Fully integrated 128-electrode CMOS chip for bidirectional interaction with electrogenic cells

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Abstract: A monolithic CMOS microelectrode array (MEA) for stimulation and recording of electrogenic cells is presented. The chip features addressability of an arbitrary electrode subset for stimulation, complete on-chip signal conditioning and A/D conversion. The array has been fabricated using an industrial 0.6-µm, 5-V CMOS technology with a 2-mask post-CMOS processing. Measurements from neuronal and cardiac cells are presented.

Keywords: CMOS microelectrode array, extracellular, electrogenic cells

Introduction

Microelectrode arrays (MEA) have become an important tool for non-invasive extracellular recording in the fields of neuroscience and biosensing [1,2]. Artificial neural networks on MEA can be trained to perform, e.g., pattern recognition [2]. Additionally, the ability to monitor the electrophysiological response of a cell, or cell culture, to a pharmacological agent in vitro extends the application area to pharma-screening. Typical system designs encompass an MEA with external signal conditioning electronics and a system control that is realized by discrete off-chip components [1]. The array size of these MEAs is limited to the number of available interconnects. Approaches which address the connectivity problem by using on-chip buffers and multiplexers are presented in the literature [3,4,5]. However, in order to analyze network functioning of neural or cardiac cells, a tool with a large number of stimulation and recording electrodes is required. Here we present a single-chip system that addresses these requirements. An 8-by-16 array of metal-electrodes is integrated on a CMOS chip. Complete on-chip signal conditioning, i.e., filtering, amplification and A/D-conversion, is provided. For stimulation, an arbitrary subset of the array can be selected.

System Description

Figure 1 shows a micrograph of the monolithic CMOS microsystem. The 6.5 x 6.5 mm² chip comprises an 8 x 16 array, each electrode of which is capable of both, stimulation and recording. A reference electrode is integrated on chip as well. The system is structured in a modular design (Figure 1 and 2). Each “pixel” of the MEA incorporates the signal transducing electrode, a fully differential band-pass filter for immediate signal conditioning, a mode storage and a buffer for stimulation. Implementing filters and buffers at each electrode offers important advantages in comparison to other CMOS MEAs published previously [3,4,5]: (i) The signal is amplified and filtered in close vicinity to the electrodes, which makes the design less sensitive to noise and distortion picked up along connection lines; (ii) a stimulation buffer makes the stimulation signal independent of the number of activated electrodes; (iii) the high-pass filter (HPF in Figure 2) removes offset and slow drifts of the biochemical signals and, thereby, allows for immediate signal amplification; (vi) the low-pass filter (LPF) limits the noise bandwidth and acts as an anti-aliasing filter for subsequent A/D-conversion.
All in-pixel circuitry components are optimised for low noise and small area. A total equivalent input noise of the pixel circuitry of 5.9 µV_{RMS} (10 Hz to 100 kHz) was measured. The electrodes are continuously read out at a sampling rate of 20 kHz per electrode. An amplification of 1000 or 3000 can be selected. The overall power consumption of the chip is 120 mW at 5 V supply, 20 mW of which are dissipated within the array. Electrogenic cells are very sensitive to temperature so that temperature changes may change cell activity and may even lead to cell death. An on-chip temperature sensor monitors the chip operating temperature. Circuits operating at low frequency (5 kHz down to 1 Hz) are sensitive to leakage currents, the effect of which has been reduced by the fully differential design of the in-pixel readout circuitry. Furthermore, electromagnetic interference is also generally reduced in a fully differential architecture.

An arbitrary stimulation pattern (with a maximum sampling frequency of 60 kHz) can be applied to any subset of electrodes. After stimulation the circuitry at each electrode can be reset (“reset” in Figure 2) to its operating point in order to suppress artifacts evoked by the stimulation pulses from the stimulated electrode itself or from neighbouring electrodes.

A digital control unit is integrated on the chip. It controls multiplexing, electrode selection for stimulation, reset of single electrodes, and it contains the successive-approximation registers of the A/D converters and the interfaces to the outside world.

Fabrication of the 6.5 x 6.5 mm² chip is realized using an industrial 0.6 µm CMOS process (XFAB, Germany). After the CMOS process, a 2-mask post-processing procedure is performed to cover the Al electrodes with biocompatible platinum. Details on the procedure are given in [6]. A pitch of 250 µm was chosen for the pixel unit. But the electrode pitch, size and shape is very flexible, since the electrodes are realized during post-processing. The processed chip is mounted on a ceramic package (Figure 3). Then poly (dimethyl siloxane) (PDMS) is used together with a glass ring to form a bath for the nutrient medium.

Figure 2: Schematic representation of the system architecture.

Figure 3: Packaged Chip. The processed CMOS chip is mounted on a ceramic package (JLCC84). Poly(dimethyl siloxane) is used together with a glass ring to form a bath for the nutrient medium.
Results

The performance of the chip was tested by sinusoidal excitation (500 Hz, 1 mVpp) of the electrolyte through a platinum wire while recording from all sensor electrodes (Figure 4a). The corner frequencies of the filter were set to 5 Hz and 10 kHz, respectively. Functionality of the stimulation circuitry has been shown by applying a saw-tooth signal to one of the electrodes. Digital commands to select one electrode for stimulation and to generate a saw-tooth stimulation signal on this electrode were sent to the chip. The saw-tooth signal was repeated at a frequency of 1.8 Hz and covered the entire code range (0 .. 255). The positive and the negative reference voltages were set to 1.5 V and 3.5 V. The recorded signal (see Figure 4b) shows good monotony and linearity. This test was repeated for different electrodes and, additionally, a larger number of electrodes. In all cases, the same result was observed.

Neural networks originating from dissociated cortical tissue of fertilized chicken eggs (gallus domesticus) at embryonic day 10 (E10) were successfully cultured on the chip. Signals from spontaneously firing cells after 56 days in vitro (DIV) were recorded (Figure 5). A sterilized elastomer cap with integrated reference electrode was placed over the dish upon recording to keep the cell culture pH constant during the measurement period. For this measurement, the cut-off frequency of the HPF was
set to 10 Hz, and that of the LPF was set to 5 kHz. The noise level in these recordings was 27 \( \mu \text{V}_{\text{RMS}} \).

The signals recorded from 30-\( \mu \text{m}-\)diameter electrodes showed signal amplitudes between 500 \( \mu \text{V} \) and 700 \( \mu \text{V} \), the signals from 40-\( \mu \text{m}-\)diameter electrodes were in a range between 200 \( \mu \text{V} \) and 300 \( \mu \text{V} \). The signal amplitudes are seemingly inversely proportional to the area of the electrodes. In our case, doubling the electrode area leads to about half the signal amplitude, which is in agreement with the models used for electrogenic cells on microelectrodes as described in [7]. The fraction of the electrode area under the cell and the fraction exposed to the culture medium constitute, in a first approximation, a capacitive divider so that the signal amplitude decreases when cells of similar size are placed on larger electrodes.

Primary neonatal rat cardiomyocytes were successfully cultured on the chip. These cells very quickly become electrically active. Recordings from the entire network can be obtained after three days in culture, whereas dissociated neurons show spontaneous spiking after about two to three weeks. A recording of the spontaneously beating cells from one electrode after 5 DIV is shown in Figure 6. In this example, the cells beat at a rate of about 10 Hz. The signals were recorded on a 20-\( \mu \text{m} \) electrode. The lower graph shows one of the spikes at an extended time scale.

In an attempt to demonstrate biosensing, quinidine, a K+ channel blocker, was added to the medium at a final concentration of 48 pmol/l. Even at this low concentration, the addition of the channel blocker led to a cessation in spontaneous beating activity. When the medium was exchanged with fresh medium, the cells recommenced beating. It is believed that it is necessary to externally stimulate the cells in order to study the resultant changes in the action potential upon exposure to a pharmacological substance. Furthermore, since the electrical activity is highly dependent on temperature and pH, it is necessary to perform such testing in a controlled environment, such as an incubator. This highlights the need for bidirectional chip capabilities, as well as a controlled environment that can potentially be achieved by using microfluidics.

**Conclusion**

An extracellular monolithic recording system with 128 platinum electrodes, fabricated in industrial CMOS technology and combined with post-CMOS processing has been presented. The circuitry is realized in a modular design, where a repeatable circuitry unit at the signal transducing electrode comprises a band-pass filter, amplifiers for immediate signal conditioning, and a buffer for stimulation.

Recorded signals from neuronal cultures at 56 DIV and primary neonatal rat cardiomyocytes at 5 DIV demonstrate both, the biocompatibility of the packaged chip and the ability of the chip to measure signals after long culturing periods.

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