bath	Multiple Vendors	
Laminar flow hood	Multiple Vendors	
Neubauer hemocytometer	Multiple Vendors	
Cell culture incubator	Multiple Vendors	
Centrifuge	Multiple Vendors	
Inverse microscope	Multiple Vendors	

Table 2: Overview of required consumables.

Item	Vendor	Catalog Number
Cryopreserved Peri.4U	Axiogenesis	Ax-C-HP02-2M
Neuro.4U Basal Medium	Axiogenesis	Ax-M-NBM250
Neuro-Supplement 1	Axiogenesis	Ax-M-PCS
50% Polyethyleneimine solution (PEI)	Sigma-Aldrich	P3143
Boric acid	Sigma-Aldrich	B7660
Sodium tetraborate	Sigma-Aldrich	221732
1.5 ml and 50 ml centrifuge tubes	Multiple Vendors	
Trypan blue solution 0.4%	Sigma-Aldrich	T8154
24-well Multiwell-MEA plates	Multi Channel Systems MCS	24W700/100F-288 24W300/30G-288
Sterile distilled water	Multiple Vendors	

Table 3: Overview of required software.

Item	Vendor	Catalog Number
Multiwell-Screen v. 1.5.5.0 and above	Multi Channel Systems MCS	http://www.multichannelsystems.com/software/multiwell-screen
Optional further analysis software of your choice	Multiple Vendors	

3 Workflow

The Peri.4U™ are thawed into Neuro.4U Basal Medium and plated in Peri.4U culture medium into 24-well MEA plates, previously coated with 0.1% PEI solution. On day 1 post-plating the entire medium has to be changed. During the subsequent culture the spent medium should be replaced 3 times a week.

Days in culture	
Day -1	Coat MEA plate with 0.1% PEI
Day 0	Thaw and plate Peri.4U™ into MEA plate
Day 1	Replace 100% of spent medium
Day 1+	Replace spent medium 3 times a week

4 Preparations

4.1 Preparing the 0.1% PEI solution

1) Prepare 100 ml of borate buffer by dissolving 310 mg boric acid and 475 mg sodium tetraborate in 100 ml distilled water.
   a) Dissolve the boric acid completely.
   b) Add the sodium tetraborate.
   c) Mix the borate buffer overnight.
2) Adjust the pH to 8.4 using HCl.

3) Prepare a 0.1% PEI solution by diluting a sufficient amount of 50% PEI solution in borate buffer.
4) Filter the 0.1% PEI solution through a 0.22 µm sterile filter unit.

4.2 Preparing the MEA plate

All tasks need to be performed under sterile conditions.

1) Add 80 µl/well of the 0.1% PEI solution to the 24-well MEA plate (Fig. 1 & 2) to coat the entire bottom of the wells.
2) Incubate for 1 h at room temperature.
3) Aspirate the PEI solution from the 24-well MEA plate and rinse the wells with >500 µL/well of sterile distilled water 4 times.
4) Air-dry the 24-well MEA plate with the lid removed in a laminar flow hood overnight.

Attention: *It is crucial to let the MEA plate air-dry overnight to reach an optimal performance.*

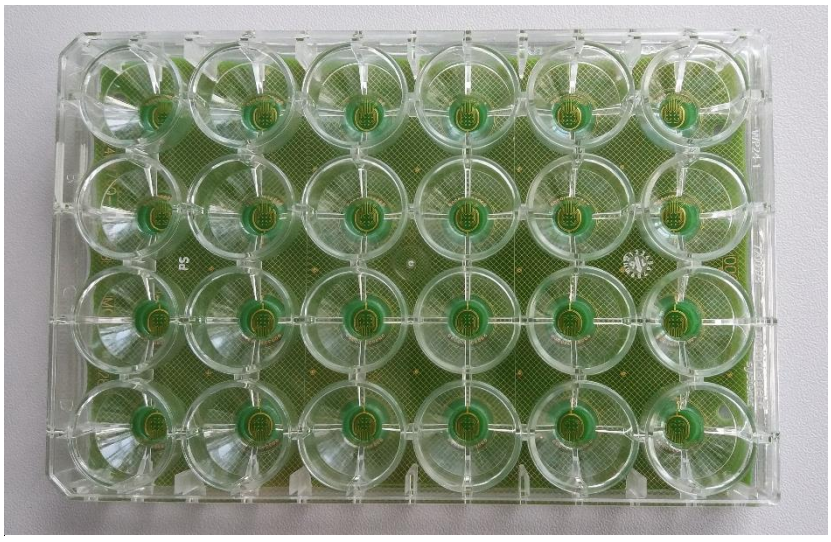

Fig. 1: 24-well Multiwell-MEA plate with gold electrodes.

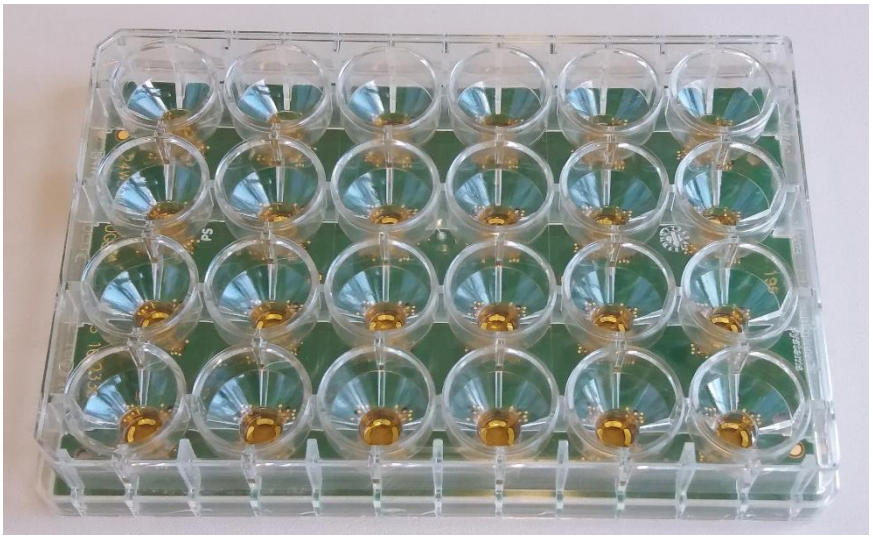

Fig. 2: 24-well glass Multiwell-MEA plate with PEDOT-coated gold electrodes.

4.3 Preparing the Medium

1) Thaw a vial containing Neuro-Supplement 1 in the refrigerator.
2) Transfer 50 ml of Neuro.4U Basal Medium to a 50 ml centrifuge tube.
3) Mix Neuro-Supplement 1 by pipetting up and down.
4) Prepare the Peri.4U™ culture medium by adding 1.25 ml of Neuro-Supplement 1 to the 50 ml centrifuge tube containing Neuro.4U Basal Medium.
5) Mix Peri.4U™ culture medium by pipetting and store at 4°C.

4.4 Preparing the storage box

1) Place a paper towel on the bottom of a storage box (Fig. 3) and add 5 - 10 ml distilled water to moisten the paper towel.
2) Put the lid of a common culture plate in the storage box to avoid that the bottom of the MEA plate gets wet.
3) Loosely place the lid of the storage box on top of it.

Fig. 3: Exemplary storage box with paper towel that can hold 3 Multiwell-MEA plates.

5 Thawing Peri.4U™

In brief: Thaw the Peri.4U™ according to their Handling Guide.

1) Equilibrate Neuro.4U Basal Medium (without supplements) to room temperature.
2) Transfer 1 ml Neuro.4U Basal Medium to a 50 ml centrifuge tube.
3) Quickly transfer cryopreserved Peri.4U™ from the vapor phase of liquid nitrogen or from a transport dewar with liquid nitrogen directly to a 37°C water bath.

   Attention: *Do not use dry ice for the transport. This might affect cell viability.*

4) Thaw the vial until the frozen cell suspension detaches from the bottom of the vial and only a small ice clump is visible (2 minutes).
5) Gently resuspend the sedimented cells by carefully swinging the vial back and forth. Avoid any pipetting of the thawed neurons.
6) Transfer the cell suspension to the 50 ml centrifuge tube using a 1000 µl pipette.

7) Gently mix the cell suspension by carefully swinging the centrifugation tube back and forth.
8) Remove a 20 µl sample of cells to confirm viability using a Neubauer hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
9) Add further 8 ml Neuro.4U Basal Medium to the cell suspension in the 50 ml centrifuge tube to get a total volume of 10 ml.
10) Centrifuge the cell suspension at 260 x g for 8 minutes at room temperature.
11) During centrifugation, count the cells and calculate the final volume of Peri.4U™ culture medium needed to resuspend the cell pellet to 14.4 x 10^6 viable cells / ml using the number of viable cells.
12) Aspirate the supernatant, being careful not to disturb the cell pellet. Tilt the centrifuge tube, while aspirating the supernatant.
13) Add the volume of Peri.4U™ culture medium to the cell pellet to resuspend the neurons in a density of 14.4 x 10^6 viable cells / ml.
14) Gently mix the cell suspension by carefully swinging the centrifugation tube back and forth.
15) Transfer the cell suspension to a sterile 1.5 ml centrifuge tube.

6 Plating Peri.4U™ into the MEA plate

The following procedure details plating the peripheral neurons into a pre-coated 24-well MEA plate.

1) Gently mix the cell suspension by swinging the 1.5 ml centrifugation tube back and forth.
2) Plate Peri.4U™ on the pre-coated MEA plate by dispensing 5 µl droplets of the cell suspension (72,000 cells / well) in the center of the electrode field of each well (Fig. 4 & 5).

Attention: *Even though gold electrodes are robust try to avoid touching the electrode area to prevent damaging. Ideally form a drop on the tip of the pipette and place this onto the electrode field.*

3) Put the lid on the MEA plate and place it in the storage box (Fig. 6) and store it in a cell culture incubator at 37°C at 5% CO_2 for 1 hour to allow the cells to attach to the plate.
4) During incubation, prepare an aliquot of Peri.4U™ culture medium with the volume that is needed to fill up the wells containing Peri.4U™ neurons and equilibrate this aliquot to room temperature.
5) Gently add 300 µl of pre-warmed Peri.4U™ culture medium down to the side of the wells of the MEA plate. For this carefully place the tip at the edge between bottom and side of the wells and add the medium, carefully lifting the pipette during dispensing.

Attention: *Adding the medium too quickly will dislodge the adhered cells.*

6) Place the MEA plate in the storage box and transfer the storage box into the incubator (avoid major vibrations). Culture the Peri.4U™ at 37°C, 5% CO_2 in a humidified atmosphere.

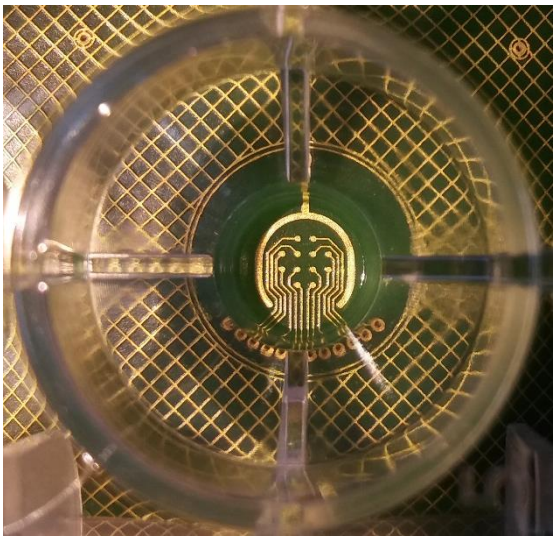

Fig. 4: Close-up view of one well of a 24-well Multiwell-MEA plate. Note the 12 electrodes in the center of the well and the circular reference electrode. Cells ideally are only plated in the center on the recording electrodes without covering the reference electrode.

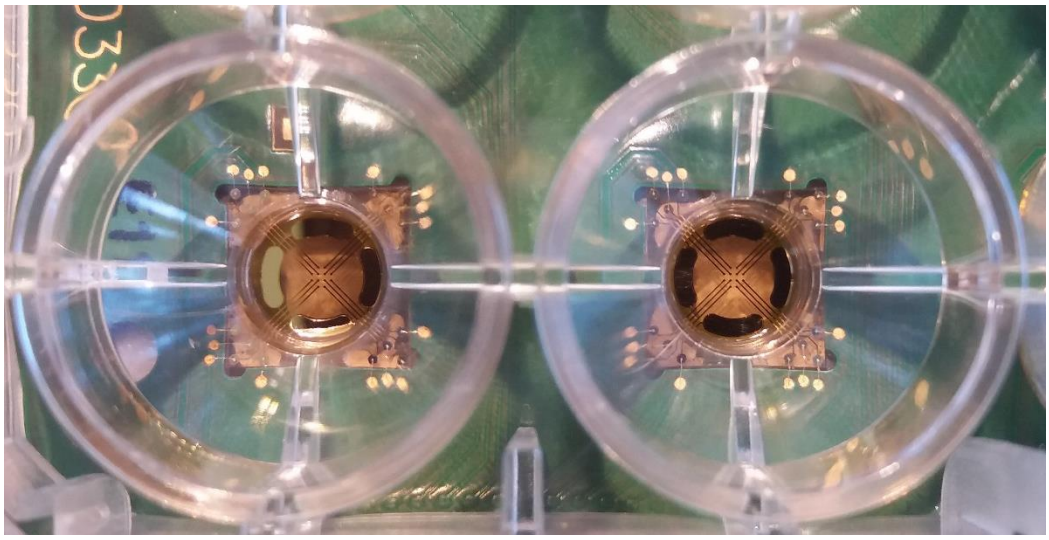

Fig. 5: Close-up view of two wells of a 24-well glass Multiwell-MEA plate. Note the 12 electrodes in the center of the well that are surrounded by 4 reference electrode. Cells ideally are only plated in the center on the recording electrodes without covering the reference electrode.

Fig. 6: Exemplary storage box with moistened paper towel, holding 3 Multiwell-MEA plates.

7 Maintaining Peri.4U™ in the MEA Plate

The following procedure details maintaining the peripheral neurons in 24-well MEA plate.

1) Prepare an aliquot of Peri.4U™ culture medium with the volume that is needed for the medium change and equilibrate the aliquot to room temperature.
2) On day 1 post-plating, change 100% of the medium (300 µl).
3) Maintain the neurons in the MEA plate by changing the medium 3 times a week: Perform one complete (100%) medium change (e.g. on Mondays) and two 50% changes (e.g. on Wednesdays and Fridays).
4) Culture the neurons in a cell culture incubator at 37°C, 5% CO_2 in a humidified atmosphere.
5) It is recommended to perform experiments for compound testing between day 5 and 12 post plating.

8 Data Acquisition

The acquisition of electrical activity of the Peri.4U™ on the Multiwell-MEA system should be executed according to the relevant manuals of MCS. The following procedure details the preparations before starting a recording of neuronal activity.

1) Turn on the MCS Multiwell-MEA System and start the Multiwell-Screen Software.
2) Wait until the temperature of the device has reached 37°C.
3) Dry the bottom of the MEA plate with a paper towel, before placing the MEA plate into the Multiwell-MEA System. Humidity on the electrodes might affect the quality of the recording by increasing the background noise.
4) Place the MEA plate in the device and choose your settings for the recording.
5) Let the cells rest for 10 min, before starting the recording.
6) Start the data acquisition.

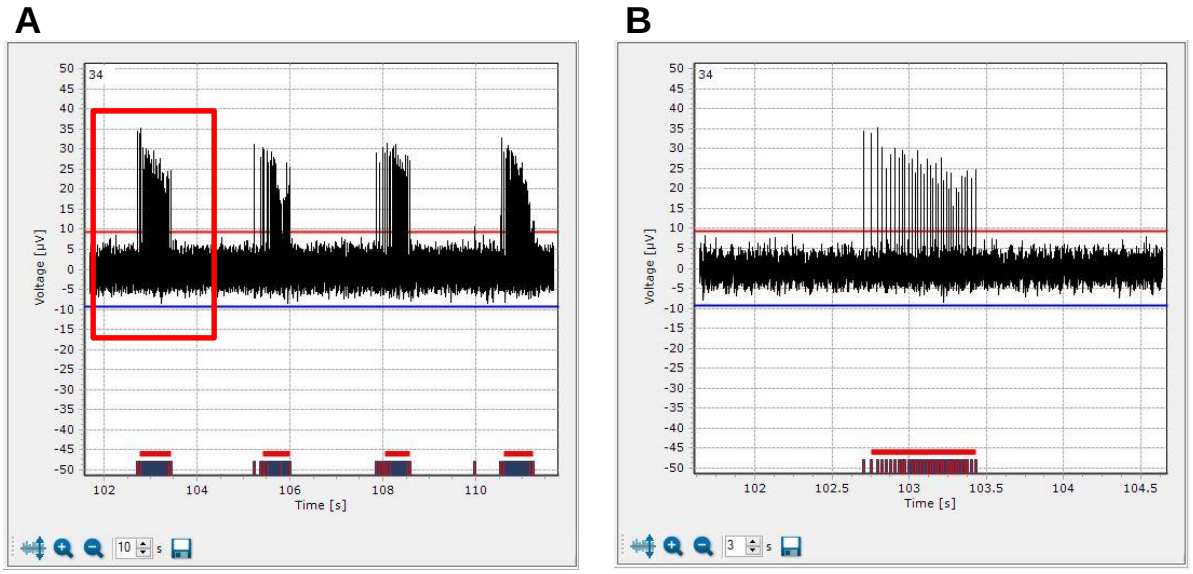

Fig. 7: Voltage traces of field action potentials of one electrode at day 12 post-plating. (A) Field action potentials grouped in four bursts. (B) Magnification of the burst marked with the red box in (A).

9 Summary

Peri.4U™ can be reanimated from cryopreservation directly into MEA plates where they rapidly recover to exhibit the expected neuronal electrical activity. The procedures presented in this application protocol point out the ease of using Axiogenesis' Peri.4U™ on the Multi Channel Systems MCS Multiwell-MEA system. The combination of these two products provides an *in vitro* system for acquiring electrophysiological activity of human peripheral neurons with a higher throughput.

Contacts:

Dr. Udo Kraushaar
udo.kraushaar@nmi.de

NMI Natural and Medical Sciences Institute at the University of Tuebingen
Markwiesenstr. 55
72770 Reutlingen, Germany