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Performance of a thin film microelectrode array for monitoring electrogenic cells in vitro

A. Mohr[a], W. Finger[a], K.J. Föhr[a], W. Göpel[b], H. Hämmerle[a], W. Nisch[a,*]

[a]*Natural and Medical Science Institute, University of Tübingen, Eberhard-Strasse 29, D-72762 Reutlingen, Germany*
[b]*Institute of Physical and Theoretical Chemistry, Center of Interface Analysis and Sensors, University of Tübingen, Auf der Morgenstelle 8, D-72076 Tübingen, Germany*

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Abstract

A planar array of microelectrodes has been developed for monitoring the electrical activity of electrogenic cells in a cell culture by an extracellular recording mode. The design, the fabrication technique, and the characterization of the array by impedance measurements is described. The device is applied to monitor extracellular signals produced by cultured chicken embryo cardiac myocytes.

Keywords: Microelectrode array; Extracellular recording; Cell culture; Cardiac cells

1. Introduction

Monitoring signals of electrogenic cells in vitro over extended periods of time is of principal interest, e.g. for investigations of neuronal networks and their changes in function, as they may be induced by external (chemical, physical) and internal parameters [1]. A particularly suitable technique for monitoring simultaneously the activity of several electrically active cells is to record extracellularly spike activity with a planar microelectrode array integrated into the bottom of a tissue culture chamber. Several different designs of microelectrode arrays have been developed in this context [1–7]. This paper describes a new design and the application of such a thin film microelectrode array. It was produced by conventional semiconductor fabrication techniques. The microelectrodes were characterized by impedance measurements, and the spatial variation of the electrical response characteristics of the platinized microelectrodes was evaluated in detail. Finally, results from extracellular recordings are described. The electrical signals were produced by cultured aggregates of cardiac myocytes of chicken embryo.

2. Design and fabrication

The device consists of an array of 60 gold microelectrodes, covered with platinum black, on a glass substrate. The circular electrodes have a diameter of 10 μm. They are arranged in an 8 × 8 matrix (missing electrodes at the corners) with a distance of 100 μm from tip to tip (Fig. 1b). The insulation layer is made of silicon nitride. The recording area of the microelectrodes is enclosed in a glass ring which forms the chamber for the cell culture (Fig. 1a). The thin film microelectrode arrays were produced by conventional semiconductor fabrication processes. Pyrex glass (Corning 7059, 2 × 2 inches) was used as a substrate, and gold was applied as an electrode material. To improve the adhesion of the 300 nm gold coating, a layer of 10 nm Cr was deposited first. The gold was patterned using standard photoresist (Shipley S1400/31) and was etched in a solution of I/KI. The Cr was etched in 1% HCl solution. This resulted in a pattern of gold conductors extending from connector pads at the edge of the glass plate to the electrode matrix at the centre. After removal of the photoresist (Shipley Remover A140), the insulation layer of Si_3N_4 (500 nm) was evaporated onto the whole surface of the substrate by a PECVD-process. Reactive ion etching (RIE) was used to pattern the insulation layer and to create openings above the tips of the conductor and for the connector pads. The arrays were

* Corresponding author.

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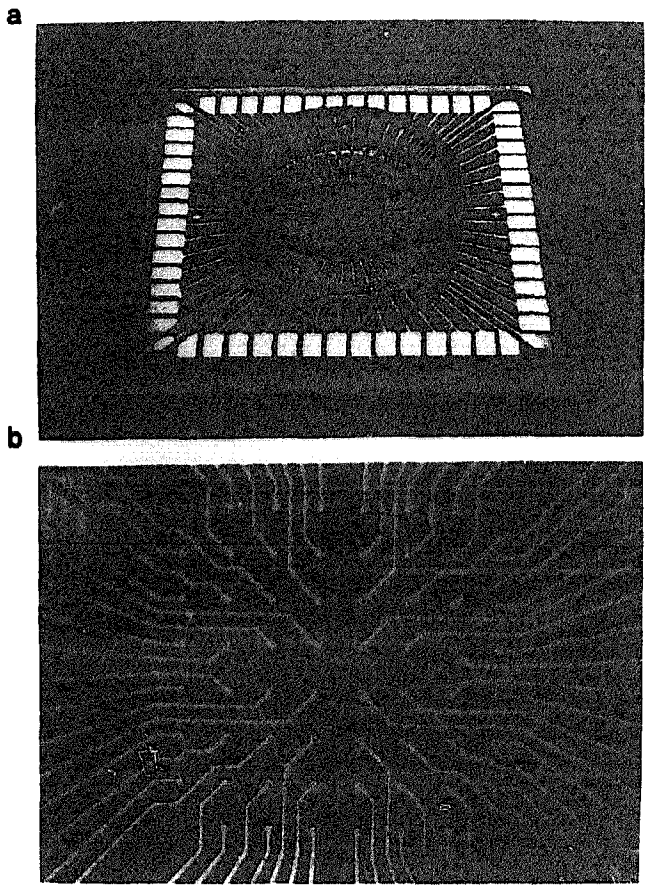


Fig. 1. The microelectrode array: the complete recording device (a) and the recording area on a finer scale (b).

O_2-plasma-etched to produce a hydrophilic surface. For details of the experimental setup, see [5,6].

3. Impedance measurements and platinization

To improve and homogenize the impedances Z of the microelectrodes, they were platinized subsequently. Impedance measurements were performed with a Hewlett Packard 4284 precision LCR meter at a constant frequency of 1 kHz and a 100 mV signal amplitude. A programmable multiplex unit connected to the array holder switched each one of the 60 microelectrodes to the LCR meter. For impedance and simulation measurements, we used 0.90% NaCl solution (physiological saline) at 22°C. Kohlrausch's solution (3% $PtCl_2$, 0.025% lead acetate) was used for platinization. Before the deposition of the platinum, typical values of $|Z|$ at 1 kHz were 4 MΩ. By electroplating of platinum, these values were adjusted to 400 kΩ (±10 kΩ). Impedances around 400 kΩ appeared to be the best compromise between the required low electrode impedance for high signal/noise recording and the stability under bioassay conditions of the platinum layer formed on the electrodes.

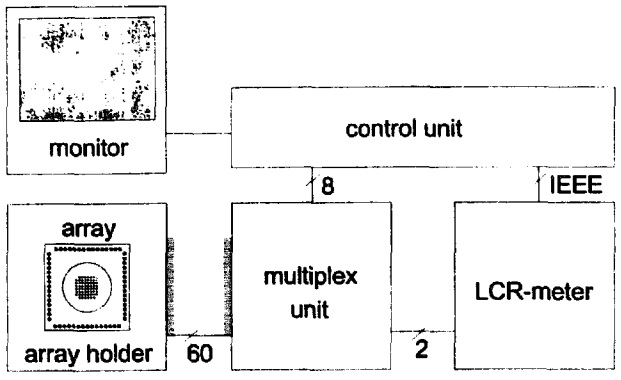


Fig. 2. Experimental setup for measuring the spatial sensitivity of the microelectrodes.

4. Simulation measurements

The influence of the geometric arrangement between cell and electrode on the extracellular recording was simulated by a spatial variation of externally applied local electric fields. For this purpose, a sinusoidal signal (1 kHz) was applied across the barrels of a double-barrelled glass micropipette (theta-style) to mimic the action potential of the cell (Fig. 2). An x,y,z-piezo actuator with a maximum lateral shift of 100 μm moved the micropipette over a single microelectrode. The response characteristics of the electrode was measured as a function of the vertical and lateral distance between this micropipette tip and the microelectrode in saline solution. The detected signal amplitude reached the noise level at a vertical and lateral distance of around 20 μm between micropipette tip and microelectrode (Fig. 3). Fig. 3 illustrates that cells which are electrically detected with one microelectrode cannot be detected at the neighbouring electrode unless parts of the cell cover both electrodes.

To maximize the amplitude of a detected extracellular

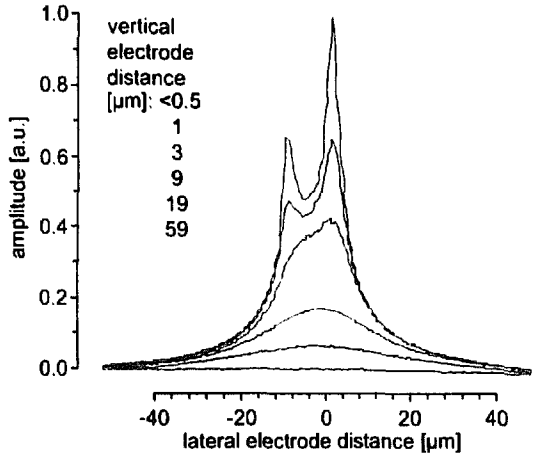


Fig. 3. Amplitude of the detected signal as a function of the vertical distance between micropipette tip and microelectrode. For details, see text.

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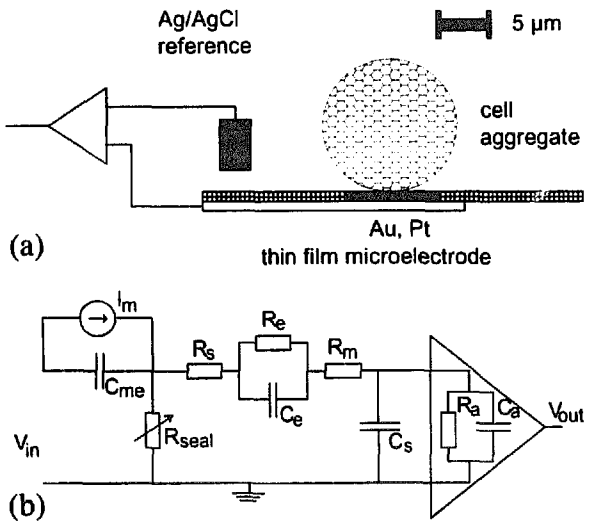


Fig. 4. Experimental setup for extracellular recording with the microelectrode array, (a) the extracellular recording device and (b) the electronic circuit diagram.

potential, the cell should evidently be positioned as close as possible to the microelectrode. In the future this will be optimized even better by the careful choice of cells, substrates, and, in particular, by arranging adhesion molecules between both. The goal of this effort is to achieve a lateral resolution which leads to additional analytical information only if the attachment of the cells to the electrodes has comparable extracellular impedance values between the membrane and the electrode.

5. Extracellular recording

The experimental setup for extracellular recordings on cell ensembles is depicted in Fig. 4. As a model system, a cardiac cell culture was taken from 11-day chicken embryos (E11). This choice was motivated by four reasons: first, embryonal cells can be kept alive much easier than adult cells. Second, cardiac cells form aggregates which

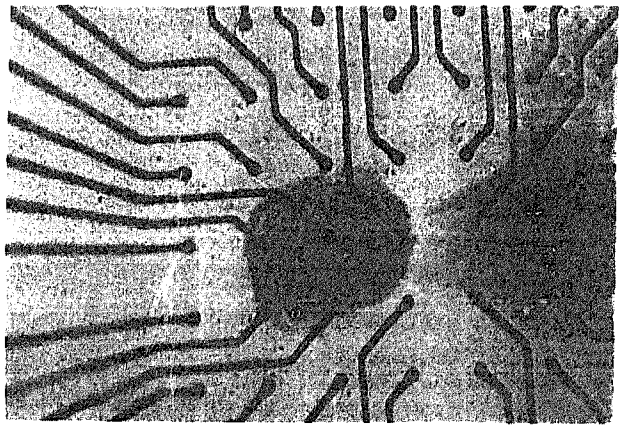


Fig. 5. Aggregate of heart muscle cells at the microelectrode array.

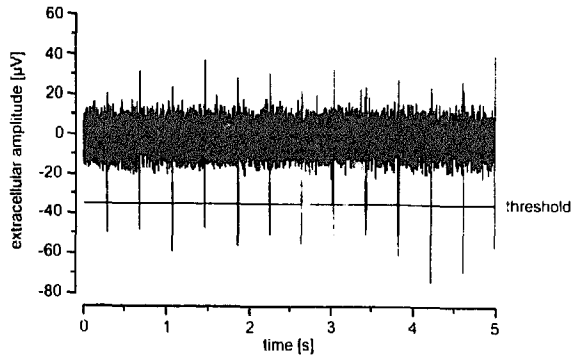


Fig. 6. Extracellular spikes of heart muscle cells measured with one electrode of the microelectrode array.

can be handled easily. Third, the whole aggregate generates a higher common action potential which can be monitored easily (about the nature of the action potential see, for example [8]). This aspect is of particular importance if one considers the comparatively small signal amplitudes of individual nerve cells [5]. The fourth reason is that the influence of drugs on heart cell activity continues to be an interesting topic for particular applications.

After 1 day of cultivation the dissociated heart muscle cells form aggregates if they are kept in suspension. Aggregates cultivated for 4 days were used for recording. In this stage of cultivation the aggregates consisted of up to 500 cells with aggregate diameters of up to 200 μm. For electrical recordings a suspension of the aggregates was transferred to the multi-electrode array. Adhesion of the aggregates was complete within about 1–2 h of incubation (Fig. 5). The extracellular recordings were performed at 37°C in a solution which contained (in mM): 136.9 NaCl, 2.68 KCl, 1.84 $CaCl_2$, 1.03 $MgCl_2$, 5.5 D-glucose, and 10 HEPES. The pH was adjusted to 7.3. A single pulse shows a peak to peak amplitude of about 120 μV (at the electrode where it is attached best) and a duration of about 1 ms. Interspike intervals are in the order of 0.4 s.

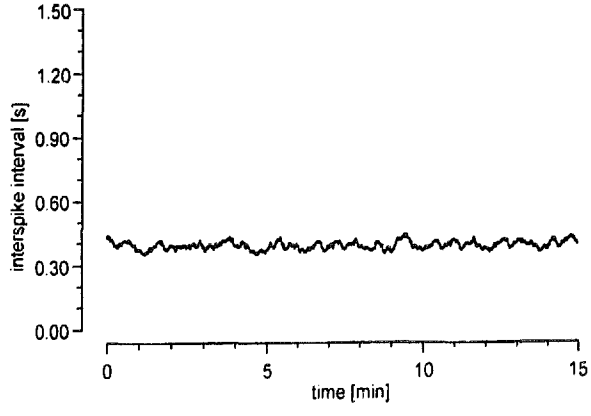


Fig. 7. Long term control measurement of the interspike interval of the heart muscle cells.

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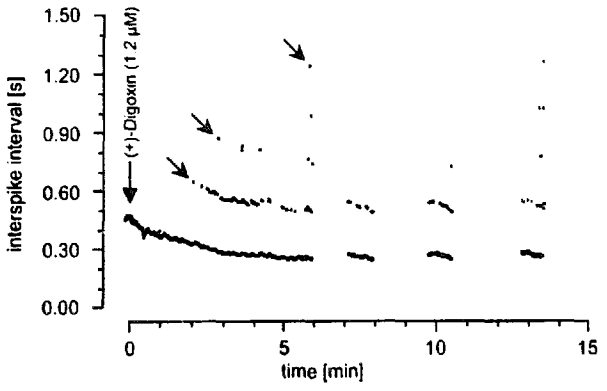


Fig. 8. Interspike interval of heart muscle cells with a heart stimulating drug.

The spike pattern of the aggregate was fairly constant with an average frequency of about 2.5 Hz (Fig. 6).

In order to estimate the influence of heart active drugs we had to gather knowledge about the long time behaviour of the cardiac cell aggregate. We achieved this by introducing a threshold into the measurement to reduce the amount of 200 kB/s raw data. Due to this threshold we only recorded spikes of an amplitude greater than 37 μV. This leads to an extension of the time scale up to 15 min. For this time interval in Figs. 7 and 8 the variation of the time distance between two spikes is shown.

In Fig. 8 the effect of the heart-active drug digoxin on the spike pattern is presented. Digoxin, a blocker of Na^+/Ca^{2+}ATPase, gives rise to a continuous increase of the spike activity, i.e. the interspike interval is decreased. After 6 min the increase of spike activity is blocked completely for a period of about 1 min. Previous to the activity block some spikes skipped as indicated by the spots representing multiple spike intervals (marked by arrows). This arrhythmic behaviour is most prominent immediately before the activity blocks occur.

6. Conclusion

The present approach to record electrical signals by multi-electrode arrays proves to be a useful tool to monitor and analyze drug effects on electrical activity from cultivated cardiac cells.

Acknowledgements

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Biographies

Wolfgang Göpel received his Ph.D. from the University of Hannover in 1971. After visiting scientist positions at the Xerox Palo Alto (CA), Xerox Webster (NY) and IBM Watson (NY) Research Centers he was appointed as Full Professor of Physics at the Center of Surface and Submicron Analysis (Bozeman, MT, USA). Since 1983 he has been the Department Head of the Institute of Physical and Theoretical Chemistry and the Director of the Center of Interface Analysis and Sensors at the University of Tübingen. Main research interests (370 original papers) include interface properties of new materials for chemical sensors, catalysts and microelectronic, molecular electronic and bioelectronic devices.

Armin Mohr graduated in 1995 in biophysics at the University of Tübingen. Most of his work was done at the Natural and Medical Science Institute. His work includes the signal transduction of electroactive cells like tectum opticum cells, nerve cells and cardiac cells. Furthermore he built up a microelectrode array for extracellular recording.

Hugo Hämmerle graduated in biology in 1982 at the University of Holhenheim and received his Ph.D. in 1985 at the University of Tübingen, where he worked as a scientific assistant until 1987. In 1987 he moved as a group leader to the NMI at the University of Tübingen. From 1990–1995 he was head of the Department of Biology. In 1995 he became Vice President and head of the Division of Life Sciences at the Natural and Medical Science Institute (NMI). His research includes multichannel recording of neural network activity with 60 microelectrode arrays, growth changes and intracellular Ca^{2+} oscillations of diploid osteoblasts and fibroblasts induced by extreme low frequent electromagnetic fields and establishment of a co-culture with lung cells as in vitro model of the blood–air barrier.

Wolfgang Finger is electrophysiologist and graduated

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at the University of Mainz in 1974. At the Department of Physiology of the Technische Universität München he received his Ph.D. in 1979, habilatated in 1984, and became a lecturer for physiology in 1985. In 1988 he moved to he NMI at the University of Tübingen and built up the Department of Biophysics. He is now head of this department and his research mainly includes signal transduction processes in among nerve cells.

Karl Josef Föhr graduated in biology in 1986 and received his Ph.D. in 1990 at the University of Ulm. In 1991 he moved as research assistant to the Department of Biophysics at the NMI, a research institute associated with the University of Tübingen. His research mainly includes patch-clamp recording from nerve and muscle cells and the measurement of intracellular Ca^{2+} transients with fluorescence microscopy.

Wilfried Nisch graduated in physics in 1982 at the University of Tübingen and received his Ph.D. in 1987 at the Institute of Applied Physics, University of Tübingen. In 1987 he became a group leader to the NMI at the University of Tübingen. Since 1990 he has been head of the Department of Applied Physics. His research includes thin film technology, microstructure technology and analysis, electron microscopy, physical electronics and bioelectronics.

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