Pancreatic beta cell line MIN6

1. Pretreatment of MED Probe surface

The most important step in cell culture on the electrode is pretreatment of the MED probe surface. In a brand-new MED probe, its contact surface including 64 electrodes is relatively hydrophobic and must be hydrophilized prior to coating with extracellular matrix. Even if the following steps are completed successfully, cell aggregation or apotosis might be happen probably due to insufficient hydrophilization. Here, an example of procedures how to hydrophilize and coat the surface with gelatin for culturing MIN6 cells will be shown.

1.1. Rinsing and sanitizing

1) Rinse the inside of the chamber of a brand-new MED Probe with sterilized distilled water (SDW) three times.
2) Fill the chamber with 70% ethanol and sanitize for 15 min.
3) Remove the 70% ethanol and rinse the inside with SDW three times. Dry out it under UV irradiation for 15 min.

1.2. Hydrophilization with 0.1% PEI solution

1) Pour 0.1% PEI solution into the MED Probe. Approximately 0.7 ml is needed to cover the surface inside the chamber including the electrodes.
2) To avoid evaporation of the 0.1% PEI solution, leave the MED Probe in a plastic petri dish over a night.
3) Remove the 0.1% PEI solution and rinse the inside with SDW at least three times.
4) Fill the inside with SDW and leave it at 4ºC (in refrigerator) at least over 72 hours.

Note1: Step 1.2. is not required for the second and the subsequent re-use of the MED Probe.
Note2: In all procedures after 1.2. 4), manage the MED Probe in a plastic petri dish while filling the inside of the dish with an appropriate amont of SDW to prevent the PEI, extracellular matrix, or cell suspension from evaporation.

Table1. The composition of 0.1%PEI solution.

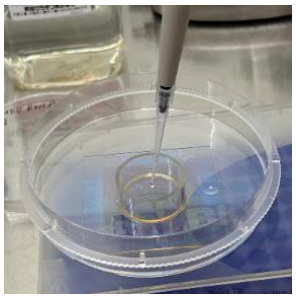
a. 25 mM borate buffer (500 ml)
1. Dissolve 4.768 g sodium tetraborate decahydrate (Sigma-Aldrich #S9640) in 450 ml of DDW.
2. Adjust the pH to 8.4 with 1N HCl.
3. Add DDW up to 500 ml and store it in the refrigerator.

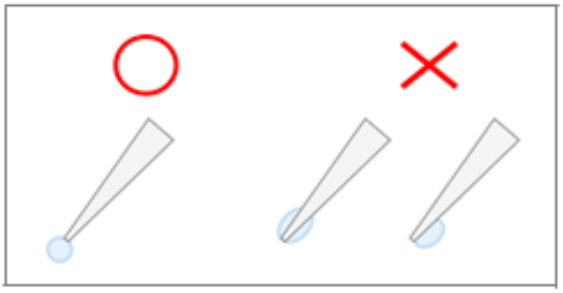
b. 1% PEI stock solution
1% PEI solution should be stocked in advance of use because the concentrate solution has a high-viscosity.
1. Dilute 1 ml of 50% poly(ethyleneimine) solution (Sigma-Aldrich #P3143) in 49 ml of 25 mM borate buffer solution and store it in the refrigerator.
2. Mix 1% PEI solution with 25 mM borate buffer solution in the ratio of 1:9 just before use.

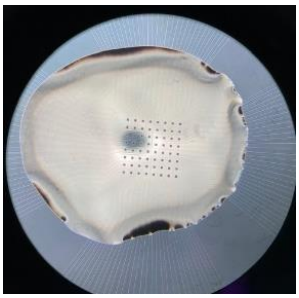
2. Culturing MIN6 cells on MED Probe

2.1. Coating with 0.1% gelatin solution

1) Rinse the inside of the MED Probe with SDW three times.
Note: At this step, check the surface of the MED Probe visually to confirm that it has lost the hydrophobicity and is moist and wet.
2) After remove SDW, drip a 4 µl of 0.1% gelatin solution (GLS250, Nitta Gelatin) on the 64 electrodes at the center of the MED Probe. Make a ball-shaped droplet at the tip of the pipette tip and drip it as like applying onto the electrodes while not damage electrodes by touching with the tip.







3) Incubate the MED Probe coated with 0.1% gelatin solution for an hour.

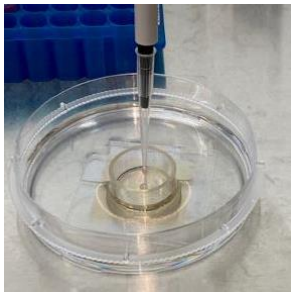
2.2. Preparing MIN6 cells suspension - detaching and collecting MIN6 cells adhered to a passage culture dish

1) Remove media from the passage culture dish.
2) Rinse the inside of the dish with 5 mL of D-PBS(-) and then remove it.
3) Fill the inside with 1 mL of trypsin solution and place it in incubator for approximately 3 min.
   Note: Check the detaching condition at every 30 sec to stop and optimize the reaction time.
4) Add 4 mL of maintenance media into the dish to stop the detaching process.
5) Cellect the cell suspension to transfer it into a 15 mL centrifuge tube.
6) Centrifuge at 1400 rpm at room temperature for 3 min.
7) Count the viable cells and prepare MIN6 cells suspension at 2 x 10^4 cells/4 µL.

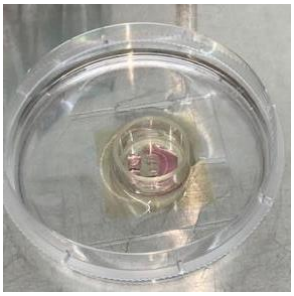
Note: The above procedure is just a case example. Please follow your laboratory's protocol to prepare.

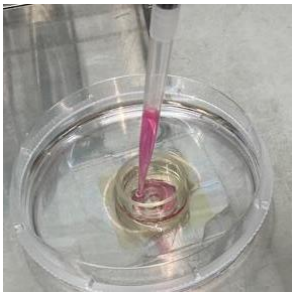
2.3. Seeding MIN6 cells suspension onto electrodes

1) Transfer the MED Probe from incubator into clean bench.
2) Seed 4 µL of cell suspension as like applying directly onto a droplet of gelatin solution on the electrodes.
   Note: Do not remove the gelatin droplet before the step.



3) Incubate the MED Probe with cells seeded at 37°C and 5% CO_2 for an hour.
4) Transfer the MED Probe from the incubator into a clean bench.
5) To prevent the cell suspension from flowing away from the electrodes, add 500 µL of maintenance medium surrounding electrodes and then add another 500 µL of maintenance medium inside the ring of the medium.





6) Incubate the MED Probe with cells seeded at 37°C and 5% CO_2 overnight.

7) Add another 500 µL of maintenance medium to make the total volume 2 mL. Change the medium into new medium completely every 3 or 4 days if necessary.
8) Perform data acquisition after the third day of culturing on the electrodes to allow the cells to proliferate and mature electrical couplings.

3. Tips for data acquisition and analysis without perfusion

3.1. Data acquisition

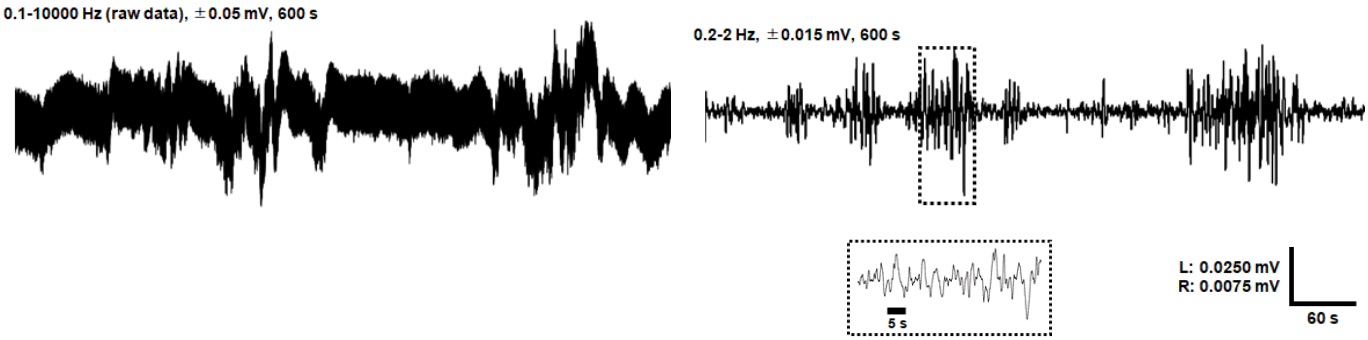
1) Replace the entire amount of maintenance medium with oxygenated physiological buffer for data acquisition (138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, 5 mM HEPES, adjusted to pH7.4 with NaOH) completely just before the data acquisition. Depending on your experimental goal, add and dissolve an appropriate amount of glucose or chemical compound into the physiological buffer to achieve a specific concentration.
Note: The physiological buffer should be aerated with 95%O_2-5%CO_2 carbogen gas, because the existence of the oxygen might affect the cellular electrical response (probably ATP/ADP ratio) severely according to our limited experience.

2) Because electrical response of beta cells usually contains very slow fluctuating component, acquire data with the frequency bandwidth ranging from 0.1 to 10000 Hz (Low cut freq: 0.1 Hz; High cut freq: 10000 Hz).
Note: The whole system must be installed in a stable environment to prevent cables from being affected by physical vibrations that induce fluctuation of baseline response when acquiring data under 0.1 Hz high-pass filter. In addition, note that pulsation caused by perfusion can also cause the fluctuation of the baseline response when an experiment with perfusing solution.

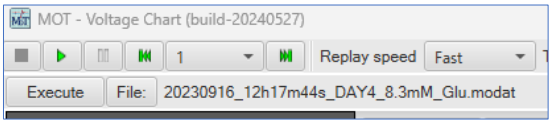
3) When evaluating different doses of glucose or compounds efficacy, replace the entire buffer into oxygenated buffer containing those as same as step 1).

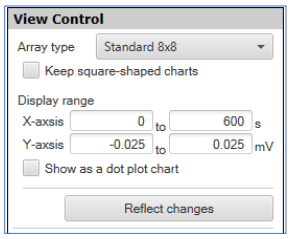
3.2. Data analysis

Electrophysiological studies in pancreatic beta cells or islet using MEA have been reported since around 2010, although they are not numerous. Most of them are from two research groups; Lang J et al. in French; Drews G and Krippeit-Drews P et al. in Germany. Especially two of these publications, one is in 2011 by Lan Pfeiffer T et al., another is in 2015 by Lebreton F et al., would be helpful for researchers to grasp the overview and begin study in pancreatic beta cells by using MEA. The French group has focus on the slow fluctuating potential of pancreatic electrical response as a marker of insulin secretory ability, called as slow potentials (SPs). It can be more apparently visualized after applying a bandpass filtering at 0.2-2 Hz to the raw data acquired under 0.1 - 10000 Hz bandwidth. Although the mechanism and physiological significance of the SPs may be controversial, this section briefly describes some steps how to quantify the SPs-like field potentials by using MED64 Offline Toolkit.

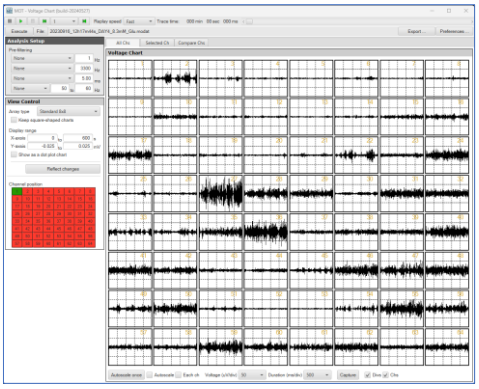


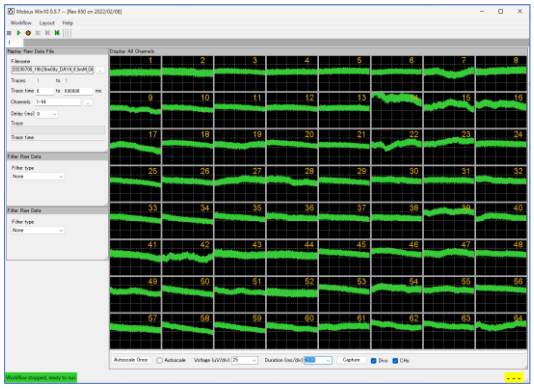
1) To view the full length of the data, use to select "Voltage Chart" tool from the Lanuncher window of the MED64 Offline Toolkit. After the main window is called, click "File:" button on the top menu to load a data file, then specify the X and the Y range to view and click "Reflect changes" button.



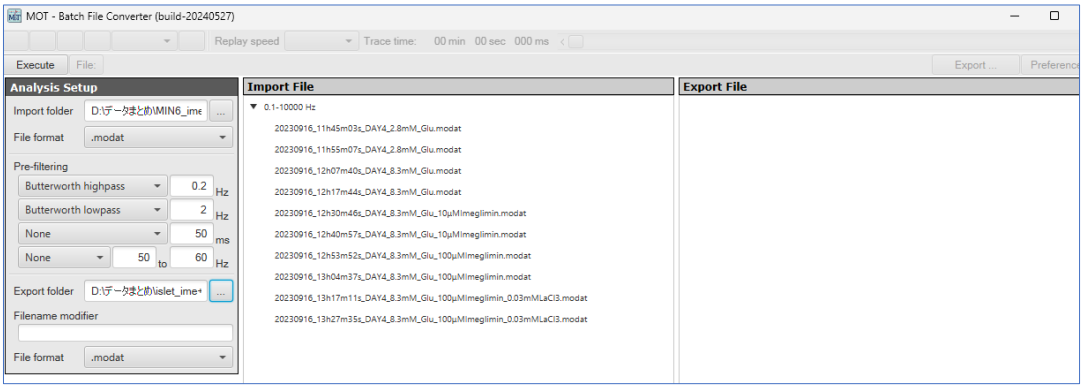


Depending on PC performance and the data file size, the full length of the data will be shown on each 64 channel on the All Chs tab window 1-2 minutes later. The MED64 System control software “Mobius” is not able to draw over 10 sec of voltage chart. Because it is design to acquire data while analyze it online, that is like a kind of specification considering overload to PC. Therefore, MED64 Offline Toolkit will be needed to view long range of data.

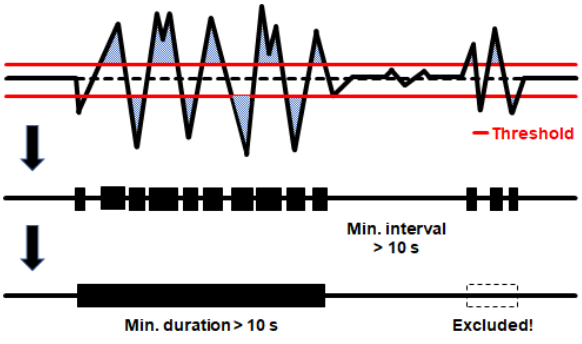


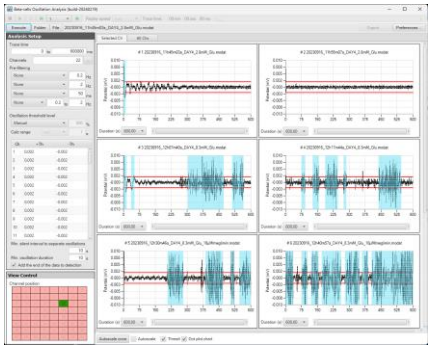


2) The “Voltage Chart” tool can not only view data but also apply high-pass or/and low-pass filter before drawing the data. But the processing time of filtering is relatively long and time consuming. Therefore, creating a set of data files after filtering by using “Batch File Converter” will be recommended prior to viewing data with the “Voltage Chart” tool. The operation of it is simple, specify an import folder containing a set of data files you would like, filter setting and an export folder, then click “Execute” button to start the processing. Depending on PC performance and the settings, files will be exported after a few minutes passed.



3) Beta-cells Oscillation Analysis tool included in MOT (after ver. 240703) enables you to detect intervals of characteristic slow and fluctuating field potentials (called as oscillation) observed in beta cells based on the simple algorithm. It can also process multiple files in an identical folder serially, although the electrode to be analyzed must be narrowed considering the big processing load.





4. Cleaning the MED Probe

The MED Probe is basically designed for a disposable use because maintaining a low electrode impedance (< 50 kΩ) is very important to recording signal with a good S/N ratio. Maintaining a low electrode impedance is also necessary to effectively stimulate neurons or cells. Impedance of the electrodes will increase with repeated use of the probes; this is due to either damage in handling or the build up of organic compounds associated with the tissue on top of the electrode (i.e. after an experiment is completed and the tissue is removed). However, the MED Probes can be reused several times if the tissue is removed gently and it is cleaned carefully. (Note: Do NOT touch the surface of the MED Probe directly, the micro-electrodes and insulation layer might be damaged.)

1) Remove the culture medium, fill the chamber with 0.25% trypsin EDTA (#25200-056, Life Technologies) immediately afterwards. Incubate it at 37°C for about an hour.
2) Detach the cells by pipetting several times.
3) Remove the trypsin EDTA by using an aspirator, and then wash two or three times with SDW.
4) Fill the MED Probe with some DDW and put it into a 90 mm dish. And then store them at the refrigerator (Note: DDW will be evaporate gradually, check the condition ad libitum and add it if necessary.)

5. Acknowledgement

This protocol was developed in collaboration with the following researchers and the results were presented at the 144th Annual Meeting of the Pharmaceutical Society of Japan (Hitomi H et al., Investigation of electrophysiological assay for evaluating drug responsesiveness of MIN6 and islets to insulin secretion using micro electrode array, 30P-am237, 2024).

Hitomi Hirose, Intractable Disease Research Center, Juntendo University
Hideo Saotome, Intractable Disease Research Center, Juntendo University

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6. References

1) Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K. Establishment of a pancreatic cell line that retains glucose inducible insulin secretion: Special reference to expression of glucose transporter isoforms. Endocrinology 127: 126-132, 1990.

2) Pfeiffer T, Kraushaar U, Dufer M, Schonecker S, Haspel D, Gunther E, Drews G, Krippeit-Drews P. Rapid functional evaluation of beta-cells by extracellular recording of membrane potential oscillations with microelectrode arrays. Pflugers Arch 462: 835-840, 2011.

3) Lebreton F, Pirog A, Belouah I, Bosco D, Berney T, Meda P, Bornat Y, Catargi B, Renaud S, Raoux M, Lang J. Slow potentials encode intercellular coupling and insulin demand in pancreatic beta cells. Diabetologia 58: 1291-1299, 2015.