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A MINIATURE MICROELECTRODE ARRAY TO MONITOR THE BIOELECTRIC ACTIVITY OF CULTURED CELLS

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SUMMARY

Electrical activity can be recorded extracellularly from contracting heart cells in vitro with the electrodes of 30-element microelectrode arrays built into the culture chambers. The arrays are fabricated in the laboratory by etching thin metal films deposited on glass coverslips; the fabrication employs techniques developed by the microelectronics industry.

Currently available and rapidly improving techniques permit the in vitro culture of an increasing variety of bio-electrically active tissues and single cells [1–9]. Perhaps the most interesting questions to be asked of such cultures are those dealing with the development and plasticity of electrical interactions among the cultured elements (tissues or single cells). Exploration of these questions would be greatly facilitated by a convenient, non-destructive method for maintaining electrical contact with an individual culture, at a large number of points, over periods of days or weeks. This report describes one approach to the development of such a method.

Thin-film microelectrode array

Inherent non-destructiveness and the avoidance of vexing problems of multiple-electrode placement would both be achieved if an array of closely-spaced, extracellular microelectrodes could be made an integral part of each culture chamber. It seemed to us that arrays with many of the desired characteristics could be constructed by etching suitable patterns into thin metal films deposited on glass coverslips and using the slips as culture-chamber bottoms.

This seemed feasible, in part, because photoetching techniques developed by the microelectronics industry can resolve patterns with virtually subcellular dimensions (<10 μm).

The array pattern which we designed as a prototype is shown in fig. 1. It contains 30 electrodes arranged in two rows 50 μm apart; the distance between electrodes within a row is 100 μm. Each electrode consists of a metal line which narrows in successive steps from the periphery of the coverslip to a final width of 7 μm at the center. Two 7 μm slits are etched into an overlying layer of insulation to expose the tips of the electrode lines. Thus square patches, ~ 50 μm^2 in area, form the electrode tips. At the periphery of the slip, each electrode terminates

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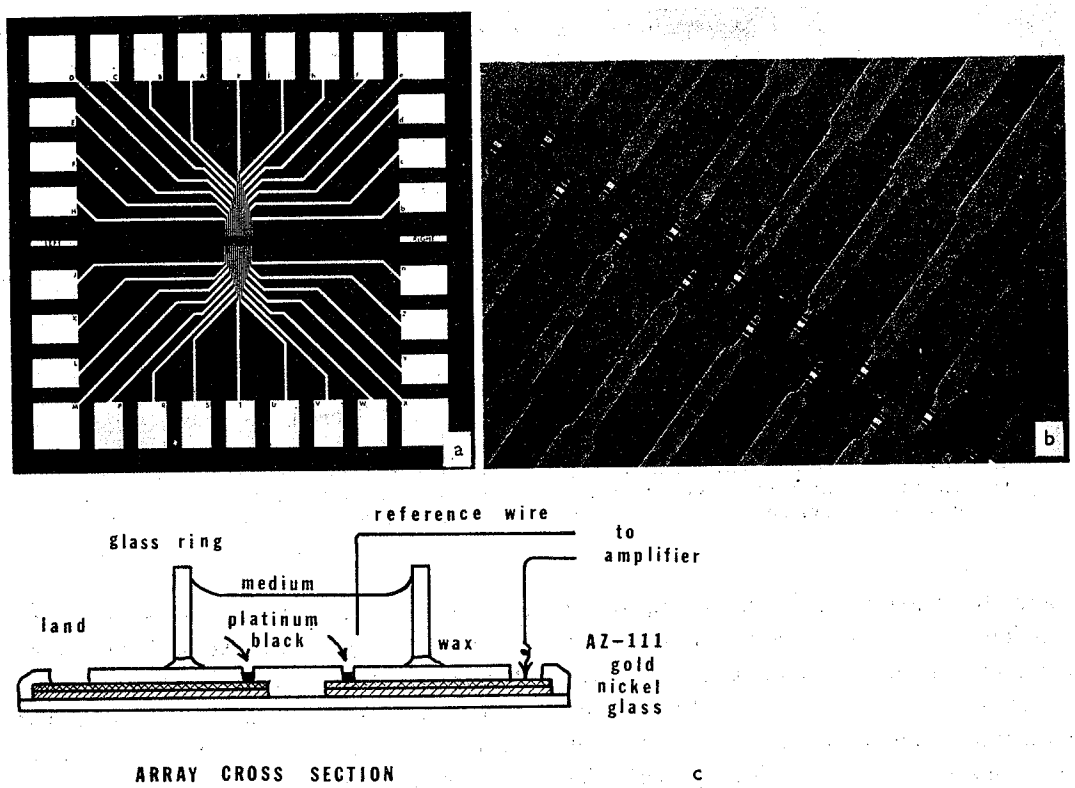

Fig. 1. The microelectrode array. (*a*), The completed array, plan view. A direct enlargement from array pattern masterplate; (*b*), the electrode tips covered with photopolymer insulation. The parallel troughs expose the underlying gold, which is eventually platinized; (*c*), diagrammatic cross-section of microelectrode array forming the floor of culture chamber.

in a large patch, or 'land', which is left uninsulated, to allow convenient connection to associated electronic equipment.

MATERIAL AND METHODS

The arrays were fabricated in our laboratory by a procedure which, briefly, was as follows [10]. Glass coverslips, 25 mm square, bearing vacuum-deposited nickel films (nominally 2 000 Å thick) were coated with an acid-resistant, photosensitive polymer and exposed to light through a photographic negative of the desired pattern. The exposed polymer was then removed by 'development' and the metal thus uncovered was etched away in acid. This left a nickel image of the photographic negative on each coverslip. The nickel was gold-plated and each slip was coated with a second photopolymer layer which served as insulation. The desired openings in this layer were formed by 'developing' the polymer after exposure through a second photographic negative of an appropriate pattern. A glass ring was then affixed to the insulated array with bees' wax, thus creating a culture chamber whose floor contained the permanently-positioned microelectrode tips. In a final step, platinum black was deposited electrochemically on the exposed tips.

During culture incubation, the entire assembly was enclosed in a small Petri dish. For the electrophysiology experiments, the assembly was held in a circuit board that made contact with the electrode lands via spring-loaded pins. In turn, the board was enclosed in a metal box which served as an electrical shield and contained switches permitting various sets of 4 electrodes to be joined, via amplifiers, to a 4-trace oscilloscope. This box was mounted in the mechanical stage of an inverted microscope. Thus it was possible to observe the cell culture and the electrodes from which recordings were being made. A platinum–iridium wire inserted into the culture medium from above was used as the indifferent (or 'reference') electrode during recording. Temperature and atmosphere composition could be controlled during recording, and medium could be changed by a perfusion arrangement.

Electrical properties of array electrodes

The 'small signal' a.c. impedances at the interface between electrode tips and culture medium and the parallel leakage through the electrode shank insulation were determined from direct observation of voltages developed across them during passage of small currents (~ 6 mA/cm^2 tip area). This was accomplished by incorporating the element under test as the feedback impedance of an operational amplifier connected in the 'inverting configuration' [11] and injecting the desired current signal at the amplifier's inverting input. The output of the amplifier was thus essentially equal to the voltage developed across the test impedance by the injected current and could be compared, with respect to phase and amplitude, to the current signal by displaying both on a dual-trace oscilloscope. The system was calibrated by substituting known complex impedances in the feedback loop. For electrode tests, the feedback path was completed by culture medium and the recording reference wire which contributed negligible impedance compared to the electrodes. Metallic resistances of the electrodes were measured directly with a VOM.

Average impedances at 1 kHz for the electrodes of three representative arrays, measured before and after the deposition of platinum black on the electrode tips, are given in table 1 (*a*). Shank leakages and tip impedances, assessed independently of one another by excluding culture medium from, or confining it to, the region of the tips, are given in table 1 (*b*). It may be noted that shank leakage represents an appreciable loss at 1 kHz for unplatinized electrodes but that platinum-blacked tips have sufficiently lowered impedance that the shank leakage may be neglected.

Values of electrode resistance and capacitance calculated from the data in table 1 (*b*), for a commonly employed equivalent circuit are shown in fig. 2. These values are comparable to those reported by others for classical bright metal and platinum-blacked microelectrodes [12–14]. The array electrodes also showed the decline of tip impedance with frequency, but relatively constant phase angle ($\phi \cong \arctan \frac{1}{2\pi} f R_t C_t$ for platinized electrodes represented by the series configuration of fig. 2, which are characteristic of metal microelectrodes. [12–14].

In stability studies, the electrode impedances were found to decrease by 30–50% during

Table 1. (*a*) *Average impedances at 1 kHz of electrodes on three arrays before and after platinization*

Array no.	Platinum blacking	Number of electrodes[a] measured	[Z] av. ±S.D.[b] (mΩ)	∅ av. ±S.D.[b] (degrees)
1001	−	23	1.8±0.5	67±6
1001	+	23	0.4±0.2	57±7
1002	−	21	2.4±0.5	78±5
1002	+	21	0.7±0.5	60±7
1007	−	23	2.2±0.4	69±4
1007	+	23	0.4±0.05	60±2

(*b*) *Average impedances at 1 kHz of separate elements of electrodes on two arrays*

Element	Number of electrodes measured[a]	[Z] av. ±S.D.[b] (mΩ)	∅ av. ±S.D.[b] (degrees)
Array no. 1005 (unplatinized)			
Tips	27	5.9±1.1	77±4
Shanks (leakage)	27	5.1±1.3	78±3
Entire electrode	27	2.4±0.4	72±3
Array no. 1007 (platinized)			
Tips	22	0.4±0.05	64±3
Entire electrode	22	0.4±0.04	64±3

[a] Obviously defective electrodes were excluded; averages before and after platinization involved the same set of electrodes on any one array.
[b] The complex impedance $Z = [Z]e^{-i\phi}$.

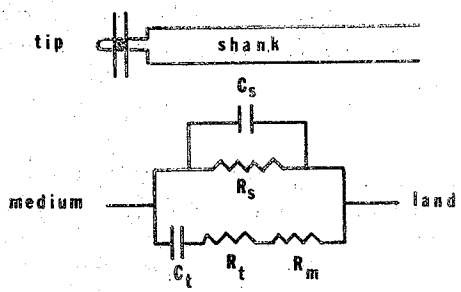

Fig. 2. An equivalent circuit describing the electrical properties of the microelectrodes. Representative values were calculated from the data of table 1 (*b*). R_m, metallic resistance, land-to-tip (~50 Ω); R_t, resistance at tip (1.3 mΩ unplatinized, 0.18 mΩ platinized); C_t, capacitance at tip (28 pf unplatinized, 440 pf platinized); R_s, resistance (leakage) across shank insulation (24 mΩ); C_s, capacitance (leakage) across shank insulation (31 pf).

the first hour of incubation with culture medium and to remain relatively constant for at least 24 h thereafter. Much longer incubation (>1 week) sometimes resulted in deterioration of the insulation layer.

The ability of platinized electrodes to withstand current densities of the order that might be required for extracellular stimulation was tested by application of 0.1 msec rectangular pulses (biphasic) from a Grass Instruments stimulator. Pulses of amplitudes up to 100 μA were passed without significant effect on tip impedances measured at 1 kHz.

Use of arrays in recording from cultured chick heart cells

Chunks of embryonic (6–10 day) chick heart tissue were dispersed with trypsin and $1–3 \times 10^5$ cells placed in each culture chamber using medium and techniques described by DeHaan [9]. This yielded rather dense distributions of cells which soon formed essentially confluent, rhythmically contracting sheets. Triphasic potentials could be recorded from electrodes whose tips underlay such sheets (fig. 2). No activity was recorded from electrodes under cell layers which were not contracting nor from electrodes in completely insulated control arrays (i.e. arrays fabricated without the slits exposing electrode tips). Contraction and recorded electrical activity could be observed as early as 3 1/2 h after the cells were plated. Peak-to-peak amplitudes of the recorded potentials ranged from 0.02 to 2.5 mV. The amplitudes of potentials obtained at a particular site sometimes varied during a recording session, often in a rhythmic fashion (fig. 3*b*), and the potentials recorded by different electrodes in the same chamber frequently differed in amplitude and distribution (fig. 3*a*, *right*). The potentials, each about 2 msec in duration, always appeared correlated with mechanical contractions which were, however, of much longer duration (~100 msec each). Perhaps the most striking feature of the activity was the precise periodicity of the potentials which is evident in slower sweep oscilloscope traces (fig. 3*b*). Periods between potentials were remarkably constant, often varying less than 1% over 20–40 beats. In some cultures, however, frequent syncopation was the rule and in others the interbeat period itself varied periodically [10]. Interbeat period was affected by changes in temperature, pH, and potassium ion concentration [10].

When simultaneous recordings were made from 3 or 4 electrodes in the same chamber, potentials sometimes appeared at virtually the same time (within 0.2 msec) on the different electrodes and at other times lagged one another by fixed intervals of 1–40 msec. These intervals often changed slowly during a recording session.

Efforts to record potentials from electrodes underlying single, contracting cells or small cell clumps have thus far failed. Most likely this failure may be ascribed to the very low extracellular impedance surrounding a single cell, or small aggregate, bathed in culture medium. Our success with contracting

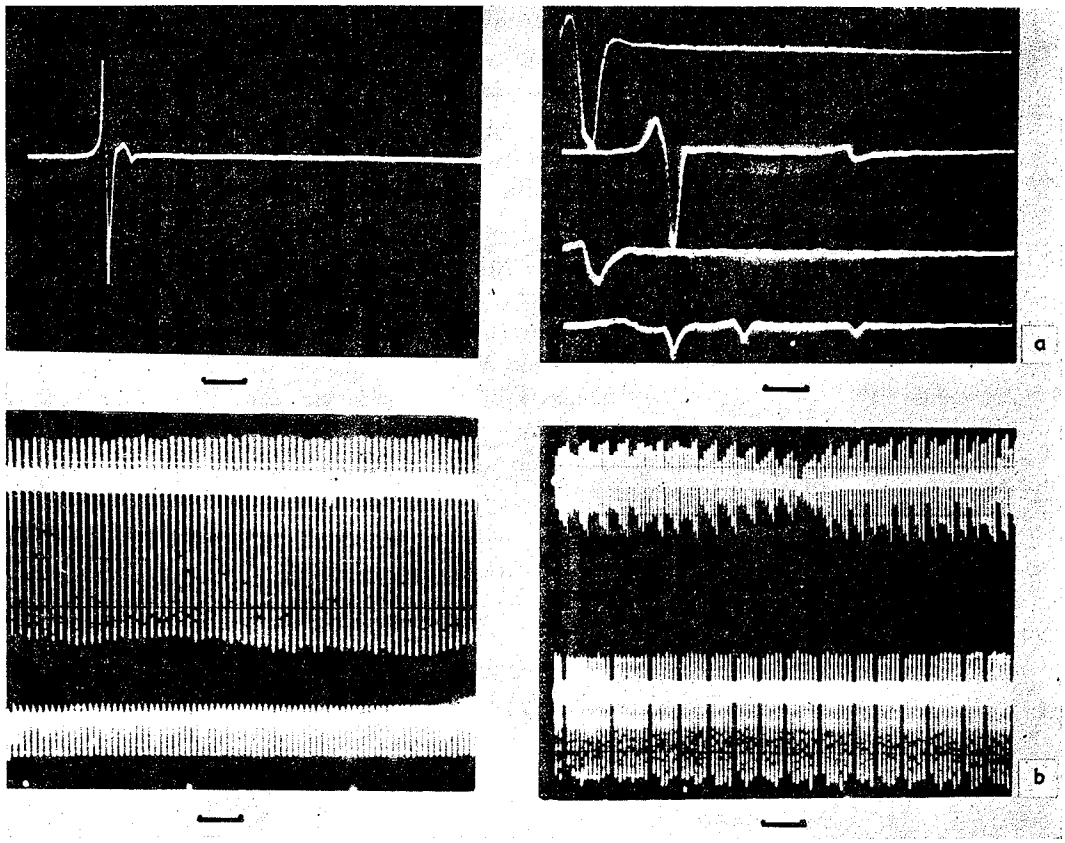

Fig. 3. Some characteristic recordings from cultured embryonic chick heart cells. The bar indicates 1 cm. The writing rate (*a*) (*left*) 10 msec/sec; (*right*) 2 msec/cm, and (*b*), 5 sec/cm. The vertical scale is 0.5 mV/cm in (*a*) except for the lower two traces of (*a*) (*right*) where the scale is 0.2 mV/cm. The vertical scales in (*b*) are 0.05 mV/cm except for the upper trace of (*b*) (*right*) which is 0.10 mV/cm. Note the time lapse between the impulses recorded at four different points in the culture in (*a*) (*right*). Note the change in amplitude of spike and the beat skipping in (*b*) (*right*).

sheets may be owed either to higher current densities produced by the large, synchronously contracting community of cells or to an increased extracellular impedance near the electrodes as a consequence of more intimate or extensive contact with the confluent cell sheet. Changes in the electrode design or a convenient way to increase extracellular impedance during recording may, in the future, make it possible to monitor single cell activity with such microelectrode arrays.

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