Assembly and preparation of MyoDish chambers

Composition

A completely assembled culture chamber comprises the following components:

- main chamber with built-in spring wire and built-in adjustable wire
- magnet fixed at the end of the spring wire (not removable)
- plastic lid from a standard 35 mm petri dish (disposable)
- 2 graphite electrodes: 1x long wire, 1x short wire
- 1 sensor board, labeled with either A, B, C, or D
- 2 screws for fixation of sensor board
  (M3x6, stainless steel, hexagon socket (DIN 912))

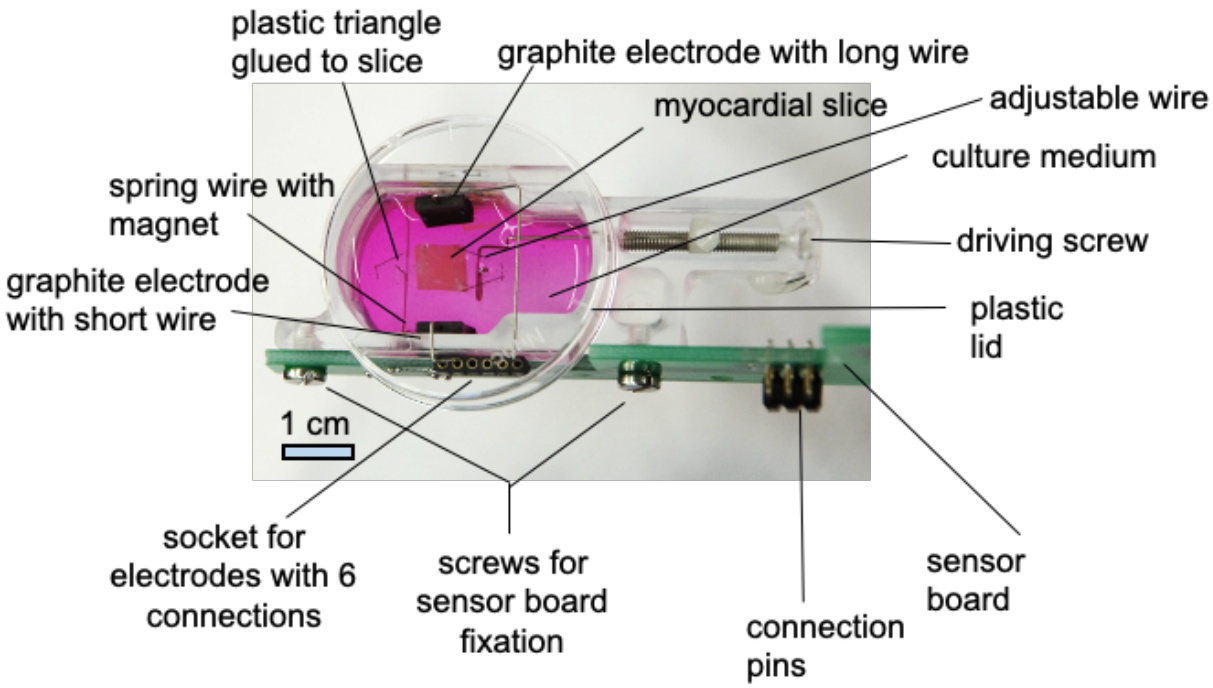

Figure 1. Photograph of completely assembled MyoDish chamber, containing culture medium and a mounted myocardial tissue slice.

Assembly and cleaning before use

- Fill culture chambers with 100% isopropyl alcohol, ideally with a sprayer, or immerse briefly in 100% isopropyl alcohol for 1 min
- Let dry under laminar flow hood (sterile cell culture bench)
- use autoclaved electrodes
  OR
  spray 100% isopropyl alcohol on electrodes and let dry under laminar flow
- Fix the sensor boards on the chambers with the screws
- Insert the dry electrodes into the chambers (1 short wire electrode and 1 long wire electrode into each chamber, see Fig. 1)
    - Note: do not use your hands, use clean forceps
    - It is possible to gently push the electrodes into the connections with a lid from the top (see next step)
    - Connect the short-wired electrode to one of the left three connections of the socket, the long-wired electrode to one of the right three connections (Fig. 1)
- Use a new or autoclaved lid from a 35 mm culture dish and place it on top of the chamber; it is possible to gently press the electrodes into the connection with the lid

Preparation of the chambers for tissue culture

- Fill 2.4 ml of culture medium (for example M199) into the cleaned and pre-assembled culture chambers
- Place the culture chambers on the MyoDish culture system (in the incubator) by connecting the connection pins with the respective socket on the MyoDish main board
- Let the medium equilibrate to the incubation conditions (e.g., 5 % CO2 and 37° C) for at least 15-20 min
- Start stimulation in the MyoDish Control Software (with 50-70 mA) and check if any of the channels signals that the stimulation current is not reached (highlighted in red at the bottom of the main window). If this is the case, check if the electrodes are connected correctly or if electrodes are defect by replacing them with new electrodes.
    - Note: If stimulation currents higher than 50-70 mA are used, the current is sometimes not reached although the electrodes are fine
- The sensor offset can now be detected. In the main form of the software, go to *Settings* → *Calibration…* , then click on *Detect offsets*, then on *Apply*.
- After the equilibration period, tissue slices can be mounted into the chambers and preload can be set to the desired value by turning the driving screw and watching the diastolic value