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A Novel Patch Micro Electrode Array for Sensing Ionic Membrane Currents

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Abstract

Ionic membrane currents play an important role during regeneration of nerve cells, embryonic development and wound healing processes. Measuring the intracellular ion currents across the cell membrane is important in understanding the cellular functions related to the ion activities. A novel patch micro electrode array (p-MEA) for measuring the ionic membrane currents without poisoning the cells due to emitting metal ions is described in this paper. Results on biocompatibility of the device are presented. We discuss the fabrication and working principle of p-MEA.

Keywords: ionic membrane currents, 3T3 Fibroblast cells, micro electrode array, patch clamp.

1. Introduction

The ionic currents produced in a cell membrane influence many biological processes, e.g. they help in wound healing and affect the formation of embryos [1]. Measuring the intracellular ion activities across the cell membrane can help in understanding the cellular functions related to that ion activities. Principally, using Alternative current does not lead to serious problems, whereas DC measurements cause electrode reactions due toxic metal ions. Patch clamp technique is widely used for measuring DC ionic

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currents from a cell membrane. A history of patch clamping technique can be found in the review article by Waltz [2]. The main disadvantages of this technique are:

- Cell membrane is ruptured.
- It is time-consuming and requires skilled labour to operate.

In order to overcome these disadvantages a novel design using micro electrode arrays (MEA) is presented. In order to study multiple cells at a time and record data simultaneously from multiple cells, MEA are more advantageous. We present a 4-channel patch MEA (p-MEA) to measure the ionic membrane currents from cells. The p-MEA comprises of a porous bio-compatible membrane hence providing electrical contact to cells but avoiding direct matter transport between electrolyte and backside of the fluidic channels and cell surface. The p-MEA is a non-destructive technique and easy to fabricate and operate. Moreover, multiple readings of ionic currents from a cell membrane can be obtained simultaneously. In this paper, we present the working principle and fabrication of the p-MEA as well as experiments showing its suitability to study cell behaviour.

2. Working principle and setup of p-MEA

An exploded view of the p-MEA is shown in Figure 1a. The size of a cell defines the design constraints on the electrode dimensions. The cells which would be investigated are 3T3 Fibroblast cells whose average size is about 50 μm in diameter. So in order to incorporate four electrodes under a cell, the dimensions are limited to 10 μm per electrode. The design parameters also require a quasi-flat surface which should be bio-compatible on which the cells can attach and grow. The flow of ions, i.e. the ionic current from the cell membrane requires the conducting medium to flow through micro-fluidic channels which should be coupled to the flat surface.

The MEA comprises three main components: a base plate which is made of a glass slide and micro-fluidic channel plate, a top plate which has a bio-compatible membrane on a Polydimethylsiloxane (PDMS) layer with opening, and the agar bridge setup for electrical stimulation of the cells. This approach allows the metallic points of the electrodes to be placed at a remote distance from the cells.

The ionic current from the cell membrane is measured by the electrodes. The electrodes act as the connectors between the cell and the data acquisition system. The top plate consists of a PDMS layer with electrode openings and a porous bio-compatible membrane. The top plate has two main functions. It first provides a flat surface for the cells to attach and grow. Secondly, the top plate acts as a seal for the channels containing the electrolyte medium. The PDMS layer which has four square holes of 10 μm acts as an electrode opening for the channels.

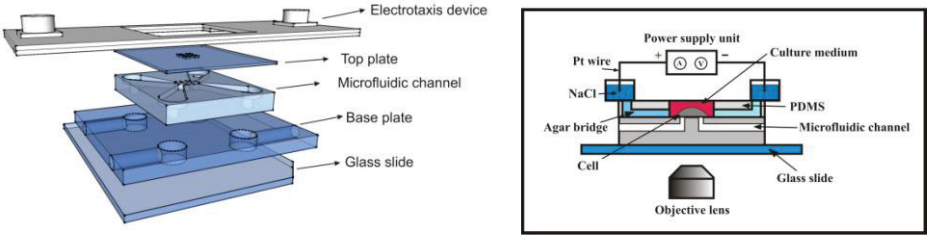


Figure 1 (a) Exploded and (b) Assembly view of p-MEA.

3. Fabrication of p-MEA

3.1. Fabrication of micro- fluidic channel plate

The micro-fluidic channel plate is used for forming the micro-fluidic channels which are filled with a conducting electrolyte medium. The current from the cell membrane is transported to the electrodes

through this medium. Figure 2 shows the four main steps involved in fabricating the channel plate. First, the micro-fluidic channel design is transferred onto a silicon wafer by laser lithography (Figure 2a). The channels are 50 mm in length and have a 40 mm width at the top which tapers down to 10 μm at the bottom. The top end would be aligned directly above the circular electrodes and the bottom end would be below the cell membrane. The second step involves etching the silicon wafer by using a Bosch process (Figure 2b) [3]. By this, a final height of 33 μm is achieved for the channel walls. The third step involves curing of MD-40 polymer material (Solvay Solexis Ltd., Italy) on the silicon wafer for 45 minutes which then is peeled off in the final step to form the channel plate (Figure 2c,d). This channel plate is used as a mould for fabricating the channels in the p-MEA as discussed in the next step.

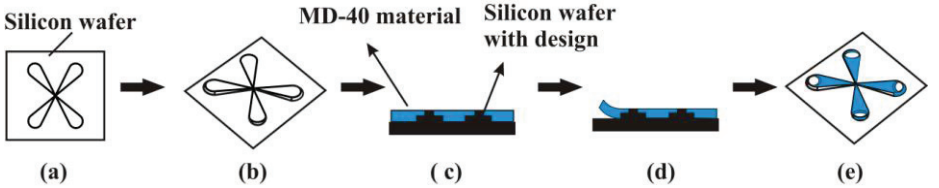


Figure 2: Fabrication steps of micro fluidic channel plate

3.2. Fabrication of the base plate

The base plate consists of a glass slide, a polydimethylsiloxane (PDMS) layer with holes for suction and the micro-fluidic channels. The PDMS and curing agent used for the base plate is prepared with a ratio of 10:1. The PDMS layer between the micro-fluidic channels and the glass slide has holes on the side for incorporating suction. The suction is used for creating a giga-ohm seal between the cell membrane and the top plate. The PDMS is cured at 100 °C for 1 hr. The micro-fluidic channel plate is disassembled to form the base plate with PDMS layer and the micro-fluidic channels.

3.3. Fabrication of the top plate

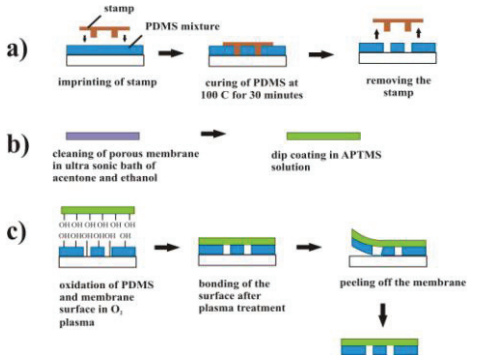
Figure 3a shows the fabrication process of the top plate. A stamp made of MD-40 material is used for imprinting into PDMS mixture of 10:1 ratio. PDMS mixture is cured for 100 °C for 30 minutes. The stamp is removed to form the base of the top plate. A 3 μm pore size membrane (polyethylene terephthalate membrane, Millipore) is dip-coated with 3-aminopropyl trimethoxysilane (APTMS) for 1 minute. Membrane treatment with APTMS is required for good bonding [4]. Both APTMS-coated membrane and PDMS layer are treated in oxygen plasma (500 W, 20 Pa, 90 seconds). Oxygen plasma creates OH bonds on the PDMS surface [5] which are favourable for bonding to the membrane as described in our previous work [6]. Then both the PDMS layer and the membrane are bonded by pressing each other.

In the final step, the stamp is peeled off manually to create the top plate. The final process in fabrication involves aligning top plate and base plate. This is done by using a manual x-y-aligner. The plasma treatment of the top plate ensures good bonding and is effective for 4-5 hours [5]. A cell culture chamber made of PDMS is placed on top of the top plate where the cells can grow in a nutrient-rich medium. The agar bridge setup is placed on top of this cell culture chamber to electrically stimulate the cells.

4. Immunofluorescence study of 3T3 fibroblast cells

Immonufluorescence study was carried out on 3T3 fibroblast cells to test the bio-compatibility of the membrane. The cells were cultured for a period of 24 hrs. The extensions of focal adhesions are strongly related to the strength of cell-material interaction. To observe the focal adhesion of 3T3 cells on the membrane, we incubated the cells with an antibody against human vinculin overnight at 4°C. In Figure 4,

it can be seen that the cell adheres well with the membrane and it is homogenously spread out. A high extent of spreading of cells implies a good cell-material interaction. To study the cell shape the cells were incubated with tetramethyl rhodamine iso-thiocyanate. Detailed fluorescence labelling procedure can be found in our previous work [7]. The 3T3 cells were able to adhere and grow on the membrane homogeneously which makes the membrane biocompatible.



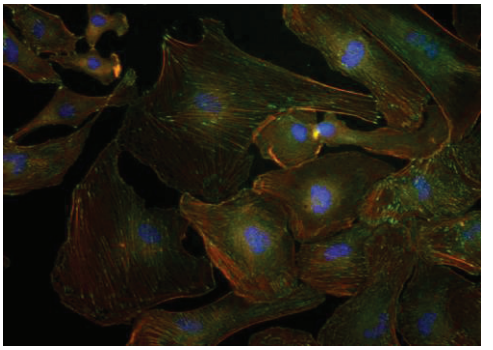


Figure 3: Fabrication steps of the topplate. Figure 4: 3T3 fibroblast cell morphology. Cells were labelled for adhesion marker proteins: vinculin (green), actin cytoskeleton (red), nucleus (blue). Scale bar 50 µm.

Conclusion

This paper describes the fabrication and working principle of a p-MEA for measuring ionic membrane currents from cell membrane. The disadvantages of a patch clamp technique such as damage to the cell membrane and skilled labour requirement can be overcome by this technique. The cell membrane is not destroyed and it is easy and simple to operate unlike the patch clamp technique. 3T3 fibroblast cells show good adhesion and growth on the device which implies the device is biocompatible. Future work will include measuring the impedance of the p-MEA and testing of gigaohm seal. In vivo measurements of ionic currents from the cells will be conducted.

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References

[1] Levin, M, Large scale biophysics: ion flows and regeneration, *Trends Cell Biol* 2007;**6**:261-270.
[2] Walz, W., 2007. Neuromethods, Patch-Clamp Analysis, 2nd ed. New Jersey: Humana Press;2007.
[3] Richter, K. *el al*, Positive etch profiles in silicon with improved pattern quality, *Plasma Process Polym* 2006;**4**:882-886.
[4] Derix, J. et al**,** Porous polyethylene terephthalate membranes in microfluidic applications, *Phys Status Solidi A* 2008;**206**:442-448.
[5] Ginn, B. T. *el al*, Polymer surface modification using microwave oven generated plasma. *Langmuir* 2003;**19**:8117-8118.
[6] Derix, J. *et al*, Biocompatible DC microelectrode array, ESTC 2008, 2nd Electronics System integration technology Conference, Greenwich, UK. Proceedings, 1, IEEE 2008, 441-445.
[7]A. Aryasomayajula, J. Derix, S. Perike, G. Gerlach, R. H. Funk, DC microelectrode array for investigating the intracellular ion changes, Biosens Bioelectron 2010;**26**:1268-1272.