1 MEA Handling

Warning: Use only liquids or cleaning solutions with a neutral pH (7) on MEAs with a silicon nitride insulation type. Otherwise, the MEAs may be irreversibly damaged.

Warning: Do not to touch the electrode field in any way during the coating or cleaning procedure. Keep all instruments, tissues, pipette tips, and so on at a safe distance from the recording area. The electrodes are easily damaged.

1.1 Pretreatment

1.1.1 Hydrophilic Surface

MEA materials tend to become hydrophobic during storage, which prevents attachment of the (hydrophilic) cells. The first step in preparing an MEA for use is therefore to ensure that the surface is hydrophilic enough for coating and cell adhesion.

To test this without contaminating the surface, place a small drop of water on the MEA surface outside the culture chamber. If the drop does not wet the surface, you likely need to perform one of the following steps, in particular when using new arrays.

Laboratories with access to electron microscopy facilities are likely to have a sputter device or a plasma-cleaning chamber (for example Harrick Scientific, Ossining, NY). MEAs can be treated in these chambers with a low-vacuum plasma for about two minutes. This will crack molecules on the surface and make them polar resulting in a more hydrophilic surface. Note that the effect wears off after a few days. The treatment gives a very clean and sterile surface that can be coated readily with water-soluble molecules.

If protein coating is acceptable in the planned experiments, there is another quick and simple way to render the surface hydrophilic.

1. Sterilize the MEAs as described below.
2. Place approximately 1 ml of a concentrated, sterile protein solution (for example, albumin, fetal calf serum or similar) onto the culture region for about 30 min.
3. Wash the culture chamber thoroughly with sterile water afterwards. The MEA can then be directly used for cell culture.

1.1.2 Sterilization

Sterilization of MEAs is not necessary for acute slices.

Silicon nitride MEAs can be sterilized with standard methods for cell culture materials using either 70 % ethanol, UV-light (about half an hour depending on the intensity), dry-heat sterilization, or vapor autoclavation.

3-D MEAs can be thermally sterilized in an oven at 56 °C with an incubation time of 8 hours.

Warning: Do **not** autoclave or sterilize **3-D MEAs** or **FlexMEAs** by **heat**. These MEA types are not heat-stable and will be irreversibly damaged.

1.2 MEA Storage

Store MEAs in distilled water in the dark (to prevent growth of algae) to maintain a hydrophilic surface. Immerse MEAs in distilled water for at least 24 hours before use.

1.3 Coating of MEAs

Coating of MEAs with various materials is used for improving the attachment and growth of cell cultures or cultured slices. Is is generally not necessary for recordings from acute slices.

In the following, some standard procedures for coating MEAs are shortly described. The behavior of MEAs differs slightly with different materials. You should try out which coating procedure proves best for your application. For more detailed information, please refer to the literature.

The listed materials are only recommendations. You can use any equivalent equipment as well.

Most coatings are stable for several uses of the MEA and do not have to be removed after use (except nitrocellulose).

1.3.1 Nitrocellulose

Coating with cellulose nitrate is a fast procedure that works with several cell types and tissues and that is also successful with slightly hydrophobic MEAs. This method has the advantage that the cells stick well to the surface. Nitrocellulose does not form a uniform layer on the MEA. Therefore, the tissue is not likely to get detached even under severe mechanical disturbance (by perfusion, for example).

Nitrocellulose coating has to be removed after use.

Note: Cellulose nitrate solutions cannot be stored indefinitely. The solution forms a visible gelatinous precipitate after extended storage of at least half a year and will not produce satisfactory adhesive coatings anymore. Prepare a fresh solution if there are visible precipitates.

1. For preparing a stock solution, dissolve a piece of 1 cm^2 cellulose nitrate filter paper in 10 ml methanol. Stock solutions may be stored at room temperature in polystyrene tubes.

2. For the working solution, add methanol in a ratio of 10:1.

3. Just before using an MEA, pipet 3–5 µl of the working solution onto the array and let it dry. It takes just a few seconds for the methanol to evaporate. Take care not to touch the electrode field with the pipet tip as this could damage the electrodes.

4. This leaves patches of cellulose nitrate on the MEA surface, which serves as a glue for the tissue to be mounted. MEAs coated with cellulose nitrate can be stored for a few days.

Literature

Ulrich Egert, Thomas Meyer; Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.) (in print)

Sources of supply

You can also use any other standard cellulose nitrate filter paper.

Product	**Supplier**
Protran Nitrocellulose Hybridization Transfer Membranes	PerkinElmer www.perkinelmer.com

1.3.2 Polyethyleneimine (PEI)

Polyethyleneimine (PEI) has been successfully employed for dissociated cell cultures and proven to enhance cell maturation in culture compared to polylysine coated plates. PEI works by changing the charge on the glass surface from negative to positive. The tissue sticks even better with this method than with the nitrocellulose method, but the polyethylenimine forms a uniform layer that can get more easily detached from the surface.

Note: It is necessary to thoroughly rinse off unbound PEI from the plates before using them to prevent the high pH (~9.5) of the solution from affecting the viability of cells or tissues.

1. Prepare a stock solution of 0.1 % PEI dissolved in distilled water or borate buffer (1.24 g boric acid, 1.9 g borax (sodium tetraborate) ad 400 ml distilled water).
2. Fill the MEA with this solution and let it stand at 4 °C over night (at least 2 h). The bottom should be sufficiently covered.
3. Thoroughly rinse with distilled water and allow to dry.
4. Sterilize with UV light for at least 1 h after coating.

Literature

- Ulrich Egert, Thomas Meyer; Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.) (in print)
- Lelong, IH, et al. (1992); J. Neurosci. Res. 32:562-568

Sources of supply

Product	Product No.	Supplier
Poly(ethyleneimine) solution	P3143	Sigma-Aldrich www.sigmaaldrich.com

1.3.3 Fibronectin

Fibronectin (Becton Dickinson, Heidelberg, Germany) is a more biological coating alternative, especially used for heart tissues. Cultures tend to be more stable with respect to adhesion, which allows longer cultivation times.

1. Prepare a stock solution of 1 mg/ml fibronectin in distilled water and store it at 4°C.
2. This solution is diluted with water to a final concentration of 10 µg/ml.
3. Cover the MEA surface with 300 µl of this solution and incubate for at least 60 min at 37 °C.
4. Wash MEAs twice in PBS and plate the cells onto the MEA immediately.

Literature

- Ulrich Egert, Thomas Meyer; Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.) (in print)

Sources of supply

Product	Supplier
BD BioCoat™ Fibronectin Cellware	BD Biosciences www.bdbiosciences.com

1.3.4 Polyornithine (plus laminin)

1. Laminin coating should be carried out just before using the MEA.
2. Incubate dishes in aqueous solution of polyornithine (500 µg/ml) for 2–3 hours or overnight.
3. Remove excess polyornithine, rinse dishes twice in distilled water before use or coating with laminin.
4. Dishes coated with polyornithine can be stored at 4 °C for several weeks until required. Poly-D-lysine can be used instead.
5. Cover pre-coated dishes with laminin at 5 µg/ml in PBS.
6. After 1 hour, remove excess laminin and rinse the dish with PBS.

Literature

- Cellular Neurobiology, A practical approach, ed. By Chad and Wheal, IRL Press, Oxford

1.3.5 Poly-D-lysine (plus laminin)

1. Poly-D-lysine has been used by several groups. Results seem to be equivalent to a coating with polyornithine.
2. Poly-D-lysine is applied to the dish.
3. After 1 hour, remove excess poly-D-lysine, rinse with distilled water and let it dry overnight.
4. Laminin is applied as described above.

Literature

- Goslin et al., 1988, Nature 336, 672-674
- Maeda et al., 1995, J.Neurosci. 15, 6834-6845
- Gross et al., 1997, Biosensors & Bioelectronics 12, 373-393

1.3.6 Concanavalin A

Concanavalin A at 1 mg/ml

Literature

- Wilson et al., 1994, J. Neurosci. Methods 53, 101–110

1.4 Cleaning of Used MEAs

1.4.1 General Recommendations for Cleaning MEAs

The cleaning procedure depends on the kind of coating and on the kind of biological preparation. In the following, a few general considerations are listed.

- If you have recorded from an acute slice without coating, you can simply rinse the MEA with distilled water and the MEA should be fine.
- If necessary, the MEA can then be cleaned with any ph-neutral cleaning agent.
- If more severe methods are needed, the MEA can also be cleaned in an ultrasonic bath for a short moment. But this method is a bit dangerous, because there are ultrasonic baths that are too strong and will destroy the MEA. The behavior should be tested with an older MEA first.
- EcoMEAs are easier to clean, because the golden electrodes are not so easily damaged.

1.4.2 Cleaning of 3-D MEAs

1. Rinse the culture chamber of the 3-D MEA thoroughly with distilled water.
2. Rinse the 3-D MEA with 70% ethanol for a few minutes.
3. Rinse the 3-D MEA with distilled water for 1 minute to remove the ethanol.
4. Air-dry the 3-D MEA, preferably under a laminar flow hood.

1.4.3 Removing Nitrocellulose Coating

Note: It is very important that you clean MEAs that have been coated with nitrocellulose and remove all biological material first before removing the coating. If you applied methanol on an uncleaned MEA, you would rather fix the cell debris on the MEA than actually remove the coating.

1. Directly after usage, biological material is rinsed off under running water and the MEA is cleaned with pH-neutral cleaning agents or enzymatically if necessary.
2. Methanol is applied for 15 to 30 min to dissolve the cellulose nitrate.
3. The MEA is then rinsed with distilled water.
4. The MEA should be stored in distilled water before reuse.