Perforated Microelectrode Arrays Optimize Oxygen Availability and Signal-to-Noise Ratio in Brain Slice Recordings

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Abstract

Complementing single electrode recordings, passive, substrate-integrated thin-film microelectrode arrays (MEAs) have become established tools to investigate spatio-temporal patterns of electrical activity and neuronal interaction in-vitro. In the neurosciences, acute brain slices with accessible and well-preserved neuronal microcircuitry have become the most widespread preparation, that can also be recorded with MEAs for spike activity and local field potentials. Oxygen and nutrients are, however, supplied to the slice tissue by diffusion only (usually from one side) and can may become limiting for tissue stability viability, and slice thickness. Since MEAs record on the face of the slice not directly exposed to the continuous stream of buffer, this might become critical. We therefore developed and compared solid and perforated MEAs for extracellular recording and stimulation, the latter to provide a second exchange surface. For each array we determined the depth profile of the local O_2-partial pressure (pO_2) in cerebellar brain slices. On impermeable MEAs, pO_2 decreased linearly with depth in the tissue. Added diffusion through the perforated MEA surface decreased the slope of the pO_2 gradient and the minimum level reached within the tissue. In addition, signal-to-noise ratios (SNR) in the recordings increased. Improved supply also allows thicker slices and thus the preservation of larger networks, i.e. more complex and in-vivo-like networks. Furthermore, drug accessibility to the recorded cells is improved, accelerating dose-response studies.

Introduction

Bio-Microsystems technology introduces new techniques for the investigation of cellular signals that yield information otherwise not or not easily accessible, with increased efficacy for routine investigations in clinical and pharmaceutical drug discovery. Miniaturization, the integration of signal conditioning circuits, the potential for multi-sensor devices and mechanical robustness open new options for biomedical research. Numerous projects have demonstrated the potential for clinical and industrial applications. Besides sensors probing molecular analytes, electrophysiological studies profit from MST developments.

Complementing single electrode approaches for intra- and extracellular recordings, microelectrode arrays have been developed to investigate spatio-temporal patterns of electrical activity and cellular interaction in excitable tissues in-vitro. Microelectrode arrays were shown to be particularly useful in studies of neuronal and of cardiac tissues and cells, either in culture systems or as acute preparations [1].

These devices for extracellular recordings consist of either silicon-based integrated circuits with biocompatible housing or of passive, substrate-integrated thin-film microelectrode arrays (MEAs). The latter, compared to the former, are technically less complex and have become commercially available in various configurations since several years. A further advantage of such microelectrode arrays is their compactness, reducing the need for extensive shielding, mechanical stabilization and micromanipulation of individual electrodes.

In the neurosciences, acute brain slices with accessible and well-preserved neuronal microcircuitry have become the most wide-spread preparation for in vitro studies seeking to investigate cellular and circuit properties, as well as their modulation by neuroactive drugs. They are used to study the dynamics of activity in neuronal networks, synaptic plasticity, pathological conditions such as epilepsy and for high-content drug assays in pharmaceutical research.

Typically, brain slices are prepared freshly for each experiment at 300-450 µm thickness and require efficient superfusion of buffer to supply oxygen, carbon dioxide, and pH-buffering. They can be maintained in a submerged configuration, i.e. with buffer streaming above the slice, or in an interface

configuration, with buffer supply from below only and exposure to humidified carbogen gas (95% O_2/5% CO_2) from above. Submerged slices can be homogeneously exposed to the buffer stream, and to drugs supplied via the buffer, and are thus more widespread for pharmaceutical testing. In such preparations, oxygen and nutrients are, however, supplied by diffusion only (usually from one side) and are therefore considered limiting for tissue stability and viability, and slice thickness. Data on O_2-availability within the tissue was, however, not available until now.

Since MEAs record on the face of the slice not directly exposed to the continuous stream of buffer, diffusion rates and distances are important. Increasing the exchange surface would stabilize the recording conditions, in particular for tissues with high metabolic activity, e.g. under elevated temperatures or application of excitatory drug. Wash-in and wash-out times for drugs under test would decrease.

One previous report indicated extended tissue survival on perforated MEAs [2], though without supplying further data on the limiting factors. Following this lead, we developed three types of MEAs and investigated the O_2-supply: (1) no perforation (60 TiN electrodes on glass, **Fig. 1A**) [3, 4], (2) perforated carrier with 4% perforation area (60 TiN electrodes on polyimide, **Fig. 1B**) and (3) perforation with 28% exchange surface (20 TiN electrodes on polyimide, **Fig. 1C**). For each array we determined the depth profile of the local O_2-partial pressure (pO_2) in cerebellar and hippocampal brain slices under varying perfusion conditions.

Methods

Perforated MEAs (pMEA) were produced with thin-film photolithography on polyimide membranes (6 µm thick), which was glued to a glass or polycarbonate carrier with Sylgard (Dow Corning). pMEAs are described in more detail in the companion report (Stett et al., this issue). pMEAs with 4% exchange surface (MEA type 2) had the same electrodes as impermeable MEAs. pMEAs with 28% exchange surface (MEA type 3) used Ø 30 µm electrodes for recording and Ø 50 µm electrodes for stimulation. All electrodes were coated with columnar TiN to increase the electrode capacitance.

Cerebellar and hippocampal brain slices were prepared as described previously [3]. Briefly, slices of 300 µm thickness (400 µm for hippocampal slices) were prepared from P18-P23 rats and maintained in artificial cerebrospinal fluid. The slices were mounted onto MEAs (rec. electrode Ø 30 µm; Multi Channel Systems, Reutlingen) coated with cellulose nitrate and were superfused with 8 exchanges of carbogen-saturated buffer in the recording chamber per minute (4 ml/min). Spontaneous spike activity was recorded with an MEA1060 system (Multi Channel Systems) at 25 kS/s (room temperature).

Cerebellar slices were placed onto the MEA such that all layers of the cerebellar lobes could be monitored for spontaneous spike activity. Hippocampal slices were positioned to record local field potentials in CA1 in response to and electrical stimulation of the Schaffer collaterals via the MEA electrodes. pO_2 was determined with a fiber-optic probe with a polymeric oxygen sensor (tip diameter Ø 30 µm; presens, Regensburg; **Fig. 2A**) inserted into the tissue.

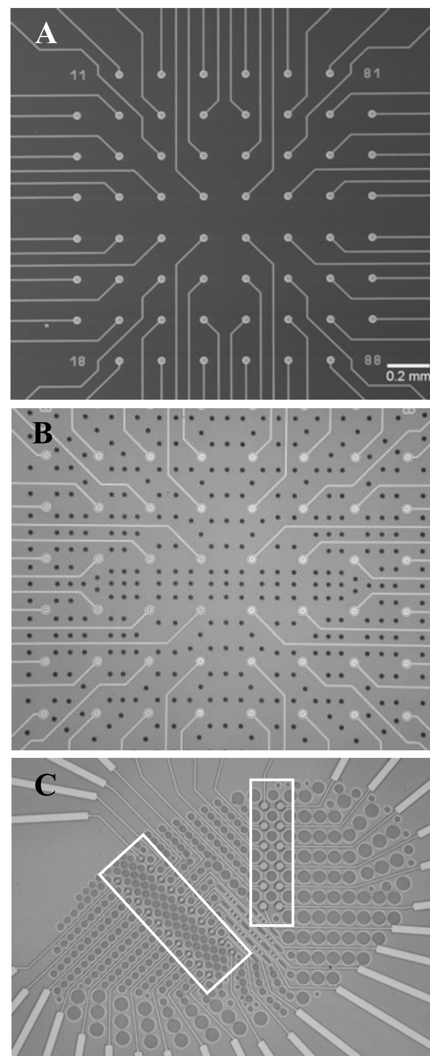

Fig. 1 MEAs with 60 (**A, B**) resp. 20 (**C**) recording electrodes (Ø 30 µm, TiN). Electrodes and leads (Au) are bright; the perforation of the PI foil in B and C is dark. The perforation comprises 4% (B) resp. 28% (C) of the relevant area. Electrode pitch in the recording region is 200 µm (A, B) and 90x200 µm (left frame in C). Separate low-impedance stimulation electrodes (right frame) and an integrated ground electrode were added in C.

Results

Spontaneous neuronal activity was recorded in cerebellar slices with all MEA types without significant differences with respect to spike shape and firing rate. O_2 availability, however, differed considerably and depended on MEA type, temperature, perfusion configuration, firing rate and tissue type.

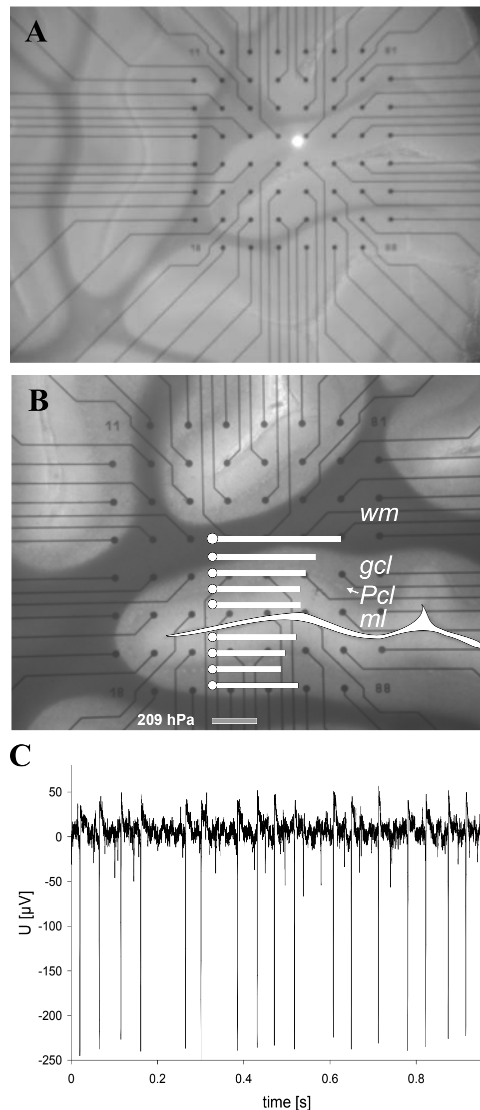

Fig. 2 A Slice on the unperforated MEA (Fig. 1A) with fiber-optic O_2-sensor positioned (bright spot). **B** O_2-partial pressure at different positions (dots) in the tissue indicating lower residual O_2 (short bars) in metabolically more active (light) than in less active regions (dark, longer bars). **C** Besides changing the accessibility for O_2, suction applied through the perforation pulls the tissue closer to the recording electrodes, thereby increasing signal amplitude (sharp peaks) and effectively the SNR. *wm* white matter, *gcl* granule cell layer, *ml* molecular layer, *Pcl* Purkinje cell layer.

On impermeable MEAs (MEA type 1) pO_2 decreased linearly from approx. 800 hPa (95% O_2/5% CO_2 saturated buffer) to 200-400 hPa at 300 µm (electrode level) in the tissue (**Fig. 3A**). Slope and minimal pO_2 depended on the superfusion rate, tissue type (influencing metabolic O_2 consumption) and neuronal activity level (**Fig. 2B**). Blocking neuronal spike activity with tetrodotoxin and thereby reducing the energy needs of the neurons increased pO_2 (data not shown).

Added diffusion through the MEA surface should decrease the slope of the pO_2 gradient and the minimum level reached within the tissue. This was implemented by a second buffer supply (termed subfusion for convenience) that was driven by a low vacuum applied to a chamber below the recording area through which an additional buffer stream was then sucked. Subfusion rate increased with the pressure applied.

With 4% perforation surface in which the tissue was exposed to the subfusion stream in the recording area (MEA type 2), the pO_2 gradient did not change significantly. SNR of single neurons spikes, however, increased greatly to levels rarely found with type 1 MEAs when suction was applied (**Fig. 2C**), presumably because of decreasing distances between cells and electrodes, and thus increasing seal resistance between the recording site and the reference electrode. This exchange surface was, however, sufficient to permit chemical stimulation of neuronal activity by increasing extracellular K^+ to 6 mM.

In contrast, the pO_2 gradient decreased by >50% with 28% perforation area (MEA type 3) and subfusion through a micromachined channel beneath the recording area. Concurrently, the minimal pO_2 increased considerably, absolute values depending on perfusion rate (**Fig. 3B**).

The electrode arrangement of type 3 MEAs is designed to optimally match the structure of slices from the hippocampus of rats and mice. Using this array we could effectively stimulate Schaffer collaterals and elicit synaptic field potential responses in CA1 at SNRs comparable to or better than in recordings with type 1 MEAs. The subfusion option simplified positioning and mounting the slice onto the electrodes.

Discussion

Perforated MEAs thus improve the recording conditions in two respects: they improve the SNR and with suitable layout greatly increase the O_2-supply in slice recordings. Superfusion from one side only creates a linear decrease of the O_2-availability that decreases with increasing temperature of the tissue (data not shown). Sparsely perforated arrays facilitate the re-

cording itself by increasing the SNR when suction is applied to the subfusion channels, which also helps to position the slice accurately. Though suitable to allow chemical stimulation, perfusion via these exchange areas did not contribute sufficiently to pO_2.
The saturation, minimal level and slope of the pO_2 depth profile of type 3 MEAs with optimized exchange area, however, shows an added supply via the perforation.

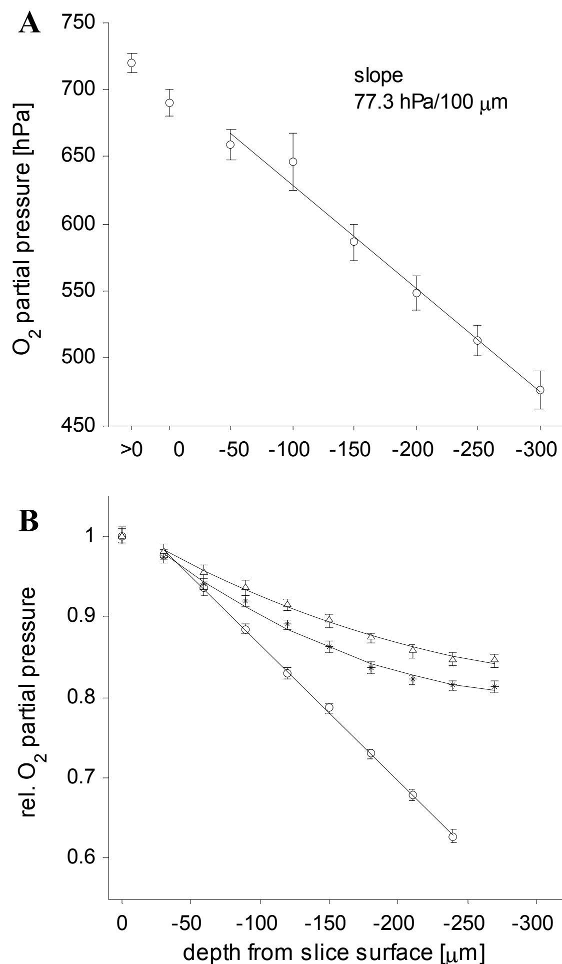

Fig. 3 **A** pO_2 depth profile in slices on unperforated MEAs. Buffer pO_2 is indicated as >0. pO_2 decreases at the nominal surface of the slice already. Within the tissue pO_2 is reduced by >10% of the surface level per 100 μm. **B** Normalized depth profiles for perforated MEAs with 28% exchange surface for different vacuum applied to the lower buffer stream, i.e. increasing perfusion rates. Higher suction increases the flow rate. The slope decreases by about 50% and becomes non-linear when the lower perfusion is turned on. The non-linearity indicates the added supply of O_2 through the MEA. (mean ± SD for 1 s).

Perforated MEAs thus open new venues for network studies and industrial applications: The increased stability of the tissue facilitates long-term studies, including tissue culture work with closed sterile chambers. Improved supply allows thicker slices and thus the preservation of larger networks, i.e. more complex and in-vivo-like networks. Finally, drug accessibility to the recorded cells is improved, accelerating dose-response studies. MEAs with 28% perforation area are produced in a compact microscope slide format (**Fig. 4**), with matching system to record from 4 arrays in parallel. Since hippocampal slices are widely used to monitor synaptic plasticity this will allow an increased throughput in drug screening studies.

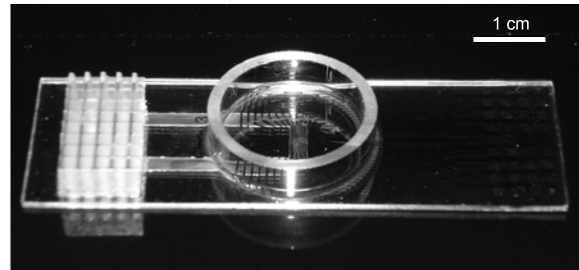

Fig. 4 Overall layout of the type 3 MEA prototype in Fig. 1C. The dimensions of the polycarbonate carrier conform to standard microscope slides. A glass ring centered on the electrodes forms the recording chamber. The perfusion channel covered by the PI film is visible below the ring and is supplied by a pump via the connector to the left.

Acknowledgements

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