MEA Application Note: Primary Culture Cardiomyocytes from Chicken Embryo
# Table of Contents

1 Material 5
   1.1 Biological Materials 5
   1.2 Technical Equipment 5
   1.3 Chemicals 5

2 Methods 6
   2.1 Preparations 6
   2.2 Harvesting Cardiac Tissue 6
   2.3 Digestion with Trypsin 7
   2.4 Plating Cells on to the MEA 8
   2.5 Alternative Coating: Polyethylenimine (PEI) 8

3 Applications 9
   3.1 QT Analysis 9
      3.1.1 Mapping 11
      3.1.2 Pacing 12
   3.2 Software 14
      3.2.1 MC_Rack 14
      3.2.2 Cardio2D 14
   3.3 Suggested MEA-Systems 14
   3.4 Microelectrode Arrays 15

4 References 15
Primary Culture Cardiomyocytes from Chicken Embryo

1 Material

1.1 Biological Materials
- 4 or more chicken eggs (*Gallus gallus*) day E13. Incubate more for replacement in case that eggs are not fertilized. Day of placement in incubator: Day 0.

1.2 Technical Equipment
- Sterile workbench
- Egg incubator at 37 %, 50 % humidity
- Incubator at 37 °C, 5 % CO₂
- Ice
- Centrifuge (for 50 ml tubes)
- 8 – 10 sterile MEAs (microelectrode arrays)
- Sharp forceps
- Large forceps
- Curved forceps
- Small scissors
- 10 ml pipette
- Petri dishes
- 50 ml Falcon tubes
- 15 ml Falcon tubes
- 1 ml Eppendorf tubes
- 100 μm nylon mesh cell strainer

1.3 Chemicals
- Media as defined below
- 0.05 % Trypsin / PBS– (Trypsin: Sigma T 7409; PBS–)
- Cellulose Nitrate
- 100 % Methanol
- 70 % Ethanol
- PBS– (without Ca/Mg)

1.4 Media
- 20 % FCS-medium (158 ml Ham' F12 (PAA E15-016), 40 ml FCS (PAA A15-151), 2 ml Pen/Strep (PAA P11-010))
- 3 % FCS medium (168 ml DMEM, 30 ml 20 % FCS-Media, 2 ml Pen/Strep
2 Methods

2.1 Preparations
1. Place four or more fertilized eggs into an egg incubator for 13 days.
2. Treat the desired number of MEAs with a plasma cleaner for 2 min.
3. Autoclave the MEAs or sterilize by UV radiation (please see MEA Manual).
4. Coat the recording area of the MEAs with cellulose nitrate solution or Fibronectin (see MEA Manual). In case of Fibronectin, MEAs need to be prepared as described in the MEA manual). This step can be done immediately before the preparation or even during the later incubation steps.
5. Prepare three petri dishes with 20 ml, 10 ml, and 2 ml DMEM medium.

2.2 Harvesting Cardiac Tissue
1. Remove the embryonic chickens from the eggs and decapitate the chickens in a petri dish filled with 20 ml DMEM medium.
2. Open the thoracic regions and isolate the hearts.
3. Gather the hearts in a petri dish filled with 10 ml DMEM medium.
4. Remove the atria and vascular tissue, wash the blood away, and transfer the hearts into a dish with fresh DMEM medium (2 ml).
5. Chop the hearts with a scissor into small pieces of 0.5 to 1 mm.
6. Collect cardiac fragments with a 10 ml pipette and transfer them into a 50 ml tube. Let the heart pieces sink down inside the pipette so that only a small volume of liquid (about 1 ml) is taken over to the tube.
2.3 Digestion with Trypsin

Incubate the heart fragments in 0.05 % Trypsin / PBS– at 37 °C, as described in the following.

1. 10 min, 10 ml Trypsin, discard supernatant.
2. 7 min, 5 – 7 ml fresh Trypsin, collect the supernatant in 20 % FCS medium (on ice).
3. Repeat step 2 until the tissue has been fully digested and you obtain a homogenous cell suspension. You can enhance and speed up the digestion by mechanic friction. For this, aspirate the suspension with a 5 ml pipette (in later steps with a 1 ml pipette).
4. Pass the collected cell suspension through a 100 μm nylon mesh cell strainer into a new 50 ml tube.
5. Centrifuge the cell suspension for 10 min at 800 rpm (or 110 g).
6. Discard the supernatant and resuspend the pellet in 1 ml 3 % FCS medium.
2.4 Plating Cells on to the MEA

Cells are plated in a high density onto the microelectrode array. Therefore, the cell culture begins to beat spontaneously after 1 – 2 days in culture.

You will obtain about 3 million cells per egg. The cell suspension should be sufficient for about 10 microelectrode arrays (MEA).

1. Count the cells to estimate the cell density.

2. Pellet the cells at 1200 rpm (or 150 g) for 1.5 min.

3. Resuspend the cells at a density of 400 000 – 500 000 cells per 10 μl in 3 % FCS medium.

4. Plate 10 μl cell suspension (equals 400 000 – 500 000 cells) directly onto the MEA, but avoid touching the electrodes with the pipette because the electrodes may be damaged.

   **Tip:** If you want to work with less cells keep the cell density in the plating suspension, but reduce the volume of the drops to 0.5 – 2 μl. Please see “Drug Testing” section for details.

5. After a few minutes (the cell suspension should not dry out), carefully fill the MEA with 1 ml 3 % FCS medium.

6. Incubate the MEAs in an incubator at 37 °C and replace the medium each second day. For incubation over the weekend, you should use as much medium as possible and place the MEA into a water-filled dish to prevent osmotic effects due to evaporation.

7. Recording can be performed from two days after the preparation on until ten days after the preparation. Please note that cell properties change slightly over culture time. Thus, compare only cultures of approximately the same age.

2.5 Alternative Coating: Polyethylenimine (PEI)

**Preparation of PEI solution**

- 1.24 g Boric acid (Sigma B0252)
- 1.9 g Borax (Sigma B0127)

  dissolve in 400 ml distilled H₂O at 80 ºC.

- Add + 0.4 ml 50 % PEI (Sigma P3143).

- Filter using sterile syringe filters.

- Store at 4 ºC.

**Coating of MEAs**

- Coat MEAs / QT-Well plates the day before the cell preparation.

- Add 200 μl PEI solution per single well MEA, 70 μl per well of a 6 well MEA or 9 well MEA or QT well plate.

- Wrap MEA or plate in parafilm to avoid evaporation.

- Store MEA or plate overnight at 37 ºC.

- Remove PEI solution.

- Rinse twice with distilled water for 2 minutes each.

- Dry MEA under laminar flow prior to plating cells.
3 Applications

3.1 QT shaped Field Potential Analysis

The drug induced prolongation of the QT interval in the ECG is considered as a significant indicator for an increased proarrhythmic potential of a drug. This effect is typically mediated by a disturbance of ventricular depolarization. Thus, a prolongation and triangulation of the action potential of a ventricular cardiomyocyte can be considered as an indicator of increased risk of ventricular tachyarrhythmia. The extracellular recording from beating cardiomyocytes using MEA electrodes records cardiac field potentials. These reflect the first derivative of the action potential as recorded by sharp electrodes or in current clamp patch clamp. The shape of the field potential is comparable to the ECG, however a careful interpretation is necessary. The illustration below shows a typical signal shape recorded with a MEA1060 amplifier. The shape of the signal might vary depending on amplifier filter settings.

![Signal shape](image1.png)

The dose dependent effect of a QT prolonging drug is shown below. The repolarization related component of the cardiac field potential is shifted towards the right and the shape is modulated reflecting a moderate triangulation.

![Signal shape](image2.png)
MEA Application Note

Literature:


3.1.1 Mapping

The propagation patterns of electrical activity in a syncytium of gap junction linked cardiomyocytes are mapped by multiple electrodes in order to reveal information on conduction velocity, possible spiral waves or re-entry cycles. The MEA offers a grid of up to 252 recording spots for high resolution mapping. These data are used either as quality markers for the cells or to study arrhythmogenic mechanisms.


3.1.2 Pacing

The pacing of cultured cardiomyocytes allows controlling the beating frequency and to modulate the origin of signal propagation. This is important to induce spiral waves or induce arrhythmia.

The change from a slightly variable beating frequency to a paced – thus very stable beating frequency is shown in the plot below.

The figure below shows a recording on a 120MEA done with MEA2100-System. The signal plotted is a local activation time plot colour coding activation times on a jet colour scale. Early is indicated by red, late by blue. The colour versus time plot creates one colour bar per beat. A reversion of the propagation pattern is observed with the begin of stimulation. (Lower right, black, was used for pacing).
As pacing requires a sufficient charge transfer it is crucial to work with electrodes with a low impedance. This typically requires at least 30 μm diameter electrodes. The 9 well MEAs have in every well two extra large electrodes with 200 mm x 50 μm. These allow to pace with a reduced voltage amplitude.

A typical pacing protocol for cardiomyocytes will require a biphasic voltage pulse of approximate +/- 2 V with a 500 μs phase duration.

Using blanking circuit systems allow efficient artifact suppression. The figure below shows not stimulated signals (orange) and paced activity (red) recorded with MEA2100 applying enhanced blanking circuit technology.
3.2 Software

3.2.1 MC_Rack

MC Rack is the versatile software that comes with all MCS recording systems. The MC_Rack manual describes all features of this software in detail. It can be used to display, record and analyze cardiac data.


3.2.2 Cardio2D

The Cardio2D software is a software for simplified mapping of cardiac signal propagation in cardiac cell preparations, slices and on isolated hearts (Langendorff) or even in vivo. The enhanced analysis is done using the Cardio2D+ software.

Please note, that only files recorded by Cardio2D can be recorded by Cardio2D+. Files recorded with MC_Rack program are not recognized by Cardio2D+.

Cardio2D works with all suggested MEA-Systems, please see chapter “System Configuration”.

A detailed description can be found in the Cardio2D manual.

3.3 Suggested MEA-Systems

System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, various system configurations are recommended for the recording from cardiomyocytes.

- **MEA2100-System**: For the MEA2100-HS60(HS2x60)-System, the same MEAs can be used as for the MEA1060 amplifiers, with the same advantages and disadvantages. In addition, for the MEA2100-HS120-System a 120-channel MEA layout is available (**120MEA200/30iR**), which provides a larger electrode field with a resolution identical to the **60MEA200/30iR**. This might be interesting for a mapping applications. The MEA2100-System allow to operate a maximum of two headstages with up to two 60 channel MEAs each. If all those slots are used with 6 well MEAs 24 wells can be tested simultaneously.

- **USB-MEA256-System**: This system includes a 256 channel amplifier and data acquisition in one device. You can record data from 252 electrodes. MEAs with 9 wells are ideal for drug testing. This is the best choice if you need high throughput and high resolution mapping.

- **USB-MEA60-Inv-System-E**: 60-channel MEA recording system for inverted microscopes. Either with USB data acquisition or MC_Card data acquisition installed in the computer. The temperature controller **TCX** regulates the temperature of the MEA and of the perfusion fluid via the perfusion cannula **PH01**. A MEA amplifier allows recording up to 60 channels from one MEA. This is the standard configuration for low-throughput academic research.
3.4 Microelectrode Arrays

Cardiomyocytes tend to form gap junction coupled cultures that are triggered by a single pacemaker. The question, which MEA type should be used, depends mostly on the size of the area of interest. We recommend an electrode diameter of 30 μm for best signal quality.

Recommended MEAs include:

- **60 channel MEA** (60MEA200/30iR-Ti) with substrate-integrated reference electrode and TiN electrodes for establishing the cell culture and recording routine (for MEA1060 amplifiers and MEA2100-HS60 or MEA2100-HS2x60 headstages).
- **Economic MEA** (60EcoMEA or 60EcoMEA-Glass) for routine recordings with a medium throughput (for MEA1060 amplifiers and MEA2100-HS60 or MEA2100-HS2x60 headstages).
- **6 Well MEAs** (60-6wellMEA) for medium throughput (for MEA1060 amplifiers and MEA2100-HS60 or MEA2100HS2x60 headstages).
- **256 channel MEA** (256MEA) for high resolution mapping (only for USB-MEA256-System).
- **9 Well MEAs** (256-9wellMEA) for high throughput (only for USB-MEA256-System).
- **120 channel MEAs** (120MEA200/30iR-Ti) for MEA2100-HS120-System only.

Ring Options

The standard glass ring is best for running a perfusion, and will also allow using the ALA MEA-Insert. A plastic ring should only be used if parallel patch clamp experiments are planned.

4 References


