Electrical stimulation of cardiomyocytes in µ-Slide I Luer Electrode

This application note describes the experimental conditions that were employed to electrically stimulate primary adult rat cardiomyocytes in the ibidi µ-Slide I Luer electrode (ibidi, 82000). With the parameter range shown in the experiments a successful stimulation of the cardiomyocytes was feasible. Also conditions were tested that lead to the formation of bubbles in the channel. Increasing the applied voltage to higher values than in the sample experiments might lead to pronounced bubble formation (electrolytic gas) and to damages of the gold electrodes in the slide. Fig 1. shows a typical image of a fluorescently stained cardiomyocyte. This picture was taken next to the electrode as the electrode itself is not compatible with fluorescence imaging.

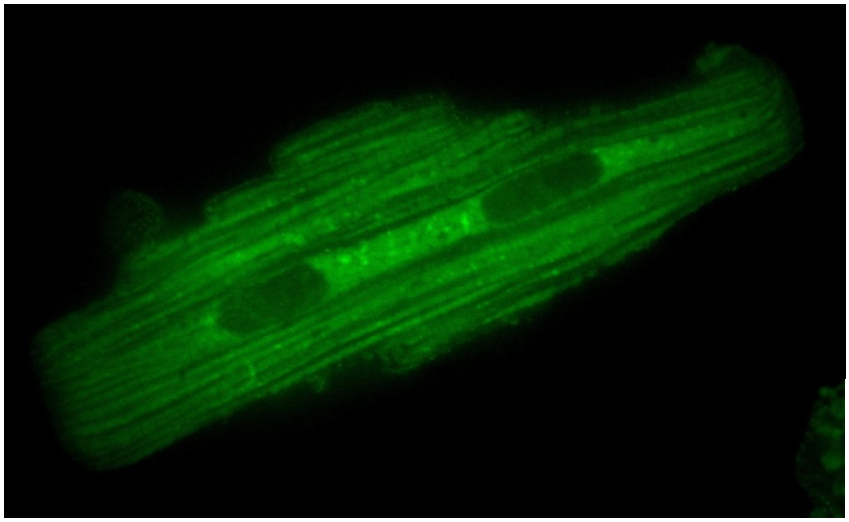

Fig 1: Primary isolated adult rat cardiomyocyte stained with a fluorescent label. The cell is 100 µm long

1. Material

Cells: Primary isolated adult cardiomyocytes of rats were used in the experiments. The isolation details can be found in: Kaestner et al. (2009) Isolation and genetic manipulation of adult cardiac myocytes for confocal imaging. J. Vis. Exp., 31, 1433 (http://www.jove.com/index/Details.stp?ID=1433) Remark: The method is not recommended for neonatal cardiomyocytes. They often beat spontaneously and can hardly be electrically paced.

Cell seeding: Primary isolated adult cardiomyocytes are not adherent cells, but would settle in the range of few minutes to 1 hour. They require coating of the slides, preferentially with a mix of extracellular matrix (ECM) proteins (details are given in the publication mentioned above). If not possible, poly-L-lysin coating should also work (but results would be not as good as ECM). Cells require a few minutes to 1h to settle. The cell density should be adjusted such that seeded cells don't touch each other. This makes all kinds of optical measurements (change in length for contraction, calcium or membrane potential) easier.

Electrical stimulation: Electrical pulses (typically rectangular) of 4-5 ms and 5-25 V at an adjustable frequency between 0.5 and 5 Hz were applied using a special pulse generator (Myopacer, IonOptix, Milton, MA, USA). The electrical contact with the pads on the slide was enabled by the ibidi contact module (ibidi, 10939).

Chip dimensions: The distances of the eight 1 mm wide and 4 mm long electrodes on the ibidi slide are show in the figure 2. The basis of the slide is a glass cover slip coated with gold electrodes.

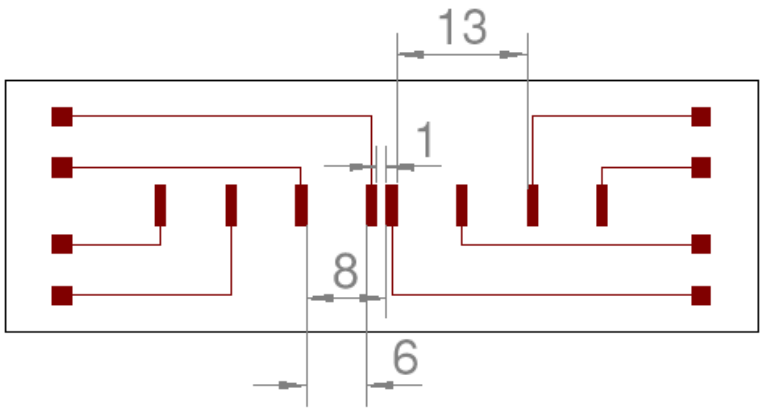

Fig 2: electrode slide with distances of the electrodes. The elctrodes are 1 mm wide

Optical measurements: Changes in cell length (contraction) or calcium imaging requires frame rates of 100 images per second, optical action potential measurements need frame rates of at least 500 images a second. The measurement duration depends on the electrolyte accumulation in the channel. With a slight laminar flow solution exchange recording durations can be extended. However minimal duration should be 10 min. Details on the optical measurements can be found in: O. Mueller et al. Cell Calcium 47 (2010) 224-233.

2. Experiments

Cardiomyocyte stimulation experiments with different electrode distances were performed at room temperature. The stimulation was recorded optically. Here only the parameters applied for the stimulation of the cardiomyocytes are shown. For typical optical evaluation results of the cardiomyocyte contractions please refer to Mueller et al. Cell Calcium 47 (2010) 224-233.

1mm electrode distance:
pulse 4ms, 1Hz, 5V. Increasing the voltage to 15 V leads to bubbles in the channel.

6mm electrode distance:
pulse 4ms, 0.5 Hz, 15V

8mm electrode distance:
pulse 4ms, 0.5 Hz, 15V

13mm electrode distance:
pulse 4ms, 0.5 Hz, 25V
pulse 4ms, 5 Hz, 15V