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MEA Application Note:
HL-1 Cardiac Cell Line
by Dr. W. Claycomb

MEA

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A major part of this information is based on the instructions provided by the laboratory of Dr. William Claycomb at Louisiana State University, New Orleans, LA, USA.

We thank Dr. Claycomb and his Coworkers for providing the cells and a detailed documentation.

Table of Contents

1 Material

1.1 Biological Materials

- 2 T25 flasks with HL-1 cell line

1.2 Technical Equipment

- Sterile workbench
- Incubator at 37 °C, 5 % CO_2
- Centrifuge (for 15 ml tubes)
- 8 – 10 sterile MEAs (microelectrode arrays), coated with Polyethylenimine (PEI) (see MEA manual)
- 50 ml Falcon tubes
- 15 ml Falcon tubes
- 10 ml pipettes
- 5 ml pipettes
- Sterile 0.2 m Acrodisc © syringe filter
- Cell culture flasks

1.3 Chemicals

- Claycomb Medium, JRH Biosciences (51800)
- Fetal bovine serum (FBS): JRH Biosciences (12103-500M), current recommended lot number 3J0229. It is important that the recommended FBS, which has been tested for this cell line, is used.
- Penicillin Streptomycin (10000 U/ml P and 10 mg/ml S), Life Technologies (15140-122)
- Norepinephrine [(±)-Arterenol], Sigma (A-0937)
- L-Ascorbic acid, sodium salt, Sigma (A-7631)
- L-Glutamine, 200 mM, Life Technologies (25030-081) (store at –20 °C)
- 95 % FBS/5 % DMSO (store at 4 °C for up to a week)
- Trypsin-EDTA, 1x, Life Technologies (25300-054)
- Trypsin inhibitor, soybean, Life Technologies (17075-029)
- Dulbecco's phosphate buffered saline (PBS)
- Fibronectin, Sigma (F-1141)
- Bacto © Gelatin, Fisher Scientific (DF0143-17-9)
- Cryovials, Corning, VWR (66021-920)
- Distilled water, cell culture grade

2 Methods

2.1 Preparations

2.1.1 Supplemented Claycomb Medium / Wash Medium

Ingredient	*Volume*	*Final concentration*
Claycomb medium	87 ml	87 %
Fetal bovine serum	10 ml	10 % (5 % for wash medium)
Penicillin/Streptomycin	1 ml	100 units/ml Penicillin,100 µg/ml Streptomycin
Norepinephrine (10 mM stock)	1 ml	0.1 mM
L-Glutamine (200 mM stock)	1 ml	2 mM

- Prepare the supplemented medium as listed above.
- Wrap the bottle with the Claycomb medium in aluminum foil since the medium is extremely light sensitive.
- You should always use fresh supplemented Claycomb medium (for up to two weeks). In case you want to use leftover medium after two weeks, you have to replenish the L-glutamine, which is chemically unstable.

2.1.2 Norepinephrine Stock Solution

The following instructions are for a 10 mM stock solution of norepinephrine. Add 1 ml of this stock solution to 100 ml medium for a final concentration of 0.1 mM. The norepinephrine stock solution needs to be freshly prepared monthly.

1. Prepare 100 ml of 30 mM ascorbic acid by adding 0.59 g ascorbic acid to 100 ml of distilled water.
2. Add 80 mg norepinephrine to 25 ml of the 30 mM ascorbic acid.
3. Filter sterilize the solution using a 0.2 m acrodisc syringe filter.
4. Prepare 1 ml aliquots in sterile microtubes with screw caps.

2.1.3 Soybean Trypsin Inhibitor

1. Dissolve 25 mg of soybean trypsin in 100 ml Dulbecco's phosphate buffered saline (PBS).
2. Filter sterilize (using a 0.2 m syringe filter) into a 100 ml bottle.
3. Store at 4 °C up to a month.

2.1.4 Pre-Coating Culture Flasks

1. Add 0.1 g Bacto-Gelatin to 500 ml distilled water in a glass bottle.
2. Autoclave. The gelatin will dissolve during the autoclavation. The final concentration of gelatin is 0.02 %.
3. Fibronectin is provided in 5 ml aliquots. Dilute 1 ml fibronectin in 199 ml of 0.02 % gelatin. Mix gently, and immediately aliquot 6 ml per 15 ml centrifuge tube.
4. Store aliquots at –20°C.

5. Before culturing cells, coat the flasks with this coating solution (2 ml / T25 or 6 ml / T75 flask).
6. Close the flasks and incubate at 37 °C over night.
7. Remove the coating solution by aspiration the next day just before adding cells to the flasks.

2.2 Culturing Cells

2.2.1 Maintenance

- Cultures are fed with supplemented Claycomb medium (5 ml/T25 flask) every day.
- To avoid feeding the cells on weekends, 10 ml of supplemented Claycomb Medium is added to each T25 flask on Friday afternoon; this medium is not changed until the following Monday morning.

2.2.2 Splitting the Cells

It is recommended that the cells be split when they reach confluency.

1. Split one of the two T25 flasks 1 : 2 (see chapters "Digestion with Trypsin" and "Passaging the Cells"), resulting in two T25 flasks. This set of two T25 flasks will be your "working" set of cells.
2. Split the other T25 flask 1 : 3, and place the contents into one T75 flask. After the cells in this T75 flask are confluent, they should be split into two T75 flasks. When the cells in these two flasks reach confluency, they can either be frozen, please see chapter "Freezing the Cells", or further split 1 : 2, resulting in 4 T75 flasks that can be frozen. We recommend two splits and freezing away four flasks.
3. Thus, within a week after receiving the HL-1 cells, you should have four cryovials of these cells that are stored frozen for future use.

2.2.3 Digestion with Trypsin

This procedure is necessary for dislodging the cells from the flask bottom before the passaging or freezing.

1. Rinse each T25 flask briefly with 3 ml of 0.05 % trypsin/EDTA warmed to 37 °C (please use 6 ml for T75) by pipetting the trypsin/EDTA onto the bottom of the flask (side opposite the cap), trying not to hit the cells directly with the enzyme.
2. Rinse gently and remove by aspiration.
3. Add another 1.3 ml trypsin/EDTA per T25 flask (3 ml per T75). Incubate at 37 °C for 2 minutes.
4. Remove and add fresh trypsin/EDTA. Incubate for an additional 3 – 8 minutes. Examine cells microscopically after 2 min.
5. Examine microscopically and, if cells are still adhered, rap the flask very gently to dislodge remaining cells from the bottom of the flask.
6. To inactivate the enzyme, add an equal amount (1.3 or 3 ml) of soybean trypsin inhibitor directly onto the cells.
7. Rinse the empty flask with 5 ml (8 ml per T75) wash medium and add this to the cells that are already in the 15 ml centrifuge tube.
8. Centrifuge at 500 g for 5 minutes.

2.2.4 Passaging the Cells

1. During the digestion or centrifugation steps (see chapter Digestion with Trypsin), remove the coating solution from each T25 flask, and add 4 ml supplemented Claycomb medium per flask. Set aside.
2. Remove the tube containing the harvested HL-cardiomyocytes from the centrifuge. Remove the supernatant by aspiration, and gently resuspend the pellet in 3 ml of supplemented Claycomb medium.
3. Transfer 1 ml into each of three (clearly labeled) coated T25 flask. Each flask contains 5 ml now.
4. If the cells are passaged on a Friday, use 2x the volume of supplemented Claycomb Medium per flask.

2.2.5 Freezing the Cells

It is useful to freeze the contents of one confluent T75 flask into one cryovial. When cells are needed, this cryovial can be thawed and placed back into one T75 flask.

Note: It is recommended that you freeze four or more vials as soon as possible after receipt of the cells to prevent a loss of the cell line due to contamination or other problems.

1. Gently resuspend the cell pellet after the Trypsin digest (see chapter "Digestion with Trypsin") in 1.5 ml of freezing medium (95 % FBS/5 % DMSO).
2. Pipette resuspended cells into a cryovial. Place the cryovial containing the cells into a Nalgene freezing jar containing room temperature isopropanol.
3. Immediately place the freezing jar into a –80 °C freezer, and freeze cells at a rate of –1 °C per minute.
4. Six to twelve hours later, transfer the vial to a liquid nitrogen Dewar vessel.

2.2.6 Thawing the Cells

1. Coat a tissue culture flask overnight in a 37 °C incubator (please see chapter "Pre-Coating Culture Flasks").
2. Remove the coating solution from the culture flask the next morning and replace with 10 ml of supplemented Claycomb medium. Place this flask back into the incubator.
3. Transfer 10 ml wash medium into an empty 15 ml centrifuge tube. Incubate tube in a 37 °C water bath.
4. Quickly thaw the cells in a 37 °C water bath (about 2 min) and transfer them into the 15 ml centrifuge tube containing the wash medium.
5. Centrifuge for 5 minutes at 500 g.
6. Remove the tube from the centrifuge and remove the wash medium by aspiration.
7. Gently resuspend the cell pellet in 5 ml supplemented Claycomb medium and add the suspension to the 10 ml medium in the T75 flask.
8. Replace the medium with 15 ml of fresh supplemented Claycomb medium 4 hours later (after cells have attached to the flask bottom).

3 Suggested MEA-System

3.1 System Configurations

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

- **MEA2100-System**: For the MEA2100-HS60(2x60)-System, the same MEAs can be used as for the MEA1060 amplifiers, with the same advantages and disadvantages. In addition, for the MEA2100-HS120 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 application. 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-MEA60-System**: **60-channel** MEA recording system for inverted or upright microscopes. The **temperature controller** TC02 regulates the temperature of the MEA and of the perfusion fluid via the **perfusion cannula** PH01. The peristaltic perfusion system PPS2 is provided, respectively. A **MEA1060 amplifier** allows recording up to 60 channels from a 60-channel MEA. This is the standard configuration for low-throughput academic research.

3.2 Microelectrode Arrays

Cardiac myocytes 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:

- **60MEA200/30iR-Ti** (with substrate-integrated reference electrode) for establishing the cell culture and recording routine.
- **60ThinMEA** for high-resolution imaging and, for example, combination with intracellular calcium measurements.
- **60EcoMEA** for routine recordings with a medium throughput.

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.

3.3 Amplifier Specifications

Though custom amplifiers with gain and bandwidth specified by the user are available, Multi Channel Systems MCS GmbH recommends the following settings for this application in **MEA1060** amplifiers.

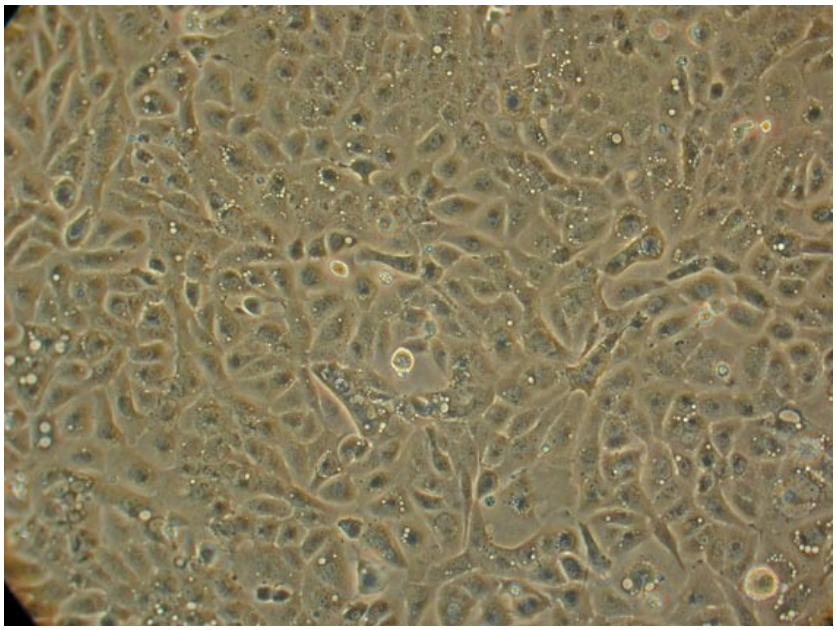
- Lower cut-off frequency: **1 Hz**
  If you select an even lower value for the lower end of the bandwidth, slow signal drifts will disturb the recordings. Lower limits of up to 10 Hz are recommended if you are not interested in Ca currents, for example for safety screening or mapping of excitation spreading.
- Upper cut-off frequency: **3 kHz**. Sufficient even for the rapid depolarization waveforms
- Gain: **1200**

In **MEA2100-Systems** the sampling rate, signal range and bandwidth can be adjusted via software control and is therefore suitable for a broad range of applications, from single unit spike recordings to field potentials from whole heart preparations.

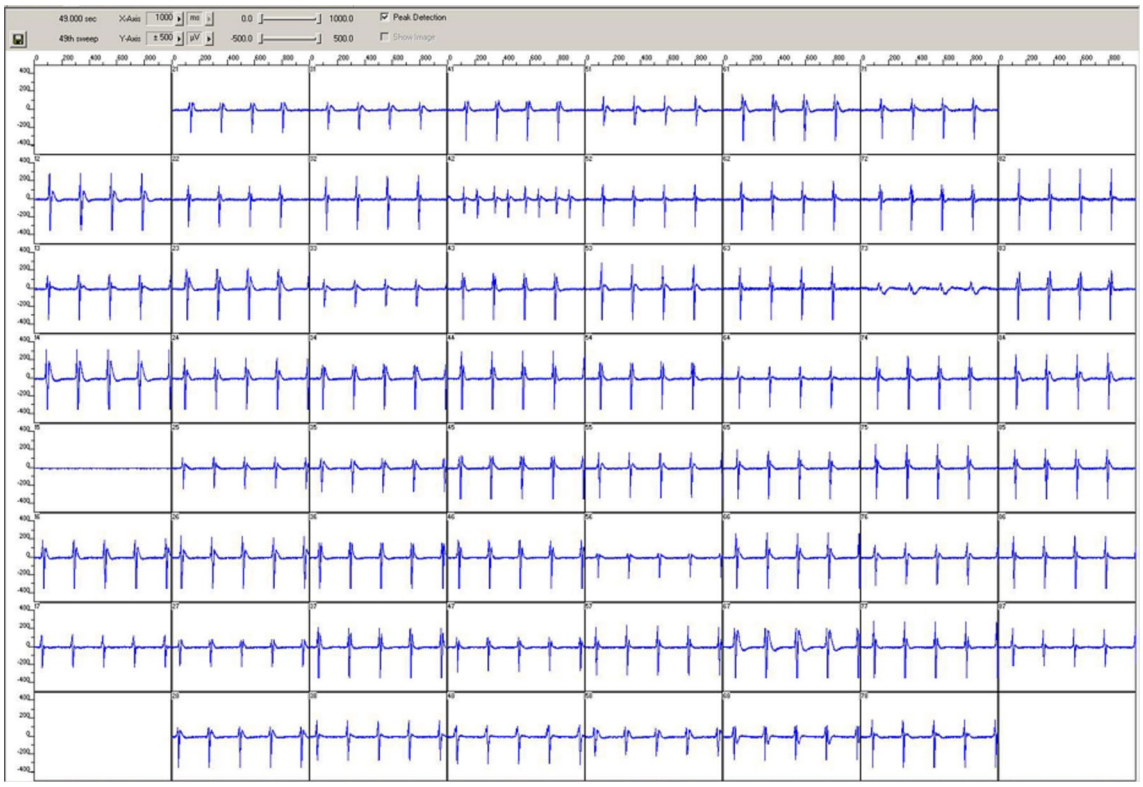
4 Application Example

The following pictures show typical signals from the HL-1 cell line recorded with the MEA-System.

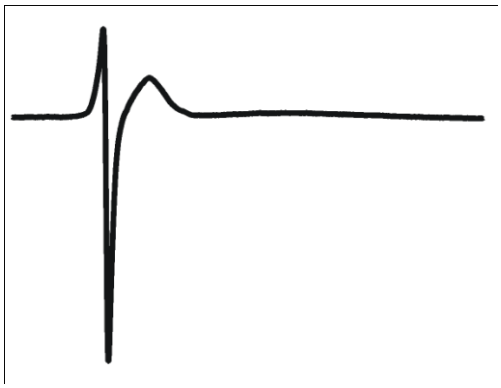
This experiment was made with 60EcoMEAs and a standard MEA1060 amplifier (bandwidth 10 Hz to 3000 Hz, gain 1200).



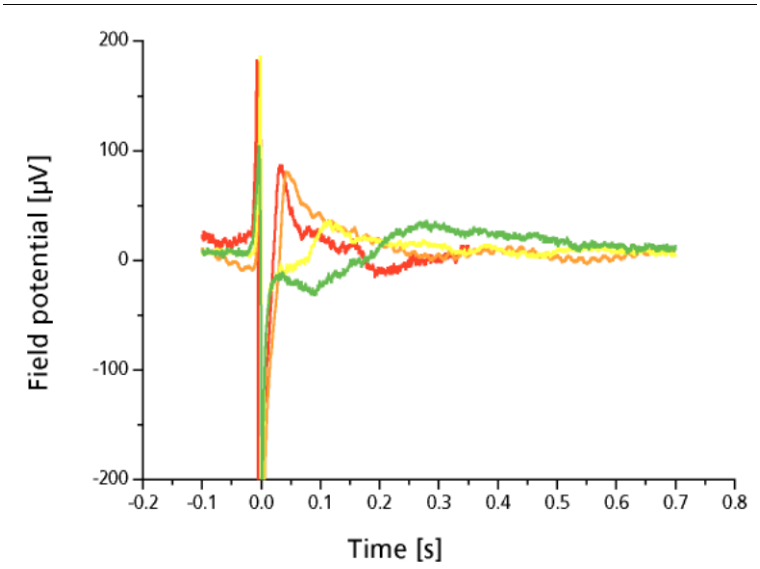
Confluent HL-1 cells, passage 59



Screen shot of a recording from HL-1 cells on a MEA with the MC_Rack program.



Typical HL-1 field potential.



Change of field potential shape under increasing concentrations of the Class IA antiarrhythmic drug Quinidine.

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5.2 Microelectrode Arrays and Cardiac Myocytes

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